NEEDLELESS SHOOTS AND LOSS OF APICAL DOMINANCE IN GREENHOUSE-GROWN LOBLOLLY PINE (*Pinus taeda* L.)

by

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(ABSTRACT)

Loblolly pine that is winter-sown in the greenhouse and spring-outplanted has been observed to exhibit growth abnormalities in the form of multiple apical and needleless shoots. Seedlings that exhibit growth abnormalities are of questionable value in the evaluation of progeny tests. The use growth data from this seedling material could result in biased and erroneus or invalid conclusions about individual tree or family performance.

To determine the causes of growth abnormality development, and to suggest possible remedies, two experiments were initiated. The first experiment examined the effects of raising five Virginia controlled-cross families in two different greenhouses and subsequently outplanting the seedlings on two contrasting sites. The second examined the effects of pre-planting exposure to 0, 4, or 6 weeks of shortened days followed by 0, 400 or 600 hours of chilling and post-planting supplemental water.

Experiment one results indicated that abnormalities were more apparent at the better growing site. Further, pre-planting hardening-off likely increased the dormancy status of the seedlings and somewhat alleviated growth abnormalities. It was determined that families varied in the expression of abnormalities. Abnormalities were only observed during the first summer after outplanting; symptoms were alleviated after overwintering.

Experiment two results indicated that treatments that influenced the dormancy status of the seedlings influenced the development of growth abnormalities. Pre-planting shortened days resulted in increased needles per total stem units for the second flush. Pre-planting chilling and post-planting supplemental water increased apical dominance.

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CHAPTER 1
INTRODUCTION

PROBLEM STATEMENT

Loblolly pine (*Pinus taeda* L.) is, commercially, the most important tree in the southeastern United States, comprising one half of the standing pine volume (Baker and Langdon, 1990). In 1985, 61.9 million acres of the southern United States were classified as pine forest. Of this 61.9 million acres, roughly one third were planted (Brown and McWilliams, 1990), often with genetically improved seedlings.

The majority of loblolly pine genetic improvement work was initiated in the 1950's, and has relied on controlled-cross progeny testing since its inception. Before 1987 most of the loblolly pine controlled-cross progeny testings established in the southeastern United States were sown in a nursery, and raised under regular nursery production regimes. These seedlings were then planted as bare root stock. However, since 1987 most of the progeny test stock has been greenhouse-grown, usually in 10 cubic inch C-10 Leach® tubes. Seedlings raised in this manner were either greenhouse-sown in the winter and outplanted the following spring, or were greenhouse-sown in the summer and outplanted the following fall.

Spring planted, controlled-cross progeny tests conducted by the Virginia Department of Forestry (VDF) have expressed growth abnormalities in the form of multiple apical and needleless shoots (personal communication, Tom Dierauf of the VDF and Peter Wallace of Westvaco Corporation, South Carolina). Mexal and Carlson (1981) have also described growth of needleless shoots and loss of apical dominance in spring outplanted loblolly pine. These abnormalities were attributed to a failure to fulfill the chilling requirement of the seedlings (Mexal and Carlson, 1981).

Description of Growth Abnormalities

Growth abnormalities are not typically observed for most of the summer, and do not develop until August. Abnormalities included the loss of apical dominance, the production of needleless shoots, the

development of shoots with primary (photosynthetic) needles, and the production of shoots with non-photosynthetic scale leaves only (failure of secondary needles to develop). Both the leader and the lateral branches were observed to lack secondary needle growth. Symptoms were often combined, forming needleless "basket whorls". "Basket whorls" often contained a stunted or absent terminal shoot, with long, needleless branches surrounding the stunted, dormant or overgrown terminal shoot (Figure 1-1). After overwintering, seedlings that expressed the growth abnormalities were observed to resume normal growth. However, several competing leaders were observed in the second year.

Cultural Treatment of Seedlings Expressing Abnormal Growth

Cultural practices for the seedlings exhibiting the abnormal growth are, for the most part, standardized within the North Carolina State University - Industry Tree Improvement Cooperative. For the Virginia Department of Forestry, seed is collected in October, and cold stratified for 30 days. In January, seed is sown into fine vermiculite in flats and allowed to germinate on heating pads (24°-27°C). After germination, seedlings are planted into Leach® tubes in a mixture of 1:1 volume:volume peat moss and vermiculite. Fertilizer is applied as Osmocoat® nine month slow release fertilizer (18:6:12 N:P:K) at a rate of 3 grams per liter of soil. Micronutrients are applied with Micromax® fertilizer, at a rate of 1.1 grams per liter of soil.

In the Virginia Department of Forestry greenhouse in New Kent, VA, Banrot® is applied weekly to prevent damping off, until seedlings harden-off. Water is supplied as needed, by misting. Daylength is set to mimic summer conditions, starting at 13:45 hours, increasing to 14:45 hours by February 26, and decreasing to 13:45 hours by April 16. This lighting regime was instituted by the VDF to overcome growth abnormalities in the form of multiple apical and needleless shoots that developed under daylengths of 16 hours.

In mid-April, seedlings are placed outside in a seed orchard (approximately 70 percent full sun), with continued irrigation. In early May, seedlings are outplanted. Virginia Department of Forestry seedlings are outplanted on the Appomattox-Buckingham State Forest, in Appomattox, Virginia on a site that has been clearcut, chopped and burned. Competing vegetation is controlled with a mixture of Oust® and Roundup®.

Figure 1-1. Commonly observed abnormalities, including loss of apical dominance and needleless shoots. This seedling was photographed in August of the first growing season. Seedlings are winter-sown in the greenhouse and outplanted in the spring.

JUSTIFICATION AND OBJECTIVES

Seedlings that exhibit growth abnormalities are of questionable value in the evaluation of progeny tests. The use or exclusion of growth data from this seedling material could result in biased and erroneous or invalid conclusions about individual tree or family performance. By determining the causes of the observed abnormal growth, procedural changes may be developed to reduce their incidence. In addition, by understanding the factors which influence abnormal growth, a conceptual model may be generated to explain its occurrence.

The specific research objectives were:

(1) To determine the influence of genetics, moisture regime, chilling, daylength and the interaction of these factors on the growth and development of containerized, greenhouse-grown, loblolly pine seedlings.

(2) To determine the influence of genetics, greenhouse of origin, planting site, and the interaction of these factors on the growth and development of containerized, greenhouse-grown, loblolly pine seedlings.

(3) To examine the physiological and morphological differences between nursery-run (1-0), loblolly pine seedlings and containerized, greenhouse-grown, loblolly pine seedlings.

(4) To develop a conceptual model explaining the presence of the observed abnormal growth.

CHAPTER 2
LITERATURE REVIEW

INTRODUCTION

Since 1975, many organizations associated with tree improvement have switched from bareroot to containerized planting stock for the establishment of progeny tests. This was primarily due to the increased survival and time of planting flexibility associated with containerized planting stock. Van Buijtenen and Lowe (1981) listed the advantages and disadvantages associated with this change in procedures. Among the advantages, van Buijtenen and Lowe listed increased uniformity of planting stock, faster rotation (a gain of one year), more plantable seedlings for a given number of seed, and greater ease of field planting for complex designs. In addition to these advantages, Mexal and Carlson (1981) indicated advantages of a shorter startup time, relatively long planting seasons (see also South and Barnett, 1986), and the possibility of three plantings per year.

There are, however, several disadvantages associated with containerized planting stock. First, the use of containerized stock distances the results of tree improvement work one step farther from operational planting. This distancing may affect family performance rankings. Second, slower initial growth has been observed on containerized seedlings. Finally, the planting season may not be lengthened in southern areas prone to droughty periods (van Buijtenen and Lowe, 1981).

Another potential disadvantage may be associated with spring-outplanting of containerized stock. The Virginia Department of Forestry (VDF) observed the late-summer development of needleless shoots and the loss of apical dominance on greenhouse-grown, controlled-cross, progeny test seedlings that were spring-outplanted. The development of this type of abnormal growth has not been observed with nursery grown progeny test stock, and may, therefore, result in different conclusions about families or individuals. This chapter will concentrate on the literature pertaining to the normal growth pattern of loblolly pine, loblolly pine morphology, and reports of growth abnormalities in the Pinaceae family.

NORMAL SHOOT GROWTH OF LOBLOLLY PINE

Definition of Terms

Doak (1935) summarized the literature to date and characterized the morphology of the genus *Pinus*. This work has been cited in many studies and will be applied here. A stem unit (*sensu* Doak) consists of a portion of stem, a modified or unmodified leaf and possibly a short shoot growing from the axil of the scale leaf. A growth increment (or flush) consists of a number of stem units that are produced in one of several growth cycles (normally a year). Bud scales are defined as closely spaced, nonphotosynthetic, scale-like organs which serve as a protective covering for the apical meristem and that collectively constitute the bud. In loblolly pine, the bud scales are characterized by frayed edges, giving the appearance of pubescence, or a wrapping around the bud. In this work, buds are defined by the lowest point at which this "wrapping" can be observed.

Doak (1935) also defined several classes of needles. The first class to be formed are the cotyledons, followed primary leaves. Primary leaves are glaucus with toothed margins, slightly broadened laterally and produced by free growth. Primary leaves may be produced in response to heavy fertilization, irrigation, or other favorable growing conditions. As the term primary leaf is more widely accepted than simple leaf, this type of leaf will be referred to as a primary leaf. Scale leaves may also be produced, with secondary needles developing in the axils of subtending scales. Scale leaves are usually less broadened laterally and the conductive tissue is less developed. In the literature, cataphyll is used interchangeably with scale leaf. For the purposes of this paper, the term scale leaf will be used.

Finally, apical control is often used to refer to the relative length and orientation of the lateral axes that do grow out. Apical dominance, as defined by Wilson (1990) is used to describe whether or not lateral buds do elongate in the first, second, or third season of growth.

Mode of Shoot Growth in Loblolly Pine

Cannell et al. (1976) described four centers of cell division in pines, including the shoot apex, sub-apical meristems, needles and sites where sub-apical meristems may form. Cell division in the main stem and branch apices produce stem units, scales, and needles. Sub-apical cell

division regulates cell numbers and, to some extent, the elongation of the internode at each stem unit. In pines, stem and needle growth occur separately, and can be considered as nearly independent events.

Lanner (1976) described three modes of shoot growth in the Pinaceae family. The first mode included those trees that produce entirely free growth. Free grwoth is the elongation of shoots by the simultaneous initiation and elongation of new shoot compenents, as well as the expansion of preformed parts. Within the first group, Lanner included tropical trees, trees that exhibit foxtail growth, and seedlings of many conifers, including northern species. The second mode included those trees that produce entirely fixed growth. Fixed growth involves the production of the stem units (that will be elongated during the next growing season, after dormancy is broken) within the terminal bud. Within the second group, Lanner included northern, single flush conifers. The third mode proposed by Lanner included those trees that produce a mixture of fixed and free growth. Loblolly pine is included in this third group. Very young loblolly pine seedlings exhibit entirely free growth. However, the amount of free growth diminishes with time, and old loblolly pine trees approach entirely fixed growth.

Loblolly pine may produce several flushes during the growing season, producing a resting (quiescent) bud when conditions are unfavorable for growth (Boyer, 1970). This resting bud may enter deep or true dormancy, usually induced by the declining fall photoperiod. This true dormancy is imposed until an intervening period of chilling has been satisfied. Boyer and South (1989) found that for first year loblolly pine seedlings raised near Auburn, Alabama, the relative state of bud dormancy increased from mid-November to mid-December, with the maximum state of bud dormancy in mid-December. Release of dormancy occurred in early January, following 600 hours of chilling. Chilling hours were defined as the number of hours between 0° and 8°C. Following release from deep dormancy, buds maintained quiescence until conditions were once again favorable for growth.

Stem elongation is determined by the number of leaf primordia produced by the apical meristem and by cell elongation. Cell elongation is dependant on the supply of photosynthate (assuming water is not limiting), both stored and produced during growth (Ford, 1980). Metabolic mobilization for shoot elongation requires the shoot becoming physiologically active prior to resuming growth. Cellular metabolism is shifted from a lower to a higher level of activity, and stored

carbohydrates may be consumed. This process is necessary regardless of the presence of a bud (Brown, 1974).

Shoot elongation is influenced by air temperature, as temperature influences the mobilization of stored carbohydrates. Further, those factors that influence photosynthesis affect shoot elongation, as photosynthesis affects the supply of newly synthesized carbohydrates (Ford, 1980).

Many environmental factors have been observed to check or curtail needle development, primarily through he reduction in the suply of photosynthate. Water stress may potentially reduce needle growth, as water stress affects the carbon balance in a seedling (Kuhns and Gjerstad, 1987) and minimizes the expansion that can occur by vacuole formation. Kuhns and Gjerstad (1987) found that as moisture stress increased, the translocation of labelled carbon to the roots and shoots of loblolly pine decreased. Mild stress resulted in increased starch production in source leaves, with severe stress reducing carbon fixation. Garrett and Zahner (1973) found the number of needles to be dependant on weather conditions of the previous year, and needle elongation of the current year was directly reduced 30% by moisture stress.

Gordon and Larson (1970) examined the role of labelled, stored food in the process of elongation. They concluded that the importance of stored food may be dependant on environmental conditions, as cold or dry weather conditions increased the time of shoot and needle elongation, therefore increasing the demand on reserves. Chung and Barnes (1980a) found that old needles decreased in dry weight, starch content, and total soluble sugars. Within the shoot being analyzed, 63% of the photosynthate production was supplied by the old needles. Growth of the new shoot accounted for 66% of the photosynthate used (Chung and Barnes, 1980b).

Cannell et al. (1976) stated that mineral nutrient deficiencies (especially boron may reduce or preclude needle growth in conifers. Chandler and Dale (1990) found that nutrient deficiencies delayed the growth of needles in 14 and 20 year old Sitka spruce (*Picea sitchensis*) and reduced needle growth.

ABNORMAL GROWTH IN THE PINACEAE FAMILY

Interruptions in the Dormancy Cycle and Dormancy Requirements

Mexal and Carlson (1981) observed growth abnormalities in spring outplanted loblolly pine that included needleless shoots and loss of apical control. These abnormalities were associated with decreased growth (when compared to fall outplanted seedlings). Growth abnormalities were attributed to a failure to fulfill the chilling requirement of the seedlings. Mexal and Carlson (1981) did not speculate whether or not pine seedlings that lacked a bud had a chilling requirement. A reduction in growth for spring outplanted loblolly pine was also observed by McKinley (1993). McKinley observed that after five years, fall-outplanted seedlings were larger in diameter, height and volume than spring outplanted seedlings. Although family rankings were not expected to change for these attributes, McKinley did not discuss the potential for shifts in form class related variables between spring and fall loblolly pine. Goodwin et al. (1981) found that for containerized loblolly pine seedlings outplanted in North Carolina, growth was greater when seedlings were outplanted in July than when outplanted in the spring or fall. Greater growth for the July-outplanted seedlings may have resulted from a lack of dormancy-induced limitations.

Growth abnormalities have been observed in nonchilled *Abies* seedlings (Tung and DeYoe, 1988). These abnormalities included a failure of the terminal bud to flush, while lateral buds flushed and elongated normally. After these same seedlings were subjected to a winter chilling (end of year 2), the terminal flushed and expanded normally. Hinesley (1982) observed that *Abies fraseri* seedlings grown in the greenhouse and outplanted in the spring grew abnormally, resuming normal growth after overwintering. Abnormalities included frost heaving, probably due to insufficient root development the previous growing season. For many trees the terminal bud failed to flush, while lateral branches flushed and elongated normally. Nonchilled seedlings that did break bud failed to elongate normally. Additionally, needles on nonchilled plants were stunted and close together. Most nonchilled plants resumed normal growth after a normal winter chilling, although several seedlings "bifurcated" or expressed "bonsai" growth. Hinesley (1982) found that abnormal growth could be overcome with 336 hours of chilling and long days or 672 to 1008 hours with a normal photoperiod.

Garber (1983) investigated the effects of chilling and shortened photoperiod on dormancy release in loblolly pine seedlings. Garber found that temperatures between 0° and 8°C were the most effective in the satisfaction of the chilling requirement of loblolly pine, and that long photoperiods (14+ hours) may substitute for chilling. Further, approximately 400 hours of chilling fulfilled the chilling requirement of seedlings, and exposure to cold increased height growth. Carlson (1985) further investigated the chilling requirement of loblolly pine seedlings. Among the most important findings, Carlson found that bud dormancy intensity was reduced on exposure to near freezing temperatures in cold storage as rapidly as the intensity is reduced in nature. At 207 hours of chilling, Carlson (1985) found that there was a two-fold range in dormancy intensity (as measured by speed of bud break after seedlings were placed in favorable growing conditions). At 1234 hours of chilling, the 20 half-sib families used in this experiment did not differ in dormancy intensity.

After exposure to treatments that increase the dormancy status of containerized seedlings, including shortened days and hardening off, an increase in height growth and field survival was observed (Mexal et al., 1979: Odlum and Colombo, 1988). Specifically, Mexal et al. (1979) found that fall outplanted loblolly pine seedlings placed outside for 42 days before planting exhibited increased cold hardiness and growth the following spring. Additionally, exposure to an 8 hour photoperiod in the greenhouse for 42 days increased survival and growth to levels comparable to the outside-hardened seedlings. Odlum and Colombo (1988) found that seedlings receiving 8 hour days before overwintering showed earlier bud growth and later budset the following year than seedlings receiving natural daylength. This resulted in greater shoot growth for those receiving 8 hour days in the greenhouse.

Lammas Growth

Lammas growth, or a late season extension of primordia that would normally elongate in the spring of the following year, was characterized by Rudolph (1964) in work with Jack pine (*Pinus banksiana*). Lammas growth was defined as late season extension of the terminal bud, while prolepsis was defined as extension of the lateral branches. According to Rudolph, lammas growth normally produced shoots with needles and a normal terminal bud that would overwinter, but often only the sterile portion of the shoot would elongate, creating "long buds" with no

secondary needles. Rudolph found the tendency to produce lammas growth was genetically controlled, with trees from more southerly provenances, from areas of higher average July temperature, and from areas with increased degree days over 10°C (50°F) producing a higher frequency of abnormal shoot growth. Photoperiod extension also increased the expression of lammas growth.

Lammas growth has been observed on *Pinus contorta* (O'Reilly and Owens, 1987), and *Picea sitchensis* (Cannell and Johnstone, 1978). O'Reilly and Owens found that northern provenances were less likely to produce polycyclic shoots than southern provenances. Generally, results indicated that provenances from more favorable growing conditions were more likely to produce lammas growth. Carvell (1954) found that *Pinus resinosa* planted well south of its natural range produced lammas growth following the relief of a late summer drought. *Tsuga heterophylla* produced lammas growth with greater frequency when grown in small cavity Styroblocks® and when lifted in November and March instead of January (O'Reilly et al., 1988).

