ABSTRACT

Rosier, Christopher L. Factors affecting the rooting of Fraser fir (*Abies fraseri*) and Virginia pine (*Pinus virginiana*) stem cuttings. (Under the direction of Dr. John Frampton)

The current research sought to determine the best methodologies to clone, through rooted cuttings, desirable genotypes of both Fraser fir and Virginia pine. Genetic improvement and subsequent production of genetically superior clones would provide Christmas tree growers with higher quality stock material capable of greatly increasing their profitability. Once desirable genotypes have been selected, propagation through the rooting of stem cuttings may provide an important means of not only preserving and maintaining, but also commercially propagating these genotypes.

The objectives of the first studies were to determine the optimal season, auxin type [IBA (indole-3-butyric acid) or NAA (1-naphthaleneacetic acid)] and auxin concentrations for promoting root initiation and subsequent root development in both species. Seven auxin concentrations (1-64 mM) of each auxin type, four combinations of both auxin types and a nonauxin control were applied to hardwood, semi-hardwood and softwood cuttings collected from three- or four-year-old Fraser firs and Virginia pines.

In three- and four-year-old Fraser firs, the effects of season and concentration were significant for numerous rooting traits, including rooting percentage, percent mortality, primary root production and total root lengths. The type of auxin, IBA or NAA, significantly affected the number of primary roots and total root length, but it did not significantly affect rooting percentage and percent mortality. The highest rooting

frequencies and lowest rates of mortality occurred when cuttings were set in June or November and treated with 4 or 16 mM auxin, respectively. Regardless of auxin type, number of primary roots and total root length varied in similar patterns across concentration; however, NAA tended to produce greater responses. Combinations of IBA and NAA did not increase rooting traits above what was achieved with a single auxin type.

In the Virginia pine study, using three- and four-year-old stock plants, there were four setting periods: September 2000, February 2001, June 2001 and October 2001. Season significantly affected numerous rooting traits including rooting percentage, percent mortality, primary root production and total root lengths. Auxin type, IBA or NAA, significantly affected total root lengths, but did not significantly affect any other trait. The highest rooting frequencies and lowest rates of mortality occurred when cuttings were set in September or June and treated with a three second dip of 8 or 16 mM auxin, respectively. Combinations of IBA and NAA did not increase rooting above what was achieved with a single auxin type

Additional, research was focused on the production and rooting of vertically oriented (non-plagiotropic) shoots from older trees of these two species. Fraser fir Christmas trees were hedged to 1 whorl (trees in the field 3 and 5 years) or 1, 3, and 5 whorls (trees in the field 7 years). Three-year-old Virginia pines were stumped to ¼, ½ and ¾ their original height. Non-cut controls of both species were also included. A second experiment was designed to create a quantitative description of the effects that crown position had on the rooting of stem cuttings collected from stumped and control trees of both species. During the summer of 2001, cuttings from both species were collected and rooted to help understand how to optimize the production and rooting of cuttings by managing the 1) stumping height, 2) crown position, 3) age of the parent tree, and 4) auxin treatments.

Stumping height and age significantly increased rooting percentage, primary root production and total root lengths in three-, five-, and seven-year-old Fraser fir Christmas trees. These rooting traits increased as the severity of the stumping treatment increased and as the age of the stock plant decreased. Auxin concentration significantly affected nearly every rooting trait assessed. Rooting percentage was significantly affected by the position from which the cuttings were collected. Overall, the higher rooting percentages (51%) and greatest number of primary roots (8.1) three-year-old stock plants were stumped to the first whorl.

In four-year-old Virginia pine Christmas trees, stumping height significantly affected rooting percentage and percent mortality. Rooting percentage increased and percent mortality decreased as the severity of the stumping treatment increased. Auxin type significantly affected rooting percentage, percent mortality, primary root production and total root lengths. In general, NAA treated cuttings rooted in higher frequencies (74%), produced a greater number of primary roots (5.5), and longer total root lengths (601 mm) than did IBA treated cuttings. Combinations of IBA and NAA did not increase rooting traits above what was achieved with a single auxin type. Rooting percentage was significantly affected by the positions from which the cutting was collected. The length of the primary needle, significantly affected by stumping height, increased as the severity of the stumping treatment increased, and was positively correlated with rooting ability.

FACTORS AFFECTING THE ROOTING OF FRASER FIR (*Abies fraseri*) AND VIRGINIA PINE (*Pinus virginiana*) STEM CUTTINGS

by

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BIOGRAPHY

Christopher L. Rosier was born August 17, 1976 in Roanoke, Virginia. He moved to Greensboro, North Carolina, in 1986, and eventually to Raleigh, North Carolina, in 1994. He attended North Carolina State University for his undergraduate studies (1994-1999), and achieved a Bachelor of Science in Forest Hydrology and a Bachelor of Science in Ecosystem Assessment. As an undergraduate student, he had several internships including work with the McHenry County Conservation District in Ringwood, Illinois, and the U.S. Forest Service at Coweeta Hydrologic Laboratory in Otto, North Carolina. Beginning in 1998, Chris began working with the North Carolina State University Slash and Loblolly Pine Rooted Cutting Program. Following completion of his bachelor degrees, Chris worked with Westvaco Corporation's Loblolly Pine Rooted Cuttings Program in Summerville, South Carolina. After working at Westvaco Corporation for eight months, Chris returned to North Carolina State University to begin his Masters of Science degree in forestry. Currently, Chris is working with Smurfit-Stone Container Corporation in Fernandina Beach, Florida, as the Orchard Supervisor. He would eventually like to return to school to pursue his PhD in forestry and tree improvement.

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General Introduction

Christmas trees are grown commercially in all 50 states, including Alaska and Hawaii (NCCTA, 2001). Currently, in the United States there are about 15,000 Christmas tree growers and over 100,000 people employed full or part-time in the industry (NCTA, 2001). Within the United States, approximately 1 million acres of land is used in Christmas tree production, yielding nearly 35 million Christmas trees annually (NCTA, 2001). Prior to the 1950s, most Christmas trees came from the forest, whereas, today over 98% of Christmas trees are plantation-grown (NCCTA, 2001). The top six Christmas tree producing states are Oregon, North Carolina, Michigan, Wisconsin, Pennsylvania and California, respectively (NCCTA, 2001)

There are approximately 2,500 growers in North Carolina, using nearly 25,000 acres of land (NCCTA, 2001). The Christmas tree industry in North Carolina is largely based in the mountainous western part of the state where Fraser fir (*Abies fraseri* [Pursh] Poir.) is grown, which represents over 96% of all the state's Christmas tree production. It is estimated that North Carolina has approximately 50 million Fraser fir Christmas trees growing at any given time (NCCTA, 2001). The most common Christmas tree species in the piedmont and coastal regions of North Carolina is Virginia pine (*Pinus virginiana* Mill.). Other Christmas tree species grown in this region include eastern white pine (*Pinus strobus* L.), Leyland cypress (x *Cupressocyparis leylandii* Dall. + Jacks.) and eastern redcedar (*Juniperus virginiana* L.).

Fraser fir

Fraser Fir is a valuable Christmas tree species that is indigenous to southwestern Virginia, eastern Tennessee, and western North Carolina. It naturally grows at elevations between 1,200 and 2,037 m and is a small to medium size tree reaching an average height of 12 m with a diameter at breast height (dbh) of 40 cm (Saravitz and Blazich, 1996). Fraser fir is highly prized in the Christmas tree industry because it produces dark green fragrant foliage, a natural conical shape, good branch structure, and needles that persist following cutting (Johnson, 1991).

Currently, Fraser fir for Christmas tree production is propagated exclusively by seed. Trees begin to produce viable seeds around age 20 to 30, and good seed crops occur every three to five years (Franklin, 1974). Seeds are first sown in a raised seedbed and following two or three years of growth, are transferred into raised transplant beds at wider spacing. Following two years of additional growth in the transplant beds, the seedlings are out-planted into commercial plantations. Following an additional seven to twelve years of growth in the field, the trees are ready for harvest.

Commercially viable asexual propagation techniques would allow for the use of clones with desirable physiological and morphological characteristics. In Fraser fir, one of greatest potential benefits would be the ability to select and propagate clones that are resistant to the introduced insect, the balsam wooly adelgid (*Adelges piceas* Ratz.), and an introduced root rot fungus, *Phytophthora cinnamomi* Rands. Additionally, the range in which Fraser fir can be grown could be extended if clones that are tolerant of lower altitudes were found and propagated.

Most of the research concerning vegetative propagation of Fraser fir has involved the use of stem cuttings, although air layering and grafting have been found to be the most successful forms of vegetative propagation. These two techniques are impractical for producing large numbers of plants, but are very beneficial in the establishment of seed orchards and clone banks. The biggest problem associated with rooting Fraser fir cuttings is plagiotrophic growth. Stem cuttings from lateral branches often root in high percentages, but plagiotrophic growth persists for several years following rooting. Orthotropic shoots can be collected from terminal leaders, but rooting percentages from older trees are usually very low.

Extensive research has been conducted to develop techniques for vegetative propagation by use of stem cuttings. Past research includes the effects of wounding, auxin treatment (Blazich and Hinesley, 1994; 1995; Hinesley and Blazich, 1980; 1981; 1984), bottom heat (Blazich and Hinesley, 1994; 1995; Hinesley and Blazich, 1981; 1984; Miller et al., 1982b), postseverance artificial chilling (Hinesley and Blazich, 1981; Miller et al., 1982b; Wise et al. ,1985b), crown level, branch order, cutting type (terminal vs lateral), cutting length (Blazich and Hinesley, 1995; Hinesley and Blazich, 1984; 1994; Garlo, 1982; Miller et al., 1982 a), photoperiod during rooting (Blazich and Hinesley, 1995; Miller et al., 1982 b), time of collection, hedging treatments, and the effects of staking on plagitrophic growth (Wise et al., 1985a,b,c; Wise, 1985; Wise et al., 1986). Since there have been several thorough literature reviews on Fraser fir vegetative propagation (Blazich and Hinesley, 1995; Hinesley and Blazich, 1984), one will not be presented here.

Virginia pine

In the piedmont and coastal regions of North Carolina, Virginia pine is one of the most common Christmas tree species. Virginia pine generally grows throughout the Mountains, and at lower elevations in the Piedmont, from central Pennsylvania southwestward to northeastern Mississippi, Alabama, and northern Georgia. It is also found in the Atlantic Coastal Plain as far north as New Jersey and Long Island, NY, and extends westward in scattered areas into Ohio, southern Indiana, and Tennessee (Harlow et al., 1996). Virginia pine is a deserving Christmas tree species due to its rapid growth (3 to 5 years to harvest), short needles, good branch structure for holding ornaments and pleasant pine scent (Frampton, 2002).

Currently, Virginia pine, for Christmas tree production, is propagated exclusively by seed. Trees begin to produce viable seeds around age four (Franklin, 1974). Seed are first sown in raised seedbeds and following one year of growth, are transplanted into the field. Following three to six years of additional growth in the field, the trees are ready for harvest. Due to the minimal amount of tree improvement in Virginia pine, several significant problems must be overcome including poor form and extreme susceptibility to the Nantucket pine tip moth (*Rhyacionia frustrana* (Comstock)). Due to these and other problems, Christmas tree growers often are only able to market about 50% of Virginia pines planted (Frampton, 2002).

Due to the high market value of Virginia pine Christmas trees (relative to forest trees) and the large genetic variation that currently exists (Belanger and Bramlett, 1975; Brown at al., 1991; Knoth et al., 2002; Meier and Goggans, 1977), genetic improvement for desirable characteristics can easily be justified. Recently, there has been an increasing interest in propagating Virginia pine asexually, through the use of rooted cuttings, to establish and propagate clones with superior Christmas tree characteristics. Asexual propagation would provide a way to capture benefits from cloning, including increased genetic gain and increased uniformity, which are major problems with Virginia pine (Zobel and Talbert, 1984). The greatest potential benefits include the ability to select and propagate clones that are resistance to the Nantucket pine tip moth and increase the uniformity of the trees produced.

Very little research involving propagation of Virginia pine through rooted cuttings has been conducted. A minimal amount of research has been conducted on the effects of auxin (Holifield et al., 1991; Snow and May, 1962), time of collection (Brown et al., 1991; Holifield et al., 1991; Snow and May, 1962), crown position (Snow and May, 1962), genotype (Brown et al., 1991), and photoperiod (Snow and May, 1962).

For the purpose of this review, these factors will be grouped into three categories: selection and collection of cutting material, treatment of cuttings, and environmental rooting conditions.

Selection and Collection of Cutting Material

Timing

The time of collection has been shown to have significant effects when rooting many tree species (Hartmann et al., 2002). Snow and May (1962) found that Virginia pine cuttings collected in December rooted much better (72%) than cuttings collected in March (19%). Root development in cuttings collected in December was found to be much slower, requiring 9 months to root compared to 7 months needed by cuttings collected in March. Holifield et al. (1991) found that cuttings collected in March rooted much better (46%) than cuttings collected in June (3%) as semi-hardwood cuttings. However, Brown et al. (1991) found no significant variation in rooting percentages by collection date, noting that cuttings collected on August 3, 1990, rooted at 46% and cutting collected on March 6, 1990, rooted at 43%.

Crown Position

The positions within the crown from which cuttings are collected have been shown to significantly affect rooting in other species (Hartmann et al., 2002). For Virginia pine, Snow and May (1962) found that for cuttings collected in March, 18% from the lower crown rooted, whereas, only 1% from the upper crown rooted.

Genotype

When rooting cuttings of Virginia pine, as with most species, a large genotypic, or tree-to-tree, variation exists. In one experiment using 25 clones, Brown et al. (1991) found large variation among trees in rooting, ranging from 13 to 88%. When considering only the 5 best clones, the overall rooting was 62%.

Treatment of Cuttings

Auxin Treatment

Treatment with an auxin is often critical when rooting cuttings of Virginia pine. The optimum concentration of auxin varies with time of collection. For cuttings, collected in December, the best results were obtained when 25 mM IBA was applied to the bases of the cuttings before setting (Snow and May, 1962). For cuttings collected in March, Snow and May (1962) found no variation in rooting percentage among auxin treatments; however, Holifield et al. (1991) found that an application of Hare's (1974) talc-based rooting powder, to the base of the cuttings, dramatically increased the rooting percent from 3% in the controls to 46% for treated cuttings. Hare's powder consisted of a talc formulation containing 1% each of the auxins IBA and 1-phenyl-3-methyl-5-pyrazolone (PPZ), 10% powdered sugar, 20% captan fungicide, and 1% of the growth retardant n-dimethylaminosuccinamic acid (B-Nine). Holifield et al. (1991) found that cuttings collected in June, as semi-hardwood cuttings, rooted at only 4%, regardless of auxin type or concentration.

Environmental Rooting Conditions

Photoperiod

Long day conditions have been shown to increase rooting percentages in some species (Hartmann et al., 2002), but little variation has been reported in Virginia pine. Snow and May (1962) reported that long day conditions slightly increased rooting for cuttings collected in December, but showed no response for cuttings collected in March; however, no data were presented.

Research Objectives

The common objective of the following series of research investigations was to determine the best methodologies to clone, through rooted cuttings, desirable genotypes of both Fraser fir and Virginia pine. Genetic improvement and subsequent production of genetically superior clones would provide Christmas tree growers with higher quality stock material that could greatly increase profitability. Once desirable genotypes have been selected, propagation through the rooting of stem cuttings would provide an important means of not only preserving and maintaining, but also commercially propagating these genotypes (Zobel and Talbert, 1984). Rooted cuttings provide a way to capture benefits from cloning including: 1) increasing genetic gain in growth quality and pest resistance, 2) increasing uniformity, and 3) allowing greater flexibility in meeting customers' needs (Frampton, 2002).

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Chapter 2

Growth Stage, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Fraser Fir

(In the format appropriate for submission to HortScience)

Growth Stage, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Fraser Fir

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Abstract. Seven concentrations of indole-3-butyric acid (IBA), seven concentrations of 1-naphthaleneacetic acid (NAA), and a nonauxin control were tested over three growth stages to determine their effectiveness in promoting adventitious rooting of stem cuttings taken from 3- and 4-year-old stock plants of Fraser fir [*Abies fraseri* (Pursh) Poir.]. Cuttings were collected in March (hardwood), June (softwood), or November (semi-hardwood) 2001, treated with auxin concentrations ranging from 0 to 64 mM, and placed under mist. Rooting percentage, percent mortality, number of primary roots, total root length, root system symmetry, and root angle were recorded after 16 weeks. Growth stage and auxin concentration significantly affected every rooting trait except root angle. NAA significantly increased the number of primary roots and total root length. However, auxin type did not significantly affect rooting percentage or percent mortality. The highest rooting percentages (99%) occurred when softwood cuttings were treated with 5 mM auxin; however, semi-hardwood cuttings also rooted at high percentages (90%) and had no mortality when treated with 14 mM auxin. Regardless of auxin type, number of primary roots and total root length varied in similar patterns across concentrations, although NAA tended to induce a greater response. To root Fraser fir stem cuttings collected from 3- and 4-year-old stock plants, it is recommended that a concentration of 5 mM NAA should be used on softwood cuttings and 14 mM IBA on semi-hardwood cuttings.

Additional index words: vegetative propagation, growth stage, indole-3-butyric acid, 1-naphthaleneacetic acid, Christmas trees, cloning, *Abies fraseri*

The Christmas tree industry in North Carolina is based primarily in the mountainous western part of the state where Fraser fir (*Abies fraseri*) is grown. Fraser fir is a valuable Christmas tree species, representing over 96% of the total state production, and is indigenous to southwestern Virginia, eastern Tennessee, and western North Carolina (Harlow et al., 1996). Production of Christmas trees is a valuable industry in the western part of North Carolina, because it provides a source of income to a largely rural region in which there are few other economic opportunities (Frampton, 2002). Fraser fir is regarded as the premier Christmas tree throughout much of the United States due to many desirable characteristics, including pleasing aromatic aroma, natural shape, dark blue-green color, strong branches for holding ornaments, and excellent post-harvest needle retention (Arnold et al., 1994; Frampton, 2002). However, Fraser fir has several significant problems including extreme susceptibility to an introduced insect, the balsam wooly adelgid (*Adelges piceas* Ratz.), and an introduced root rot fungus, *Phytophthora cinnamomi* Rands.

Asexual propagation by stem cuttings could help meet future demand for elite Fraser fir Christmas trees. Once selected, desirable genotypes could be propagated by stem cuttings for both preservation or archival purposes and commercial use (Zobel and Talbert, 1984). Rooted cuttings may provide a way to capture several benefits from cloning including 1) increased genetic gain, 2) increased uniformity, and 3) allowing greater flexibility in meeting customers' needs (Zobel and Talbert, 1984).

Many factors have been shown to influence rooting of stem cuttings of Fraser fir, including auxin concentration (Blazich and Hinesley, 1994; 1995, Hinesley and Blazich, 1980; 1981; 1984) and growth stage of the stock plants from which cuttings are taken (Wise et al., 1985a; 1985b). These factors have proven to be important influences on the rooting success of many other woody species as well (Hartmann et al., 2002).

There are three general classes of stem cuttings based on developmental growth stages of stock plants: hardwood, semi-hardwood and softwood cuttings. Hardwood cuttings are collected in late fall, winter, or early spring when stock plants are fully dormant. Early rooting research for Fraser fir utilized hardwood cuttings collected from dormant stock plants. Hardwood cuttings rooted at the highest percentages following a 1-3 s dip of the basal 2 cm of the cuttings in 25 mM indole-3-butyric acid (IBA) (Hinesley and Blazich, 1980; 1981). Although hardwood cuttings did root well, early budbreak of the cuttings is a problem, apparently producing resource allocation conflicts between their root systems and elongating shoots. As a result, shoot growth is usually weak and out of synchrony with its natural growth cycle. Thus, an additional year is required for the hardwood cuttings to begin vigorous shoot growth synchronized with the natural photoperiod (Wise, 1985).