Constant Daylength and Temperatures

Slee et al. (1976) described needleless shoots, loss of apical dominance, die back, stem forks, and basket whorls on *Pinus caribaea* (carib pine) grown in lowland areas of Malaysia. Slee (1977) attributed the presence of the deformities to relatively constant daylength and temperatures near the equator that resulted in the production and, later, the abortion of reproductive strobili.

Slee *et al.* (1976) describe unpublished studies in which high temperatures in controlled experiments caused the failure of needle development in both Carib and slash pine. Carib pine one meter in height under a 16 hour photoperiod and day/night temperatures of 36/31, 36/28, and 30/25 C° exhibited needles that failed to penetrate the fascicle sheath. This failure could cover an entire shoot. According to Slee, this needle failure was related to high night temperature and/or total light energy.

Tropical Abnormalities

Foxtailing has been observed on many species of pine planted in the tropics, including *Pinus canariensis, P. caribaea, P. cembroides, P. echinata, P. elliottii, P, kesiya, P. merkusii, P. oocarpa, F. palustris, P radiata, P. taeda,* and *P. tropicalis* (Kozlowski and

Greathouse, 1970). Foxtailing is the result of continuous apical activity, or free growth. This form of growth may be extensive on lowland tropical sites with consistently high temperatures and nonseasonal rainfall (Kozlowski and Greathouse, 1970). Because of the constant favorable conditions, new needles are constantly formed at the apex and can be seen at various stages of expansion directly below the apex. The terminal bud consists of only primary needles subtending secondary needle fascicles (USFS, 1966). No lateral branches are formed during foxtail growth. Kozlowski and Greathouse (1970) found the tendency to produce foxtail growth to be strongly heritable.

False foxtails have been observed in *Pinus elliotii* and *Pinus palustris* growing in Hawaii. False foxtail growth is similar in appearance to foxtail growth, but results from the failure of lateral branches to develop. Consequently, sterile scale zones are apparent in association with the aborted or failed lateral branch buds (Kozlowski and Greathouse, 1970).

Out-of-Phase Dormancy

Out-of-phase dormancy (OPD) has resulted in strobilus production in three-year-old loblolly pine seedlings (Greenwood, 1978). During the juvenile phase, the final resting bud that overwinters forms in September. As trees mature, the overwintering bud may form two months earlier, allowing for the production of male and female strobili (Greenwood, 1978). The continued development of the strobili is dependant on an intervening cold period. Greenwood delayed the development of the overwintering bud until midwinter, at which time the young trees were moved from the greenhouse to the outdoors. Dormancy was rapidly induced. This late-set bud did not break until two months following normal budbreak. This allowed for two months of conditions favorable for the production of strobili (Greenwood, 1977).

Long shoot growth behavior is influenced by OPD. Greenwood (1981) found that OPD halved the number of cycles of growth, and halved total elongation. According to Peter Wallace (Westvaco Corporation, South Carolina), trees subjected to OPD first cease growth for several months, and later may exhibit needleless shoots and loss of apical dominance. Further, trees subjected to OPD were observed to never entirely recover apical dominance and were slower growing.

The seedlings produced by the VDF may be out of phase, as they have been exposed to continually favorable growing conditions for nine months and have been subjected to two cycles of increasing and decreasing daylength. With the addition of timed release fertilizer and a relatively constant supply of water, the seedlings have been subjected to continuously favorable growing conditions for a nine month period.

Based on the information presented in this chapter, it is hypothesized that the growth abnormalities observed by the VDF may have been influenced by preplanting handling, or by the site on which they were planted. It is also hypothesized that the growth abnormalities may be the result of the interruption of the normal yearly dormancy cycle of the loblolly pine seedlings. Additionally, differential responses of specific controlled crosses to these factors are likely. Two experiments were established to examine these hypotheses. These experiments are presented in detail in the following two chapters.

CHAPTER 3
EXPERIMENT 1

GROWTH ABNORMALITIES IN GREENHOUSE-GROWN LOBLOLLY PINE I: FAMILY AND PREPLANTING TREATMENT GROWTH EFFECTS

INTRODUCTION

Virginia Department of Forestry (VDF) control crossed loblolly pine (*Pinus taeda*) tube stock planted in the greenhouse in January and outplanted in May have been observed to produce growth abnormalities after outplanting. Growth abnormalities develop over the summer, and are clearly noticeable by August. Abnormalities included the loss of apical dominance and the production of needleless shoots, or shoots with primary needles or sterile scales only (failure of secondary needles to develop). Needleless shoots included both the leader and lateral branches. Symptoms were observed in combination with the formation of a basket whorl. Basket whorls often contained a stunted or absent terminal, with long, needleless branches surrounding the terminal. After overwintering, seedlings that expressed growth abnormalities were observed to resume normal growth. Additionally, families were observed to vary in the degree of expression of the growth abnormalities.

Reports of abnormal growth in pine seedlings are rare. Mexal and Carlson (1981) observed growth abnormalities in spring outplanted loblolly pine that included needleless shoots and loss of apical dominance. These abnormalities were associated with decreased growth (when compared to fall outplanted seedlings). Growth abnormalities were attributed to a failure to fulfill the chilling requirement of the seedlings. Lammas growth, or a late season extension of primordia that often produced needleless shoots, was first characterized by Rudolph (1964). Slee et al. (1976) described needleless shoots, loss of apical dominance, die back, stem forks, and basket whorls on *Pinus caribaea* grown in lowland areas of Malaysia. Slee (1977) attributed the presence of the deformities to relatively constant daylength and temperatures

near the equator. Out-of phase dormancy (OPD) influences long shoot growth behavior (Greenwood 1981). According to Peter Wallace (Westvaco, South Carolina), trees subjected to OPD first cease growth for several months, and later may exhibit needleless shoots and loss of apical dominance. Hinesley (1982) observed that attempts to bypass the normal chilling requirements of *Abies fraseri* resulted in stunting, loss of apical dominance, terminal bud abortion, lack of symmetry and other morphological abnormalities.

Preplanting treatment influences seedling development after outplanting, and may have influenced the development of growth abnormalities. This experiment was established to determine the effects of preplanting seedling growing regime, planting site and family differences on the development of the abnormal morphology. Specifically, five full-sib families of loblolly pine (all Virginia provenances provided by the VDF, including specific controlled crosses 620 x 698, 681 x 693, 639 x 602, 684 x 661, and 636 x 695) were grown in the VDF greenhouse in New Kent, Virginia and in the Virginia Polytechnic Institute and State University greenhouse in Blacksburg, Virginia. These seedlings were subsequently outplanted at two very different sites.

METHODS AND MATERIALS

Study Sites

Seedlings were planted at the Reynolds Homestead Forest Resources Research Center near Critz, Virginia and the Appomattox - Buckingham State Forest near Appomattox, Virginia. Both planting sites are in the Piedmont physiographic provence. The Appomattox Buckingham State Forest site had been clearcut, and site prepared by chopping and burning the previous September. The previous cover was mixed oak and pine. Oust® was applied approximately 2 hours before planting at a rate of 0.224 kg/ha (3.2 oz / acre). A 1.5% solution of Roundup® was also applied at this time. The soils of the Appomattox site are a clayey, kaolinitic, thermic, Typic Hapludult of a Cecil series.

The Reynolds Homestead site was a highly eroded old field, which had been mowed and chemical treated with Oust 1 week before planting at a rate of 0.224 kg/ha (3.2 oz / acre). A 1.5% solution of Roundup was also applied at this time. The soils of the Reynolds site are a clayey, kaolinitic, thermic, Typic Hapludult of a Wickham series.

Seedling Production and Experimental Design

On November 25, 1991 seed was first washed in a 5% bleach solution, then stratified by soaking at 4°C for two days, drained and returned to 4°C until sowing. On January 4, 1992 (day 0), stratified loblolly pine seed was sown into fine vermiculite in aluminum germination trays and placed on heating pads (24° to 27°C) to speed germination. After germinating, seedlings were planted into 10 cubic inch C10 Leach® tubes. All germinals were planted between days 9 and 16. One hundred seedlings of each of 5 full-sib families were raised in either the Virginia Department of Forestry greenhouse near New Kent, Virginia or the Virginia Polytechnic Institute and State University Reynolds Homestead Agriculture Experiment Station greenhouse in Critz, Virginia.

The growth medium consisted of a 1:1 (v:v) mixture of peat moss and vermiculite. Fertilizer was applied as Osmocoat® brand nine-month slow-release fertilizer consisting of 18:6:12 N:P:K at a rate of 3 grams of fertilizer per liter of growth medium. Micronutrients were applied as Micromax® micronutrients at a rate of 1.1 grams per liter of growth medium.

In the greenhouses, Banrot® was used to prevent seedling losses to damping-off fungi. Lighting was set to mimic daylength over the course of the summer, starting at 13:45 (day 0), increasing to 14:45 on day 46, then decreasing to 13:30 by day 109. Daylength was extended in the New Kent greenhouse with 100 Watt incandescent light bulbs. In the Reynolds Homestead greenhouse, daylength was extended with 400 Watt sodium vapor lamps, providing a photosynthetic photon flux density of greater then 50 $\mu M\ m^{-2}\ sec^{-1}$. Seedlings were watered daily to approximately field capacity.

New Kent seedlings were placed outside in a seed orchard (30% full sun) on day 98. Reynolds seedlings were placed in a lathe house (12-15% full sun) on day 110. Seedlings were watered daily.

On day 134, seedlings were planted at the Appomattox - Buckingham site into ten blocks, with factorial combinations of 2 greenhouses and 5 families. Seedlings were planted as 5 tree row plots, with the experimental unit the mean value of the three center seedlings. Seedlings were planted at a 1m x 1m spacing. On day 136, seedlings were planted at the Reynolds Homestead Research Center in the same manner.

On day 143, 37.9 grams of Dursban® were applied to the Appomattox site (2.67 oz./gal water as 1/2 gal) to prevent infestation by pales

weevil (*Hylobius pales*). Dursban was applied to the Reynolds site on day 165. In addition, the Reynolds site was retreated with Roundup on day 178 to reduce herbaceous competition. A summary of greenhouse and outplanting procedures is shown in Table 3-1.

Data Collection

Seedlings were examined twice monthly, with root collar diameter recorded to the nearest 0.01 mm, flush height and sterile region height (to the nearest mm), the number of sterile scales and the number of needles were counted for each flush. To avoid confusion, a nursery marker was used to mark sterile scales as they were counted (which were only counted after needle elongation). The height of lateral branches were recorded to the nearest cm. As seedlings were measured repeatedly, only new growth was examined and recorded. Water potentials were measured on a single fascicle on three seedlings per block, with a pressure bomb (Plant Moisture Stress Instruments®, Corvallis, OR).

Trees were harvested on March 29, 1993, after overwintering and before budbreak of the second season. One tree was harvested from each family-treatment combination from 5 blocks, for a total of 50 trees. Reynolds Homestead trees were harvested on April 8, 1993. Root collar diameter, total height, the height of the tallest branch, and the percent needle cover of the last flush (estimated) were recorded for each seedling. Seedlings were then oven dried at 70°C to constant final weight and needle and stem dry weight were determined to the nearest 0.01g.

Statistical Analysis

The mean values for the three measured seedlings was calculated and used as the experimental unit. Analysis was conducted on the mean values of the three seedlings using a General Linear Models Procedure (SAS Inst., 1988). Specifically, a two way ANOVA was used to determine the effects of family and greenhouse of origin. The two sites (Appomattox and Reynolds Homestead) were analyzed separately, as growth differences between the two sites were extreme. The number of needles per total stem units was arc-sine transformed for analysis. The general statistical model used for stem data at each planting location for each date of examination was:

Table 3-1. Schedule of events for seedlings raised in the Virginia Department of Forestry greenhouse in New Kent, VA or in the Reynolds Homestead Research Center greenhouse in Critz, VA. Seedlings were planted at either the Appomattox - Buckingham State Forest in Appomattox, VA or on the Reynolds Homestead. Both the date and the number of days since planting are presented. (n/a = not applicable)

Greenhouse of Origin	**New Kent**	**Reynolds Homestead**
Seed sown	0	0
Seedlings planted	9	9-16
Daylength at 13:42	9	9
Daylength at 14:45	46	46
Daylength at 13:56	98	98
Daylength at 13:27	n/a	109
Hardened-off	98	110

Planting Site	**Appomattox-Buckingham**	**Reynolds Homestead**
Site prepared	134	124
Seedlings planted	134	136
Dursban	143	165
Retreated with Roundup	n/a	178
Harvest	449	458

$$Yijk = \mu_{ijk} + b_i + \alpha_j + ß_k + \alpha ß_{jk} + E_{ijk}$$

where:			
	μ =	overall mean	df
	b =	block effect	9
	α =	greenhouse	1
	ß =	family	4
	αß=	greenhouse x family	4
	E =	random error	81

Duncan's new multiple range test was used as a follow-up procedure to separate means that were found to be different in the general linear model. Repeated measurements analysis was not utilized in this analysis as variance increased over the course of the growing season. However, variance within a given sampling date was homogeneous. For the variables measured, the population fit a standard normal distribution, as determined by the Univariate Procedure (SAS Inst., 1988).

To determine if the loss of apical dominance and production of needleless shoots was more prevalent in the fastest growing trees, the ratio of branch to apical height was regressed against the final weight of the aboveground portion of the seedlings. Analysis was conducted using a Regression Procedure (SAS Inst., 1988).

Heritability

Seedlings that were raised in New Kent and planted at Appomattox were used in heritability analysis (Zobel and Talbert, 1984). Individual tree narrow sense heritabilities were calculated for full-sib families, specifically for the variable final ratio, which represents the loss of apical dominance, and is indicative of tree form. Variance components were calculated with a VARCOMP procedure (SAS Inst., 1988). The specific analysis of variance table used in this analysis was:

Source	df	MS	EMS
Block	9	---	---
Fam	4	MSF	$s^2_W+Ss^2_{RF}+SFs^2_F$
Rep*Fam	36	MSRF	$s^2_W+Ss^2_{RF}$
Subsample	100	MSS	s^2_W
Total	149		

where: S = Trees in plot = 3
R = Replications = 10
F = Families = 5
W = Within plot variation

and: $s^2_{RF} = (MSRF-MSS)/S$
$s^2_W = MSS$
$s^2_F = (MSF-MSRF)/TR$
$h^2 = 2s^2_F/(s^2_W + 4s^2_{RF} + s^2_F)$

Second Growing Season

Seedlings planted at the Appomattox-Buckingham State Forest in May of 1992 were evaluated again on July 23, 1993. For this evaluation, one seedling from each treatment-block combination in five blocks was randomly selected. The tallest branch and apical height were measured to the nearest cm, and the flush that elongated immediately following the first winter was analyzed for the number of needles per total stem units.

RESULTS AND DISCUSSION

Site Differences

After one full growing season in the field, seedlings planted at the Appomattox-Buckingham State Forest were over 400 percent larger than seedlings planted at the Reynolds Homestead Research Center (39.4 versus 7.7 grams, respectively). At the Appomattox site 98% completed 2 flushes, 76% completed 3 flushes, and 29% completed a fourth flush. In contrast, at the Reynolds Homestead, only 85% completed 2 flushes, and 13% completed 3 flushes. No seedlings planted at the Reynolds Homestead completed a fourth flush.

Loss of apical dominance and the expression of needleless shoots became apparent in late July and in August of the first growing season. As abnormalities became more apparent with each flush, the expression of abnormalities was much more apparent at the Appomattox site. Because of the gross differences in seedling size and development between the two sites, statistical analysis was conducted separately.

Water potential was measured repeatedly and averaged over the growing season. The cumulative water potential deficit between June 18 and September 20 was -18.7 MPa days. This may partially account for the growth differences between the two sites. Soil temperatures were likely higher at Appomattox, as the burned, blackened soil absorbed more solar radiation. These temperature differences likely led to a greater degree-day accumulation and more growth at the Appomattox site. The

burn of the previous year may have released more nutrients to the developing seedlings than the herbicide-killed grasses at the Reynolds Homestead planting site. Armson (1977) states that fire results in increased amounts of calcium, magnesium, potassium, and phosphorous, all in the available state. Additionally, root growth may have been restricted at the Reynolds site. The soil at the Reynolds site was highly eroded, and the seedlings were planted into the sandy clay B-horizon.

At the Reynolds Homestead an initial slowing of growth was observed after outplanting, as was reported by McKinley (1993) and by van Buijtenen and Lowe (1981). At Appomattox, this initial slowing of growth was not apparent. Seedlings planted at Appomattox that did not have a bud at outplanting set a bud within 2-4 weeks but this bud elongated quickly. The seedlings that did have a bud at the time of outplanting also elongated rapidly. Seedlings planted at the Reynolds Homestead were much slower to set a bud after outplanting, with many seedlings continued to grow slowly for 3 weeks before setting a bud.

Influencing Agents

Survival was high on both sites (approaching 100%), and losses to tipmoth (*Rhyacionia frustrana*) were light (2% and 1.5% at Appomattox and Reynolds, respectively). Deer damage was heavy at Reynolds during the winter of 1992-1993, affecting 24% of the trees.

Controlled cross 636 x 695 was affected by an unidentified stem canker that caused foliar discoloration, stunting of growth, stem splitting, and death in several seedlings. Upon outplanting, seedlings that exhibited the symptoms of the unidentified canker were excluded from measurement. Over the summer symptoms were not usually obvious, but may have resulted in reduced growth for family 636.

APPOMATTOX SEEDLINGS

Greenhouse Effects

At the time of outplanting, height growth did not differ between greenhouses (overall mean of 23.5 cm) (Table 3-2). However, New Kent seedlings had significantly larger root collar diameters, were more yellowed in appearance, and were more likely to have a bud than seedlings produced at the Reynolds Homestead (Table 3-2). These differences between the two greenhouses may be attributed to the

Table 3-2. Outplanting height, root collar diameter (RCD), and the frequency of a terminal bud at outplanting for five families of 127-day-old loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center in Critz, VA and outplanted at the Appomattox-Buckingham State Forest near Appomattox, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Greenhouse	Height (cm)	RCD (mm)	Bud %
New Kent	23.54 a	3.557 a	80.7 a
Reynolds	23.37 a	3.283 b	40.7 b
Overall Mean	23.45	3.420	60.7
p-value	0.7038	0.0001	0.0001

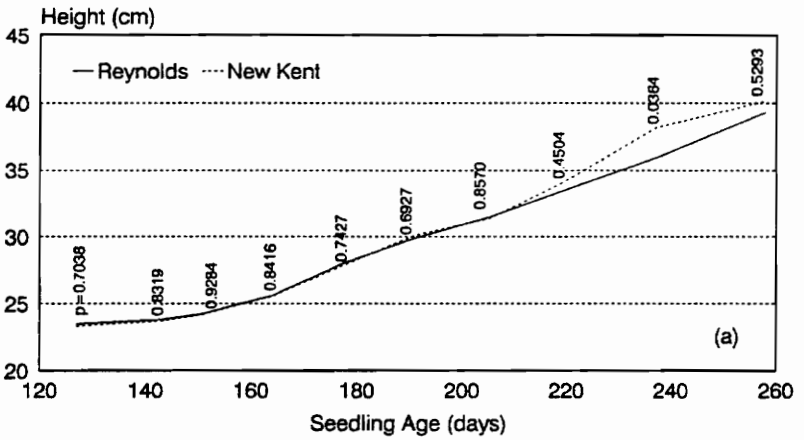
difference in the hardening-off period the seedlings were subjected to. New Kent seedlings were placed outside 2 weeks earlier than Reynolds seedlings and were subjected to more direct sunlight in the seed orchard than the Reynolds seedlings that were placed in the lathehouse. Studies have indicated that seedlings grown outside have larger root collars than greenhouse-grown seedlings (Retzlaff et al. 1990).

Repeated examination of the Appomattox seedlings during the growing season revealed that height growth was episodic. Seedlings expressed cyclical patterns of rest, followed by budburst and active growth. Although this was expected on an individual seedling basis (Lanner, 1977), the trend was evident when averaged over seedling source (Figure 3-1a) and even more evident when averaged for family 681 (Figure 3-1b). Height growth during the growing season was not significantly different for seedlings raised in either the Reynolds Homestead or the New Kent greenhouse. However, 237 days after sowing mean total heights were significantly different between greenhouses. This difference was not apparent on the last sampling date, 258 days after sowing (Figure 3-1a). This is an interesting growth pattern, as pre-planting treatments generally produce differential results at the time of outplanting which decrease over a growing season.

Elongation of Reynolds seedlings (that were subjected to the more mild hardening-off treatment) slowed near the end of the growing season, even though conditions were still favorable for growth. In contrast, Appomattox seedlings (subjected to the more severe hardening off period) elongated steadily to the end of the growing season. Late season growth differences may likely be attributed to the presence of a quiescent bud on the New Kent seedlings at the time of outplanting. It is hypothesized that the more severe hardening off period for the New Kent seedlings increased the dormancy status of the terminal bud, partially substituting for a normal yearly dormancy cycle. This allowed for terminal growth late in the normal growing season.

Significantly larger root collars were observed for the New Kent seedlings for 50 days following outplanting (Figure 3-2). However, the rate of root collar diameter increase was greater for the Reynolds seedlings, and the Reynolds seedlings had increasingly larger (although non-significant) root collars near the end of the growing season (Figure 3-2).

The degree of apical dominance was determined by the division of branch height by apical height as measured from the ground. A ratio of



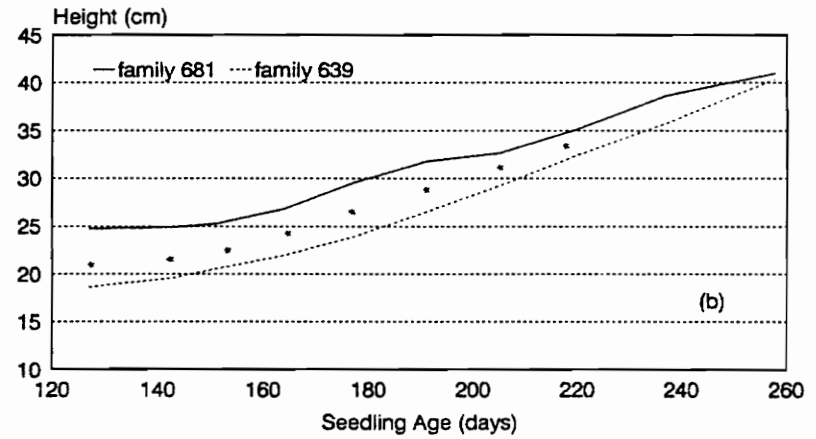


Figure 3-1. Height growth for loblolly pine seedlings planted at the Appomattox-Buckingham State Forest near Appomattox, VA. Seedlings were raised in either the Virginia Department of Forestry greenhouse or in the Reynolds Homestead Research Center greenhouse (a). p-values are presented for each sampling date. (b) depicts the performance of the best and poorest performing families. * indicates that these families are significantly different at alpha = 0.05.

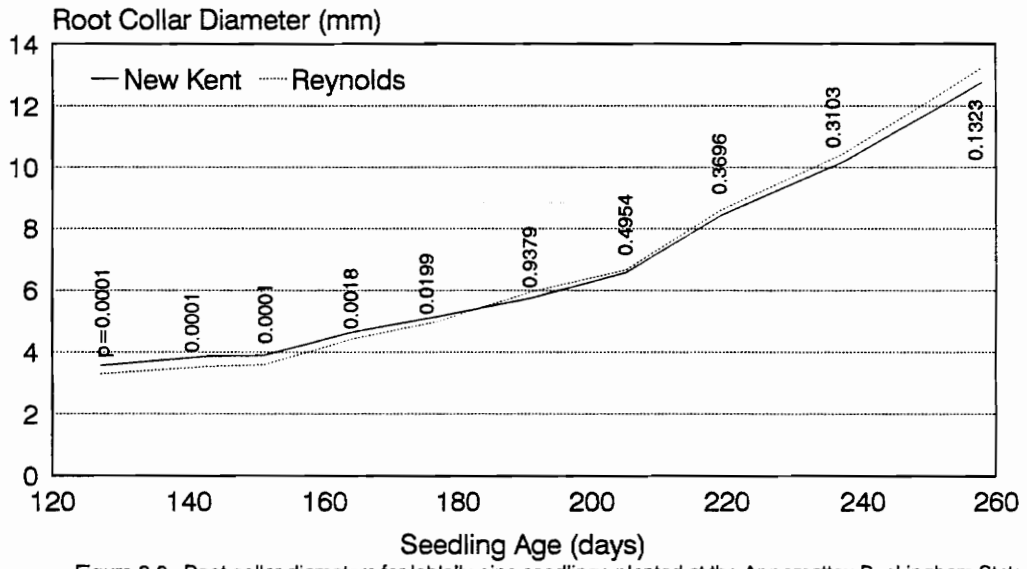


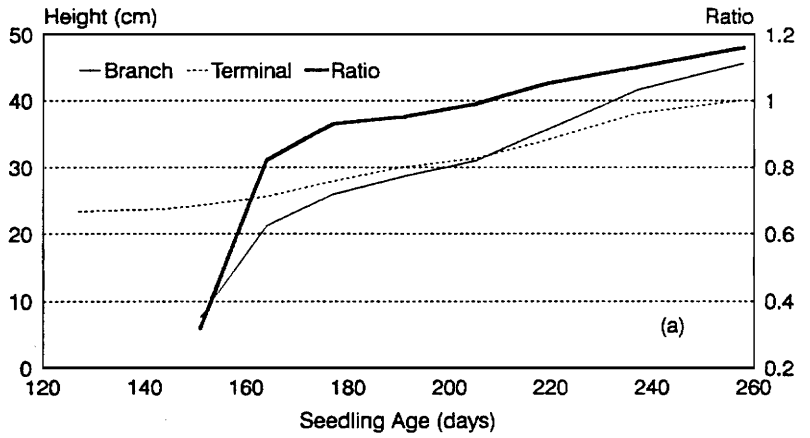
Figure 3-2. Root collar diameters for loblolly pine seedlings planted at the Appomattox-Buckingham State Forest near Appomattox, Virginia. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, Virginia or in the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were outplanted on May 18, 1992, 134 days after sowing. P-values are presented for each sampling date.

greater than one indicated a loss of apical dominance. At outplanting, New Kent seedlings expressed a significantly greater ratio of branch to apical height than the Reynolds seedlings (0.31 vs. 0.52, p = 0.0001). However, New Kent seedlings had significantly lower branch to apical height ratios with the first flush, and near the end of the growing season (1.08 vs 1.16, p = 0.0057) (Figure 3-3a and Figure 3-3b). The increased branch to apical height ratio at the end of the growing season for the Reynolds seedlings resulted from declining height growth of the terminal apex while the lateral branches continued growth (Figure 3-3a). New Kent seedlings exhibited a decrease in branch growth rate during this same period (Figure 3-3b). New Kent seedlings lost apical dominance with the third flush, but the rate of terminal growth following the flush was similar to the rate of branch growth (Figure 3-3b).

The end-of-growing season harvest of the aboveground portion revealed no significant differences between greenhouses for total shoot weight (overall mean = 39.4 g). Increased height growth (Figure 3-1a), and decreased branch growth (Figure 3-3b) suggest a shift in biomass allocation to shoot growth from branch growth that appears to be associated with the extended hardening off (increased levels of dormancy) of the New Kent seedlings.

Needle production was examined at the end of the year, as needle growth occurs after shoot elongation. Needle development was measured by counting the total number of secondary needles and dividing this number by the total number of stem units for each flush. Data is presented for only the first, second and third flush, as only 56% of seedlings (after the average was determined for three seedlings in each row plot) produced a fourth flush. Ninety-five percent of the seedlings planted at the Appomattox-Buckingham State Forest produced 3 flushes (after three seedling averages were taken). The five experimental units that failed to produce a third flush were all controlled cross 636 x 695, which was affected by an unidentified stem canker. Greenhouse differences were significant in the second flush (0.621 for New Kent seedlings vs. 0.519 for Reynolds seedlings) but not in the third flush (Table 3-3).

Differential needle growth in the second flush may be attributable to the quiescent bud present at the time of outplanting for the New Kent seedlings (Table 3-4). Needle formation likely continued within the resting bud, although active elongation was delayed. Seedlings raised



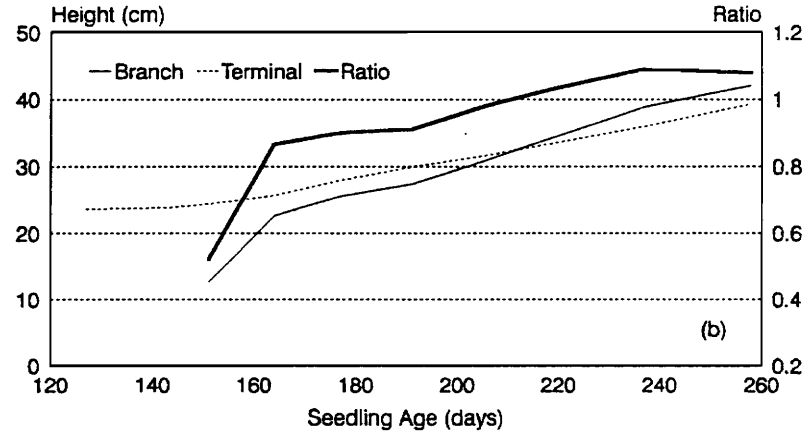


Figure 3-3. Branch and terminal height growth and the ratio of branch to terminal height of loblolly pine seedlings planted at the Appomattox- Buckingham State Forest near Appomattox, Virginia. Seedlings were raised in the Reynolds Homestead Research Center (a) or in the Virginia Department of Forestry greenhouse in New Kent, Virginia (b).

Table 3-3. Needles per total stem units for the second and third flush for five families of 258-day-old loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center greenhouse in Critz and outplanted at the Appomattox-Buckingham State Forest near Appomattox, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Greenhouse	Needles/Unit Flush 2	Needles/Unit Flush 3
New Kent	0.621 a	0.344 a
Reynolds	0.519 b	0.371 a
Overall Mean	0.570	0.358
p-value	0.0001	0.3088

Table 3-4. Outplanting height, root collar diameter (RCD), and the frequency of a terminal bud at outplanting for five families of 127-day-old loblolly pine. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center greenhouse in Critz and outplanted at the Appomattox-Buckingham State Forest near Appomattox, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Bud %
620 x 698	24.77 a	3.88 a	75.1 ab
636 x 695	25.26 a	3.74 ba	66.7 b
681 x 693	24.76 a	3.73 ba	86.8 a
684 x 661	23.83 a	3.62 bc	60.0 b
639 x 602	18.66 b	3.50 c	15.1 c
Overall Mean	23.46	3.42	60.7
p-value	0.0001	0.0039	0.0001

in the Reynolds Homestead greenhouse set a bud soon after outplanting, which elongated at approximately the same time as the New Kent seedlings (Figure 3-1a). This produced a shorter period between budset and elongation for needle production in the Reynolds seedlings, resulting in the production of fewer needles per total stem units. For the third flush, buds were set and elongated at approximately the same time (figure 3-1), which likely resulted in nonsignificant differences in needle production.

Family Effects

Family differences were apparent in seedling form, growth and development, and in the expression of growth abnormalities. Differences were apparent at outplanting, and throughout the growing season. At the time of outplanting, statistically significant family differences were apparent for height growth, root collar diameter, and the percentage bearing a terminal bud (Table 3-4). The most noticeable features at outplanting were the very slow growth of the 639 x 602 cross and the canker susceptibility of the 636 x 695 cross.

At the end of the growing season, height growth differences between families were not significant except for family 636, which was significantly shorter (4-5 cm) than the other families (Table 3-5), likely as a result of the stem canker that affected only this family. Root collar diameter differences were also apparent, with family rankings changing appreciably from the time of outplanting (Table 3-5 and Table 3-4).

The ratio of branch to apical height increased with each successive flush, and dominance loss was usually observed with the third flush. At the end of the growing season, family differences were apparent for the ratio of branch to apical height (Table 3-5). The ratio of branch to apical height for family 639 was significantly less than the ratio for the other four families. Family 681 expressed the poorest ratio. Overall, family 639 was as tall and completed the same number of flushes as the other families.

Family differences were observed for needle production for each flush at the end of the growing season. The number of needles per total stem units was observed to decrease from 0.570 to 0.358 from the second to the third flush (Table 3-5). Although the fourth flush could not be statistically analyzed, the mean number of needles per total stem units was 0.334 (similar to the third flush).

Table 3-5. Height, root collar diameter, aboveground weight, the ratio of the tallest branch to apical height, and the number of needles per total stem units for the second and third flush for five families of 258-day-old loblolly pine. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center greenhouse in Critz and outplanted at the Appomattox-Buckingham State Forest near Appomattox, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Weight (g)	Final Ratio	Needles/ Unit Flush 2	Needles/ Unit Flush 3
620 x 698	43.39 a	13.99 a	35.524 a	1.132 ab	0.608 a	0.324 ab
636 x 695	34.14 b	12.43 b	35.975 a	1.132 ab	0.540 b	0.215 b
681 x 693	41.03 a	13.41 ab	44.092 a	1.155 a	0.586 ab	0.382 a
684 x 661	39.78 a	12.41 b	40.513 a	1.126 ab	0.568 ab	0.364 a
639 x 602	40.42 a	12.78 b	41.124 a	1.042 b	0.548 b	0.470 a
Overall Mean	39.75	13.00	39.446	1.117	0.570	0.358
p-value	0.0005	0.0063	0.8788	0.1220	0.0681	0.0333

Loss of Dominance: a Comparison of the Best and Worst Performers

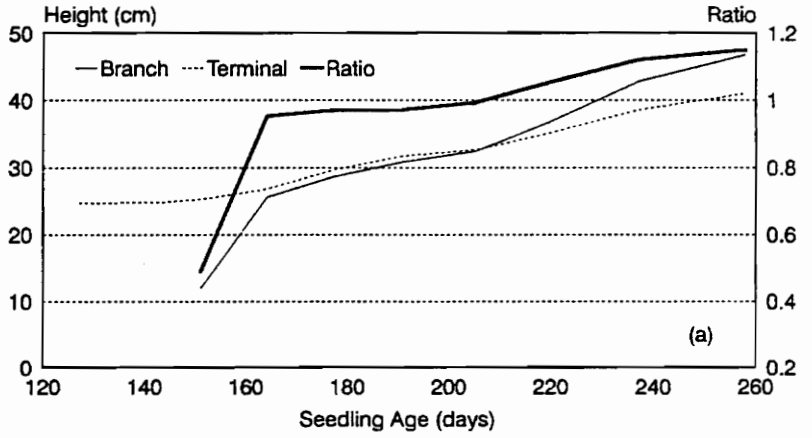
Family 639 exhibited the most favorable ratio of branch height to apical height at the end of the measurement period, and family 681 exhibited the poorest ratio (Table 3-5). A comparison of the growth patterns of these two families reveals some striking differences. Both families lost apical dominance approximately 219 days after outplanting, concurrent with the elongation of the third flush (Figure 3-4a and Figure 3-4b). In agreement with Boyer (1970), branches and the apical meristem elongated at approximately the same time. However, branches grew more rapidly than the terminal during the elongation of the third flush, especially for family 681 (Figure 3-4a).

Initially, branch growth in family 639 was more rapid than apical meristem growth, resulting in the loss of apical dominance. However, apical expansion proceeded at a steady rate, eventually overtaking branch heights at the end of the measurement period (Figure 3-4b).

Over the course of the growing season, family 639 seemed to be much less sensitive to environmental fluctuations and seedling growth proceeded at a very steady rate (Figure 3-1b). Although the average seedling in family 639 completed at least three flushes, seedlings in this family did not set and break bud in unison, resulting in a very smooth growth curve. In contrast, family 681 was very sensitive to changes in the environment. Individual seedlings in family 681 appeared to cycle through budset and elongation at approximately the same time (Figure 3-1b). However, this cycling could not be completely explained by changes in the water potential status of the seedlings (Figure 3-5).

At the end of the measurement period, the growth curve of family 639 appears to be accelerating, indicating a potential lack of sensitivity to decreasing daylength (Figure 3-1b). In contrast, the growth curve of family 681 appears to be slowing at the end of the growing season, likely in response to decreasing daylengths. The branches of family 681 have also responded to decreasing daylength. However, this response was not as strong as the response of the terminal apex (Figure 3-5). The lack of response of the lateral branches and the strength of the response of the terminal apex increased the end-of-growing season ratio of branch to apical height for family 681 to 1.15.