Softwood cuttings are collected in the spring or early summer, once apical buds have broken and shoot elongation has ceased. In some species, softwood cuttings have differentiated axillary buds that continue to develop during rooting but, in Fraser fir, these buds do not break during the rooting stage (Blazich and Hinesley, 1994; Powell, 1982). Lack of budbreak reduces the resource allocation problems associated with early budbreak in hardwood cuttings. For cuttings collected from 14-year-old stock plants of Fraser fir, the best rooting occurred when cuttings were collected from the lower crown prior to lignification, wounded, and treated with 22 mM IBA (Wise et al., 1985a). Due to the lack of budbreak, softwood cuttings rooted more frequently and had lower rates of mortality than hardwood cuttings (Wise et al., 1985a).

A third type of cutting, semi-hardwoods, are typically collected in the fall when shoot elongation has ceased and stem tissue has completely lignified. Previous research has shown that semi-hardwood cuttings of Fraser fir collected in October and receiving no artificial chilling failed to root or break bud (Miller, 1982). However, the chilling requirements for rooting this type of cutting were met more quickly than those for budbreak. Visible terminal bud activity was negligible for cuttings artificially chilled at 4 ^{0}C < 6 weeks; however, bud activity increased sharply to a maximum following 10 weeks chilling. If semi-hardwood cuttings are artificially chilled longer than 8 weeks, early budbreak becomes a problem (Miller et al., 1982).

Chilling semi-hardwood Fraser fir cuttings for 4 to 8 weeks prior to being inserted into the rooting medium, in combination with wounding and auxin treatment, increased rooting percentages, as well as the number and length of primary roots (Hinesley and Blazich, 1981; Miller et al., 1982; Wise et al., 1985b). Overall, for cuttings collected in early October from 14-year-old stock plants, the best rooting responses occurred for cuttings that were severed from the upper crown, chilled for 4 weeks, and treated with 22 mM IBA. For cuttings collected from the lower crown, the best rooting occurred for those chilled for 4 weeks and treated with 7 mM IBA (Wise et al., 1985b). Following 1 year of growth (one flush), these cuttings still exhibited plagiotropic growth, but were comparable in size to hardwood cuttings with 2 years of growth (two flushes) (Wise et al., 1985b).

Although previous research has examined rooting of softwood, semi-hardwood, and hardwood stem cuttings of Fraser fir, only one NAA (1-naphthaleneacetic acid) concentration has been evaluated and, no optimum IBA concentration has been reported for softwood stem cuttings. Therefore, the present study evaluated a greater range of concentrations of both NAA and IBA for rooting of all three types of cuttings than previous studies. Specifically, the objectives of the current study were to determine the optimal growth stage, auxin type (IBA or NAA), and auxin concentrations for promoting adventitious root initiation and subsequent root development of stem cuttings from 3- to 4-year-old stock plants of Fraser fir.

Materials and Methods

Fraser fir stem cuttings were harvested at the North Carolina Division of Forest Resources' Linville River Nursery near Crossnore. Tips of primary axes were collected from either 4-0 (hardwood) or 3-0 (softwood or semi-hardwood) seedlings. Following collection, the cuttings were placed immediately into seedling bags, transported to Raleigh, N.C., on ice, and stored at 4 ^{o}C. The cuttings for the first two rooting experiments were harvested on 17 Mar. 2001 (hardwood) and 29 June 2001 (softwood), and inserted into the rooting medium the day following collection. Semi-hardwood cuttings were removed 17 Oct. 2001, cold-stored for 4 weeks, and inserted into the rooting medium 13 Nov. 2001. Cuttings were collected from the primary axes of the seedlings to avoid the plagiotropic growth habitat often present in cuttings from secondary or tertiary axes.

IBA and NAA were tested for their ability to stimulate adventitious rooting of cuttings in three different growth stages. Seven IBA and seven NAA concentrations (1, 2, 4, 8, 16, 32, or 64 mM) and a solvent control were utilized. For each concentration, the auxin was dissolved into a 50% isopropyl alcohol/ deionized water solution. The prepared auxin solutions were then stored at 4 ^{o}C in opaque bottles and were used within 2 d.

Rooting was conducted in a propagation greenhouse located at the Horticulture Field Laboratory, Raleigh. Before being inserted into the rooting medium, the cuttings were re-cut from the bases to a length of 9 cm and auxin was applied for 3 s to the basal 1.5 cm. Cuttings for the control treatment were dipped into a 50% isopropyl alcohol/deionized water solution. Needles were not removed before or after the auxin was applied. Based on preliminary research, no wounding treatment was applied to the basal portions of the cuttings (Rosier, 2003 Chapter 6). The treated cuttings were air-dried for a minimum of 15 min before being inserted to a 3 cm depth into a rooting medium of 3 horticultural perlite : 2 peat (by volume). Cuttings were rooted in individual 164 cm^3 Ray Leach SC-1- Super Cells (Steuwe and Son, Inc., Corvallis, Ore.).

During the rooting period, cuttings were maintained under intermittent mist using a Grower Junior™ (McConkey Co., Sumner, Wash.) overhead boom irrigation system. Frequency of mist application was controlled using relative humidity (RH) and time of day. As RH in the rooting greenhouse decreased, the frequency of mist application increased; however, mist application was less frequent during the night decreasing from an average of one mist cycle every 6 min during the day to an average of one mist cycle every 60 min at night. Flow rate per mist cycle was held constant over the 4-month rooting period at $0.05\ L \cdot m^{-2}$. Average day/night temperatures in the greenhouse were 25 ± 2/19 ± 2 °C , 26 ± 2/21 ± 2 °C, and 24 ± 3/19 ± 2 °C, for hardwood, softwood, and semi-hardwood cuttings, respectively. All cuttings were rooted under ambient light conditions and so were subjected to natural growth seasonal photoperiod and irradiance levels.

Rooting percentage, percent mortality, number of primary roots, total root length, root system symmetry, and root angle were recorded following 16 weeks in the greenhouse. The latter four traits were assessed only on cuttings that rooted. A cutting was considered rooted if a minimum of one root ≥ 1 mm in length was present. A cutting with two roots was considered to have a symmetrical root system when the angle between the roots was > 135°, using the stem center as the vertex (Frampton et al., 1999). Cuttings with three or more roots were scored as symmetrical if the angle between the two roots that were farthest apart was < 135°. Cuttings with one root were scored as having asymmetrical root systems. Root angle was used to determine the percentage of positively geotropic roots for each cutting. Root angle was defined as the percentage of roots that formed an angle < 60° with an extension of the cutting's stem. The experimental design for cuttings collected at each growth stage was a randomized complete block. There were eight blocks of eight cuttings per treatment within each block for a total of 960 cuttings per growth stage. The entire study was surrounded by a row of border cuttings.

Analyses of variance (ANOVAs) were conducted using the General Linear Models procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Growth stage, replication within growth stage, auxin, concentration, concentration squared, and all the two- and three-way interactions other than those involving replication were sources of variation. Transformations of the dependent variables (rooting traits) were investigated to increase homogeneity among treatment variances. Several transformations were explored including the square root, the arcsine of the square root, and a range of transformations using the Box and Cox (1964) procedure that varied λ from -1.00 to +1.00 in increments of 0.25. The transformation, or no transformation, yielding the smallest differences among treatment variances was selected for each dependent variable. Once the most appropriate dependent variable transformation was selected, log and square root transformations of the independent variables (concentration and concentration squared) were compared to no transformation. The model yielding the highest R^2 value is reported for each dependent variable.

Despite employing the most appropriate data transformations and including squared terms for concentration, the least-squares regression approach failed to adequately define the relationship between concentration and the variables assessed. Therefore, nonlinear regression techniques were employed by regressing each dependent variable on treatment means, concentration or auxin type by concentration, using the NLIN procedure of the Statistical Analysis System Version 8.1 (SAS Institute, Inc., 1999). The double exponential model (Rawlings et al., 1998), modified by two constants (A and B), was employed:

$$\text{Trait} = \theta_1(Be^{(-\theta2*\text{Concentration})}-e^{(-\theta1*\text{Concentration})})/[A(\theta_1-\theta_2)]$$

where,

e = base of natural logarithm,

θ_1,θ_2, A and B = regression parameters, and

concentration = auxin concentration in mM.

Results

ANOVAs and transformations of the independent and dependent variables for all rooting traits are presented in Table 1. Since no significant differences were noted for root angle, these data are not presented.

Rooting Percentage. The main effects of growth stage, concentration, and concentration squared were significant for rooting percentage (Table 1). Significant interactions were detected between growth stage and concentration as well as between concentration squared and growth stage (Table 1). However, no significant differences were detected for auxin type ($P = 0.31$) or its interactions. Thus, IBA and NAA were averaged for nonlinear regression analysis.

Using the predicted optima from the nonlinear regression analysis, the greatest overall rooting percentage for softwood (99%) and hardwood (48%) cuttings occurred when 5 mM and 7 mM auxin, respectively, (Fig. 1) were applied. The nonlinear regression equation predicted that the greatest overall rooting percentage occurred for semi-hardwood cuttings (90%) treated with 14 mM auxin (Fig. 1). At supraoptimal rates, rooting percentages for softwood and semi-hardwood cuttings decreased more rapidly than for hardwood cuttings.

Percent Mortality. The main effects of growth stage, replication within growth stage, concentration, and concentration squared were significant (Table 1). A significant interaction was detected between concentration and growth stage as well as between concentration squared and growth stage (Table 1). Because no significant differences were detected for auxin type (P = 0.92) or its interactions, IBA and NAA were averaged for nonlinear regression analysis. In hardwood and softwood cuttings, percent mortality decreased from the control to 2 mM and then increased as the concentration of auxin increased. The highest mortality, 22% and 13%, for hardwood and softwood cuttings, respectively, occurred at 64 mM (Fig. 1). There was no mortality in the semi-hardwood cuttings at any auxin concentration.

Number of Primary Roots. The main effects of growth stage, concentration, and concentration squared were significant for primary root production (Table 1). Significant interactions were detected between concentration and growth stage, concentration squared and growth stage, concentration and auxin, and between concentration squared and auxin (Table 1). Due to these interactions, separate nonlinear regression analyses were performed for IBA and NAA. Regardless of the growth stage, when cuttings were treated with IBA, the predicted number of primary roots produced continued to increase with increases in auxin concentration (Fig. 2). The number of primary roots increased from 5.1, 4.1, and 2.8 in the controls to 10.4, 8.8 and 8.9 in cuttings treated with 64 mM IBA for softwood, semi-hardwood, and hardwood cuttings, respectively. Regardless of growth stage, primary root production increased rapidly at lower auxin concentrations and began to level off between 8 mM and 16 mM, increasing slightly to 64 mM. When hardwood cuttings were treated with NAA, the predicted number of primary roots increased from the control (3.1) to 8 mM (6.1) and then increased slightly with increases in NAA concentration up to the predicted maximum at 64 mM (7.9) (Fig. 2). For semi-hardwood and softwood cuttings, the predicted maximum number of primary roots, 9.5 and 9.7 respectively, occurred following treatment with 9 mM auxin.

Total Root Length. The main effects of growth stage, replication within growth stage, concentration and concentration squared were significant (Table 1). Significant interactions were detected between concentration squared and growth stage, concentration and auxin, as well as concentration squared and auxin (Table 1). Due to these interactions, separate nonlinear regression analyses were performed for IBA and NAA. When semi-hardwood and softwood cuttings were treated with IBA, predicted optima from the nonlinear regression for total root length was reached at 12 mM (713 mm) and 10 mM (800 mm), respectively, before decreasing slightly with increases in IBA concentration (Fig. 3). Using the predicted values, total root length for hardwood cuttings increased with increases in IBA concentration from the control (19 mm) to 64 mM (179 mm), with most of the increase occurring between 0 and 4 mM. When semi-hardwood and softwood cuttings were treated with NAA, the predicted optima for total root length was reached at 8 mM (766 mm) and 7 mM (773 mm), respectively, before decreasing (Fig. 3). Predicted estimates for total root length in hardwood cuttings increased gradually from the control (8 mm) to 64 mM NAA (222 mm), with most of the increase occurring between 0 and 8 mM.

Root Symmetry. Root symmetry was significantly affected by growth stage, replication within growth stage, concentration, concentration squared, and by the interaction between concentration squared and growth stage (Table 1). Because no significant differences were detected for auxin type and its interactions, IBA and NAA were averaged for nonlinear regression analysis. For semi-hardwood and softwood cuttings, root symmetry reached a maximum before decreasing with increasing auxin concentrations (Fig. 4). Semi-hardwood cuttings reached a predicted maximum at 13 mM (76%) and softwood cuttings reached a predicted maximum at 5 mM (91%). In hardwood cuttings, predicted root symmetry values increased with increasing auxin concentrations ranging from 0% in the controls to 55% at 64 mM, with most of the increase occurring between 0 and 8 mM.

Discussion

Growth Stage Differences. In the present study, as in previous investigations involving rooting of Fraser fir stem cuttings, large influences were observed regarding the growth stage of the stock plants from which cuttings were taken (Wise, 1985; Wise et al., 1985a; 1985b). Most likely, these differences reflect underlying physiological conditions of the stock plants and the cuttings at the time of cutting collection. In the current study, hardwood cuttings were collected in March from fully dormant stock plants in which chilling requirements for budbreak had been satisfied. Consequently, when placed into the warm greenhouse environment, apical budbreak on most (68%) hardwood cuttings occurred prior to rooting. As a result, resources such as mineral nutrients and carbohydrates likely were diverted from root formation, as suggested by lower rooting percentages, higher mortality, fewer and shorter roots, and poorer root symmetry than softwood and semi-hardwood cuttings. Early budbreak resulting in resource allocation problems has been reported in previous studies utilizing hardwood cuttings of Fraser fir (Hinesley and Blazich, 1980;1981).

Apical buds of softwood cuttings collected in June were not completely developed and displayed little shoot elongation (1%) following insertion of the cuttings into the rooting medium. Thus, more cutting resources required for root growth were likely available for root initiation, root growth, and development. Softwood cuttings rooted at higher frequencies, had lower mortality, produced more and longer roots, and had the highest frequency of root symmetry of the three types of cuttings assessed. Softwood cuttings in the present study rooted at higher frequencies than those reported previously from the lower crowns of 14-year-old trees [0% (control) to 53% (22 mM IBA)] (Wise et al., 1985b) most likely due to a combination of greater juvenility and improved greenhouse environmental conditions.

Hartmann et al. (2002) suggest that softwood cuttings may have higher metabolic activity and increased levels of endogenous auxin production than other types of cuttings. In the present investigation, softwood cuttings reached optimal rooting at a lower auxin concentration (5mM) than the other two cutting types (Fig. 1). When treated with concentrations > 4 mM, rooting percentages, root production, root lengths, and root symmetry began to decrease rapidly for softwood cuttings, especially in NAA-treated cuttings. Furthermore, at concentrations > 16 mM, mortality began to increase steadily (Fig. 1). Detrimental total auxin levels were reached at lower concentrations of exogenously applied auxin for the softwood cuttings relative to the two other cutting types, suggesting that their endogenous auxin levels were indeed highest.

Semi-hardwood cuttings collected in October were sufficiently chilled under artificial conditions to break cambial but not apical bud dormancy (Miller et al., 1982; Wise et al., 1985b). Semi-hardwood cuttings generally did not break bud (4%) prior to rooting. They were intermediate between the other two cutting types for all traits assessed except percent mortality, although not always statistically different from softwood cuttings. Rooting percentages in this and a previous study using semi-hardwood Fraser fir cuttings from the lower crown of 14-year-old trees (98% maximum at 22 mM IBA) (Wise et al.,1985b) were similar, suggesting that the effects of maturation on rooting semi-hardwood cuttings are small.

Hartmann et al. (2002) suggest that as the annual growing cycle progresses, endogenous auxin production begins to decrease and, therefore, higher levels of exogenous auxin are needed to promote optimal rooting. This is most apparent when the semi-hardwood cuttings are compared with the softwood cuttings, in which optimal rooting percentages occurred using 14 and 5 mM auxin, respectively (Fig. 1). For the semi-hardwood cuttings, rooting percentage, root production, root lengths, and root symmetry exhibited slower rates of decline with increasing concentrations of applied auxin relative to the softwood cuttings and no mortality occurred.

Auxin Type and Concentration Effects. The type of auxin, IBA or NAA, did not significantly affect rooting percentage or percent mortality (Table 1). However, relative to IBA, NAA produced significantly more and longer roots at auxin concentrations that were optimal for percent rooting. Application of NAA has also been shown to significantly increase root production in hypocotyl cuttings of eastern white pine (*Pinus strobus* L.) (Goldfarb et al., 1998). Diaz-Sala et al. (1996) suggest that NAA was more effective than IBA in adventitious root induction in hypocotyls cuttings of loblolly pine due to its slower rate of metabolism. They found that rooting was not directly correlated with auxin differences in availability at the rooting site or in its transport capacity. Their findings are supported by Greenwood et al. (1974) who concluded that free auxins, not their metabolites, are essential for adventitious root formation in sugar pine (*Pinus lambertiana* Dougl.).

Auxin concentration significantly affected every rooting trait assessed (Table 1). Inclusion of a greater range and more concentration levels provided a clearer description of trends than in previous research (Wise et al., 1985a; 1985b). Percent rooting and survival increased with increases in auxin concentration up to an optimum, before decreasing steadily (Fig. 1). Percent survival always peaked at lower concentrations than rooting percentage. The number of primary roots and total root lengths increased to an optimum and then, depending on the auxin type, either showed little change (IBA) or decreased slightly (NAA). Regardless of the growth stage or type of auxin, primary root production and total root lengths were positively related and reached optima at approximately the same concentration. Root symmetry, not affected significantly by auxin type, also increased rapidly to an optimum before decreasing with increases in auxin concentration, and paralleled primary root production (Fig. 4). This may be expected since a greater number of primary roots increase the likelihood that symmetry will occur.

High concentrations of auxin also affected the origin of root initiation on the stem. In cuttings treated with low levels of auxin, roots appeared to emerge from the callus formed below the cut end of the stem, although this was not determined microscopically. This phenomenon was most pronounced in the hardwood cuttings. At concentrations = 16 mM NAA or 32 mM IBA, the bases of hardwood cuttings appeared necrotic. Following tissue dieback, callus was slow to form and occurred just above the wounded tissue. Subsequent adventitious root formation consisted of numerous roots measuring a few millimeters in length that originated along the stem above the callus. A similar trend has been reported for hypocotyl cuttings of eastern white pine (Goldfarb et al., 1998). This resulted in a high number of primary roots by the hardwood cuttings while total root lengths were low.

For most traits assessed, significant interactions were detected between growth stage and concentration (and its quadratic term). These interactions are accounted for by the previously discussed phenomena: 1) budbreak and resource depletion before rooting in the hardwood cuttings and 2) a rapid decline in rooting performance after reaching optima for the softwood cuttings, perhaps due to high endogenous auxin levels.

While not presented herein, a study investigating combinations of auxin types was conducted simultaneously with the current study (Rosier, 2003 Appendix I). Eight factorial combinations of three concentrations (0, 4, and 16 mM) of both IBA and NAA were tested. Generally, these combinations produced negative effects on the rooting traits assessed relative to the use of a single auxin. Auxin was detrimental when 16 mM was applied in any combination. Future research should address combinations of auxin concentrations lower than those evaluated in this separate study.