At the time of outplanting, family 681 was significantly larger than family 639 in terms of root collar diameter (3.73 mm vs. 3.50 mm, respectively), but was taller than family 639 (24.8 cm vs 18.7 cm, respectively) (Table 3-4). Family 681 soon developed significantly larger diameters and continued to exhibit greater heights than family



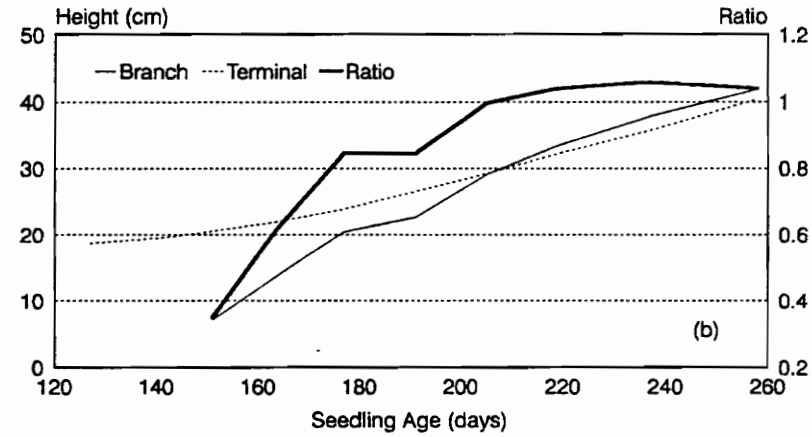


Figure 3-4. Branch and terminal height growth and the ratio of branch to apical height for the poorest (a) or the best (b) performing family of loblolly pine tubelings planted at the Appomattox- Buckingham State Forest near Appomattox, Virginia. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, Virginia or in the Reynolds Homestead Research Center in Critz, Virginia.

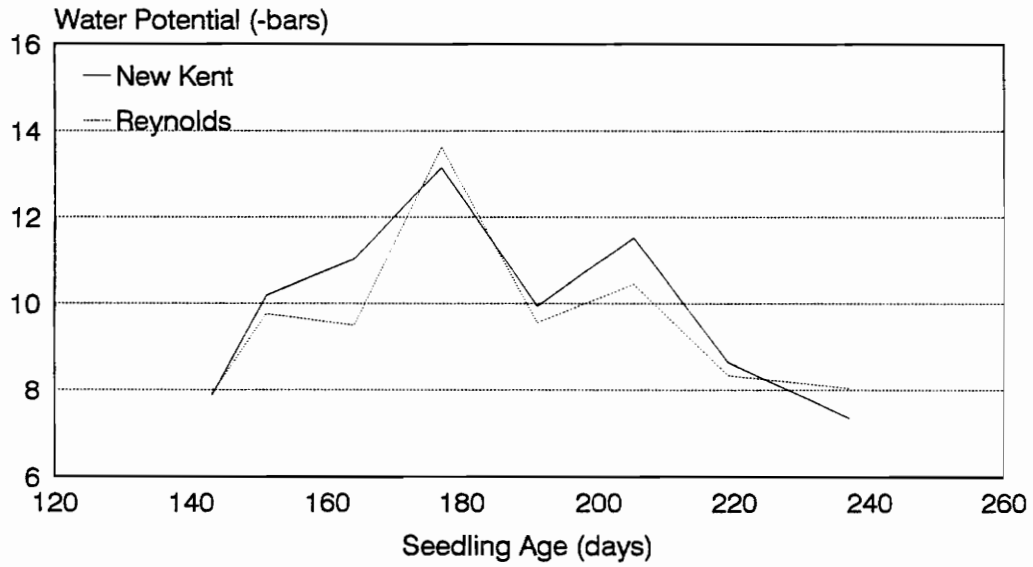


Figure 3-5. Water potentials for loblolly pine seedlings planted at the Appomattox-Buckingham State Forest near Appomattox, Virginia. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, Virginia or in the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were outplanted on May 18, 1992, 134 days after sowing.

639 (Figure 3-1b). However, near the end of the growing season these differences were not apparent. Statistical differences in diameter were not apparent due to increased variance in root collar diameter near the end of the summer. Family 639 was nearly the same height (40.4 cm) as family 681 (41.0 cm) on the last sampling day (Figure 3-1b), and there were no differences between these two families for end-of-growing-season above-ground biomass .

Heritabilities

Heritabilities were calculated for seedlings planted at the Appomattox site, and were calculated separately for seedlings raised in the New Kent greenhouse and for those raised in the Reynolds greenhouse. Two important trends emerged from this analysis. First, for the New Kent seedlings, the ratio of branch to apical height is more strongly inherited than root collar diameter (0.05) and is less strongly inherited than height (0.13) (Table 3-6). The second important trend is that the regime under which the seedlings are raised, specifically the amount of hardening-off may have influenced the heritability of the ratio of branch to apical height. It is hypothesized that the extended hardening-off period of the New Kent seedlings resulted in an overall decrease in the ratio of branch to apical height and therefore resulted in a greater expression of family differences.

REYNOLDS HOMESTEAD SEEDLINGS

Seedling growth and development at the Reynolds Homestead was much slower than at the Appomattox site. Height growth at the Reynolds Homestead at the end of the growing season (28 cm) corresponded with 50 days of height growth after outplanting at Appomattox (Table 3-7). The mean end-of-growing season root collar diameter at Reynolds was greater than the mean root collar diameter after 80 days at Appomattox (Table 3-7), following a rapid increase in root collar diameter at the end of the growing season (Figure 3-6).

As previously stated, growth rates and patterns of growth differed drastically between the two sites. Height growth at the Reynolds Homestead was obviously episodic (Figure 3-7), and mostly stopped after the elongation of the second flush. Nearly all seedlings planted at the Reynolds Homestead set buds soon after outplanting. The second flush elongated approximately 25 days after outplanting (day 159) and elongation continued for 35 days (to day 194). After this period, seedlings elongated little for the remainder of the summer. One

Table 3-6. Family heritability for the ratio of branch to apical height (Final Ratio), height and root collar diameter (RCD) for five families of 258-day-old loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center in Critz, VA and outplanted at the Appomattox-Buckingham State Forest near Appomattox.

Greenhouse	Final Ratio	Height	RCD
New Kent	0.0702	0.1266	0.0494
Reynolds	0.0045	0.0798	0.1059

Table 3-7. Comparison of height and root collar diameter growth (RCD) for five families of loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center in Critz, VA and outplanted at either the Appomattox - Buckingham State Forest in Appomattox, VA or the VPI & SU Reynolds Homestead Research Center in Critz, VA.

Location	**Appomattox**		**Reynolds**	
Date	**Height (cm)**	**RCD (mm)**	**Height (cm)**	**RCD (mm)**
177 days after sowing	27.96	5.06	26.53	4.67
205 days after sowing	31.47	6.62	27.25	5.10
258 days after sowing	39.88	13.00	28.17	7.90

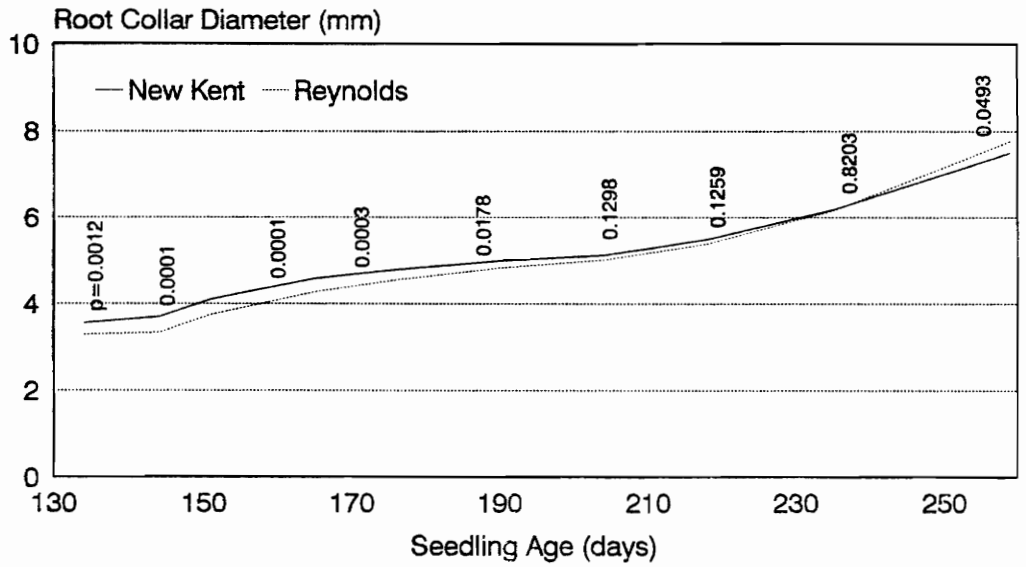


Figure 3-6. Root collar diameters for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, Virginia or in the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were outplanted on May 20, 1992, 136 days after sowing. P-values are presented for each sampling date.

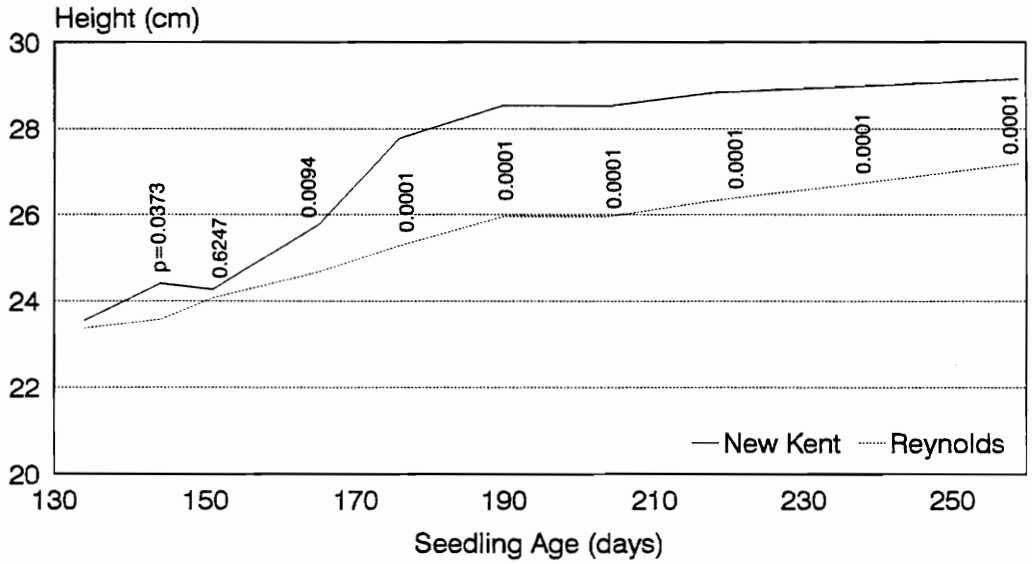


Figure 3-7. Heights of loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were raised in either the Virginia Department of Forestry greenhouse in New Kent, Virginia or in the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were outplanted on May 20, 1992, 136 days after sowing. P-values are presented for each sampling date.

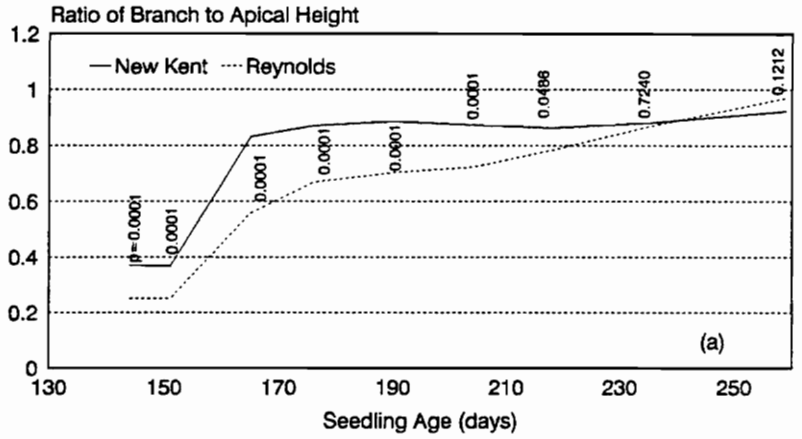
possible contributing factor for the cessation of growth at the Reynolds Homestead may have been the lower (more negative) mid-day water potentials as compared to the Appomattox site recorded from 39 to 120 days after outplanting. A mean peak of -17.7 bars was observed 63 days after outplanting. This probably limited the extension of the first flush. Continued dry conditions may have limited the development of the third flush until the end of the growing season. In contrast, the minimum (most negative) mean water potential recorded at Appomattox was -13 bars.

The decreased growth rate of the seedlings and the lack of elongation of flushes limited the expression of growth abnormalities at the Reynolds Homestead. The average seedling at the Reynolds Homestead did not lose apical dominance (Figure 3-8a, Figure 3-8b) and produced only one flush after outplanting.

Greenhouse Effects

Seedlings raised in the Reynolds Homestead greenhouse generally lacked a bud at outplanting (Table 3-2), and many seedlings exhibited free growth after outplanting, resulting in a more favorable branch to apical height ratio (Figure 3-8a). However, after the extended dry period, nearly all seedlings ceased growth and developed a quiescent bud (Figure 3-7). There were no statistical differences in the end-of-measurement-period ratio of branch to apical height for seedlings raised in the Reynolds Homestead or New Kent greenhouse (Figure 3-8a). However, the Reynolds Homestead seedlings expressed numerically smaller ratios, consistent with the findings of the Appomattox planting site (Figure 3-3a, Figure 3-3b). Seedlings raised in the New Kent greenhouse exhibited a rapid increase in branch height (Figure 3-8a). However, branch height did not exceed apical height. In contrast, the seedlings raised in the Reynolds Homestead greenhouse exhibited a steady increase in branch growth (Figure 3-8a).

Needle production was affected by where the seedlings were raised ($p = 0.0001$). Seedlings produced at the New Kent greenhouse produced 0.616 needles per total stem units. In contrast, the Reynolds greenhouse seedlings produced only 0.430 needles per total stem units. This was probably due, in part, to the greater percentage of seedlings with a terminal bud at the time of outplanting, which led to a longer period of time for primordia to differentiate into needles.



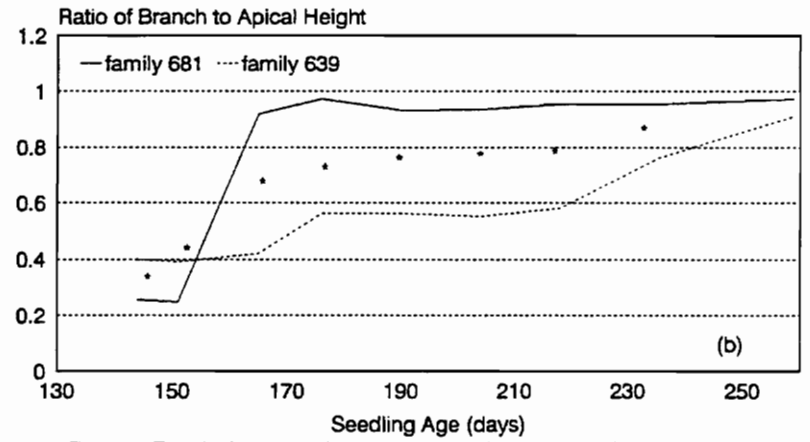


Figure 3-8. The ratio of branch to apical height for loblolly pine planted at the Reynolds Homestead Research Center in Critz, VA. Seedlings were raised in either the Virginia Department of Forestry greenhouse or in the Reynolds Homestead Research Center greenhouse (a). p-values are presented for each sampling date. (b) represents the ratio of the best (639) and poorest performing family (681. * indicates that these families are significantly different at alpha = 0.05.

Family Effects

Family differences were apparent for height growth at the Reynolds Homestead planting site. Family 620 x 698 expressed the greatest height growth and family 639 x 602 expressed the least height growth (Table 3-8). With the exception of family 636 x 695, the height rankings of the families from the time of outplanting varied little between planting sites. That is, the mechanism that caused growth abnormalities and decreased terminal growth that was evident in the Appomattox seedlings was not expressed to the same extent in the seedlings outplanted on the Reynolds Homestead. Weight and root collar diameters were not significantly different at the end of the measurement period for families outplanted on the Reynolds Homestead (Table 3-8).

The number of needles per total stem units for the second flush was not significantly different between families (Table 3-8). An interesting relationship emerges when family rankings are considered for the percentage of seedlings with a bud at outplanting for each family (Table 3-4). With the exception of family 636, family rankings for the number of needles per total stem units corresponded with the family rankings for percentage with a bud at outplanting. That is, the greater the percentage of seedlings with a bud at outplanting, the greater the needle growth for the second flush. Apparently, needle primordia were produced within the resting bud.

Loss of Dominance: A Comparison of the Best and Worst Performers

For seedlings planted at the Reynolds Homestead, family differences were not significant at the end of the measurement period for the ratio of branch to apical height (overall mean of 0.95) (Table 3-8). Overall, families performed similarly at the Reynolds Homestead and at the Appomattox - Buckingham State Forest. Family 639 x 602 exhibited the most favorable final ratio (0.914), and family 681 x 693 exhibited the poorest final ratio (0.975). However, height growth patterns of these two families were similar at the Reynolds Homestead. This disagreement with the Appomattox results may have been influenced by the more negative water potentials observed after outplanting at the Reynolds Homestead. At the Reynolds site family 639 exhibited free growth for a short period, then apparently set a bud and grow at the same rate as family 681. Overall, family 639 was always significantly shorter than family 681 at the Reynolds Homestead.

Family 681 appeared to produce more rapid root collar diameter growth throughout the growing season. However, at the end of the

Table 3-8. End of growing season height, root collar diameter (RCD), aboveground weight, the ratio of branch to apical height, and needles per total stem units for the second flush for five families of 258-day-old loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center in Critz, VA and outplanted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Weight (g)	Final Ratio	Needles/ Unit Flush 2
620 x 698	30.23 a	8.14 a	7.830 a	0.960 a	0.555 a
636 x 695	28.37 b	7.66 c	9.117 a	0.954 a	0.468 a
681 x 693	28.73 b	7.78 ab	8.047 a	0.975 a	0.597 a
684 x 661	28.77 b	7.39 bc	6.502 a	0.942 a	0.509 a
639 x 602	24.75 c	7.19 c	7.226 a	0.914 a	0.486 a
Overall Mean	28.17	7.63	7.744	0.948	0.523
p-value	0.0001	0.0003	0.3850	0.7639	0.2278

measurement period, families were not statistically different following a rapid increase in root collar diameter at the end of the growing season.

As the second flush of family 681 elongated, branch height and apical height increased at the same rate, causing no change in the ratio of these two parameters (Figure 3-8b). In contrast, family 639 did not exhibit a bud at the time of outplanting, and individual seedlings exhibited free growth for varying periods. The steadily increasing branch height over the summer (and its associated ratio increase) is the result of the formation of terminal and lateral branch buds associated with the terminal buds (Figure 3-8b). The steady free growth and slow formation of second flush branches of family 639, resulted in significantly different ratios of branch to apical height between these two families throughout most of the growing season (Figure 3-8b). However, at the end of the measurement period, all of the seedlings in family 639 had developed a second flush, resulting in a lack of a significant difference between the two families.

Site Comparisons: Family Rankings

Family rankings varied somewhat between the Appomattox-Buckingham State Forest and the Reynolds Homestead (Table 3-9). Height growth rankings were inconsistent for all five families on both sites, with the largest differences in rank between the two sites for family 639 and for family 684. Family 639 exhibited slow and steady growth at the Appomattox site (Figure 3-1b), and was the third tallest family by the end of the measurement period. At the Reynolds Homestead growth was reduced for all families and family 639 exhibited the least height growth. Root collar diameter growth followed the same general trends as height growth (Table 3-9). Above-ground biomass rankings varied between the two planting sites (Table 3-9).