In conclusion, stem cuttings collected from 3- and 4-year-old seedlings of Fraser fir root best when in the softwood or semi-hardwood growth stage and treated with 5 mM NAA or 14 mM IBA, respectively. At these concentrations, little mortality occurs in either cutting type and root production is either approaching or has reached an optimal level. For hardwood cuttings taken from 3- to 4-year-old seedlings, early budbreak leading to resource depletion before rooting is a problem and leads to a decrease in rooting performance and shoot growth relative to the other two types of cuttings.

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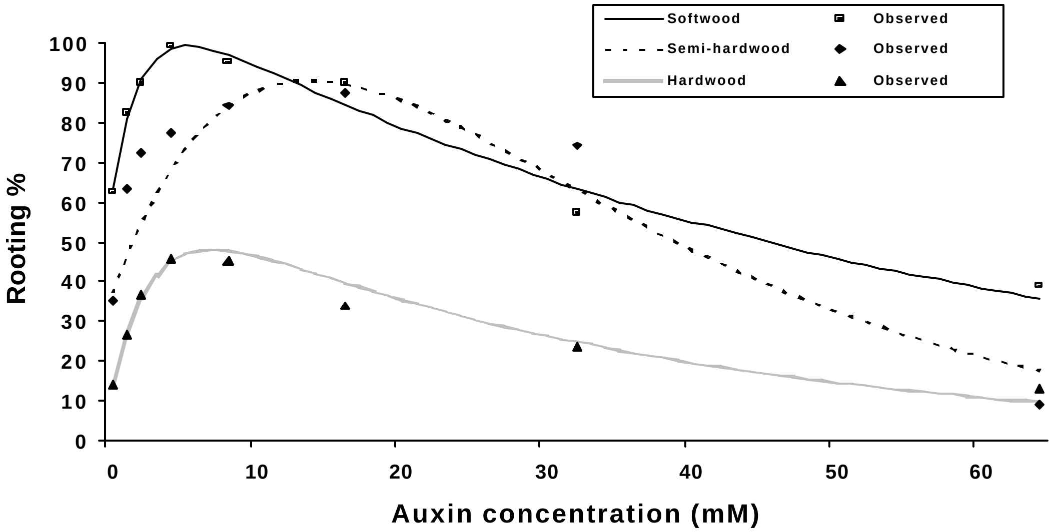
Table 1. ANOVA for traits assessed in a rooting trial of Fraser fir stem cuttings collected from 3- and 4-year-old seedlings.

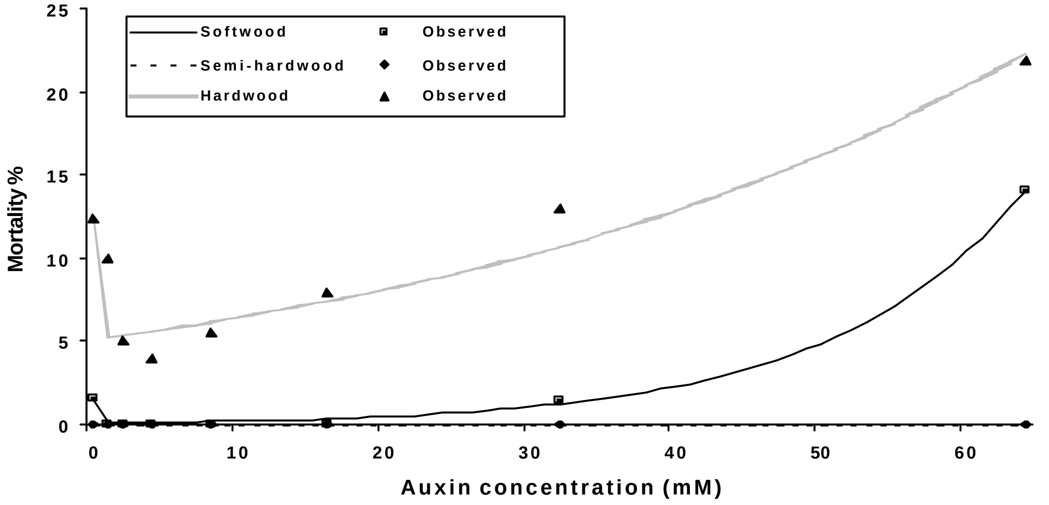
	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine Square root	Arcsine Square root	None	Log	None	None
X Transformation	Square root	Log	Log	Log	Log	None
Growth stage	*	*	*	*	*	NS
Rep (Growth stage)	NS	*	NS	*	*	NS
Auxin	NS	NS	NS	NS	NS	NS
Growth stage x Auxin	NS	NS	NS	NS	NS	NS
Concn [Z]	*	*	*	*	*	NS
C^2	*	*	*	*	*	NS
Concn x Growth stage	*	*	*	NS	NS	NS
C^2 x Growth stage	*	*	*	*	*	NS
Concn x Auxin	NS	NS	*	*	NS	NS
C^2 x Auxin	NS	NS	*	*	NS	NS
Concn x Growth stage x Auxin	NS	NS	NS	NS	NS	NS
C^2 x Growth stage x Auxin	NS	NS	NS	NS	NS	NS

[Z] Concn=auxin concentration, C^2 =auxin concentration squared.

NS, * Nonsignificant or significant at $P < 0.05$.



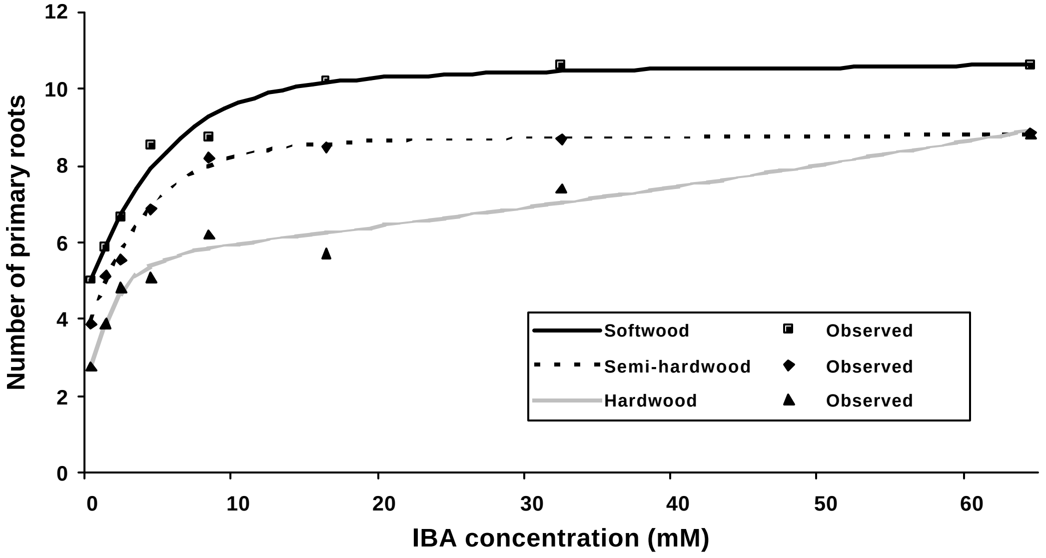
	q_1	q_2	**A**	**B**	***R^2***
Softwood	0.5085	0.0180	2.0880	2.2663	0.97
Semi-hardwood	0.0603	0.0601	0.4889	1.0006	0.90
Hardwood	0.3600	0.0296	2.1750	1.2733	0.91



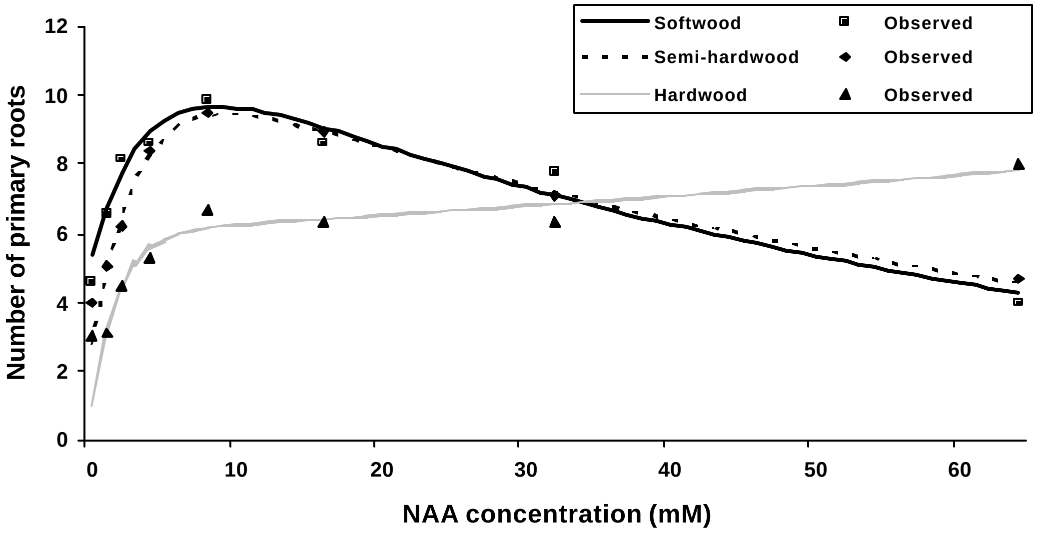
	q_1	q_2	**A**	**B**	***R^2***
Softwood	-0.0756	56.4883	-1.1963	-12.9914	0.99
Semi-hardwood [z]	-	-	-	-	-
Hardwood	-0.0230	22.4097	-0.0200	-1.435	0.88

[z] No mortality occurred in the semi-hardwood cuttings.

Fig. 1. Effects of growth stage and auxin concentration on rooting percentage (upper) and percent mortality (lower) of Fraser fir stem cuttings taken in 2001. Auxin concentration responses are averaged over IBA and NAA. *R^2* values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).

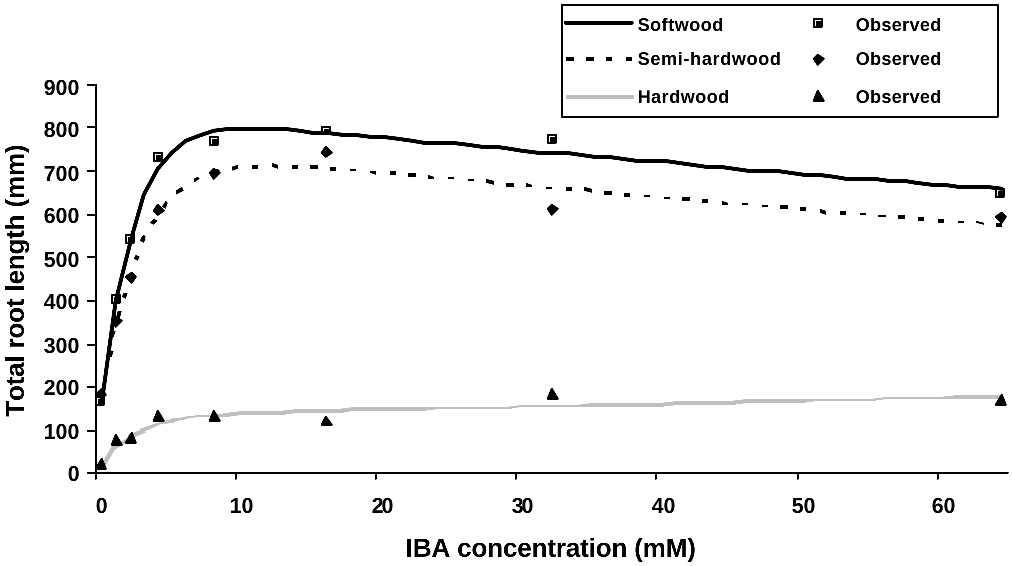


	q_1	q_2	A	B	R^2
Softwood	0.2000	-0.00053	0.1889	1.9481	0.97
Semi-hardwood	0.2468	-0.00036	0.2144	1.8516	0.99
Hardwood	0.5226	-0.00741	0.3556	2.0020	0.96

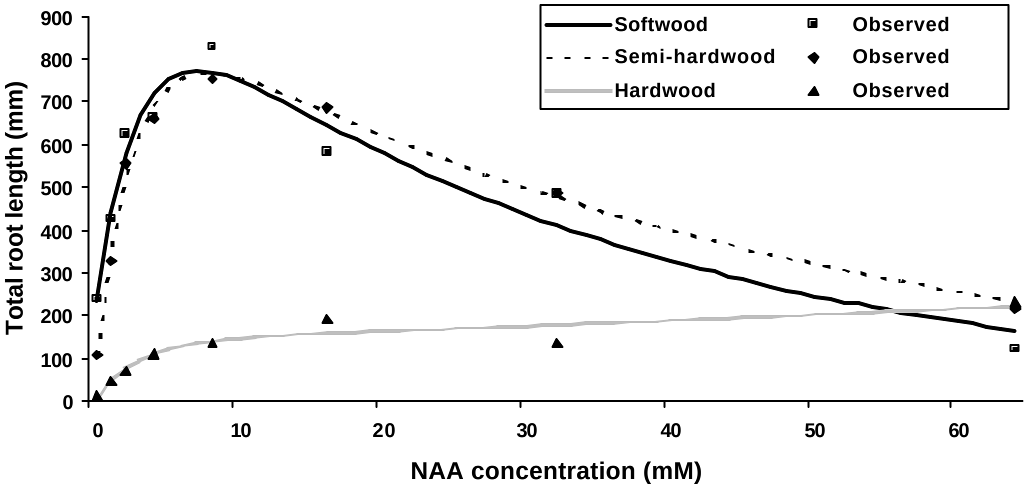


	q_1	q_2	A	B	R^2
Softwood	0.2789	0.0158	0.1660	1.8404	0.95
Semi-hardwood	0.3197	0.0141	0.1226	1.3202	0.99
Hardwood	0.5468	-0.00412	0.1994	1.2057	0.96

Fig. 2. Effects of growth stage and IBA (upper) or NAA (lower) concentration on primary root production of Fraser fir stem cuttings taken in 2001. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).

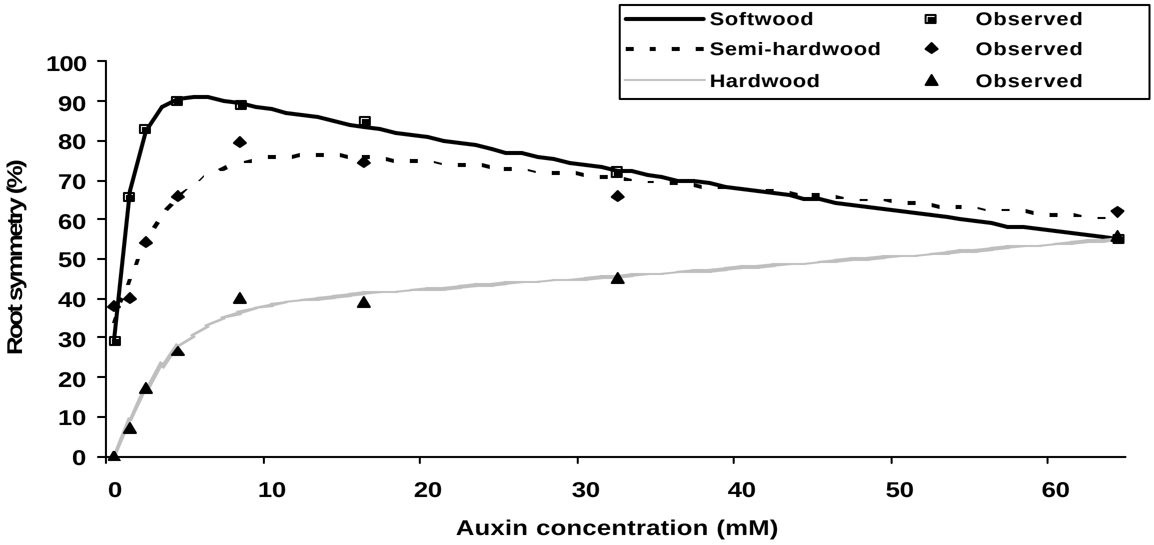


	q_1	q_2	A	B	R^2
Softwood	0.4340	0.00382	0.00149	1.2401	.98
Semi-hardwood	0.3424	0.00438	0.00174	1.3084	0.96
Hardwood	0.3850	-0.00443	0.00853	1.1612	0.88



	q_1	q_2	A	B	R^2
Softwood	0.3536	0.0285	0.00138	1.2997	0.93
Semi-hardwood	0.3704	0.0222	0.00123	1.1198	0.99
Hardwood	0.3196	-0.00689	0.00729	1.0621	0.88

Fig. 3. Effects of growth stage and IBA (upper) or NAA (lower) concentration on the total root lengths of Fraser fir stem cuttings taken in 2001. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).



	q_1	q_2	**A**	**B**	***R^2***
Softwood	0.8657	0.00869	1.5086	1.4335	0.99
Semi-hardwood	0.2855	0.00492	2.0964	1.6989	0.93
Hardwood	0.3112	-0.00591	2.4997	0.9612	0.99

Fig. 4. Effects of growth stage and auxin concentration on root system symmetry of Fraser fir stem cuttings rooted in 2001. Auxin concentration responses are averaged over IBA and NAA. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).

Chapter 3

Growth Stage, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Virginia Pine

(In the format appropriate for submission to HortScience)

Growth Stage, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Virginia Pine

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Abstract. Seven concentrations of indole-3-butyric acid (IBA), seven concentrations of 1-naphthaleneacetic acid (NAA) and a nonauxin control were tested over three growth stages to determine their ability to promote adventitious rooting of stem cuttings collected from 3- and 4-year-old stock plants of Virginia pine (*Pinus virginiana* Mill.). Cuttings were harvested in Sept. 2000 (semi-hardwood), Feb. 2001 (hardwood), June 2001 (softwood) or Oct. 2001 (semi-hardwood), treated with auxin concentrations ranging from 0 to 64 mM and placed under intermittent mist in a greenhouse. Rooting percentage, percent mortality, number of primary roots, total root length, root symmetry, root angle, and root diameter were assessed following 16 weeks. Growth stage significantly affected every rooting trait except root symmetry and diameter. Auxin type significantly affected total root length and root diameter, while auxin concentration significantly affected every rooting trait except root angle. The highest predicted rooting percentages (46%) occurred when semi-hardwood cuttings were collected in Sept. 2000 and treated with 7mM auxin. For cuttings collected within the same growing season (2001), the highest predicted rooting percentage (33%) occurred when softwood cuttings were treated with 6 mM auxin. Mortality was generally highest in semi-hardwood cuttings; however, semi-hardwood cuttings set in 2001 produced the greatest number of roots and root lengths. Root diameter was significantly greater when NAA was applied, compared to IBA, especially at higher concentrations. To root Virginia pine stem cuttings collected from 3- and 4-year-old stock plants, it is recommended that a concentration of 6 mM IBA be used on softwood cuttings.

Additional index words: vegetative propagation, growth stage, indole-3-butyric acid, 1-naphthaleneacetic acid, Christmas trees, cloning, *Pinus virginiana*

In the Piedmont and Coastal Plain of North Carolina, Virginia pine (*Pinus virginiana*) is the most commonly cultivated Christmas tree species. Virginia pine is desirable as a Christmas tree because of its rapid growth (3 to 6 years to harvest), short needles, good branch structure for holding ornaments, and pleasant pine aroma (Frampton, 2002). Other desirable traits include good survival following planting, vigorous response to shearing, and ability to grow despite poor soil conditions (Belanger and Bramlett, 1975). However, Virginia pine exhibits several significant problems including poor form, extensive variability among seedlings, somewhat poor post-harvest needle retention, and extreme susceptibility to the Nantucket pine tip moth [(*Rhyacionia frustrana* (Comstock)] (Frampton, 2002). Due to these problems, Christmas tree growers in eastern North Carolina are only able to market about 50% of the Virginia pines planted (Frampton, 2002).

Currently, commercial growers of Virginia pine Christmas trees are limited to using seeds that have been collected from orchards originally established to enhance forest production and pulpwood characteristics (Belanger and Bramlett, 1975; Holifield et al., 1991). Due to the high market value of Christmas trees, genetic improvement of Virginia pine for desirable Christmas tree characteristics can be justified. Past research has shown that desirable traits such as height, stem straightness, density of foliage, symmetry and branches per whorl are under genetic control (Belanger and Bramlett, 1975; Brown at al., 1991; Meier and Goggans, 1977). Furthermore, the retail value of Virginia pine Christmas trees are under strong genetic control (Knoth et al., 2002). Thus, selection and production of genetically superior clones using rooted stem cuttings could provide growers with higher planting stock quality and uniformity resulting in greatly increased profits (Zobel and Talbert, 1984).

Little research involving propagation of Virginia pine by stem cuttings has been reported. Limited reports have focused on the effects of auxin treatment, growth stage (i.e., time of collection), and crown position (Brown et al., 1991; Holifield et al., 1991; Snow and May, 1962).

Growth stage and auxin treatments have shown significant effects when rooting stem cuttings of many trees species (Hartmann et al., 2002). However, due to limited research, the effect of growth stage on Virginia pine rooting is not clearly understood. Snow and May (1962) found that hardwood cuttings of Virginia pine collected in December rooted efficiently (72%), but had slow root initiation, requiring 9 months. Better results were obtained applying a solution of 25 mM IBA (indole-3-butyric acid), rather than a no auxin control treatment to the cutting bases (Snow and May, 1962). When using a rooting powder (Hare, 1974), Brown et al. (1991) found that, for clones obtained from a Virginia pine field study, the average rooting percentage for semi-hardwood cuttings collected in August was 45%; however, the best clone rooted at 88%. In that study, the authors also found that the average rooting percentage for hardwood cuttings collected in early March was 43%, with the best clone rooting at an average of 75%.

Additional research may promote the use and benefits of Virginia pine stem cuttings. Specifically, the objectives of the current study were to determine the optimal growth stage, auxin type [indole-3-butyric acid (IBA) or 1-naphthaleneacetic acid (NAA)], and auxin concentrations for promoting adventitious root initiation and subsequent root development of stem cuttings from 3- to 4-year-old stock plants of Virginia pine.

Materials and Methods

Virginia pine cuttings were collected from an open-pollinated progeny test established during Fall 1997 and located at the North Carolina State University Horticultural Crops Research Station near Clinton, N.C. The stock plants had been grown from seeds and cultured as Christmas trees (Johnson, 1991). The cuttings were harvested on 14 Sept. 2000 (semi-hardwood), 23 Feb. 2001 (hardwood), 12 June 2001 (softwood) and 3 Oct. 2001 (semi-hardwood). Two terminal branch cuttings per tree were randomly selected from the upper third of the crown and cut to a minimum length of 13 cm. Following collection, the cuttings were wrapped immediately in moist paper towels, transported to Raleigh, N.C., on ice, and stored overnight at 4 ^{o}C. All cuttings were set (inserted into the rooting medium) the day following collection.

IBA and NAA were evaluated for their ability to stimulate adventitious rooting of Virginia pine cuttings collected during three different growth stages. Seven IBA and seven NAA concentrations (1, 2, 4, 8, 16, 32, or 64 mM) and a solvent control were utilized. For each concentration, the auxin was dissolved into a 50% isopropyl alcohol/ deionized water solution. The prepared auxin solutions were then stored at 4 ^{o}C in opaque bottles and used within 2 d.

Rooting was conducted in a propagation greenhouse located at the Horticulture Field Laboratory, Raleigh. Before being set into the rooting medium, the cuttings were re-cut from the bases to a length of 9 cm and auxin was applied for 3 s to the basal 1.5 cm. Cuttings for the control treatment were dipped into a 50% isopropyl alcohol/deionized water solution. Needles were not removed before or after the auxin was applied. The treated cuttings were air-dried for a minimum of 15 min before being inserted to a 3 cm depth into a rooting medium of 3 horticultural perlite : 2 peat (by volume). Cuttings were rooted in individual 164 cm^3 Ray Leach SC-1- Super Cells (Steuwe and Son, Inc., Corvallis, Ore.).

During the rooting period, cuttings were maintained under intermittent mist using a Grower Junior™ (McConkey Co., Sumner, Wash.) overhead boom irrigation system. Frequency of mist application was controlled using relative humidity (RH) and time of day. As RH in the rooting greenhouse decreased, the frequency of mist application increased; however, mist application was less frequent during the night decreasing from an average of one mist cycle every 6 min during the day to an average of one mist cycle every 60 min at night. Flow rate per mist cycle was held constant over the 4-month rooting period at 0.05 $L \cdot m^{-2}$. Average day/night temperatures in the greenhouse were 25.5 ± 2/20.0 ± 2 °C for semi-dormant cuttings collected in 2000, and 25.0 ± 2/19.4 ± 2 °C, 26.1 ± 2/20.5 ± 2 °C, and 24.4 ± 2/19.4 ± 2 °C, respectively, for hardwood, softwood and semi-dormant cuttings collected in 2001.

Rooting percentage, percent mortality, number of primary roots, total root length, root system symmetry, root angle, and root diameter were recorded following 16 weeks in the greenhouse. The latter five traits were assessed only on cuttings that rooted. A cutting was considered rooted if a minimum of one root ≥ 1 mm in length was present. A cutting with two roots was considered to have a symmetrical root system when the angle between the roots was > 135°, using the stem center as the vertex (Frampton et al., 1999). Cuttings with three or more roots were scored as symmetrical if the angle between the two roots that were farthest apart was < 135°. Cuttings with one root were scored as having asymmetrical root systems. Root angle was used to determine the percentage of positively geotropic roots for each cutting. Root angle was defined as the percentage of roots that formed an angle < 60° with an extension of the cutting's stem. Root diameter was measured on a sub-sample (n=240) of each auxin type. These cuttings were randomly selected over all seven auxin concentrations within each of the four setting periods. Using a digital caliper, root diameter was measured 1 cm from the base of the cutting to the nearest 0.5 mm.

The experimental design for cuttings collected at each growth stage was a randomized complete block. There were eight blocks of eight cuttings per treatment within each block for a total of 960 cuttings per growth stage. For each growth stage, the entire study was surrounded by a row of border cuttings.

Analyses of variance (ANOVAs) were conducted using the General Linear Models procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Growth stage, replication within growth stage, auxin type, auxin concentration, auxin concentration squared, and all the two- and three-way interactions other than those involving replication were sources of variation. Transformations of the dependent variables (rooting traits) were investigated to increase homogeneity among treatment variances. Several transformations were explored including the square root, the arcsine of the square root, and a range of transformations using the Box and Cox (1964) procedure that varied λ from -1.0 to +1.0 in increments of 0.25. The transformation, or no transformation, yielding the smallest differences among treatment variances was selected for each dependent variable. Once the most appropriate dependent variable transformation was selected, log and square root transformations of the independent variables (concentration and concentration squared) were compared to no transformation. The model yielding the highest R^2 value is reported for each dependent variable.

Despite employing the most appropriate data transformations and including squared terms for concentration, the least-squares regression approach failed to adequately define the relationship between concentration and the variables assessed. Therefore, nonlinear regression techniques were employed by regressing each dependent variable on treatment means, concentration or auxin type by concentration, using the NLIN procedure of the Statistical Analysis System Version 8.1 (SAS Institute, Inc., 1999). The double exponential model (Rawlings et al., 1998), modified by two constants (A and B), was employed:

$$\text{Trait} = \theta_1(Be^{(-\theta 2^*\text{Concentration})} - e^{(-\theta 1^*\text{Concentration})})/[A(\theta_1 - \theta_2)]$$

where,

e = base of natural logarithm,

θ_1,θ_2, A and B = regression parameters, and

concentration = auxin concentration in mM.

The least-squares linear regression approach using the General Linear Model procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999) adequately defined the relationship between concentration and root diameter. The model employed was:

Root Diameter = Intercept + Log (Concentration) x A + Log^2(Concentration) x B

where,

A and B = regression parameters.

Results

ANOVAs and transformations of the independent and dependent variables for all rooting traits are presented in Table 1.

Rooting Percentage. Growth stage, replication within growth stage, concentration, concentration squared as well as the interaction between growth stage and concentration squared significantly affected rooting percentage (Table 1). No significant differences were detected for auxin type ($P = 0.56$), thus IBA and NAA were averaged for the nonlinear regression analysis. For semi-hardwood cuttings set in 2000, the greatest overall predicted rooting percentage (47%) occurred when cuttings were treated with 6 mM (Fig. 1). Hardwood and softwood cuttings set in 2001 reached their maximal predicted rooting percentages (23% and 32%, respectively) at 7 mM. The highest predicted rooting percentage (24%) in semi-hardwood cuttings set in 2001 occurred at 17 mM.

Percent Mortality. Percent mortality was significantly affected by growth stage, replication within growth stage, concentration, and concentration squared (Table 1). Because no significant differences were detected for auxin type ($P = 0.73$) or its interactions, IBA and NAA were averaged for the nonlinear regression analysis. Predicted percent mortality decreased from 16% in the controls to 10% at 2 mM and then increased with increasing concentrations, reaching a maximum of 42% at 64 mM auxin (Fig. 1).

Number of Primary Roots. The main effects of replication within growth stage, concentration and concentration squared significantly affected primary root production (Table 1). A significant interaction was detected between growth stage and concentration

(Table 1). No significant differences were detected for auxin type (*P* = 0.41) or its interactions, so that IBA and NAA were averaged for the nonlinear regression analysis. For semi-dormant cuttings set in 2000, the greatest predicted number of primary roots (4.6) occurred at 6 mM (Fig. 2). For cuttings rooted in 2001, primary root production increased rapidly to 8 mM and either leveled off (softwood and semi-hardwood cuttings set in 2001) or continued to increase (hardwood cuttings) with increases in auxin concentration. At 8 mM, predicted primary root production was 4.2, 4.3, and 6.5 for hardwood, softwood, and semi-hardwood cuttings set in 2001, respectively.

Total Root Length. The main effects of growth stage and concentration as well as the interaction between growth stage and auxin type significantly affected total root length (Table 1). Interactions between concentration squared and growth stage also were significant (Table 1). Due to these interactions, separate nonlinear regression analyses were conducted for each auxin type.

When IBA was applied, predicted total root lengths for semi-hardwood cuttings set in 2000 and 2001 increased rapidly to a maximum (194 mm at 6 mM IBA and 473 mm at 17 mM IBA, respectively), before decreasing slightly with increases in auxin concentration (Fig. 3). When IBA was applied to softwood cuttings, predicted total root lengths increased rapidly from 24 mm in the control to 369 mm at 20 mM before leveling off and reaching a maximum of 412 mm at 64 mM. In the hardwood cuttings, predicted total root lengths continued to increase with increases in auxin concentration from the control (0 mm) to 64 mM concentration (304 mm).

When NAA was applied to the bases of the semi-dormant cuttings, predicted total root lengths peaked at 10 mM NAA in 2000 (274 mm) and 22 mM NAA in 2001 (369 mm) before decreasing slightly with increases in auxin concentration (Fig. 3). In the softwood cuttings, predicted total root length continued to increase slightly with increases in auxin concentration from the control (111 mm) to 64 mM (615 mm). For hardwood cuttings, predicted total root length increased rapidly from the control (0 mm) to 8 mM NAA (170 mm) before increasing slightly with increasing concentrations to 64 mM NAA (192 mm).

Root Symmetry. Concentration and concentration squared significantly affected root symmetry (Table 1). No significant differences were detected for auxin type or its interactions; therefore, both IBA and NAA were averaged for the nonlinear regression analysis. Root symmetry increased rapidly from the control (4%) to 8 mM (41%) before leveling off and only slightly increasing with increases in auxin concentration up to a maximum of 44% at 64 mM (Fig. 4).

Root Angle. Growth stage was the only source of variation that was significant for root angle (Table 1). The highest predicted percentage of positively geotropic roots (87%) occurred in the softwood cuttings. There was little difference between the predicted values for hardwood (79%) and semi-hardwood cuttings set in 2001 (79%), however, semi-hardwood cuttings collected in 2000 produce the fewest number of positively geotropic roots (66%).

Root Diameter. The main effects of auxin, concentration, and the interaction between auxin and concentration significantly affected root diameter (Table 1). When IBA was applied to the bases of cuttings, the predicted average for root diameter increased from 1 mM (0.6 mm) to 32 mM (1.5 mm) before decreasing at 64 mM (1.4 mm) (Fig. 5). When NAA was applied to the bases of cuttings, the predicted average for root diameter increased slowly from 1 mM (1.1 mm) to 16 mM (1.4 mm) before rapidly increasing at 64 mM (2.7 mm).

Discussion

Maximal rooting percentage for the semi-hardwood cuttings set in 2000 was about twice that of the semi-hardwood cuttings set in 2001, and considerably higher than those of the hardwood and softwood cuttings set in the 2001. One possible explanation for the difference in rooting percentage may be environmental and climatic conditions at the time of collection. Maturation of the stock plants is another likely factor contributing to the difference in rooting between the 2 years. Marino (1982) noted that, excluding hedged material, the threshold age at which maturation increases and rooting decreases in some southern pine species occurs around age three. If this is true in Virginia pine, then this phenomenon, at least partially, explains the difference in rooting between the cuttings set in 2000 (3-year-old material) and those set in 2001 (4-year-old material). For this reason, growth stage comparisons discussed in the following section are focused on only the 4-year-old material.

Growth Stage Differences. In this study, growth stage of the stock plants from which stem cuttings were taken greatly affected rooting. This phenomenon has been reported previously for cuttings of several other *Pinus* L. spp. (Boeijink and van Broekhuizen, 1974; Cameron, 1968; Kiang et al., 1974; Lanphear and Meahl, 1963) and is attributable to morphological and physiological characteristics of the stock plant at the time of cutting collection (Hartmann et al., 2002).

The Virginia pine hardwood cuttings were collected during February when the stock plants were fully dormant and shoot chilling requirements apparently had been met.

Consequently, when placed into the warm greenhouse environment, apical budbreak on most (84%) hardwood cuttings occurred prior to rooting, resulting in shoot elongation throughout the rooting period and lower rooting percentages than the other cutting types (Fig. 1). Snow and May (1962) also found that hardwood cuttings set in March rooted much poorer (19%) than semi-hardwood cuttings (72%) collected from material of the same age. Thus, early budbreak (prior to rooting) of hardwood Virginia pine cuttings may be an underlying problem affecting rooting ability. Although not previously reported in Virginia pine, budbreak prior to rooting has been reported to occur in hardwood cuttings of several conifer species, including Fraser fir (*Abies fraseri* [Pursh] Poir.) (Hinesley and Blazich, 1980; 1981; Rosier, 2003 Chapter 2).

For softwood cuttings, apical buds were not fully developed when removed from the stock plants; therefore, shoot elongation generally did not occur (4 %) following setting into the greenhouse. Lack of apical budbreak presumably meant more cutting resources were available for root initiation and development. As a probable consequence, softwood cuttings exhibited the highest rooting percentages when compared with the other cutting types collected during the same year (Figs. 1 and 2).

Softwood cuttings generally have higher metabolic activity and increased levels of endogenous auxin production relative to cuttings in other growth stages, and hence require less exogenous auxin to promote rooting (Hartmann et al., 2002). In this study, softwood cuttings reached optimal rooting at a lower auxin concentration (7 mM) than the semi-dormant cuttings (17 mM) before rooting declined rapidly, most likely due to increased mortality (data not presented), especially in NAA-treated cuttings (Fig. 1).

This pattern suggests that the endogenous auxin levels of the softwood cuttings were indeed higher than those of the semi-hardwood cuttings.

Semi-hardwood cuttings collected in 2001 produced the greatest number of roots and lengths, and rarely broke bud (<1 %) prior to rooting. Hartmann et al. (2002) suggest that as the annual growing cycle progresses, levels of exogenous auxin production begin to decrease. Higher levels of exogenous auxin consequently are needed to achieve an optimum for desired rooting characteristics. Semi-hardwood cuttings are also less likely to be affected detrimentally by higher levels of exogenous auxin due to slower rates of metabolism and lower levels of endogenous auxin. This is most apparent when primary root production and root lengths of the semi-hardwood cuttings and softwood cuttings are compared. The semi-hardwood cuttings tended to produce a larger root system and appeared less detrimentally affected by higher levels of exogenous auxin (Fig. 2 and 3).

Since callus formation and root initiation in the semi-hardwood cuttings were still occurring following the 16-week rooting period, some (n=196) callused but non-rooted cuttings were re-set into fresh medium and rooting percentage was reexamined after an additional 6 weeks. About half (52%) of these cuttings had at least one root measuring 1 mm in length, suggesting that a rooting period longer than 16 weeks may be required for semi-hardwood Virginia pine cuttings. Previous research in which Virginia pine cuttings were collected from 8- to 9-year-old material required 7 to 9 months before root development was evident, although environmental conditions were not described (Snow and May, 1962).

Auxin Type and Concentration Effects. Auxin type, IBA or NAA, did not significantly affect rooting percentage or primary root production (Table 1). Although auxin type did not significant affect root production, NAA treated Virginia pine cuttings produced a great number of roots. These findings support previous research with succulent loblolly pine (*Pinus taeda* L.) cuttings collected from hedged stock plants where NAA significantly increased root production (B. Goldfarb, personal communication). The type of auxin used significantly affected total root lengths, but varied depending on the growth stage; however, NAA generally induced longer root systems at optimal concentrations for rooting (Fig. 3). Goldfarb et al. (1998) have also shown that the application of NAA significantly increased root production in hypocotyl cuttings of eastern white pine (*Pinus strobus* L.). In one study, it was suggested that NAA was more effective than IBA in adventitious root induction due to its slower rate of metabolism, and it was determined that rooting was not directly correlated with auxin differences in availability at the rooting site or in its transport capacity (Diaz-Sala et al., 1996). Their findings are supported by Greenwood et al. (1974) who concluded that free auxin, not its metabolites, is essential for adventitious root formation in sugar pine (*Pinus lambertiana* Dougl.).

Auxin type and concentration significantly affected root diameter, with cuttings treated with NAA always producing larger diameters when compared to equal concentrations of IBA (Fig. 5). As the concentration of NAA increased, it was also observed that secondary root production began to decrease. When 32 mM NAA was applied, very little secondary root growth was evident and at 64 mM NAA secondary root formation ceased. As the concentration of IBA increased, secondary root formation decreased slowly, but was always present even at the highest concentrations. The fact that NAA treated cuttings produced larger root diameters may be related to the fact that

NAA is metabolized at a much slower rate and therefore is present in the cutting for a longer period of time (Diaz-Sala et al., 1996). Further research will be needed investigate what significance, if any, this phenomenon has on survival and field growth following planting.

Auxin concentration significantly affected nearly every rooting trait assessed (Table 1). Inclusion of a wide range and many levels of concentrations provided a clear picture of dosage responses. Percentage rooting increased significantly and mortality decreased significantly with increases in auxin concentration up to an optimum before higher concentrations of auxin detrimentally affected these traits (Fig. 1). Percent survival always peaked at lower concentrations than rooting percentage. Root production increased rapidly to an optimum and then continued to increase slightly with increases in auxin concentration. An exception was semi-hardwood cuttings set in 2000, which decreased slightly following the optimum (Fig. 2). Total root lengths increased rapidly at lower concentrations and then, depending on the auxin type, either showed little change (IBA) or slightly decreased (NAA) (Fig. 3). Regardless of the growth stage, primary root production and total root lengths in IBA treated cuttings were positively correlated and reached optima at approximately the same concentration. However, for NAA treated cuttings, these traits generally decreased more rapidly at higher auxin concentrations. Root symmetry, not significantly affected by auxin type, also increased rapidly to an optimum before decreasing with increases in auxin concentration.