The rankings for the ratio of branch to apical height was very consistent between the two planting sites. Family 639 expressed the strongest apical dominance at the Reynolds Homestead and at Appomattox (Table 3-9). Family 681 was the poorest performer at both sites. The rankings for the number of needles per total stem units varied between the two sites, primarily because of the influence of budset at outplanting that determined the number of needles per stem units on the second (highest) flush on the Reynolds Homestead (Table 3-9).

Table 3-9. End of growing season rankings for five families of 258-day-old loblolly pine.loblolly pine raised in either the Virginia Department of Forestry greenhouse in New Kent, or the VPI & SU Reynolds Homestead Research Center in Critz, VA and outplanted at either the Appomattox Buckingham State Forest, near Appomattox, VA (left number) or at the Reynolds Homestead Research Center in Critz, VA (right number). Presented are the height, root collar diameter (RCD), weight, ratio of branch to apical height (Branch/Apical Height) and the number of needles per total stem units for the last complete flush (Needles/Stem Unit).

Family	Height	RCD	Weight	Branch/Apical Height	Needles/Stem Unit
620 x 698	1 / 1	1 / 2	5 / 3	3 / 4	4 / 2
636 x 695	5 / 4	4 / 1	4 / 1	4 / 3	5 / 5
681 x 693	2 / 3	2 / 3	1 / 2	5 / 5	2 / 1
684 x 661	4 / 2	5 / 4	3 / 5	2 / 2	3 / 3
639 x 602	3 / 5	3 / 5	2 / 4	1 / 1	1 / 4

SECOND YEAR GROWTH AT APPOMATTOX

Seedlings at the Reynolds site were not examined in the second year because of the lack of growth abnormalities at this site. However, examination of the Appomattox seedlings in the second growing season revealed that there were relatively few abnormalities that were present in the second growing season. Seedlings had regained apical dominance and needle growth appeared to be normal. Apical dominance apparently was regained either by the elongation of the stunted terminal bud of the previous year, or through the assumption of dominance by a lateral branch such that there was only a slight deviation from the original axis. The only apparent long term consequence of the abnormalities of the previous year was low forking. Of the seedlings examined, 22% had low forking with two or more leaders of approximately the same height.

The average ratio of branch to apical height of the seedlings in the second year was 1.02. The ratio had improved from 1.12 recorded at the end of the previous growing season. A ratio approaching 1.0 is expected, as lateral branches and terminal apices elongate at approximately the same time on recurrently flushing loblolly pine seedlings. With the elongation of each new flush and overall increasing height, branches and the apical meristem are nearly the same height as measured from the ground.

The ratio of branch to apical height was not significantly different for families ($p = 0.4558$) or for nursery of origin ($p = 0.5889$). Family and nursery of origin differences present at the end of the first growing season were no longer significant. Family rankings for the ratio of branch to apical height shifted, with family 681 maintaining the poorest ratio (1.08). Family 639 (formerly ranked the highest) ranked third of five (1.03).

Total height (overall mean of 118.2 cm) did not differ between families ($p = 0.5564$) or for the greenhouse in which the seedlings were raised ($p = 0.9830$).

The flush that overwintered and elongated the following spring was examined. The greenhouse in which the seedlings were raised did not produce significant differences in the mean height of the flush that elongated in the spring (overall mean of 36 cm, $p = 0.2711$). Family differences in the number of needles per total stem units were not significantly different (overall mean of 0.97, $p = 0.1103$). Although differences were not significant, family rankings for the number of needles per total stem units produced interesting results. Family 681 produced the least apical elongation of the highest flush and

consequently had the poorest branch to apical ratio in 1992. In the spring of 1993, family 681 produced the longest flush arising from an overwintering bud. In 1992 family 639 produced the greatest apical elongation of the highest flush, and consequently had the most favorable branch to apical ratio. In the spring of 1993 family 639 produced a short flush (only family 684 produced a shorter flush).

Needle production was increased for the stem that elongated in the spring of 1993. The mean number of needles per total stem units for the flush that elongated in the spring of 1993 was 0.875, compared to a mean of 0.358 for the third flush of the previous year. Greenhouse of origin and family differences were not significant ($p = 0.3899$ and 0.1358, respectively). Family rankings had changed compared to measurements taken for the third flush at the end of the growing season, but did not appear to follow a pattern.

The total number of stem units for the first flush that elongated in the spring of 1993 indicated that the family that elongated the least the previous year produced the greatest number of stem units the following year. Family differences in the total number of stem units were apparent ($p = 0.0179$). Family 681 ranked the highest, with 213 total stem units. Family 639 ranked fourth, with 132 total stem units.

These results indicate that seedlings planted in tubes in a greenhouse in January, and outplanted in May are exhibiting a form of lammas growth as described by Rudolph (1964). Rudolph described lammas growth as a borrowing of stem units that would normally elongate in the spring of the following year. Lammas growth is normally associated with more northern, single flush conifers, but may have become more important in the loblolly pine seedlings that have been exposed to constantly favorable growing conditions for the equivalent of two growing seasons. These seedlings have likely become very sensitive to decreasing daylength. As daylength decreased, the dormancy level of the apex increased, requiring less of a stimulus to produce a resting bud. The resting bud may have formed in response to mid- to late-summer drought. Cessation of terminal growth resulted in increased carbon allocation to branches and to diameter growth. As conditions were still favorable for growth near the end of the growing season, the sensitive terminal apex elongated, but only partially. In this way, these loblolly pine seedlings resembled a more northern conifer that would elongate in response to late season favorable growing conditions.

The absence of needle growth late in the growing season was likely the result of the elongation of only the sterile portion of the new

shoot. After overwintering the remainder of the flush elongated, along with the additional needles that formed under the bud. This may explain the increased number of needles per total stem units in the flush that elongated in the spring.

CONCLUSIONS

Seedlings that are greenhouse-sown in January and outplanted in May may produce growth abnormalities during the first growing season. Abnormalities include stunted apical growth that leads to the loss of apical dominance, shoots that lack needles, and large increases in root collar diameters at the end of the summer.

Large differences in the growth patterns of seedlings raised in the New Kent and Reynolds greenhouses were apparent. The largest difference in seedling handling between the two greenhouses was the length and degree of the hardening-off period. It is concluded that pre-planting hardening-off may have increased the dormancy status of the seedlings, somewhat alleviating symptoms. After one year in the field, family differences in the degree of expression of growth abnormalities were large, and were comparable in magnitude to height and diameter growth. Additionally, growth abnormalities were expressed to a greater degree at sites that produced greater growth.

The loss of apical dominance was likely the result of dormancy induced cessation of growth of the terminal apex, without a corresponding cessation of growth in the lateral branches. Needleless shoots were likely produced when terminal apices elongated only partially, "revealing" the sterile portion of the shoot.

Effects of growth abnormalities appear not to be long lasting. After overwintering, second year growth resumed as normal, with the only obvious long-term effects of the growth abnormalities of the previous year being competing leaders.

CHAPTER 4
EXPERIMENT 2

GROWTH ABNORMALITIES IN GREENHOUSE-GROWN LOBLOLLY PINE II: IMPOSED DORMANCY TREATMENTS AND SUPPLEMENTAL WATER

INTRODUCTION

Virginia Department of Forestry (VDF) control crossed loblolly pine (*Pinus taeda*) tube stock planted in the greenhouse in January and outplanted in May have been observed to produce growth abnormalities the summer after outplanting (see Chapter 3).

Loblolly pine normally exhibits a mixture of fixed and free growth, producing several flushes during the growing season, and first-year loblolly pine seedlings have been observed to exhibit entirely free growth (Lanner 1976). Older loblolly pine has been observed to exhibit entirely free growth under constantly favorable environmental conditions, resulting in foxtail growth (Kozlowski and Greathouse, 1970). However, if acted upon by stresses such as water or nutrient shortages or shortening daylength, loblolly pine establishes a bud. If the stress that initiated budset is relieved, resting buds have been observed to flush (Boyer, 1970). Under normal conditions, short days trigger the establishment of a bud that progresses into deep dormancy and overwinters. This overwintering bud flushes only after an intervening chilling requirement has been satisfied. Release from dormancy has been observed in early January under normal conditions, following 600 hours of chilling (Boyer and South, 1989).

Mexal and Carlson (1981) observed growth abnormalities in spring outplanted loblolly pine that included needleless shoots and loss of apical dominance. These abnormalities were associated with decreased height growth (when compared to fall outplanted seedlings). Growth abnormalities were attributed to a failure to fulfill the chilling

requirement of the seedlings. After exposure to treatments that increase the dormancy status of containerized seedlings, including shortened days and hardening off, an increase in height growth and field survival has been observed (Mexal et al., 1979: Odlum and Colombo, 1988, Chapter 3). Hinesley (1982) observed that attempts to bypass the normal chilling requirements of *Abies fraseri* resulted in stunting, loss of apical dominance, terminal bud abortion, lack of symmetry and other morphological abnormalities.

Seedlings that have been winter planted in the greenhouse and outplanted in May have been exposed to nine months of continuously favorable growing conditions. However, during this period, the seedlings have been subjected to increasing and decreasing daylength. It was hypothesized that treatments that influence the dormancy status of the loblolly pine seedlings may also influence the development of abnormal growth. This experiment was established to determine the effect of imposed dormancy treatments and supplemental water on the development of the abnormal growth. Specifically, each of 6 families (VDF specific controlled crosses 620 x 698, 681 x 693, 639 x 602, 684 x 661, 636 x 695 and 641 x 664) received factorial combinations of shortened daylength (0, 4, 6 weeks), and chilling hours (0, 400, 600 hours), with post-planting supplemental water supplied as a split plot. Controlled cross 641 x 664 was raised under natural daylength conditions (received no supplemental daylength treatment). This family was raised in this manner to partially examine the hypothesis that increasing and later decreasing daylength in the greenhouse influenced seedling morphology.

METHODS AND MATERIALS

1992 PLANTINGS (INTERACTIONS CONFOUNDED WITH END DATE)

Study Sites

The Reynolds Homestead Research Center site was a highly eroded old field, which had been mowed and chemical treated with Oust® before planting at a rate of 0.224 kg/ha (3.2 oz / acre). A 1.5% solution of Roundup® was also applied before planting, with 30 gallons of mix (11.4 liters) used. The soils of the Reynolds site are a clayey, kaolinitic, thermic, Typic Hapludult of an Altavista series.

Seedling Production and Experimental Design

Seed was handled as in Chapter 3, and planted (days 9-16 after sowing) in the Virginia Polytechnic Institute and State University greenhouse in Blacksburg, VA. Seedlings were grown under conditions described in Chapter 3.

Dormancy treatments consisted of factorial combinations of short days (0, 4 or 6 weeks) and subsequent chilling (0, 400 or 600 hours). Short days were implemented by placing the seedlings under PAK Midnight Photo Period Fabric® for all but eight hours per day. Temperatures under the shadecloth were not regulated. Records from 1993 show that temperatures did not rise more than 4°F after covering, and that warmer temperatures were recorded under the shadecloth for 2 hours after covering. Morning temperatures under the shadecloth increased more rapidly than outside the shadecloth, generally reaching 3° warmer than ambient air temperature before removal. Greenhouse temperatures were regulated with a pad cooler, and ranged from 70°F to 90°F. Chilling was implemented by placing the seedlings in a refrigerator at 3°C with no supplemental light. For a complete schedule of applied treatments, refer to Table 4-1.

On day 132, all seedlings receiving one treatment only were placed in the lathehouse where they were exposed to ambient temperatures, 12-15 % full sun and kept well watered. Because of time constraints and to prevent confounding from storage and additional hardening off, seedlings were planted at the Reynolds Homestead Research Center at two different times. On the first date (day 141 - 142), all seedlings that were subjected to one dormancy treatment (either short days only or chilling only) were planted. On the second date (day 158 - 159), all seedlings subjected to both daylength and chilling treatments were planted. To partially reconcile the two planting dates and to compare growth and development of greenhouse grown stock and nursery-run stock, 1-0 loblolly pine seedlings (produced by the Virginia Department of Forestry, New Kent, Virginia) were planted with the greenhouse stock.

Seedlings were planted in four blocks, and supplemental water was imposed as a split-plot. Within each split-plot, were factorial combinations of all family (6), daylength (3) and chilling (3) treatments. Seedlings were planted in six tree plots, on a 0.5 x 0.5 meter spacing. To prevent watering of non-watered plots, a three meter strip was left between split plots. Measurements were taken on three seedlings in each six tree plot, with the mean used as the experimental

Table 4-1. Schedule of events for loblolly pine seedlings raised in the Virginia Polytechnic Institute and State University greenhouse in Blacksburg, VA, exposed to dormancy treatments and outplanted at the Reynolds Homestead Research Center in Critz, VA.

Treatment	Day Trt Began	Day Trt Ended
6 Weeks Short Days	91	132
4 Weeks Short Days	91	118
600 Hours Chilling	94	118
400 Hours Chilling	101	118
4 Weeks + 400 Hours	118	135
4 Weeks + 600 Hours	120	145
6 Weeks + 400 Hours	132	149
6 Weeks + 600 Hours	132	157
Site prepared	124	
Seedlings Planted (Early)	141-142	
Seedlings Planted (Late)	158-159	
Watering Commenced	185	
Dursban	165	
Retreated with Roundup	178	

unit. The remaining three seedlings were harvested over the course of the summer, for allometry and starch analysis.

Watering commenced on day 185, as it was unnecessary before that. Well watered plots received 2.54 cm (1 inch) of water per week, applied with a soakhose.

On June 18, 1992 (day 165), 37.85 grams of Dursban® were applied (37.85g of product as 2.67 oz./gal water in 1/2 gal) to prevent pales weevil (*Hylobius pales*) infestation. In addition, the study site was retreated with Roundup on day 178 to reduce herbaceous competition.

Data Collection

Seedlings were examined twice monthly, with root collar diameter recorded to the nearest 0.01 mm, flush height and sterile region height (to the nearest mm), the number of sterile scales and the number of needles were counted for each flush. To avoid confusion, a nursery marker was used to count sterile scales (which were only counted after needle elongation and flush completion). The number and height of lateral branches were recorded. Water potentials were measured on a single fascicle on one seedling per treatment per block (family selected randomly), with a pressure bomb (Plant Moisture Stress Instruments®, Corvallis, OR). As seedlings were measured repeatedly, only new growth was examined and recorded.

Seedlings harvested for starch analysis and allometry were removed bi-monthly, with three to two seedlings harvested per split-plot for each dormancy treatment (family selected randomly). Three seedlings were harvested for each treatment early in the growing season to ensure enough ground product for starch analysis. Later in the growing season, two seedlings were harvested for each treatment. Harvesting began with early planted seedlings on day 138, and was completed on day 208. Late planted seedlings were harvested on day 161, and on day 245.

Harvested seedlings were placed in an ice chest in the field to slow metabolic activity, washed, root and shoot separated, placed in poly bags and placed in a freezer at -20°C. After freezing, seedlings were placed in paper bags and oven dried for at least 48 hours at 70°C. Dry weights were recorded for roots, shoots and needles to the nearest 0.0001 g.

After weights were obtained, seedlings were ground in a Wiley mill to pass through a 30 mesh screen. Watered and unwatered seedlings were combined, resulting in four blocks of each dormancy treatment

combination. Starch analysis proceeded according to methods described by Rose et al. (1991), and included acetone extraction of chlorophyll, starch extraction, solubilizitation in hot ethanol, followed by colorometric determination with anthrone. Samples were analyzed with a Bausch and Lomb® spectrophotometer with wavelength set at 625 nm. Early planting dates June 5, July 2, and July 30, and late planting dates June 13, and August 6 were analyzed (separately).

Statistical Analysis - Early Plantings

The null hypotheses tested were that dormancy inducing treatments had no influence on the growth of the seedlings, that supplemental water would not influence the development of the growth abnormalities, that families would not produce growth differences, and that the interactions between dormancy treatments, water, and family were not significant.

Total final height, the needles per cm for each flush, the number of secondary needles per total stem units for each flush after the first flush, and the ratio of branch to total height for each flush and for the whole seedling were calculated. 1-0 nursery run seedlings planted late were compared with seedlings that were planted early with a two sample t-test.

The mean value of the three seedlings was analyzed and analysis was conducted using a General Linear Models Procedure (SAS Inst., 1988). A three way ANOVA was used to determine the effects of short days, water and family, and again for chilling, water and family. The statistical model used for the early plantings on each date of examination was:

$$Yijk = \mu_{ijkl}+b_i+\alpha_j+ß_k+\delta_l+b\alpha_{ij}+\alpha ß_{jk}+\alpha\delta_{jl}+ß\delta_{kl}+\alpha ß\delta_{jkl}+E_{ijkl}$$

where:

μ =	overall mean	df
b =	block effect	3
α =	water	1
ß =	dormancy	2
δ =	family	5
$b\alpha$ =	whole plot error	3
αß =	water*dormancy	2
$\alpha\delta$ =	water*family	5
ßδ =	dormancy*family	15
αßδ =	water*dormancy*family	15
E =	random error	92

Allometry data analysis was conducted using a General Linear Models Procedure (SAS Inst., 1988). A two way ANOVA was used to determine the effects of short days and water, and again for chilling and water. For allometry, the effects of family were ignored. The statistical model used for the early plantings on each date of examination was:

$$Yijk = \mu_{ijk} + b_i + \alpha_j + ß_k + b\alpha_{ik} + \alpha ß_{jk} + E_{ijk}$$

where:

μ =	overall mean	df
b =	block effect	3
α =	water	1
ß =	dormancy	2
bα =	block*water	3
αß =	water*dormancy	2
E =	random error	12

Starch analysis was conducted using a General Linear Models Procedure (SAS Inst., 1988). A one way ANOVA was used to determine the effects of short days and water, and again for chilling and water. The number of needles per total stem units was arc sine transformed before analysis. For starch analysis, the effects of family and water were pooled.

The statistical model used for the early plantings on each date of examination was:

$$Yijk = \mu_{ijk} + b_i + \alpha_j + ß_k + b\alpha_{ik} + \alpha ß_{jk} + E_{ijk}$$

where:

		df
μ =	overall mean	
b =	block effect	3
α =	dormancy	2
E =	random error	6

Statistical Analysis - Late Plantings

The statistical model utilized for the examination of late plantings was similar, but included both daylength and chilling terms (each with one degree of freedom) and the two interaction of these terms Duncan's new multiple range test was used as a follow-up procedure to separate means that were found to be different in the general linear model. Repeated measurements analysis was not utilized in this analysis as variance increased over the course of the growing season.

1993 PLANTINGS (INTERACTIONS CONFOUNDED WITH START DATE)

In the 1992 plantings, interactions between preplanting shortened days and chilling could only be examined incompletely. Further, the effects of dormancy treatments on the growth of seedlings planted on a site with much more favorable growing conditions were unknown. These factors prompted the establishment of an experiment that examined the effects of shortened daylength and subsequent chilling on the growth and development of seedlings that were outplanted at the Appomattox-Buckingham State Forest. Dormancy treatments included factorial combinations of 0, 4, and 6 weeks of shortened days (8 hour daylength) and 0 or 400 hours of chilling (3°C) before outplanting.

Seed was sown in fine vermiculite on January 4, 1993, and seedlings were raised as described in Chapter 3. Three families of controlled cross loblolly pine were used for this experiment, including specific VDF crosses 620 x 698, 681 x 693 and 639 x 602. Germinals were pooled and five seedlings were selected from this pool of families for each treatment-block combination. Temperatures in the greenhouse ranged from 68°F to 90°F. Seedlings were watered daily to field capacity.

Treatment initiation was staggered to ensure that seedlings subjected to different dormancy treatments would end treatment simultaneously. Seedlings that were to receive 6 weeks of shortened days and 400 hours of chilling were moved to eight hour days 71 days after sowing, 4 weeks of shortened days and 400 hours 89 days after sowing, 6 weeks of shortened days 88 days after sowing, 4 weeks of shortened days 102 days after sowing, and 400 hours of chilling only 113 days after sowing. Temperature under the shadecloth was not regulated, but records were maintained. Seedlings were covered at 5:30 P.M. and uncovered at 9:30 A.M. Temperatures under the shadecloth generally did not rise more than 4°F after covering, and warmer temperatures were recorded under the shadecloth for 2 hours after covering. Morning temperatures under the shadecloth increased more rapidly than outside the shadecloth, generally reaching 3° warmer than ambient before removal.

Treatment ended for all seedlings on day 129. Seedlings were subsequently transferred to a lathehouse for hardening-off, and were outplanted on day 146. Lathehouse conditions were as previously stated.

Seedlings were outplanted at the Appomattox-Buckingham State Forest near Appomattox, Virginia in a plot adjacent to Experiment One (planted in 1992). The site was prepared as before, with a mixture of

Roundup® and Oust®, and seedlings were planted at a one meter by one meter spacing. Five tree row plots in each of ten blocks were planted for each treatment.

The middle three trees were measured in each row at outplanting, twice during the growing season and at the end of the 1993 growing season. Variables measured included: the height of each flush, branch height, root collar diameter, and the number of needles per total stem units for each flush after the first flush. Additionally, the frequency of a bud was determined at the time of outplanting.

Means were computed for the three trees in each row plot and used as the experimental unit. The data was analyzed as a two-factor analysis of variance, with 10 blocks, 3 levels of shortened days (0, 4 and 6 weeks of shortened days), and 2 levels of chilling (0 and 400 hours at 3°C). The number of needles per total stem units was arcsin pretransformed for analysis.

RESULTS AND DISCUSSION

1992 PLANTINGS (INTERACTIONS CONFOUNDED WITH END DATE)

Differences in Early and Late Planted 1-0 Seedlings

Early and late planted 1-0 stock did not significantly differ at the end of the growing season for root collar diameter, height, and the ratio of branch to apical height. The number of needles per total stem units could not be analyzed, as too few seedlings produced a second flush to permit analysis. Of the 40 seedlings that were early planted, 93% produced a second flush, and 20% produced branches with the second. Of the late planted seedlings, only 41% of the seedlings produced a second flush. Of these, only 6% of the seedlings produced branches with the second flush.

The 18 day difference in planting apparently had a strong influence on lateral branch growth with the second flush. For this reason, the main effects of daylength and chilling were analyzed separately. The interaction of these two factors was examined incompletely with the analysis of late planted seedlings (Table 4-2).

Differences in 1-0 Seedlings and Greenhouse Stock

Greenhouse and 1-0 stock were very different at outplanting, resulting from the VDF production methods that include top-clipping. At

Table 4-2. Experimental design and outline of analysis for loblolly pine seedlings subjected to dormancy treatments. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Early planted refers to trees planted on May 25 and 26, 1992. Late planted seedlings were planted on June 11, 1992.

Treatment	0 Weeks Short Days	4 Weeks Short Days	6 Weeks Short Days
0 Hours Chill	Early Planted	Early Planted	Early Planted
400 Hours Chill	Early Planted	Late Planted	Late Planted
600 Hours chill	Early Planted	Late Planted	Late Planted

outplanting, the VDF seedlings had no central leader, limiting the applicability of seedling measures. At outplanting, 1-0 stock had significantly greater dry mass than greenhouse stock (2.00 vs. 1.38 g). However, the ratio of root to shoot were the same for both seedlings (0.24).

By the end of the growing season, greenhouse stock weighed an average of 5.55 g, significantly greater than the mass of 1-0 stock (4.07 g), and the root to shoot ratio for greenhouse stock had increased to 0.30 (vs. 0.20 for 1-0 stock). At the end of the growing season, greenhouse stock had significantly larger root collar diameters (7.99 mm vs. 5.06 mm, $p = 0.0001$), and were significantly taller (28.3 cm vs. 24.3 cm, $p = 0.0001$). Late in the growing season, greenhouse stock had branch to apical height ratios similar to 1-0 stock (mean of 0.88).

Starch levels of greenhouse stock did not differ from 1-0 stock early in the summer (mean = 0.056 g/g). However, greenhouse stock was significantly greater than 1-0 stock at mid-summer (0.096 vs. 0.078 g/g, $p = 0.0446$) and in late summer (0.068 vs. 0.052 g/g, $p = 0.0600$).

Influencing Agents

Survival was variable across treatments for the early plantings, (Table 4-3). Tipmoth affected only 2% of all seedlings. As in the previous experiment, controlled cross 636 x 695 was affected by an unidentified stem canker. Seedlings that exhibited the symptoms of the unidentified canker were excluded from treatment, but symptoms that appeared after treatment began were included in analysis. During the growing season symptoms were not obvious, but may have resulted in growth reductions for this family.

SHORTENED DAYLENGTH

Main Effects

Imposed short days had an effect on the morphology of the seedlings at outplanting (Table 4-4). Seedlings subjected to shortened days were significantly shorter than control seedlings (23.7 cm vs. 19.4 cm for 4 weeks or 18.4 cm for 6 weeks) and had reduced height growth in the lateral branches at outplanting (6.7 cm vs. 5.0 cm for 4 weeks or 4.7 cm for 6 weeks). Decreased height and lateral branch growth resulted in an unchanged ratio of branch to apical height control and treated seedlings.

Table 4-3. Percent mortality during the summer of 1992 for loblolly pine nursery run (1-0) seedlings, control tube stock, and tube stock subjected to dormancy treatments. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA.

Treatment	Percent Mortality
1-0	5
Control	4.9
4 weeks short days	3.5
6 weeks short days	11.1
400 hours chill	4.2
600 hours chill	2.1

Table 4-4. Outplanting height, root collar diameter (RCD), the height of the tallest branch and the ratio of branch to apical height for five families of 141-day-old loblolly pine seedlings subjected to 0, 4, or 6 weeks of eight-hour days. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Weeks of Shortened Days	Height (cm)	RCD (mm)	Tall Branch (cm)	Branch:Apical Height
0 weeks	23.7 a	2.94 a	6.7 a	0.279 a
4 weeks	19.4 b	2.81 a	5.0 b	0.255 a
6 weeks	18.4 b	2.93 a	4.7 b	0.250 a
Overall Mean	20.5	2.89	5.4	0.261
p-value	0.0001	0.2123	0.0133	0.5811

The percentage of budset at outplanting was not significantly different for seedlings subjected to 0 (63%), 4 (54%) or 6 (55%) weeks of shortened days. This is likely the result of limited rooting volume available for the seedlings in the Leach tubes. As the control seedlings became root-bound, they set terminal buds. Seedlings subjected to short days were significantly shorter than controls (Table 4-4), but were not more likely to have set a bud, indicating that the shortened days did not improve budset.

After outplanting, seedling growth differences were apparent in the short day treatment. Seedlings that were subjected to shortened days elongated more rapidly after outplanting than did control seedlings (Figure 4-1). This trend did not continue, however, and after 45 days, elongation proceeded at approximately the same rate to the end of the growing season. On the last measurement day, seedlings that had been subjected to 6 weeks of short days were significantly shorter than controls (28.5 cm vs. 26.3 cm) (Table 4-5).

Root collar diameter did not differ between control and short-day seedlings at outplanting (Table 4-4). During most of the growing season, seedlings that received 6 weeks of short days did not differ from control seedlings but seedlings subjected to 4 weeks of short days were significantly smaller than controls. The increased root collar diameters of the seedlings receiving six weeks of short days in combination with the reduced height growth of these seedlings suggests a shift in carbon allocation from height growth to diameter growth with shortened days. This is in agreement with the findings reported in Chapter 3, in which late growing season carbon allocation shifted from elongation of stem to diameter growth. Near the end of the growing season, all short-day seedlings differed significantly from control seedlings following a comparatively rapid increase in diameter growth by the control seedlings (Table 4-5).

Seedlings that were subjected to short days had significantly shorter lateral branches than control seedlings at outplanting (Table 4-4). However, rapid elongation of the second flush produced lateral branches that were taller than conrol lateral branches. The ratio of branch to apical height steadily increased for control seedlings as lateral buds associated with the second flush were produced. The end result of this steady increase was a late growing season lack of significant differences in the ratio of branch to apical height (Figure 4-2).

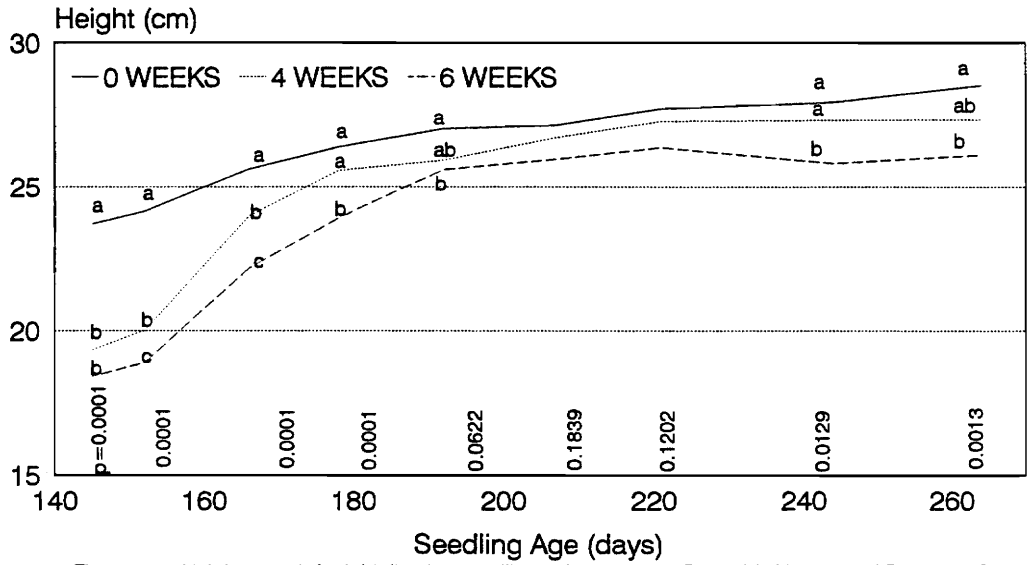


Figure 4-1. Height growth for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,4 or 6 weeks of shortened (8 hour) days prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing.
P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

Table 4-5. Root collar diameter (RCD), the height of the tallest branch and the ratio of branch to apical height for five families of 264-day-old loblolly pine seedlings subjected to 0, 4, or 6 weeks of eight-hour days. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Weeks of Shortened Days	Height (cm)	RCD (mm)	Tall Branch (cm)	Branch:Apical Height
0 weeks	28.5 a	8.06 a	25.7 a	0.899 a
4 weeks	28.0 ab	7.47 b	23.1 a	0.810 a
6 weeks	26.3 b	7.33 b	22.5 a	0.842 a
Overall Mean	27.6	7.58	23.8	0.851
p-value	0.0251	0.0007	0.0863	0.1957

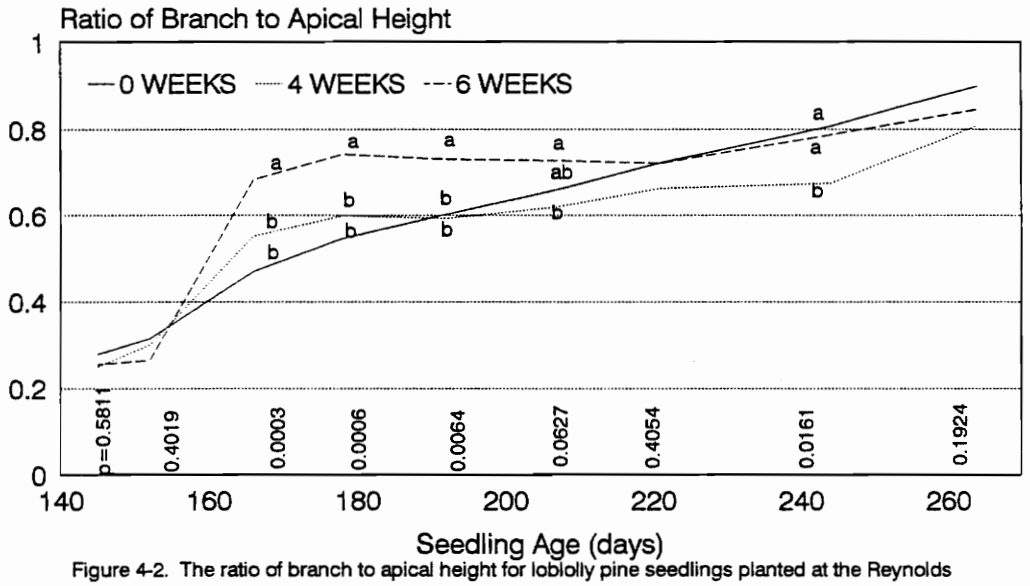


Figure 4-2. The ratio of branch to apical height for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,4 or 6 weeks of shortened (8 hour) days prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing. P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

Of the six families planted in this experiment, only three (Virginia Department of Forestry specific crosses 681 x 693, 620 x 698 and 684 x 661) consistently produced a second flush to allow analysis. Seedlings that were subjected to six weeks of shortened days produced significantly more needles per total stem units than control seedlings (0.77 vs. 0.49 for controls). This shift in the number of needles per total stem units can not be solely attributed to the presence of a bud on the short-day seedlings, as there was no significant difference in budset between these seedlings and controls at the time of outplanting. The daylength treatment may have increased needle production in the second flush.

On a whole-plant basis, shortened days significantly changed the concentration of starch within the seedlings (Figure 4-3). Early in the growing season, seedlings exposed to 6 weeks of shortened days had significantly higher starch reserves than did control seedlings. By the middle of the growing season, significant differences were not apparent, and starch concentrations had increased over the early growing season value. By the last starch sampling date, the seedlings exposed to 6 weeks of shortened days had significantly less starch reserves than did control seedlings. Starch concentrations in the roots of control trees generally did not differ from seedlings subjected to shortened days.

Adams *et al.* (1986) examined starch concentration in the roots of loblolly pine trees and found that starch concentrations were highest in roots collected in March. Concentrations were lowest in autumn. It may be interpreted that seedlings that received 6 weeks of shortened days were "reset" to a normal cycle. That is, starch concentrations were relatively higher in these seedlings after they were removed from shortened days, somewhat corresponding to midwinter starch levels in "normal" trees. Over the summer, the starch concentration of these seedlings dropped, approaching much lower values than control seedlings by late summer. This can be thought of as being comparable to late summer values of "normal" trees.

Shoot dry weights were significantly reduced by shortened days immediately following treatment ($p = 0.0893$). Four weeks of shortened days only slightly reduced shoot weight (From 1.86g to 1.75g). With an additional two weeks of shortened days, shoot dry weights were reduced to 1.07g. Large reductions in total dry weight were observed with 6 weeks of shortened days, with only slight reductions in total weight with 4 weeks of shortened days ($p = 0.0926$).

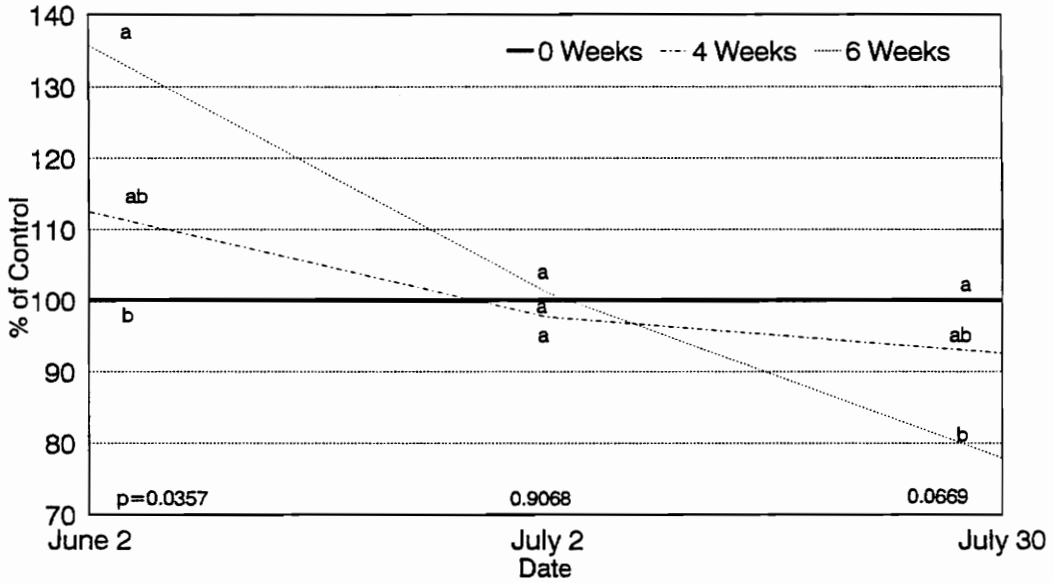


Figure 4-3. Whole-plant starch concentrations (in grams of starch per gram of plant material) presented as a percent of control for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0, 4 or 6 weeks of shortened (8 hour) days prior to planting. Seedlings were outplanted on May 21, 1992, 137 days after sowing. P-values are presented for each sampling date. Values followed by the same letter on a given date are not significantly different at alpha = 0.05.

Near the end of the summer, root and total dry weights, as well as the root to shoot ratio for seedlings subjected to shortened days were not significantly different from control seedlings. However, shoot weight was reduced for seedlings that were subjected to shortened days ($p = 0.0579$). Control seedlings shoot weights averaged 5.36g near the end of the summer, and seedlings subjected to 6 weeks of shortened days weighed only 3.89g.