Higher concentrations of auxin (> 16 mM) did not significantly affect the number of roots produced, but affected the origin of root initiation on the stem. When cuttings were treated with low levels of auxin, roots generally emerged directly from the callused basal area of the stem. A similar trend has been reported for hypocotyl cuttings of eastern white pine (Goldfarb et al., 1998). This phenomenon was most pronounced in the hardwood cuttings. At auxin concentrations = 32 mM, regardless of auxin type, the bases of the hardwood cuttings began to die back and cutting mortality rapidly increased. Callus formation was slow to occur above the dead tissue and subsequent root growth consisted of numerous roots measuring a few millimeters in length that extended up the stem. Due to this pattern, primary root numbers in the hardwood cuttings were high while the total root lengths, regardless of auxin type, were low.

For nearly every trait assessed, growth stage and concentration (and its quadratic term) were significant (Table 1). These significant main effects are accounted for by the previously discussed phenomena: 1) maturation, environmental and climatic conditions and their effects on rooting percentages for cuttings set in different years, 2) budbreak and its relationship to resource depletion before rooting in the hardwood cuttings, 3) the large variation in total root lengths, especially in NAA treated cuttings, and 4) the differences in optima among the four setting periods.

While not presented herein, a study investigating combinations of auxin types was conducted simultaneously with the current study (Rosier, 2003 Appendix II). Eight combinations of three concentrations (0, 4, and 16 mM) of both IBA and NAA were tested in a factorial fashion. Generally, these combinations produced negative effects on the rooting traits assessed relative to the use of a single auxin. Auxin was detrimental when 16 mM was applied in any combination. Future research should address combinations of auxin concentrations lower than those evaluated.

In conclusion, stem cuttings collected from 3- and 4-year-old Virginia pines rooted best when softwood stem cuttings were used and 7 mM NAA was applied to their bases. At these concentrations, mortality was low and root production was either approaching or had reached an optimal level. Early budbreak leading to apparent resource depletion before rooting was a problem in the hardwood cuttings, and led to a decrease in rooting performance relative to the other two types of cuttings.

Results from the current study should provide a basis for the advancement of cutting propagation for Virginia pine; however, further research is needed to develop a protocol that will increase the rooting percentage and enhance root development. Future studies should also focus on subsequent field growth following rooting. One of the main deterrents to rooting pine cuttings is their dependency on the age of the stock plant. This is a common problem in progeny testing where trees are allowed to grow over an extended period of time, and by the time their genetic potential is determined, the material is too mature to root. Rooting and subsequent growth of cuttings have been shown to be affected not only by maturation, but also the crown position from which cuttings are collected (Zobel and Talbert, 1984). To circumvent these problems associated with maturation, hedging of stock plants may provide a way to produce multiple juvenile shoots that root readily. Hedging of stock plants has been demonstrated in several other pine species including loblolly pine (*Pinus taeda* L.) and radiata pine (*Pinus radiata.* Don) (e.g. Bolstad and Libby, 1982; Fielding, 1954; Libby et al., 1972; Menzies, 1986).

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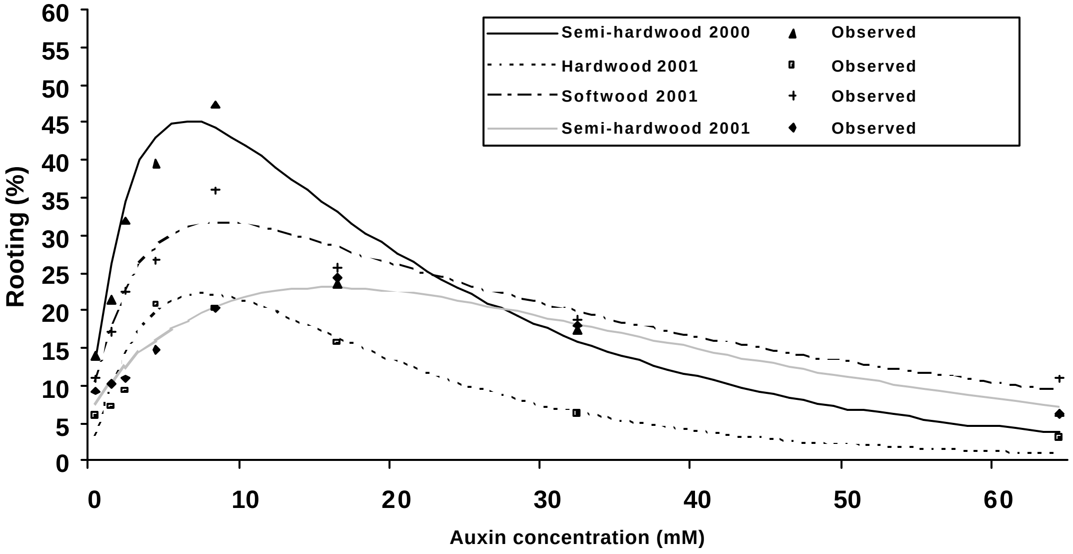
Table 1. ANOVA for traits assessed in a rooting trail of Virginia pine stem cuttings collected from 3- and 4-year-old stock plants.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle	Root diameter
Y Transformation	Arcsine Square Root	Square Root	None	Square Root	Log	None	None
X Transformation	Log	None	Log	Log	Log	None	Log
Growth stage	*	*	NS	*	NS	*	NS
Rep (Growth stage)	*	*	*	NS	NS	NS	-[Y]
Auxin	NS	NS	NS	NS	NS	NS	*
Growth stage x Auxin	NS	NS	NS	*	NS	NS	NS
Concn[Z]	*	*	*	*	*	NS	*
C^2	*	*	*	NS	*	NS	NS
Concn x Growth stage	NS	NS	NS	NS	NS	NS	NS
C^2 x Growth stage	*	NS	*	*	NS	NS	NS
Concn x Auxin	NS	NS	NS	NS	NS	NS	*
C^2 x Auxin	NS	NS	NS	NS	NS	NS	NS
Concn x Growth stage x Auxin	NS	NS	NS	NS	NS	NS	NS
C^2 x Growth stage x Auxin	NS	NS	NS	NS	NS	NS	NS

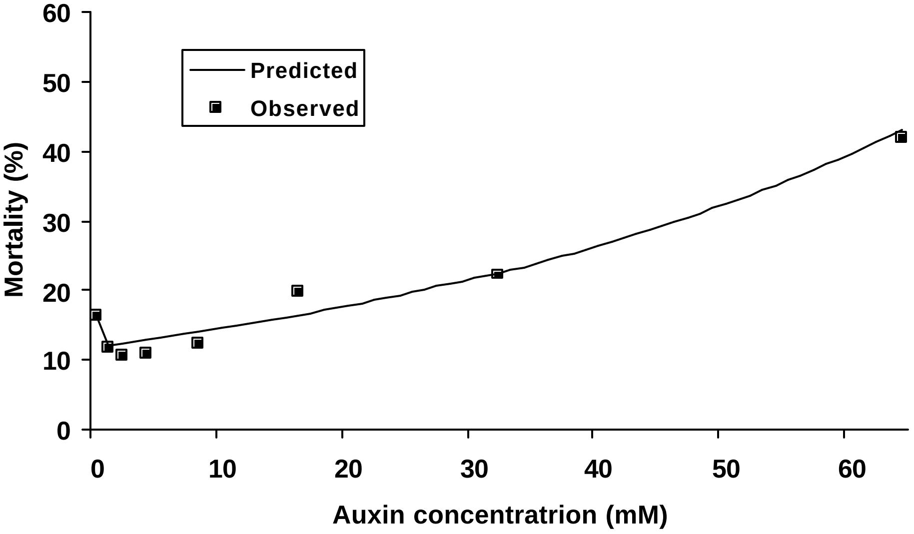
[Z] Concn = auxin concentration, C^2 = auxin concentration squared.

[Y] Not measurable due to nature of the sub-sample.

[NS, *] Nonsignificant or significant at $P<0.05$, respectively.

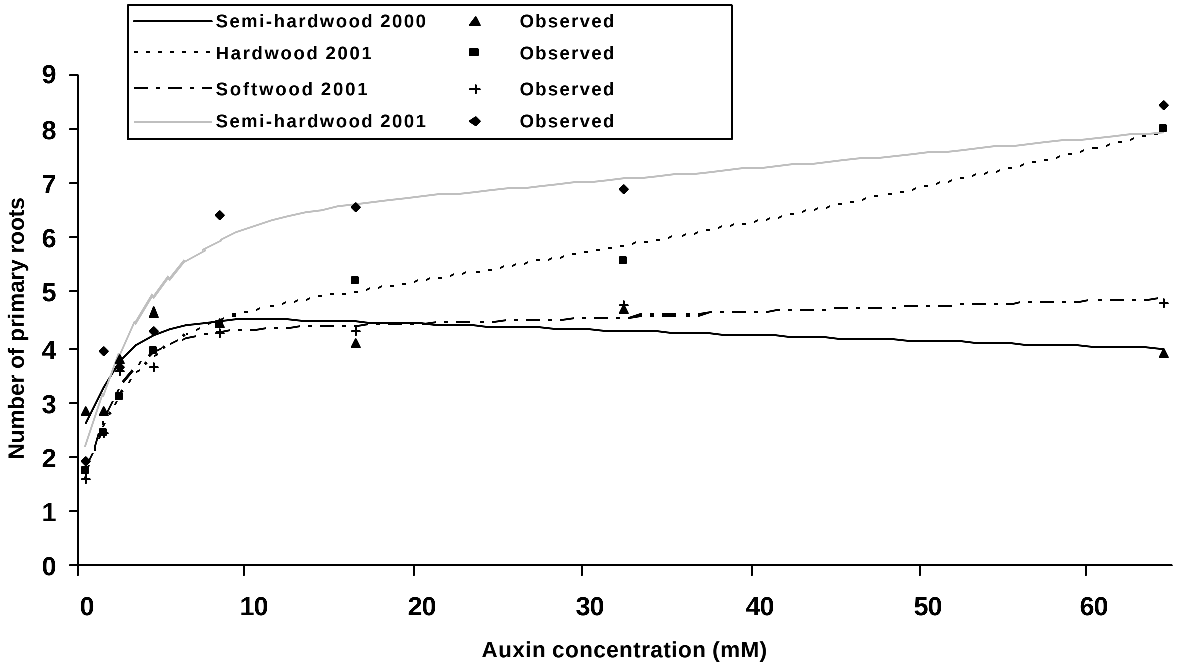


	q_1	q_2	**A**	**B**	***R^2***
Semi -hardwood 2000	0.3354	0.0458	2.2203	1.2370	0.91
Hardwood 2001	0.0624	0.2376	0.7588	0.9278	0.88
Softwood 2001	0.3055	0.0231	3.4991	1.3363	0.94
Semi -hardwood 2001	0.0355	0.0869	1.0000	0.8783	0.97



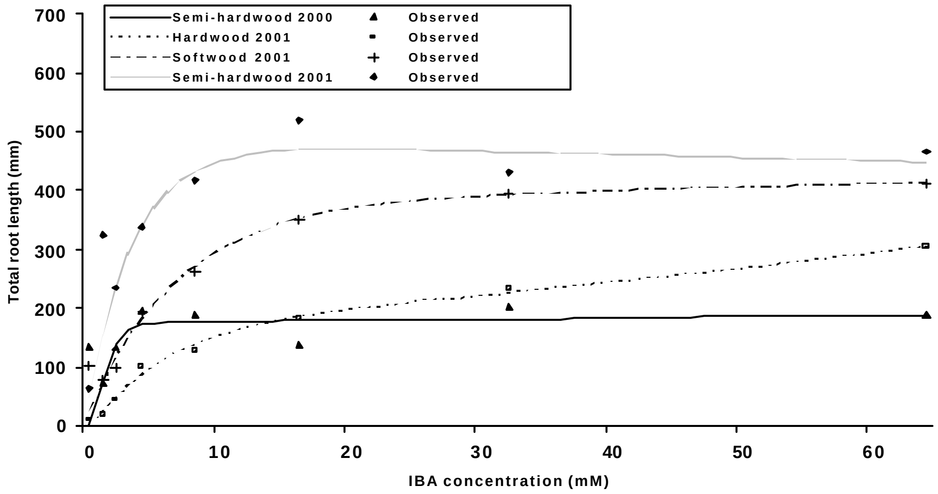
	q_1	q_2	**A**	**B**	***R^2***
Predicted	-0.0202	17.822	-0.0096	-0.3844	0.91

Fig. 1. Effects of growth stage and auxin concentration on rooting percentage (upper) and percent mortality (lower) of Virginia pine stem cuttings taken in 2000 and 2001. Auxin concentration responses are averaged over IBA and NAA. For percent mortality, growth stage responses are also averaged over the four setting periods. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).

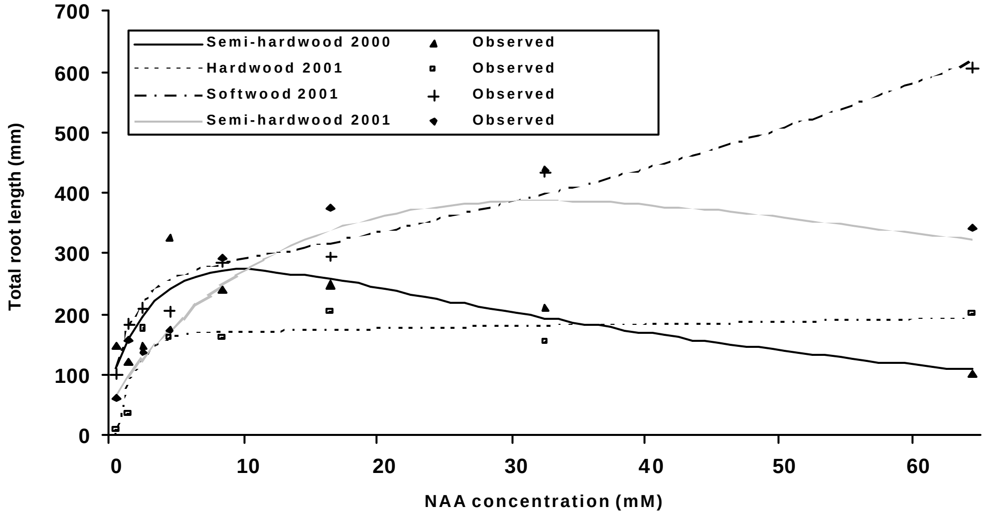


	q_1	q_2	**A**	**B**	***R***^{2}
Semi-hardwood 2000	0.4136	0.00244	0.4881	2.2662	0.87
Hardwood 2001	0.3612	-0.00962	0.3761	1.6554	0.99
Softwood 2001	0.4785	-0.00205	0.3714	1.5896	0.97
Semi-hardwood 2001	0.2507	-0.00362	0.2421	1.5466	0.95

Fig. 2. Effects of growth stage and auxin concentration on primary root production in Virginia pine cuttings collected in 2000 and 2001. Auxin concentration responses are averaged over IBA and NAA. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).

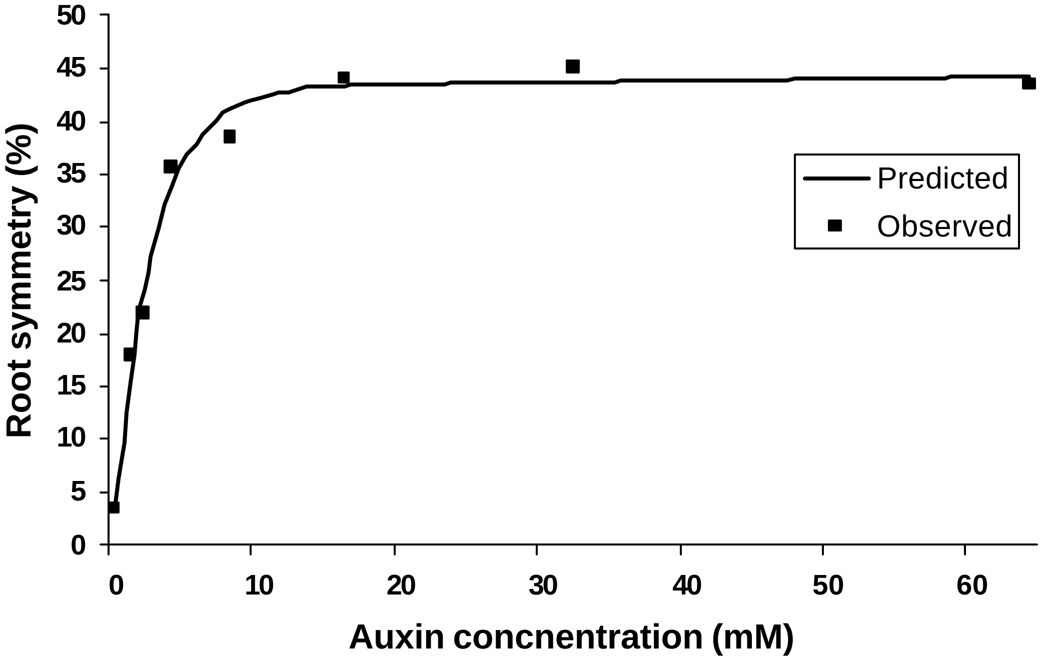


	q_1	q_2	**A**	**B**	***R*²**
Semi-hardwood 2000	-0.00117	1.0790	-0.00000621	1.7513	0.82
Hardwood 2001	0.1715	-0.00923	0.00541	0.9604	0.99
Softwood 2001	0.1409	-0.00123	0.00278	1.0681	0.99
Semi-hardwood 2001	0.2794	0.00111	0.00232	1.1114	0.86



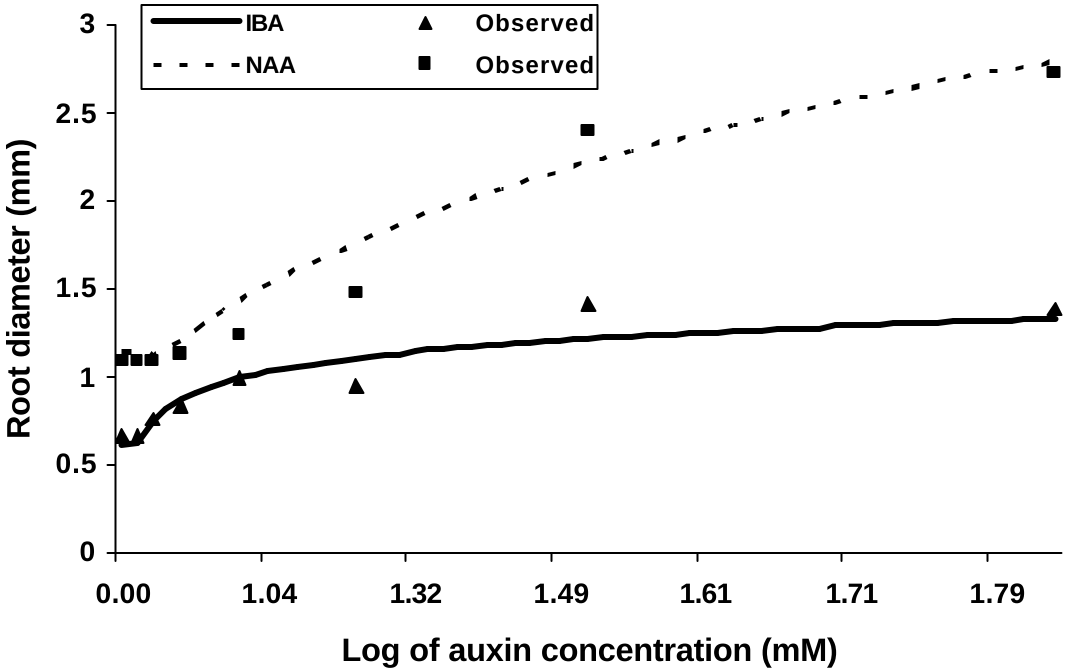
	q_1	q_2	**A**	**B**	***R*²**
Semi-hardwood 2000	0.2526	0.0185	0.00452	1.4770	0.74
Hardwood 2001	0.7110	-0.00212	0.00586	0.9878	0.85
Softwood 2001	0.5924	-0.0138	0.00682	1.7769	0.97
Semi-hardwood 2001	0.0653	0.0100	0.00182	1.1217	0.95

Fig. 3. Effects of growth stage and IBA (upper) or NAA (lower) concentration on the total root lengths of Virginia pine stem cuttings taken in 2000 and 2001. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model for each cutting type (see text for equation).



	q_1	q_2	**A**	**B**	R^2
Predicted	0.3648	-0.00040	2.5129	1.0845	0.98

Fig. 4. Effect of auxin concentration on the percent of symmetrical roots of Virginia pine cuttings. Auxin concentration responses are averaged over IBA and NAA. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model (see text for equation).