Family Effects

Different controlled crosses produced very different growth responses. As in Chapter 3, family 639 x 602 exhibited slower early growth and the most favorable end of the growing season ratio of branch to apical height ratio. Family 681 x 693 was consistently among the fastest growing seedlings in terms of height and root collar diameter growth and expressed the least favorable ratio of branch to apical height.

Families 620 x 698, 684 x 661, and 681 x 693 were among the largest in terms of height (ave. 22.5 cm) and root collar diameter (ave. 3.2 mm) at outplanting (Table 4-6). Family 639 x 602 and family 641 x 664 were significantly smaller (ave. 17.2 cm and 2.6 mm) than families 620, 684 and 681 (Table 4-6). The ratio of branch to apical height did not differ between families at outplanting, with the exception of family 641, which had a significantly reduced branch to apical height ratio. Family 641 was raised in the greenhouse under natural light only and received no additional daylength.

Budset differences were apparent between families (range from 85% for family 620 to 11% for family 639) (Table 4-6). Budset roughly corresponded to seedling height, with the tallest families having the greatest percentage of budset.

Family 639 and family 681 were the best and poorest performers, respectively, in Experiment 1 (Chapter 3) at both the Reynolds Homestead and Appomattox - Buckingham State Forest planting sites. In this planting, family 639 expressed the most favorable end-of-growing-season ratio of branch to apical height (0.647) while family 681 (ratio = 0.913) was not significantly different from the poorest performing family (family 620, ratio = 0.979).

The root collar diameter and apical height of family 639 were always significantly smaller during the growing season. Both families

Table 4-6. Outplanting height, root collar diameter (RCD), height of the tallest branch, the percentage budset and the ratio of branch to apical height for five families of 141-day-old loblolly pine seedlings subjected to 0, 4, or 6 weeks of shortened days. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Tall Branch (cm)	Budset %	Branch:Apical Height
620 x 698	22.5 a	3.22 a	6.4 ab	84.7 a	0.287 a
684 x 661	23.2 ab	3.21 a	6.1 ab	66.7 bc	0.267 a
681 x 693	21.8 ab	3.10 a	7.5 a	65.3 c	0.330 a
636 x 695	21.1 b	2.68 b	6.0 ab	82.0 ab	0.287 a
639 x 602	16.5 c	2.63 b	4.7 b	11.1 e	0.282 a
641 x 664	17.8 c	2.53 b	1.9 c	33.3 d	0.116 b
Overall Mean	20.5	2.89	5.436	57.2	0.261
p-value	0.0001	0.0001	0.0001	0.0001	0.0001

elongated rapidly between 7 and 45 days following planting. After this period, and coincident with a mid-season mild drought, both families ceased height growth for the remainder of the growing season.

Family 641 (the family that did not receive supplemental lighting during greenhouse growth) was significantly smaller in terms of height and diameter at outplanting than all families except family 639. This difference persisted until the end of the growing season (Table 4-7). Family 639 produced a significantly better end of the growing season ratio of branch to apical height than did family 641, and family 641 did not differ significantly from other families with the exception of the poorest performing family (family 620, ratio = 0.979).

This information, coupled with the observation of growth abnormalities by the Virginia Department of Forestry before the lighting regime was established suggests that pre-planting lighting had little influence on seedling growth and the development of the loss of apical dominance after outplanting.

Needle production was analyzed for three families, as only three families completed a second flush by the end of the measurement period. Of these families, cross 681 x 693 produced 0.76 needles per stem unit, significantly higher than either family 684 x 661 (0.61) or family 620 x 698 (0.57). Seedlings grown at Appomattox produced results that were quite different from Experiment 1; these three families did not differ for the second or third flush at either Appomattox or the Reynolds Homestead.

Supplemental Water

Supplemental watering had very little effect on the growth and development of the loblolly pine seedlings. Well-watered and control seedlings did not produce differences in height, root collar diameter or in branch height growth. However, supplemental water reduced shoot dry weights at the end of the summer ($p = 0.0536$) from 4.85g to 4.36g. In addition, there was a significantly different end-of-growing-season ratio of branch to apical height, with watered seedlings measuring 0.790, and control seedlings measuring 0.910 ($p = 0.0403$).

Significant differences in the ratio of branch to apical height were observed between watered and control seedlings 23 days after watering began, and continued to the end of the growing season. The difference in the ratio of branch to apical height was likely the result of extended apical growth into the mid-summer drought.

Table 4-7. Height, root collar diameter (RCD), height of the tallest branch, and the ratio of branch to apical height for five families of 264-day-old loblolly pine seedlings subjected to 0, 4, or 6 weeks of shortened days. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Tall Branch (cm)	Branch:Apical Height
620 x 698	29.1 a	7.97 ab	28.5 a	0.979 a
684 x 661	29.2 a	7.58 bc	24.3 ab	0.832 ab
681 x 693	29.4 a	8.25 a	26.5 a	0.910 ab
636 x 695	28.1 a	8.04 ab	26.3 a	0.930 ab
639 x 602	24.7 b	7.00 cd	16.3 c	0.647 c
641 x 664	25.1 b	6.73 d	20.7 bc	0.805 b
Overall Mean	27.60	7.59	23.80	0.851
p-value	0.0001	0.0001	0.0001	0.0001

PREPLANTING CHILLING

Main Effects

Seedlings that were chilled for 600 hours had a significantly lower percentage of budset (7% vs. 63%) (Table 4-8). Unchilled seedlings and seedlings that were chilled for 400 hours were free to continue growth but instead set buds as the seedlings occupied the planting medium volume of the Leach tubes. Seedlings that were placed in the refrigerator simply ceased growth.

Seedlings receiving chilling had significantly smaller root collar diameters at outplanting (2.94 for controls vs. 2.47 for 400 hours or 2.07 for 600 hours). Root collar diameter growth proceeded at the same rate for chilled and non-chilled seedlings, resulting in significant differences throughout the growing season (Figure 4-4).

Seedlings that were subjected to 600 hours of chilling were significantly shorter than control seedlings and seedlings subjected to 400 hours (Table 4-8). Height differences between chilling treatments persisted throughout the growing season (Figure 4-5). There was no difference in apical height at the end of the growing season between control seedlings and seedlings subjected to 400 hours of chilling.

The pattern of branch height growth for seedlings that received 600 hours of chilling was quite different than the pattern of branch growth exhibited by control seedlings and the seedlings that received 400 hours of chilling (Figure 4-6). The difference in growth patterns was likely due to the lack of a bud on the seedlings receiving 600 hours of chilling (Table 4-8). Control seedlings and seedlings receiving 400 hours of chilling flushed shortly after outplanting. This flush resulted in seedlings with significantly taller branches than the seedlings receiving 600 hours of chilling. This difference was apparent through the last measurement day.

The reduction in branch height growth (resulting from the suppression of bud formation) in seedlings chilled for 600 hours decreased the branch to apical height ratio. At outplanting the branch to apical height ratio did not differ between treatments (Table 4-8). However, 21 days after outplanting seedlings receiving 600 hours of chilling expressed an improved branch to apical height ratio (Figure 4-7). This difference persisted throughout the growing season.

Table 4-8. Outplanting height, root collar diameter (RCD), height of the tallest branch and the ratio of branch to apical height and the frequency of a terminal bud for five families of 141-day-old loblolly pine seedlings subjected to 0, 400, or 600 hours of preplanting chilling. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Hours of Chilling	Height (cm)	RCD (mm)	Tall Branch (cm)	Branch: Apical Height	Bud %
0 hours	23.7 a	2.94 a	6.6 a	0.279	63.2 a
400 hours	21.6 b	2.47 b	5.8 ab	0.269	56.0 a
600 hours	17.1 c	2.07 c	4.4 b	0.258	7.3 b
Overall Mean	20.8	2.49	5.6	0.269	42.1
p-value	0.0001	0.0001	0.0094	0.7841	0.0001

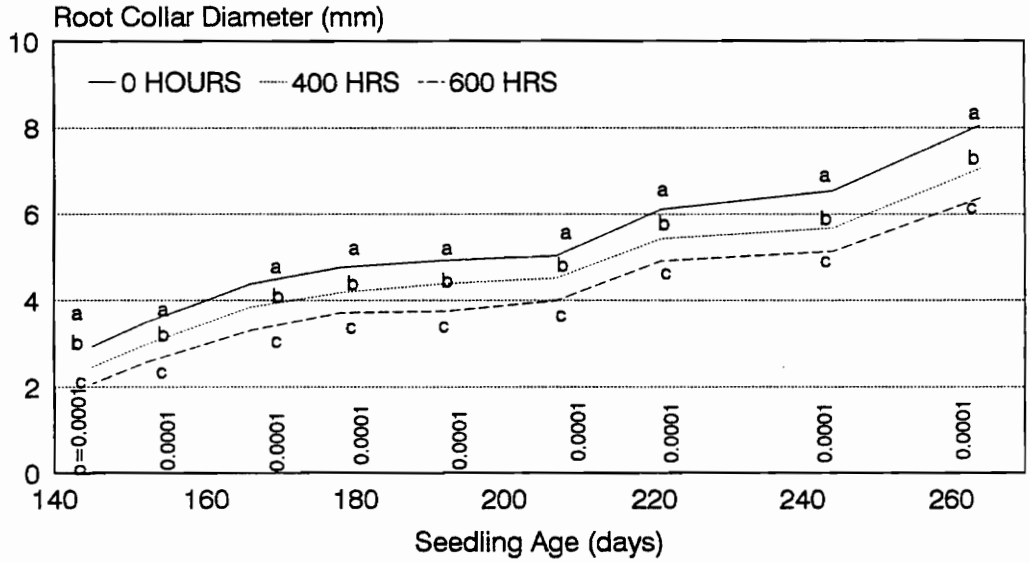


Figure 4-4. Root collar diameter growth for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,400 or 600 hours of chilling (3 C) prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing. P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

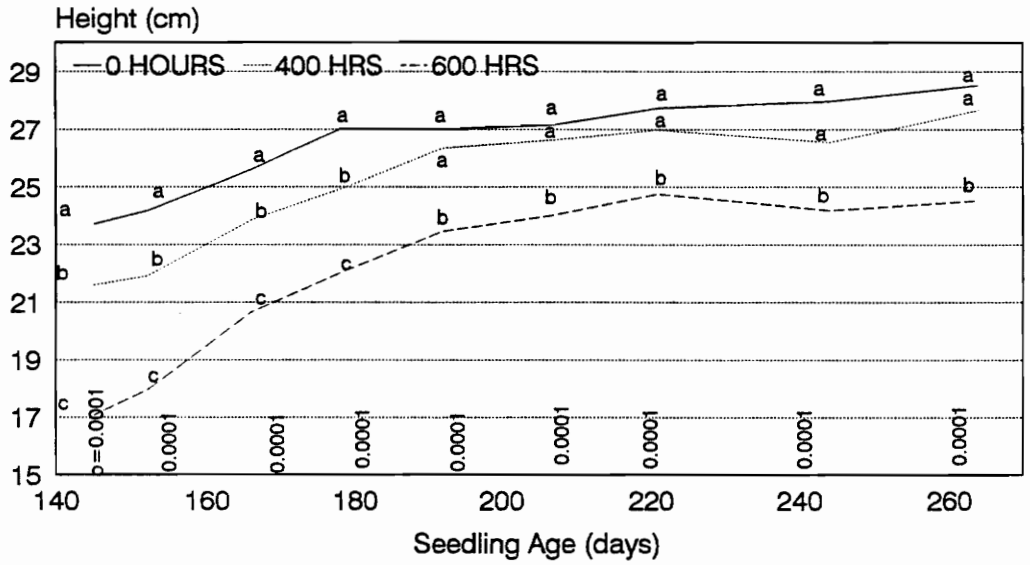


Figure 4-5. Height growth for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,400 or 600 hours of chilling (3 C) prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing.
P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

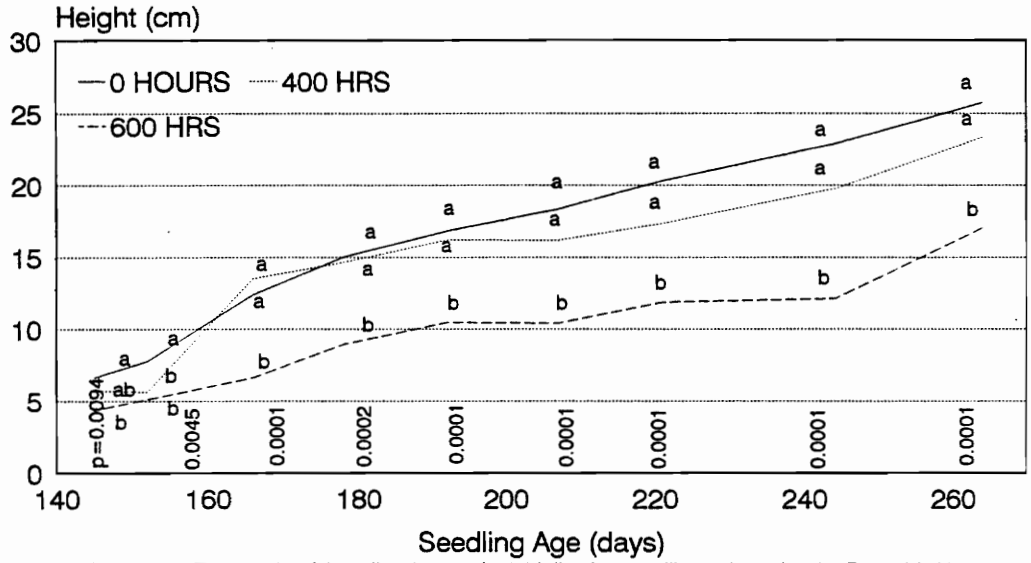


Figure 4-6. The height of the tallest branch for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families were subjected to 0,400 or 600 hours of chilling (3 C) prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing.

P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

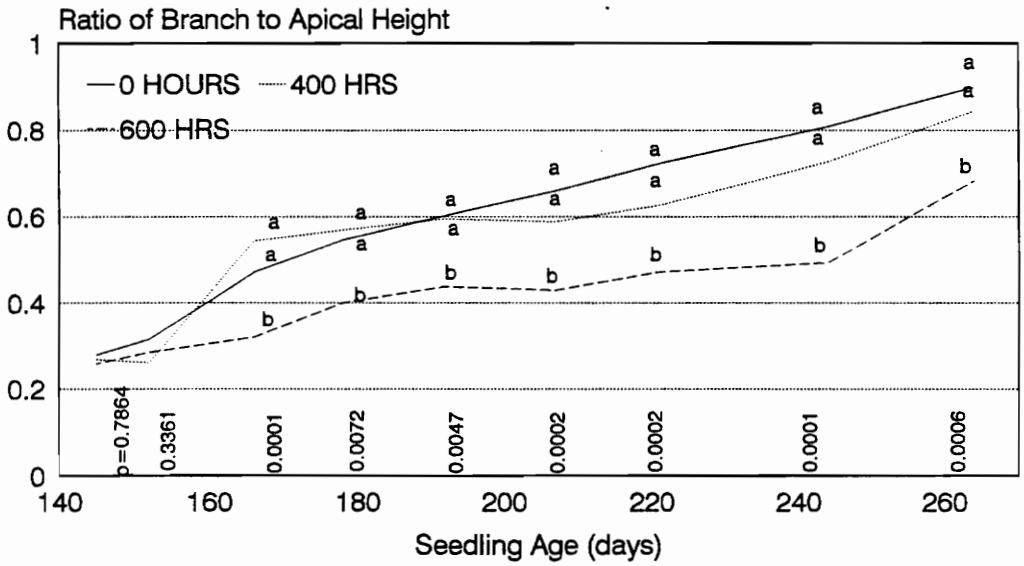


Figure 4-7. The ratio of branch to apical height for loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,400 or 600 hours of chilling (3 C) prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing. P-values are presented for each sampling date. Points on a sampling date marked with the same letter are not significantly different at alpha = 0.05.

At outplanting, starch concentrations in the roots of control trees did not differ from seedlings receiving pre-planting chilling. However, on the mid-summer sampling date, starch concentration in the roots of control trees was significantly higher than in the roots of seedlings chilled for 600 hours (Table 4-9). Late in the growing season, there were no differences in root starch concentrations for chilled seedlings. Chilling apparently had some effect on root starch concentrations, but not to the extent of shortened days.

Family Effects

Family responses of chilled seedlings were similar to the responses of seedlings subjected to shortened days. As before, family 681 was consistently the largest in terms of root collar diameter and height, and expressed the poorest form. Family 639 was consistently the smallest, and expressed the best form.

Differences between these two families were apparent at the beginning of the season (Table 4-10) and were expressed at the end of the season. Root collar growth and height growth indicated consistent differences between the best and poorest family, with the significant differences present at outplanting persisting throughout the measurement period. Again, it is apparent that height growth proceeded until the mid-summer drought and that few seedlings elongated the bud set during this drought. However, branch growth proceeded following the drought, resulting in increased ratios of branch to apical height (Figure 4-8).

Family 681 had the greatest percentage of budset (60%) at outplanting. As the buds set before outplanting elongated seven days after outplanting the height of the tallest branch rapidly increased. This elongation greatly influenced the ratio of branch to apical height. Differences that arose between families with this elongation persisted to the end of the measurement period.

Supplemental Water

The application of supplemental water prolonged apical growth by delaying budset and decreased branch growth (Figure 4-9). Watering delayed budset during the drought event and allowed for continued apical growth. Branch growth was suppressed either hormonally or by a shift in carbon allocation to the still-elongating bud.

Table 4-9. Root starch concentrations (g/g of plant tissue) at three sampling dates during the 1992 growing season for five families of loblolly pine seedlings subjected to 0, 400, or 600 hours of preplanting chilling. Seedlings were outplanted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Chilling Hours	June 2	July 2	July 30
0 hours	0.060 a	0.961 a	0.077 a
400 hours	0.045 a	0.089 ab	0.069 a
600 hours	0.043 a	0.083 b	0.072 a
Overall Mean	0.049	0.089	0.073
p-value	0.2110	0.0685	0.7984

Table 4-10. Outplanting height, root collar diameter (RCD), height of the tallest branch, the percentage budset and the ratio of branch to apical height for five families of 141-day-old loblolly pine seedlings subjected to 0, 400, or 600 hours of preplanting chilling. Seedlings were planted at the Reynolds Homestead Research Center in Critz, VA. Values in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Family	Height (cm)	RCD (mm)	Tall Branch (cm)	Budset %	Branch:Apical Height
620 x 698	22.8 a	2.76 a	6.7 a	56.9 a	0.291 a
684 x 661	23.1 a	2.62 a	6.0 a	43.5 ab	0.265 a
681 x 693	22.4 a	2.68 a	7.5 a	59.7 a	0.319 a
636 x 695	21.8 a	2.37 b	5.8 a	52.1 a	0.268 a
639 x 602	16.1 c	2.28 b	5.6 a	6.9 c	0.355 a
641 x 664	18.6 b	2.25 b	2.0 b	33.3 b	0.113 b
Overall Mean	20.8	2.49	5.6	42.1	0.2686
p-value	0.0001	0.0001	0.0001	0.0001	0.0001

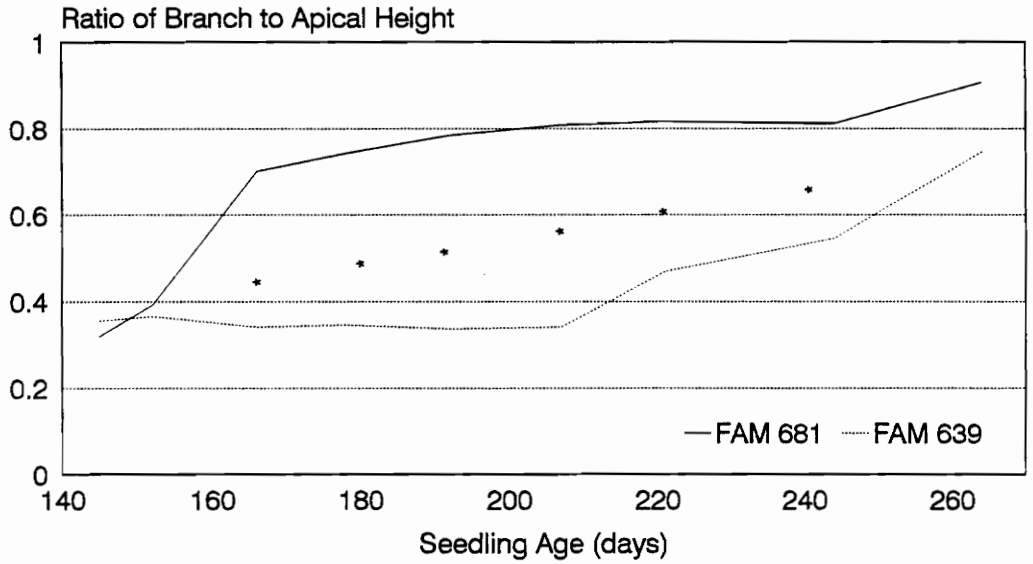


Figure 4-8. The ratio of branch to apical height of the best (family 639) and poorest (family 681) performing of six families of loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Seedlings were subjected to 0, 400, or 600 hours of chilling (3 C) prior to outplanting. Seedlings were outplanted on May 21, 1992, 141 days after sowing. * indicates family differences at alpha = 0.05 for a given sampling day.

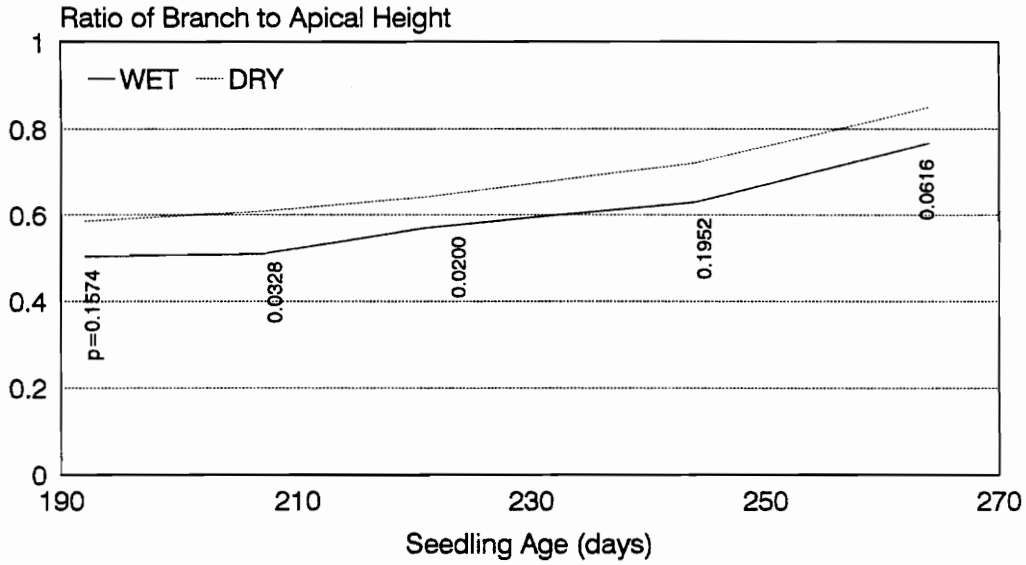


Figure 4-9. The ratio of branch to apical height of loblolly pine seedlings planted at the Reynolds Homestead Research Center in Critz, Virginia. Six families of seedlings were subjected to 0,400 or 600 hours of chilling (3 C) prior to planting. Seedlings were outplanted on May 21, 1992, 141 days after sowing. Watered plots received one inch of water per week beginning on July 8, 195 days after sowing. P-values are presented for each sampling date.

1993 PLANTINGS (INTERACTIONS CONFOUNDED WITH START DATE)

At outplanting, it was found that shortened daylength significantly reduced root collar diameters. Control seedlings were significantly larger than seedlings subjected to 6 weeks of shortened days (3.28 mm vs. 3.05 mm, $p = 0.0391$), but were not larger than seedling subjected to 4 weeks of shortened days. Chilled seedlings had significantly smaller root collar diameters (3.56 mm vs. 2.76 mm, $p = 0.0001$). Seedling heights were similarly affected. Chilling significantly reduced seedling heights by 25 percent (27.2 cm vs. 20.3 cm, $p = 0.0001$). Shortened days reduced height growth (28.0 for controls, 24.1 for 4 weeks, and 19.2 for 6 weeks, $p = 0.0001$), producing significant differences between 0, 4 and 6 weeks of shortened days. For root collar diameters and for height growth, interactions between daylength and chilling were insignificant.

Seedlings that were exposed to shortened days for 6 weeks had significantly lower budset (51% vs. 26%, $p = 0.0016$). Similarly, seedlings that were chilled lacked terminal buds (66% vs. 25%, $p = 0.0001$). Again, this effect is likely a result of seedling size, not the influence of dormancy treatments. Seedlings that received 6 weeks of shortened days were transferred to short days before seedlings filled the Leach tubes. As growth was reduced with short days, roots were less restricted, and the degree of budset was reduced. A significant interaction was observed between shortened days and chilling ($p = 0.0253$), with 91% of seedlings that were subjected to 4 weeks of shortened days and 0 hours of chilling having a bud. This is likely the result of two more weeks of long days in the seedlings subjected to 4 weeks of shortened days (as compared with the seedlings subjected to 6 weeks of short days) in combination with shortened days influencing budset.

By the end of the growing season, 95% of the seedlings (after the means of three tree row plots were calculated) produced a second flush, and 52% of the seedlings produced a third flush.

Shortened days did not affect root collar diameter growth, but height growth was significantly reduced (from 28.4 cm to 25.4 cm, $p = 0.0480$, Table 4-11). Chilling significantly reduced diameter growth ($p = 0.0022$) from 7.61 mm to 6.58 mm, reduced the height growth of the apical meristem from 28.3 cm to 25.0 cm ($p = 0.0016$) and lateral branch growth ($p = 0.0001$) (Table 4-11).

Table 4-11. Root collar diameter (RCD), height and the height of the tallest branch at the end of the growing season for loblolly pine seedlings subjected to factorial combinations of 0, 4 or 6 weeks of shortened days and 0 or 400 hours of preplanting chilling. Seedlings were planted at the Appomattox-Buckingham State Forest, near Appomattox, VA. Seedlings were outplanted on May 30, 1993, 146 days after sowing.

Dormancy Treatment	RCD (mm)	Height (cm)	Tall Branch (cm)
Short Days			
0 Weeks	6.74 a	28.4 a	29.0 a
4 Weeks	6.99 a	26.1 ab	25.2 b
6 Weeks	6.88 a	25.4 b	23.8 b
p-value	0.3790	0.0480	0.0212
Chilling Hours			
0 Hours	7.61 a	28.3 a	29.2 a
400 Hours	6.58 b	25.0 b	22.9 b
p-value	0.0022	0.0016	0.0001

The ratio of the height of the tallest branch to the height of the terminal apex was affected by dormancy treatments. Shortened daylength decreased the ratio of branch to apical height (from 1.02 for controls to 0.90 for 6 weeks, $p = 0.1057$, Table 4-12). Chilling significantly decreased the ratio of branch to apical height from 1.03 for control seedlings to 0.89 for chilled seedlings ($p = 0.0042$, Table 4-12).

The analysis of the number of needles per total stem units was examined for the second flush only. Of the ten blocks planted, two blocks exhibited reduced growth, and included seedlings that did not complete a second flush. To produce a balanced ANOVA, these two blocks were omitted from analysis. Additionally, as values generally fell between 0.25 and 0.75, values were arcsin pretransformed. It was found that dormancy treatments did not affect needle production in the second flush (Table 4-12). However, these results may have been confounded by the presence of a terminal bud at the time of outplanting (see Chapter 3). Although main effects were insignificant, a significant interaction ($p = 0.0808$) was observed between shortened days and chilling for the number of needles per total stem units (Figure 4-10).

Two treatments had resulted in increased needle production in the second flush. Seedlings subjected to 4 weeks of short days plus 400 hours of chilling and seedlings subjected to 6 weeks of shortened days produced a high proportion of needles per total number of stem units (0.389 and 0.421, respectively) (Figure 4-10). Seedlings that were subjected to 6 weeks of shortened days plus 400 hours of chilling produced fewer needles per total stem units (0.236). This interaction corresponds with the pattern observed for budset at the time of outplanting, suggesting the presence of a bud was the overriding factor influencing needle production in the second flush.

Generally, results were similar to the results obtained for seedlings subjected to shortened days and chilling before outplanting at the Reynolds Homestead Research Center. At the Reynolds Homestead, chilling decreased the ratio of branch to apical height and short days increased the number of needles per total stem units. Shortened daylength and chilling favorably influenced the ratio of branch to apical height. The combination of both factors increased the number of needles per total stem units in the second flush. However, it is likely that the presence of a bud at outplanting was the major factor in the determination of needle production in the second flush. Very dry

Table 4-12. The ratio of branch to apical height (for 10 blocks) and the number of needles per total stem units for the second flush (8 blocks) for loblolly pine seedlings subjected to factorial combinations of 0, 4 or 6 weeks of shortened days and 0 or 400 hours of preplanting chilling. Seedlings were planted at the Appomattox-Buckingham State Forest, near Appomattox, VA. Seedlings were outplanted on May 30, 1993, 146 days after sowing.

Dormancy Treatment	Branch/ Apical Height	Needles/ Stem Units
Short Days		
0 Weeks	1.02 a	0.21 a
4 Weeks	0.96 ab	0.30 a
6 Weeks	0.90 b	0.33 a
p-value	0.1057	0.4020
Chilling Hours		
0 Hours	1.03 a	0.30 a
400 Hours	0.89 b	0.26 a
p-value	0.0042	0.2760

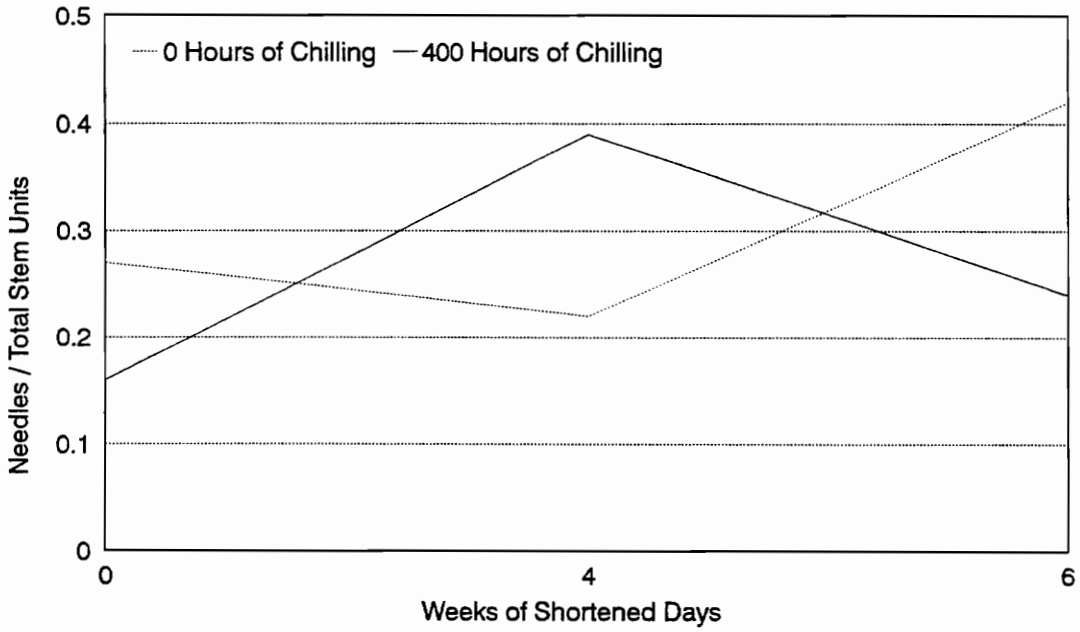


Figure 4-10. The number of needles per total stem units for the second flush at the end of the first growing season for factorial combinations of loblolly pine seedlings subjected to 0, 4 or 6 weeks of shortened days followed by 0 or 400 hours of preplanting chilling. Seedlings were planted at the Appomattox-Buckingham State Forest near Appomattox, Virginia. Seedlings were outplanted on May 30, 1992, 146 days after sowing.

conditions reduced seedling growth at the Appomattox site relative to the 1992 growing season, resulting in fewer flushes produced in each seedling. Because of this, the influence of dormancy treatments on needle production could not be determined for the third flush.

CONCLUSIONS

Preplanting short days reduced seedling size, which persisted to the end of the growing season. However, by late in the growing season, it was apparent that the seedlings that were subjected to 6 weeks of shortened days produced more secondary needles per total stem units than controls. Differences in the ratio of the tallest branch to apical height were not apparent. Starch concentrations were higher at outplanting for seedlings that were subjected to shortened days, but by late summer, starch concentrations were higher for control seedlings.

Chilling reduced seedling size and weights more than shortened days, but had the beneficial effect of decreasing the ratio of branch to apical height. Seedling starch and the number of needles per total stem units were unaffected by chilling.

Supplemental water maintained height growth (and discouraged branch growth) through a mid-summer drought. This resulted in a decreased ratio of branch to apical height.

As expected, family differences were apparent for most growth parameters, and results were generally similar to those found in Chapter 3. It should be noted that family 641, which did not receive supplemental daylength while in the greenhouse was not significantly different from the other families for the growth parameters measured.

Treatments that influenced seedling dormancy ameliorated the growth abnormalities that were observed in control seedlings. This indicates that the observed growth abnormalities were the result of abnormalities in the normal seedling dormancy cycle. Seedlings planted in the greenhouse were grown under continually favorable growing conditions for nine months, while the normal field growing season in Virginia may last for five months. It is hypothesized that in late summer (when abnormalities were first observed) apical meristems ceased (or decreased) growth in response to decreased daylength (Garber, 1983; Boyer and South, 1989). The cessation in growth of apical meristems would not be observed under normal conditions until September (Boyer, 1970). However, seedlings were grown under favorable conditions for an

extended period may have been very sensitive to shortened days.

Shortened daylengths prior to planting likely resulted in fall-like conditions that increased the dormancy status of the seedlings. The level of dormancy may be interpreted with examination of the level of starch in the seedlings (Ericsson, 1979; Adams *et al., 1986*). Adams *et al.* (1986) found that root starch concentrations were lowest during Autumn and highest in mid to late winter. At outplanting, seedlings placed under short days had higher whole plant (but not root) levels of starch. During late summer, control seedlings had higher levels of starch than seedlings placed under short days. Higher levels of starch in late summer may be related to deeper levels of dormancy in control seedlings, which fits the proposed conceptual model (Figure 4-11) explaining the growth abnormalities. The role of shortened days and starch concentration in needle production in subsequent flushes is unknown, and may be a topic for further study.

Chilling has been demonstrated to release seedlings from dormancy (Boyer and South, 1986; Boyer and South, 1989; Garber, 1983; Carlson, 1985), and it has been suggested that chilling releases dormancy regardless of the presence of a bud (Boyer and South, 1989). Additionally, Mexal and Carlson (1981) suggested that the "champagne glass" growth they observed on loblolly pine seedlings was the result of a failure to fulfill the chilling requirement of the seedlings. It is suggested that 600 hours of preplanting chilling satisfied the seasonal chilling requirement of the seedlings; although the seedlings were not necessarily in a dormant state before they were chilled. Satisfaction of the annual chilling requirement before planting produced seedlings that were less sensitive to shortening days later in the summer, allowing continued apical growth. Continued apical growth resulted in more favorable ratios of branch to apical height in chilled seedlings.

Month	Daylength	Normal Development	Abnormal Development	"Reset" Seedlings
January	increasing		germination	germination
February				
March				
April		germination	tube volume induces budset	chilling begins / chilling requirement satisfied
May			outplanting & elongation of second flush	outplanting and growing degree days accumulate
June	longest day			elongation of flush 1 or 2
July	decreasing	mid-summer drought - apical growth stops	mid-summer drought - apical growth stops	
August		growth of flush 2	apex responds to decreasing daylength - partial elongation of flush 3	
September		decreasing daylength causes budset		decreasing daylength causes budset
October				
November		cold causes dormancy	cold causes dormancy	cold causes dormancy
December	shortest day			
January	increasing	chilling requirement fulfilled	chilling requirement fulfilled	chilling requirement fulfilled

Figure 4-11. Conceptual model explaining the development of the growth abnormalities.

RECOMMENDATIONS

Spring outplanted greenhouse-grown tube stock produced growth abnormalities during the 1992 and 1993 growing seasons in the form of multiple apical and needleless shoots. It was found that the expression of these abnormalities was greater at the better planting site. However, after overwintering growth abnormalities were not apparent, and the only apparentlong-lasting abnormalities were low forking on several trees. It could not be determined if the development of growth abnormalities affected family or individual performance ratings.

It was determined that a longer and/or more severe hardening-off period prior to outplanting increased the dormancy status of the seedlings and lessened the expression of growth abnormalities. A more severe hardening-off period increased apical dominance and increased the needle growth in the second flush.

Pre-planting shortened days apparently had an influence on needle development in the second flush, primarily by influencing budset and needle development under the resting bud that was initiated by short days and by root-binding in the leach tubes. The influence of shortened days on needle development in the third flush could not be determined. Pre-planting chilling increased apical dominance during both the 1992 and 1993 growing season. It was found that 400 hours at 3°C significantly increased apical dominance at the end of the growing season.

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