	Intercept	A	B	***R*²**
IBA	0.6213	0.4258	0.0190	0.91
NAA	1.1448	-0.0293	0.6369	0.90

Fig. 5. Effects of auxin type and concentration on root diameter of Virginia pine cuttings. R^2 values and estimated regression coefficients (intercept, A, B) are presented for the best model (see text for equation).

Chapter 4

Stumping Height, Crown Position, Age of Parent Tree, and Auxin Influence Rooting of Stem Cuttings of Fraser Fir

(In the format appropriate for submission to HortScience)

Stumping Height, Crown Position, Age of Parent Tree, and Auxin Influence Rooting of Stem Cuttings of Fraser Fir

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Abstract. Two experiments were conducted to develop a protocol for rooting stem cuttings from 3-, 5-, and 7-year-old Fraser fir (*Abies fraseri* [Pursh] Poir.) Christmas trees. The first experiment tested the effect of stumping treatments and tree age on shoot production and subsequent adventitious root production. One auxin concentration [4 mM indole-3-butyric acid (IBA)] and a nonauxin control were tested. Stock plants were stumped to the first whorl (trees in the field 3 and 5 years) or the first, third, and fifth whorls (trees in the field 7 years). Intact (nonstumped) controls were also included for each age. The second experiment was designed to create a quantitative description of the effects that crown (leaves and above ground branches of a tree) position have on the rooting of stem cuttings collected from stumped and nonstumped trees. The exact position was determined by measuring the distance from the stem, height from the ground and the degrees from north. Crown positions were recorded as cuttings were collected and then cuttings were tested for rooting response. The rooting traits assessed in both experiments included rooting percentage, percent mortality, number of primary roots, total root length, root symmetry, and root angle. In the first experiment, rooting percentage, primary root production, and total root length increased as the age of the stock plant decreased and the severity of the stumping treatment increased. Application of auxin significantly increased rooting percentage, root production, root lengths, and root symmetry while decreasing mortality. Overall, the highest rooting percentages (51%) and the greatest number of primary roots (8.1) occurred when 3-year-old stock plants were stumped to the first whorl and treated with 4 mM IBA. The greatest total root lengths (335 mm) occurred in the 3-year-old stock plants. In the second experiment, rooting percentage was significantly affected by the position from which the cuttings were collected. Cuttings collected lower in the crown and closer to the main stem rooted more frequently than cuttings collected from the outer and upper crown.

Additional index words: vegetative propagation, crown position, indole-3-butyric acid, maturation, Christmas trees, cloning, *Abies fraseri*

Fraser Fir (*Abies fraseri*) is a valuable Christmas tree species that is indigenous to southwestern Virginia, eastern Tennessee, and western North Carolina. It grows naturally at elevations between 1,200 and 2,037 m and is a small to medium size tree reaching an average height of 12 m with a diameter at breast height of 40 cm (Saravitz and Blazich, 1996). Fraser fir is highly prized in the Christmas tree industry because it produces dark green fragrant foliage, a natural Christmas tree shape, good branch structure, and needles that persist following cutting (Arnold et al., 1994; Frampton, 2002; Johnson, 1991). However, Fraser fir has several significant problems including extreme susceptibility to an introduced insect, the balsam wooly adelgid (*Adelges piceas* Ratz.), and an introduced root rot fungus, *Phytophthora cinnamomi* Rands.

Recently, there has been a renewed interest in propagating Fraser fir asexually, through the use of rooted cuttings, to select and propagate clones with superior Christmas tree characteristics (Frampton, 2002). Asexual propagation would provide a way to capture benefits from cloning, including increased genetic gain and uniformity, and allow greater flexibility in meeting customers' needs (Zobel and Talbert, 1984). Once selected, desirable genotypes could be propagated and used for both clonal preservation and commercial production. Other potential benefits include the ability to combine desirable characteristics by, for example, selecting and propagating clones that are resistant to both the balsam wooly adelgid and phytophthora root rot. Due to the high market value of Christmas trees and the large genetic variation that currently exists, genetic improvement of Fraser fir for desirable Christmas tree characteristics is justified.

While considerable research has been reported on propagation of Fraser fir by stem cuttings, very little work has focused on the problems associated with maturation, which are among the major limitations to the effective use of vegetative propagation (Zobel and Talbert, 1984). Cuttings collected from younger trees will often root readily, but as the tree matures, rooting becomes increasingly difficult (Hartmann et al., 2002; Zobel and Talbert, 1984). This is the case in Fraser fir where maturation decreases rooting competence, and also increases the plagiotropic growth of rooted cuttings (Wise, 1985; Wise et al., 1985c).

Previous research with radiata pine (*Pinus radiata*. Don) (Bolstad and Libby, 1982; Fielding, 1954; Libby et al., 1972, Menzies, 1986), Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Black, 1972), and Norway spruce (*Picea abies* L.) (Bentzer, 1993) demonstrated that continuous hedging of a stock plant provides a way to increase cutting production and maintain juvenility. Wise et al. (1985c) demonstrated that decapitating, or stumping, mature stock plants of Fraser fir could produce multiple orthotropic shoots. In that study, as the severity of the decapitation treatment increased, the number of orthotropic shoots produced and rooting percentages increased. Further research could provide a more precise protocol that may lead to increased cutting yields and greater rooting. Therefore, the present investigation evaluated a wider range of ages and a greater range of stumping treatments.

Two experiments were designed to investigate the possibility of increasing production and rooting of vertically oriented (orthotropic) shoots from 3-, 5, and 7-year old trees receiving several stumping treatments. *Experiment 1*, was designed to test the effects of age and stumping treatments on shoot production and subsequent adventitious root formation. Fraser fir stock plants were stumped to the first whorl (trees in the field 3 and 5 years) or the first, third, and fifth whorls (trees in the field 7 years). Intact (nonstumped) controls were also selected from each age. *Experiment 2*, was established to describe quantitatively the effects of stumping treatments and crown (leaves and above ground branches of a tree) position on the rooting ability of Fraser fir stem cuttings. Crown positions were recorded as cuttings were collected and then cuttings were tested for rooting response.

Materials and Methods

Field Treatments and Cutting Collection. Five North Carolina Fraser fir Christmas tree growers, four from Avery County and one from Mitchell County, provided trees. Each of the five growers donated 55 trees, of which 15 were 3 years in the field, 15 were 5 years in the field, and 25 were 7 years in the field, for a total of 275 study trees. Before stumping treatments were imposed, total tree height was measured for each study tree, and soil and foliage samples were collected and analyzed for nutrient contents. Following analysis, it was determined that the trees' nutrient levels were at or above average (Campbell, 2000). Within the 3- and 5-year-old age classes at each site, 10 of the original 15 trees were stumped to the first above ground whorl and the remaining 5 trees were left as nonstumped controls. Within the 7-year-old age class at each site, 10 trees where stumped to the first whorl, 5 trees where stumped to the third whorl, 5 trees were stumped to the fifth whorl and 5 trees where left as nonstumped controls.

Stumping treatments were applied to the primary axes on 12-15 Mar., 2001. Consequently, the previous year's growth on each branch was removed from the 3-year-old trees, two years' growth on each branch was removed from the 5-year-old trees and four years' growth on each branch was removed from the 7-year-old trees. Prior to collection, the total number of newly flushed orthotropic shoots 6 cm or longer were counted for each tree. The total number of newly flushed orthotropic stump sprouts (shoots originating directly from the stump) were counted separately. Cuttings were collected on 29 July 2001 for *Experiment 1*, and 4-5 Aug, 2001 for *Experiment 2*. In *Experiment 1*, 50 cuttings were collected from every tree. In *Experiment 2*, the exact position of 24 cuttings per tree was determined by measuring the distance from the stem, height from the ground and the degrees from north. Following collection, the cuttings were wrapped immediately in moist paper towels, transported to Raleigh, N.C. on ice, and stored overnight at 4 ^{o}C. All cuttings were set the day following collection.

Auxin Preparation. In *Experiment 1*, a nonauxin control was compared to an application of 4 mM IBA. In *Experiment 2*, all cuttings were treated with 4 mM IBA. The auxin solutions were prepared individually by dissolving a known amount of IBA powder in 70% isopropyl alcohol and then diluting the solution with deionized water to make a 50% isopropyl alcohol/water solution. Auxin solutions were stored at 4 ^{o}C in an opaque bottle and used within 2 d of preparation.

Test Design and Greenhouse Management. Rooting was conducted in a propagation greenhouse located at the Horticulture Field Laboratory, Raleigh. Before being inserted into the rooting medium, the cuttings used in *Experiment 1* were selected from within the same grower, age, and stumping treatment. For both experiments, the cuttings were re-cut to a length of 6 cm and auxin was applied for 3 s to the basal 1.5 cm of the cuttings. Cuttings for the control treatment in *Experiment 1* were dipped into a 50% isopropyl alcohol/deionized water solution. Needles were not removed before or after the auxin had been administered. Based on preliminary research, no wounding treatment was applied to the basal portions of the cuttings (Rosier, 2003 Chapter 6). The auxin-treated cuttings were air-dried for a minimum of 15 min before being inserted to a 2 cm depth into a rooting medium of 3 horticultural perlite : 2 peat (by volume). Cuttings were rooted in individual 164 cm^3 Ray Leach SC-1- Super Cells (Stuewe and Sons, Inc., Corvallis, Ore.).

During the rooting period, cuttings were maintained under intermittent mist using a Grower Junior™ (McConkey Co., Sumner, Wash.) overhead boom irrigation system. Frequency of mist application was controlled using relative humidity (RH) and time of day. As RH in the rooting greenhouse decreased, the frequency of mist application increased; however, mist application was less frequent during the night, decreasing from an average of one mist cycle every 6 min during the day to an average of one mist cycle every 60 min at night. Flow rate per mist cycle for this study was held constant over the 4-month rooting period at 0.05 $L^{\bullet}m^{-2}$. Average day/night temperatures in the greenhouse were 26.1 ± 2/20.5 ± 2 ^{o}C.

Following 16 weeks in the greenhouse, rooting success was measured by calculating rooting percentage, percent mortality, number of primary roots, total root length, root symmetry and root angle. The latter four traits were assessed only on cuttings that rooted. A cutting was considered rooted if at least of one root ≥ 1 mm in length was present. A cutting with two roots was considered to have a symmetrical root system when the angle between the roots was > 135^{o}, using the stem center as the vertex (Frampton et al., 1999). Cuttings with three or more roots were scored as symmetrical if the angle between the two roots that were farthest apart was < 135^{o}. All cuttings with one root were scored as having asymmetrical root systems. Root angle was used to determine the percentage of positively geotropic roots for each cutting. Root angle was defined as the percentage of primary roots that formed an angle $< 60^{o}$ with an extension of the cutting's stem.

The experimental design for *Experiment 1* was a randomized complete block. There were six blocks with eight cuttings per treatment per block for a total of 3840 cuttings, with the entire study being surrounded by a border row. The experimental design for *Experiment 2* was a completely randomized design with the entire study surrounded by a border row.

Statistical Analysis for Experiment 1. Two separate analyses were conducted. The first compared the nonstumped controls with trees stumped to one whorl for all three ages. The second analysis compared all treatments in the 7-year-old stock plants. In the former case, analyses of variance (ANOVAs) were conducted using the general linear models procedure (GLM) of the Statistical Analysis System version 8.1 (SAS Institute, Inc. 1999) with grower, replication, tree age, stumping, auxin, and all the two-, three-, and four-way interactions not involving replication as sources of variation. In the latter case, grower, stumping, replication, auxin, grower x auxin, grower x stumping, stumping x auxin, and grower x stumping x auxin were sources of variation. Transformations of the dependent variables (rooting traits) were investigated to increase homogeneity among treatment variances. Several transformations were explored including the square root, the arcsine of the square root, and a range of transformations using the Box and Cox (1964) procedure that varied λ from -1.00 to +1.00 in increments of 0.25. The transformation, or no transformation, yielding the smallest differences among treatment variances was selected for each dependent variable. Tree means were examined and treatment least-squares means for all the rooting traits were separated using pair-wise t-tests with Tukey-Kramer experiment-wise error rate adjustments.

Statistical Analysis of Experiment 2. Cutting positions were converted to a 3-dimensional (x,y, z) co-ordinate system. The x-axis ran from ? /2 to 3? /2 radians (east to west), the y-axis ran from 0 to ? radians (north to south) and the z-axis was the cutting height from the tree base. The x, y and z values for each tree were standardized to a mean of zero and a standard deviation of one to compare the cutting position among trees within the same stumping treatments using the following equation:

$$\text{Variable} = (d_i - d)/(S_d)$$

where,

d_i = x, y, or z distance of the cutting

d = mean x, y, or z distance of all cuttings on tree

S_d = Standard deviation of x, y, or z of all cuttings on tree

Within each stumping treatment, discriminant analyses were employed to classify rooted and non-rooted cuttings. Ten dependent variables were used: x, y, z, x^2,y^2 ,z^2, xy, xz and yz. Step-wise techniques using forward selection and allowing variables to enter the model at $P \leq 0.15$ were conducted and a full model was also employed for comparison. In addition, a full model was run for each of the five individual trees within each stumping treatment.

Results

Experiment 1

ANOVAs and transformations of the dependent variables for all rooting traits are presented in Table 1 (all three age classes) and Table 2 (7-year-old trees only).

Rooting Percentage. In the analysis comparing the three age groups, the main effects of grower, replication, tree age, stumping and auxin were significant (Table 1). The two-way interactions between tree age and stumping, stumping and auxin as well as the three-way interaction between tree age, stumping and auxin were significant. Rooting percentages by grower ranged from 22% to 36% with a mean of 28% (data not presented). When examining the main effect of tree age, rooting percentage increased with decreases in age from 21% in the 7-year-old-tree to 25% and 35% in the 5-, and 3-year-old trees, respectively (Table 3). Auxin treated cuttings rooted more frequently (31%) than nonauxin treated cuttings (23%). Across all ages and auxin treatments, rooting percentages were significantly lower in the nonstumped trees (16%) than when stock plants were stumped to the first whorl (38%). The highest rooting percentage occurred when 3-year-old stock plants were stumped to the first whorl regardless of whether they had been treated with (51%) or without auxin (50%). When 5-year-old stock plants where stumped to the first whorl, the rooting percentages were greater in auxin treated cuttings (36%) than in the nonauxin treated controls (28%).

In the analysis of the 7-year-old stock plants only, the main effects of replication, stumping and auxin were significant (Table 2). Rooting percentage increased as the severity of the stumping increased from 10% in the nonstumped controls to 31% when stock plants were stumped to the first whorl (Table 4). The application of auxin significantly increased rooting percentages from 15% in the nonauxin controls to 23% when auxin was applied.

Percent Mortality. Across all three ages, the main effects of grower, replication, stumping, and the interaction between tree age and stumping were significant (Table 1). Percent mortality in the stock plants for the five growers ranged from 10% to 28% with a mean of 16% (data not presented). Across all ages and auxin treatments, percent mortality was lower (13%) in the stumped trees than in the nonstumped controls (17%) (Table 5). Percent mortality for cuttings collected from 3-, 5-, and 7-year-old stock plants stumped to the first whorl were 9%, 14%, and 16%, respectively. For cuttings collected from nonstumped controls, percent mortality was not significantly different among the ages sampled.

Within the 7-year-old stock plants only, the main effects of grower, replication and auxin were significant (Table 2). For the main effect of grower, percent mortality in the stock plants ranged from 10% to 28% with a mean of 15% (data not presented). The application of auxin decreased mortality from 18% in the nonauxin treated cuttings to 11% when auxin was applied.

Number of Primary Roots. In the analysis comparing the three age classes, the main effects of replication, tree age, stumping and auxin were significant. The interaction between tree age and stumping as well as the interaction between tree age, stumping and auxin were significant (Table 1). Primary root production was greater when stock plants were stumped and increased slightly as the age of the stock plant decreased (Table 6). The greatest number of roots was produced when 3-year-old stock plants were stumped to the first whorl and treated with 4 mM IBA (8.1). The fewest number of roots (2.8) occurred when cuttings collected from nonstumped 7-year-old stock plants and were not treated with auxin. For the main effects of stumping, primary root production decreased from 7.1 when stock plants where stumped to the first whorl to 4.2 in the nonstumped controls. Across all stumping and auxin treatments, primary root production was 6.1, 5.5 and 5.4 for cuttings collected from 3-, 5-, and 7-year-old stock plants, respectively. The application of auxin significantly increased root production from 5.1 when no auxin was applied to 6.2 when cuttings were treated with auxin.

When analyzing the 7-year-old stock plants only, the main effects of replication, stumping and auxin were significant (Table 2). Primary root production increased as the severity of the stumping treatment increased from 3.6 in the nonstumped controls to 7.1 when stock plants were stumped to the first whorl (Table 7). Primary root production for stock plants stumped to the third and fifth whorls were 4.8 and 5.4, respectively. The number of primary roots significantly increased from 4.6 in the nonauxin controls to 5.8 when auxin was applied.

Total Root Length. For 3-, 5-, and 7-year-old stock plants, the main effects of tree age, stumping and auxin were significant (Table 1). For the main effect of age, total root lengths were 335, 287 and 312 mm for 3-, 5- and 7-year-old stock plants. Total root lengths increased from 188 mm in the nonstumped controls to 434 mm when stock plants were stumped to the first whorl. The application of auxin significantly increased total root lengths from 247 mm in the nonauxin treated cuttings to 375 mm when auxin was applied.

In the analysis of the 7-year-old stock plants only, the main effects of replication, stumping and auxin were significant (Table 2). Total root lengths were greater in auxin treated cuttings and increased as the severity of the stumping treatment increased (Table 9). Across all ages and auxin treatments, total root lengths increased from 156 mm in the nonstumped controls to 259, 306, and 467 mm when stumped to the fifth, third and first whorls, respectively. Following the application of auxin, root lengths significantly increased from 221 mm in the nonauxin controls to 373 mm in auxin treated cuttings.

Root Symmetry. In the analysis for all three age classes, the main effects of stumping and auxin as well as the interaction between stumping and auxin were significant (Table 1). Root symmetry increased from 43% in the nonstumped controls to 70% when stock plants were stumped to the first whorl. When no auxin was applied, root symmetry was 58 and 17% in stock plants stumped to one whorl and the nonstumped controls, respectively. With the application of auxin, root symmetry increased from 55% in the nonstumped controls to 80% when trees were stumped to the first whorl. For the overall main effect of auxin, root symmetry was greater (71%) following the application of auxin when compared to the nonauxin controls (45%).

When comparing stumping treatments within the 7-year-old stock plants, the main effects of stumping and auxin were significant (Table 2). Root symmetry was greater when cuttings were treated with auxin and increased as the severity of the stumping treatment increased. Root symmetry increased from 0% in the nonstumped controls to 47, 56 and 71 % when stock plants were stumped to the fifth, third and first whorls, respectively. Auxin significantly increased root symmetry from 36% in the nonauxin controls to 70% with application.

Root Angle. When comparing stumping treatments over all three ages, the main effects of replication and auxin were significant (Table 1). Cuttings treated with auxin produced a greater percentage of geotropic roots (83%) than the nonauxin treated cuttings (61%). No significant effects occurred when comparing treatments within the 7-year-old stock plants (Table 2).

Shoot production. The number of shoots produced, greater than or equal to 6 cm, was larger in the nonstumped trees and decreased as the severity of the stumping treatment increased. On the other hand, the number of cuttings initiated directly from the main stump, stump sprouts, increased as the severity of the stumping treatment increased. For example, in the 7-year-old stock plants, shoot production was 1194 in the nonstumped controls and decreased to 167, 126 and 83 in stock plants stumped to the fifth, third and first whorls, respectively (Table 10). However, the number of stump sprouts increased from 0 in the nonstumped controls to 3, 6 and 10 when stock plants were stumped to the fifth, third and first whorls, respectively (Table 10). For rooting percent, no significant difference was detected between orthotropic stump sprouts and nonstump sprouts.

Experiment 2

Effect of Stumping and Crown Position. Results from the step-wise discriminant analysis to classify rooted and non-rooted cuttings based on their crown position and stumping treatments along with rooting percentages by age and stumping height are presented in Table 11.

The effect of height (z) consistently explained more of the variation in every age class and stumping height, with the exception of the 7-year-old nonstumped controls; however, in this treatment, height did explain a significant portion of the variation (Table 11). In the 3-year-old stock plants, the partial squared canonical correlations were 0.16

and 0.12 for height in the nonstumped controls and stock plants stumped to the first whorl, respectively. The partial squared canonical correlations for 5-year-old stock plants were 0.23 and 0.14 for height in the nonstumped controls and stock plants stumped to the first whorl, respectively. For 7-year-old stock plants that were not stumped, x^2 explained the largest amount of the variation (0.12). In the remaining 7-year-old stock plants, the partial squared canonical correlations were 0.20, 0.16 and 0.34 for height in stock plants stumped to the first whorl, third and fifth whorls, respectively.

Three-dimensional (x,y,z) representations of the effects of crown position on the rooting percentage of cuttings collected from stumped and nonstumped control trees of various ages are presented in Fig. 1. In general, rooting percentages increased as the age of the stock plant decreased and the severity of the stumping treatment increased. In the nonstumped controls of the 3- and 7-year old trees, rooting percentage increases as the distance from the base of the stem decreases (Fig. 1). This phenomena is less obvious in the stock plants stumped to the first whorl, most likely due to the limited distance from the base of the stock plant to where the stumping treatment was applied.

Discussion

Stumping Height and Position Effects. In the current study, as in a previous investigation involving rooting of Fraser fir stem cuttings (Wise et al. 1985c), large influences were observed regarding stumping treatments applied to the stock plants. Previous research demonstrated that decapitating 14-year-old Fraser fir stock plants promoted orthotropic shoot production and increased adventitious rooting; however, the numbers of cuttings produced were low (Wise et al., 1985c). In that study, trees were decapitated and, in a few instances, lateral branch growth was removed. As the severity of the decapitation and hedging treatment increased, rooting percentages and orthotropic shoot production increased. Based on these findings, more severe stumping treatments were employed in the current study in hopes of increasing juvenile orthotropic shoot production.

In the current study, as the severity of the stumping treatment increased, rooting percentage, primary root production, total root lengths and root symmetry generally increased. It was observed that shoot elongation, a characteristic associated with vigor (Haines et al., 1992) also increased as the severity of the stumping treatments increased. Reduced bud formation may also have been a factor contributing to the increased performance in many rooting traits in cuttings collected from the stumped trees. Cuttings collected from trees that had been stumped exhibited minimal signs of differentiated axillary bud formation and, in most cases, the cuttings appeared to still be actively growing. Even in the 7-year-old trees, bud development was found to increase as the severity of the stumping treatment decreased (data not presented). Previous research suggests that the optimal time to collect cuttings is in the summer before the axillary buds have set, while the cuttings are still actively growing (Rosier, 2003 Chapter 2). On the other hand, cuttings collected from nonstumped control trees already had pronounced axillary bud formation, the stem tissue had hardened and the trees appeared to be preparing for dormancy.

Branch order and crown position have been shown to greatly affect the plagiotrophic tendencies and rooting ability of Fraser fir cuttings. Miller (1982a) found that cuttings from first- and second-order lateral branches root more frequently and produce a greater number of roots than cuttings collected from the primary axis, but plagiotropic growth was more pronounced. Blazich and Hinesley (1995) suggest that this effect is due to the retention of juvenile characteristics in lower order branches. Cuttings collected from the primary axis, as terminal shoots, grow orthotropically and produce symmetrical trees, but rooting percentages are very low (Blazich and Hinesley, 1994; Miller et al., 1982a).

In the current study, the position from which the cutting was collected significantly affected rooting percentage. In general, the variation explained by position ranged from squared canonical correlations of 0.8 to 0.9 for individual trees with a maximum of 0.97. However, the large amount of tree-to-tree variation averaged out to obscure this relationship in the overall analyses. For rooting percentage, the height (z) from which the cutting was collected was almost always the most important variable, regardless of age or stumping height. These findings are not surprising and support previous research which has shown that maturation increases as the distance from the base of a tree increases (Zobel and Talbert, 1984; Hartmann et al., 2002).

A comparison of rooting percentage was also made between cuttings that initiated directly from the stem (stump sprouts) and cuttings that initiated from every where but the main stem. No significant differences were detected for any of the rooting traits. One likely explanation for the lack of significance may be related to the small number of stump sprouts generated.

Age Effects. In general, rooting percentage, primary root production, and total root length significantly increased and mortality significantly decreased as the age of the stock plant decreased. The current findings support previous research which has shown that the rooting ability of Fraser fir decreases with stock plant age, or maturation, regardless of where on the stock plant the cuttings are collected (Hinesley and Blazich, 1980). Plagiotropism, another characteristic associated with maturation, also becomes more pronounced as the age of the stock plant increases. Following rooting, many of the cuttings in this study were potted and observations were made the following growing season. Plagiotropism appeared to be more pronounced in the older material and increased as the severity of the stumping treatments decreased.

Auxin Effects. Treatment with an auxin is greatly beneficial when rooting cuttings of many species, including Fraser fir (Blazich and Hinesley, 1994; 1995; Hinesley and Blazich, 1980; 1981; 1984). In the current study, the application of auxin significantly increased rooting percentage, primary root production, total root length, and root symmetry. However, to control the size of the study, only one auxin type and concentration was compared to a nonauxin control. The type and concentration of auxin selected were based on previous research (Rosier, 2003 Chapter 2).

Grower Effects. In general, the grower effect was minimal and only significantly affected rooting percentage and percent mortality. The most likely explanation for the differences noted in these two traits may be damage of the stock plants caused by insects. At one of the 7-year-old sites, a majority of the newly flushed succulent cuttings were severely damaged by red spider mites (*Oligonychus ununguis*) and Balsam twig aphids (*Mindarus abietinus*). Subsequently, mortality was greater and the rooting percentages were lower in these cuttings. This was the only site that was detrimentally affected by insects; however, minimal damage from these insects were noted on one other site.

In conclusion, it was determined that the highest rooting percentages (51%) and the greatest number of primary roots (8.1) occurred when 3-year-old stock plants were stumped to the first whorl and the induced cuttings were treated with auxin. The longest root lengths (335 mm) occurred in 3-year-old stock plants. Overall, rooting percentages, primary root production, and total root lengths significantly increased as the age of the stock plant decreased and the severity of the stumping treatments increased. Application of 4 mM IBA, when compared to a nonauxin control, significantly increased every rooting trait assessed. Rooting percentages were also significantly affected by crown position and decreased as the height from the base of the tree increased. Results from the current study demonstrate that stumping mature Fraser fir Christmas trees for orthotropic shoot production can be effective and should provide a basis for the advancement of cloning desirable Fraser fir Christmas trees. Future research involving hedging and subsequent rooting from mature Fraser fir stock plants should focus on increasing the severity of the stumping treatments. Currently, no stock plant morality was observed suggesting that more severe stumping treatments could be tolerated.

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Table 1. ANOVA for traits assessed in a rooting trial of Fraser fir stem cuttings. This analysis included 3-, 5- and 7-year old stock plants. Treatments included trees stumped to the first whorl and intact controls.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine-Square Root	Arcsine-Square Root	None	None	Log	None
Grower	*	*	NS	NS	NS	NS
Replication	*	*	*	NS	NS	*
Tree age	*	NS	*	*	NS	NS
Stumping	*	*	*	*	*	NS
Auxin	*	NS	*	*	*	*
Grower x Tree age	NS	NS	NS	NS	NS	NS
Grower x Stumping	NS	NS	NS	NS	NS	NS
Grower x Auxin	NS	NS	NS	NS	NS	NS
Tree age x Stumping	*	*	*	NS	NS	NS
Tree age x Auxin	NS	NS	NS	NS	NS	NS
Stumping x Auxin	*	NS	NS	NS	*	NS
Grower x Tree age x Stumping	NS	NS	NS	NS	NS	NS

Table 1. (Continued)

Grower x Tree age x Auxin	NS	NS	NS	NS	NS	NS
Grower x Stumping x Auxin	NS	NS	NS	NS	NS	NS
Tree age x Stumping x Auxin	*	NS	*	NS	NS	NS
Grower x Tree age x Stumping x Auxin	NS	NS	NS	NS	NS	NS

NS, * = Nonsignificant or significant at $P < 0.05$, respectively.

Table 2. ANOVA results for traits assessed in a rooting trial of Fraser fir stem cuttings. This analysis was included 7-year old stock plants stumped to the first, third and fifth whorls and intact controls.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine-Square Root	Arcsine-Square Root	None	SQRT	Log	None
Grower	NS [z]	*	NS	NS	NS	NS
Replication	*	*	*	*	NS	NS
Stumping	*	NS	*	*	*	NS
Auxin	*	*	*	*	*	NS
Grower x Stumping	NS	NS	NS	NS	NS	NS
Grower x Auxin	NS	NS	NS	NS	NS	NS
Stumping x Auxin	NS	NS	NS	NS	NS	NS
Grower x Stumping x Auxin	NS	NS	NS	NS	NS	NS

NS, * = Non-significant or significant at $P < 0.05$, respectively.

Table 3. Effect of stumping and IBA concentration on the rooting percentage of Fraser fir stem cuttings collected from stock plants of different ages.

Age in field (yr)	Stumping treatment				
	1 Whorl		Nonstumped controls		
	Control	Auxin	Control	Auxin	**Overall average**
3	50 aA[z]	51 aA	15 aB	24 aB	**35**
5	28 bB	36 bA	15 aC	22 aB	**25**
7	25 bB	37 bA	7 bD	13 bC	**21**
Overall average	**34**	**41**	**12**	**20**	***27***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 4. Effect of IBA concentration on the rooting percentage of Fraser fir stem cuttings collected from 7-year-old stock plants stumped to different heights.

Stumping treatment (remaining whorls)	Auxin treatment		
	Control	Auxin	**Overall average**
1	25 aB [z]	37 aA	**31** a
3	14 bB	22 bA	**18** b
5	12 bB	20 bA	**16** b
Nonstumped control	7 cB	13 cA	**10** c
Overall average	**15** B	**23** A	***19***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 5. Effect of stumping and IBA concentration on the percent mortality of Fraser fir stem cuttings collected from stock plants of different ages.

Age in field (yr)	Stumping treatment		
	1 Whorl	Nonstumped controls	**Overall average**
3	9 bB [z]	18 aA	**14**
5	14 aA	15 aA	**15**
7	16 aA	18 aA	**17**
Overall average	**13**	**17**	***15***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 6. Effect of stumping and IBA concentration on the number of primary roots of Fraser fir stem cuttings collected from stock plants of different ages.

Age in field (yr)	Stumping treatment				
	1 Whorl		Nonstumped controls		
	Control	Auxin	Control	Auxin	**Overall average**
3	7.3 aB[Z]	8.1 aA	3.9 aD	5.1 aC	**6.1**
5	5.9 cB	7.2 bA	3.8 aD	5.1 aC	**5.5**
7	6.9 bA	7.2 bA	2.8 bC	4.3 bB	**5.4**
Overall average	**6.7**	**7.5**	**3.5**	**4.8**	***5.7***

[Z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 7. Effect of stumping and IBA concentration on primary root production of Fraser fir stem cuttings collected from 7-year-old stock plants.

Stumping treatment (remaining whorls)	Auxin treatment		
	Control	Auxin	**Overall average**
1	6.9 aA[z]	7.2 aA	**7.1** a
3	4.6 bB	6.2 bA	**5.4** b
5	4.1 bB	5.5 bA	**4.8** b
Nonstumped control	2.8 cB	4.3 cB	**3.6** c
Overall average	**4.6** B	**5.8** A	***5.2***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 8. Effect of stumping and IBA concentration on the total root lengths of Fraser fir stem cuttings collected from stock plants of different ages.

Age in field (yr)	Stumping treatment				
	1 Whorl		Nonstumped controls		
	Control	Auxin	Control	Auxin	**Overall average**
3	422 aB[z]	518 abA	151 aD	246 abC	**335** a
5	276 bB	453 bA	147 aC	273 aB	**287** b
7	388 abB	546 aA	96 aD	216 bC	**312** ab
Overall average	**362** B	**506** A	**131** D	**245** C	***311***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 9. Effect of stumping and IBA concentration on total root length of Fraser fir stem cuttings collected from 7-year-old stock plants.

Stumping treatment	Auxin treatment		
	Control	Auxin	**Overall average**
1	388 aB[z]	546 aA	**467** a
3	213b B	398 bA	**306** b
5	188 bB	330 bA	**259** b
Nonstumped control	96 cB	216 cA	**156** c
Overall average	**221** B	**373** A	***297***

[z] Values followed by different uppercase letters (within rows) or lowercase letters (within columns) are significantly different at the 5% level.

Table 10. Effect of stumping on shoot production and estimated rooted cutting yeilds. The number of sprouts initiating directly from the stump are also provided.

	Stumping treatment							
	1 Whorl		3 Whorls		5 Whorls		Nonstumped controls	
Years in field (yr)	Total number of shoots (Stump sprouts)	Estimated number of rooted cuttings	Total number of shoots (Stump sprouts)	Estimated number of rooted cuttings	Total number of shoots (Stump sprouts)	Estimated number of rooted cuttings	Total number of shoots (Stump sprouts)	Estimated number of rooted cuttings
3	32 (3)	17	--	--	--	--	279 (0)	67
5	65 (5)	16	--	--	--	--	892 (0)	196
7	83 (10)	31	126 (6)	28	167 (3)	33	1194 (0)	155

Table 11. Discriminant analysis results of cutting position on rooting of stem cuttings of Fraser fir. x, y, and z = standardized distances from the base of the donor tree (n = 5 trees for each analysis).

Tree age	Stumping treatments	Mean rooting Percentage	Squared canonical correlation		
			Variables	Partial	Total
3-year-old	1 Whorl	49	Z	0.12	0.15
			XY	0.03	
	Nonstumped	20	Z	0.16	0.27
			Z^2	0.05	
			XZ	0.03	
			YZ	0.03	
5-year-old	1 Whorl	39	Z	0.14	0.18
			X	0.04	
	Nonstumped	18	Z^2	0.23	0.39
			Z	0.09	
			Y	0.03	
			X	0.03	
7-year-old	1 Whorl	28	Z	0.20	0.27
			Z^2	0.05	
			Y	0.02	
	3 Whorls	20	Z	0.16	0.31
			X	0.06	
			X^2	0.03	
			XZ	0.02	
			XY	0.02	
			YZ	0.02	
	5 Whorls	17	Z	0.34	0.61
			Z^2	0.16	
			XZ	0.07	
			Y	0.04	
	Nonstumped	10	X^2	0.12	0.27
			Z^2	0.07	
			Z	0.04	
			XZ	0.05	

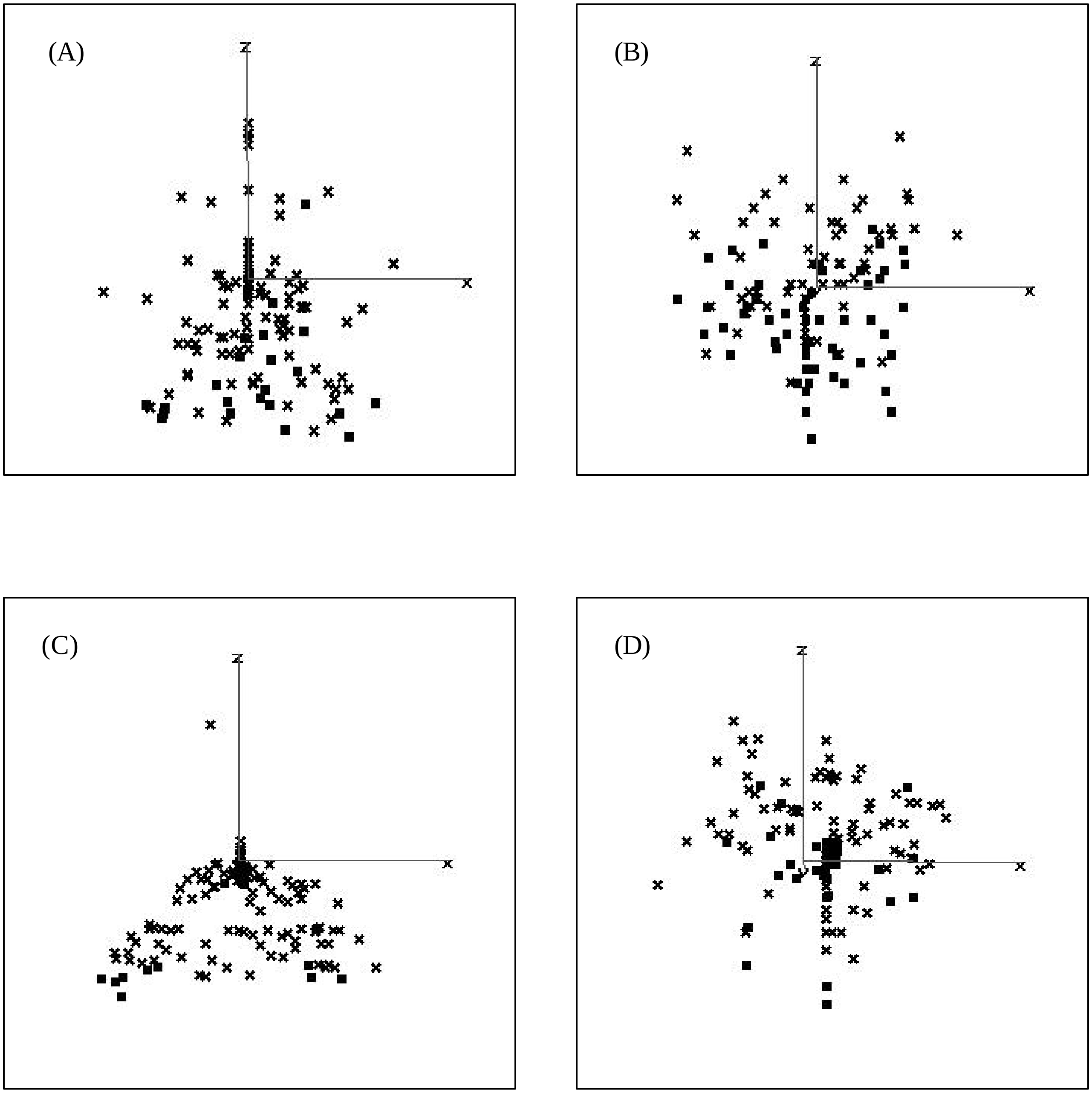

Fig. 1. Effects of position on the rooting of cuttings collected from (A) nonstumped 3-year-old stock, (B) 3-year-old stock plant stumped to the first whorl, (C) Nonstumped 7-year-old stock plant, and (D) 7-year-old stock plant stumped to the first whorl where ¦ = rooted and **x** = non-rooted. Positions were standardized among trees within each stumping treatment (n = 5).

Chapter 5

Stumping Height, Crown Position, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Virginia Pine

(In the format appropriate for submission to the
Southern Journal of Applied Forestry)

Stumping Height, Crown Position, Auxin Type, and Concentration Influence Rooting of Stem Cuttings of Virginia Pine

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***Abstract*.** Two experiments were conducted to develop a protocol for rooting stem cuttings from 4-year-old Virginia pine (*Pinus virginiana* Mill.) Christmas trees. The first experiment tested the effects of stumping treatments, auxin types, and concentrations on shoot production and adventitious root initiation. The efficiency of five indole-3-butyric acid (IBA) concentrations, five 1-naphthaleneacetic acid (NAA) concentrations, and a nonauxin control were tested on cuttings from three stumping treatments and a nonstumped control. A second experiment created a quantitative description of the effects of crown positions on the rooting of stem cuttings collected from stumped and nonstumped trees. The exact position was determined by measuring the distance from the stem, height from the ground and the degrees from north. Crown position was recorded as cuttings were collected and then cuttings were tested for rooting response. The rooting traits assessed included rooting percentage, percent mortality, number of primary roots, total root length, root symmetry, root angle and primary needle length. Stumping height significantly affected rooting percentage and percent mortality. Rooting percentage increased and percent mortality decreased as the severity of the stumping treatment increased. Auxin type significantly affected every rooting trait except root symmetry and root angle. Overall, NAA treated cuttings from trees stumped to ¼ original height and treated with 4 mM rooted in higher frequencies (74%) and produced a greater number of primary roots (5.5) and longer total root lengths (601 mm) than the other treatments. Rooting percentage was significantly affected by the crown position from which the cutting was collected. Primary needle length significantly increased as the severity of the stumping treatment increased and was positively correlated with rooting ability. Based on these findings, it is recommended that 4-year-old Virginia pine trees be stumped to ¼ original height and the resultant cuttings treated with 4 mM NAA.

Additional index words: vegetative propagation, maturation, crown position, indole-3-butyric acid, 1-naphthaleneacetic acid, cloning, *Pinus virginiana*

In the Piedmont and Coastal Plains of the southeast United States, Virginia pine (*Pinus virginiana* Mill.) is one of the most commonly grown Christmas tree species. Virginia pine is a desirable Christmas tree species for several reasons, including rapid growth (3 to 6 years to harvest), short needles, good branch structure for holding ornaments, and pleasant pine scent (Frampton, 2002). However, Virginia pine has several significant problems including poor form, large phenotypic tree-to-tree variation, and extreme susceptibility to the Nantucket pine tip moth (*Rhyacionia frustrana* Comstock). Due to these and other problems, Christmas tree growers often are only able to market about 50% of the Virginia pines planted (Frampton, 2002).

Recently there has been increasing interest in propagating Virginia pine asexually, by the use of rooted cuttings, to exploit clones with desirable Christmas tree characteristics (Frampton, 2002). Asexual propagation would provide a way to capture benefits from cloning, including increased genetic gain and uniformity (Zobel and Talbert, 1984). Other potential benefits include the ability to combine desirable characteristics such as rapid growth, uniform and full crown density, and resistance to the Nantucket pine tip moth. Previous research involving Virginia pine has shown that its retail value as a Christmas tree (Knoth et al., 2002), height, stem straightness, density of foliage, symmetry and branches per whorl are under strong genetic control (Belanger and Bramlett, 1975; Meier and Goggans, 1977). Due to the high market value of Christmas trees relative to forest trees and the large phenotypic variation that currently exists, genetic improvement of Virginia pine for desirable Christmas tree characteristics can be justified.

Little research involving propagation of Virginia pine by rooted stem cuttings has been conducted. Existing results include the effects of auxin (Holifield et al., 1991; Rosier, 2003 Chapter 3; Snow and May, 1962), time of collection (Brown et al., 1991; Holifield et al., 1991; Rosier, 2003 Chapter 3; Snow and May, 1962), crown position (Snow and May, 1962), genotype (Brown et al., 1991), and photoperiod (Snow and May, 1962).

Time of collection and application of auxin have been shown to have significant effects when rooting stem cuttings from sheared Virginia pine trees. Rosier (2003 Chapter 6) found that season and the concentration of auxin applied within the season significantly affected numerous rooting traits in 4- and 5-year-old Virginia pines including rooting percentage, mortality, primary root production and total root length. The highest rooting percentages and lowest rates of mortality occurred when cuttings were set in September or June and treated with a three-second dip of 6 or 7 mM auxin, respectively. In that study, auxin type significantly affected only one rooting trait, total root length. These findings were surprising because previous research in loblolly pine (*Pinus taeda* L.) (Diaz-Sala et al., 1996) and eastern white pine (*Pinus strobus* L.) (Goldfarb et al., 1998) suggest differences among auxin types, specifically 1-naphthaleneacetic acid (NAA), compared to indole-3-butyric acid (IBA), increased primary root production and, in most cases, rooting percentage.

One of the major limitations in the effective use of vegetative propagation is the developmental process known as maturation (Zobel and Talbert, 1984). Maturation is defined as the physiological and morphological changes that occur with increases in tree age (Greenwood and Hutchison, 1993). In many conifer species, maturation increases the time for root initiation to occur, decreases rooting ability, and decreases growth rate of rooted cuttings following rooting (Zobel and Talbert, 1984). Younger trees will often root readily, but as the tree matures, rooting becomes increasingly difficult (Hartmann et al., 2002; Zobel and Talbert, 1984). This is a common problem in progeny testing where trees are allowed to grow over an extended period of time, but by the time their genetic potential is determined, the material is too mature to root.

The position within the crown from which a cuttings is collected has been shown to significantly affect rooting in many tree species (Hartmann et al., 2002). Cuttings collected from the lower crown tend to root in higher percentages because they are physiologically more juvenile than cuttings collected from branches higher in the crown (Zobel and Talbert, 1984). These differences become much more pronounced as the stock plants mature. For Virginia pine, Snow and May (1962) found that, for cuttings collected in March, 18% from the lower crown rooted, whereas only 1% from the upper crown rooted.

Hedging is a treatment to maintain a plant at a fixed height. The hedging process is an important stock plant management technique that maintains juvenility, allows for increased shoot production, allows for easier cutting collection and reduces sexual reproduction (Hartmann et al., 2002). Previous research with loblolly pine (*Pinus taeda* L.) (e.g., Cooney, 1999), radiata pine (*Pinus radiata.* Don) (e.g. Bolstad and Libby, 1982; Fielding, 1954; Libby et al., 1972; Menzies, 1986), Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Black, 1972) and Norway spruce (*Picea abies* L.) (Bentzer, 1993) has demonstrated that continuous hedging of a stock plant provides a way to increase cutting production and maintain juvenility.

At the time of selection, typically 4 to 5 years old, Virginia pine Christmas trees do not produce juvenile shoots with a high rooting capacity. Although previous research has shown that the continuous hedging of juvenile stock plants maintains the production of juvenile shoots, it is not known whether stumping a mature tree will induce juvenile shoots that root better than cuttings collected from intact trees. Further research is needed to develop a protocol that will increase cutting yield, improve rooting percentage, and determine optimal hedging treatments for mature (4 to 6 years old) stock plants of Virginia pine.

Therefore, two experiments were conducted to examine the possibility of increasing production and rooting of vertically oriented (nonplagiotropic) shoots from mature trees of the same age stumped to various heights. *Experiment 1* tested the effect of stumping treatments, auxin types (IBA or NAA) and auxin concentrations on shoot production and subsequent rooting ability. Five auxin concentrations (2-12 mM) of each auxin type and a nonauxin control were tested on cuttings generated from nonstumped trees and from trees stumped to ¼, ½, ¾ original height. *Experiment 2* tested the effects of stumping treatments and crown position on the rooting ability of Virginia pine stem cuttings.

Materials and Methods

Field Design and Cutting Collection. Two experiments were designed in which Virginia pine stock plants were stumped to ¼ original height, ½ original height and ¾ original height. Intact (nonstumped) control trees were also identified for comparisons. The trees were part of a 4-year-old progeny test located at the North Carolina State University Horticultural Crops Research Station near Clinton, N.C. The stock plants had been grown from seed and cultured as Christmas trees (Johnson, 1991). Before stumping treatments were applied, total height was measured for each study tree, and soil and foliage samples were collected for mineral nutrient analyses. Analyses revealed deficiencies in nitrogen and potassium (Campbell, 2000). On 5 May, 2001, a soluble solution of 20-20-20 fertilizer was applied to the base of each tree at a rate of 36.7 $kg \bullet ha^{-1}$.

Stumping treatments were applied to the primary axes on 16-17 Mar., 2001. Once the primary axes had been stumped, the previous year's growth and all remaining terminal buds were removed from the remaining branches. Prior to collection, the total number of newly flushed shoots, 9 cm or longer, per tree was counted. For *Experiment 1*, 144 trees were selected from the borders of the progeny test, and assigned to 8 replications with each replication containing 4 controls, 6 trees stumped to ¼ height, 4 trees stumped to ½ height and 4 trees stumped to ¾ height. For *Experiment 2,* four additional trees from seven of the original eight blocks in *Experiment 1* (one of each stumping type and the nonstumped control) were identified for a total of twenty-eight study trees.

Cuttings were collected on 4 June (*Experiment 1*) and 6-7 June (*Experiment 2*), 2001. In *Experiment 1*, 45 cuttings were collected from all trees, except the trees stumped to ¼ height, from which 25 cuttings were collected. In *Experiment 2,* the exact positions of 50 cuttings per tree were determined by measuring the distance from the stem, height from the ground and degrees from north. In *Experiment 2,* along with measuring the exact position of each of the cuttings, the length of the most proximal primary needle of each cutting was measured to the nearest 1 mm. Following collection, the cuttings were immediately wrapped in moist paper towels, transported to Raleigh, N.C., on ice, and stored overnight at 4 ^{o}C. All cuttings were set the day following collection.

Auxin Preparation. IBA and NAA were tested for their ability to increase adventitious rooting and enhance root development in *Experiment 1*. No auxin (a control), five IBA and NAA concentrations (2, 4, 6, 8, and 12 mM) were examined. In *Experiment 2,* all cuttings were treated with 4 mM IBA. The auxin solutions were individually made by dissolving a known amount of auxin powder into 70% isopropyl alcohol then diluting the solution with deionized water to make a 50% isopropyl alcohol/water solution. Solutions were stored at 4 ^{o}C in an opaque bottle and used within 2 d of preparation.

Test Design and Greenhouse Management. The experiments were conducted in a propagation greenhouse located at the Horticulture Field Laboratory, Raleigh, N.C. Before preparation and setting, the cuttings from different trees within the same stumping treatment used in *Experiment 1* were mixed. In both experiments, the cuttings were re-cut to a length of 9 cm and auxin was applied for 3 seconds to the basal 1.5 cm of each cutting. Cuttings for the control treatment in *Experiment 1* were dipped into a 50% isopropyl alcohol/deionized water solution. Needles were not removed before or after the auxin was administered. All cuttings were air-dried for a minimum of 15 minutes before setting into the rooting medium to a depth of 3 cm. The medium was a mixture of 3 horticultural perlite: 2 peat moss (by volume).

During the rooting period, the cuttings were subjected to intermittent mist using a Grower Junior™ (McConkey Co., WA) irrigation boom. Frequency of mist application was controlled using relative humidity (RH) and time of day. As RH in the greenhouse decreased, the frequency of mist application increased; however, frequency of mist application was less during the night, decreasing from an average of one mist cycle every 6 min during the day to an average of one mist cycle every 60 min at night. Flow rate was held constant during the first 3 weeks at $0.06 \cdot m^{-2} \cdot$ per mist cycle, and at $0.05\ l \cdot m^{-2}$ per mist cycle for the 9 remaining weeks. The average day/night temperatures in the greenhouse were 26.1 ± 2/20.5 ± 2 ^{o}C, respectively. All cuttings were rooted under ambient light conditions; i.e., natural seasonal photoperiod and irradiance levels.

The experimental design for rooting *Experiment 1* was a randomized complete block. There were seven blocks (blocks were not maintained from the field), with eight cuttings per treatment per block for a total of 2464 cuttings. The entire study was surrounded by a border row. The experimental design for *Experiment 2* was a completely randomized design with a total of 1400 cuttings. In both studies, rooting data were collected following 16 weeks in the greenhouse.

Rooting success was measured by calculating rooting percentage, percent mortality, number of primary roots, total root length, root symmetry and root angle. The latter four traits were assessed only for cuttings that rooted. All analyses for *Experiment 1* were conducted using plot means (n=8). A cutting was considered rooted if a minimum of one root measuring ≥ 1 mm in length was present. Root systems were considered symmetrical when the angle between two roots was more than 135 degrees, using the stem center as the vertex (Frampton et al., 1999). Systems with three or more roots were symmetrical if the angle between the two roots that were farthest apart was < 135 degrees. Cuttings with one root were scored as having asymmetrical root systems. Root angle was a rooting characteristic developed to determine the percentage of geotropic roots of a cutting. Root angle was the percentage of primary roots that formed an angle of < 60 degrees with an extension of the cutting's stem.

Statistical Analysis for Experiment 1. Analyses of variance were conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Stumping, replication, auxin, concentration, concentration squared, and all of the two- and three-way interactions not involving replication were potential sources of variation in *Experiment 1*. Transformations of the dependent variables (rooting traits) were investigated to increase homogeneity among treatment variances. Several transformations were explored: the square root, the arcsine-square root and a range of transformations using the Box Cox (1964) procedure where λ was varied from -1.00 to 1.00in increments of 0.25. The transformation, or no transformation, yielding the smallest differences among treatment variances was selected for each dependent variable. Once the most appropriate dependent variable transformation had been selected, log and square root transformations of the independent variables, concentration and concentration squared, were compared to no transformation. The model yielding the highest r-square value was selected for each dependent variable.

Despite employing the most appropriate data transformations and including squared terms for concentration, the least-squares regression approach failed to adequately define the relationship between concentration and the variables assessed. Therefore, nonlinear regression techniques were employed by regressing each dependent variable on treatment means, concentration or auxin type by concentration, using the NLIN procedure of the Statistical Analysis System Version 8.1 (SAS Institute, Inc., 1999). The double exponential model (Rawlings et al., 1998), modified by two constants (A and B), was employed:

$$\text{Trait} = \theta_1(\text{B}e^{(-\theta 2*\text{Concentration})}-e^{(-\theta 1*\text{Concentration})})/[\text{A}(\theta_1-\theta_2)]$$

where,

e = base of natural logarithm,

θ_1,θ_2, A and B = regression parameters, and

Concentration = auxin concentration in mM.

Statistical Analysis for Experiment 2. Cutting positions were converted to a 3-dimensional (x,y,z) coordinate system. The x-axis ran from ?/2 to 3?/2 radians (east to west), the y-axis from 0 to ? radians (north to south) and the z-axis was the cutting height from the tree base. The x, y and z values for each tree were standardized to a mean of zero and a standard deviation of one in order to compare the cutting position among trees within the same stumping treatments using the following equation:

$$\text{Variable} = (d_i - d)/(S_d)$$

where,

d_i = x, y, or z distance of the cutting,

d = mean x, y, or z distance of all cuttings on tree, and

S_d = standard deviation of x, y, or z of all cuttings on tree

Within each stumping treatment, discriminant analyses were employed to classify rooted and non-rooted cuttings. Ten dependent variables were used: primary needle length, x, y, z, x^2,y^2 ,z^2, xy, xz, and yz. Step-wise techniques using forward selection and allowing variables to enter the model at $P \leq 0.15$ were conducted. A full model was also employed for comparison. In addition, a full model was run for each of the 8 individual trees within each stumping treatment. Using both a linear and quadratic term, mean tree primary needle lengths were regressed on each rooting response variable. Treatment least-squares means for primary needle lengths, rooting percentage, and total root lengths were separated using pair-wise t-tests with Tukey-Kramer experiment-wise error rate adjustments.

Results

Experiment 1

ANOVAs and transformations of the independent and dependent variables for all rooting traits are presented in Table 1. Since no significant differences were identified for root angle, these data are not presented.

Rooting Percentage. The main effects of stumping, replication, concentration and concentration squared were significant (Table 1). Significant interactions were detected between auxin and concentration, between auxin and concentration squared, as well as between stumping and concentration squared (Table 1). As the severity of the stumping treatment increased, rooting percentages increased from 27%, 50%, 63%, and 64% for nonstumped controls and trees stumped to ¼, ½, ¾ original height, respectively. With the exception of the control trees, when auxin (IBA or NAA) was applied to the bases of the cuttings, rooting percentage reached a peak and then either leveled off or decreased slightly (Fig. 1). When IBA was applied to the bases of the cuttings, the highest predicted rooting percentage (73%) occurred where trees had been stumped to ½ original height and treated with 2 mM. Using the predicted optima from the nonlinear regression analysis, the greatest rooting percentage for trees stumped to ¼ (60%) and ¾

(59%) original height, occurred when 3.5 mM IBA and 5 mM IBA, respectively, were applied. In the nonstumped control trees, predicted rooting percentage steadily increased from 14% in the nonauxin treated cuttings to 36% at 12 mM IBA. When NAA was applied to the base of the cuttings, the highest predicted rooting percentage (76%) using the nonlinear regression analysis occurred when trees where stumped to ¼ original height and treated with 4 mM (Fig. 1). In the remaining stumping treatments, the greatest predicted rooting percentage for trees stumped to ½ (70%) and ¾ (65%) original height, occurred when 4 mM NAA and 3 mM NAA were applied, respectively. When NAA was applied to the nonstumped trees, the rooting percentage slowly increased from 14% when no auxin was applied to 31% at 12 mM.

Percent Mortality. The main effects of stumping, replication, concentration and concentration squared were significant (Table 1). For the main effect of stumping, mortality increased as the severity of the stumping treatment decreased from 10% when stock plants were stumped to ¼ original height to 23% in the nonstumped controls. Mortality was 13% and 19% when stock plants were stumped to ½ and ¾ original height, respectively. Across all stumping and auxin type treatments, predicted percent mortality decreased from the nonauxin control (12%) to 2 mM (6%) and then increased with increases in auxin concentration up to 12 mM (22%) (Fig. 2).

Number of Primary Roots. The main effects of replication, concentration and concentration squared were significant (Table 1). Significant interactions were also detected between auxin and concentration, and between auxin and concentration squared. Using the nonlinear regression analysis, the predicted number of primary roots increased with increases in auxin concentration, regardless of the type of auxin applied (Fig. 3).

When IBA was applied, the predicted number of primary roots produced increased only slightly from 2.5 in nonauxin treated cuttings to 3.1 for cuttings treated with 12 mM. When NAA was applied, however, the predicted number of primary roots produced dramatically increased from 2.5 in the nonauxin treated cuttings to 5.5 at 12 mM.

Total Root Length. The main effects of replication, auxin and concentration were significant (Table 1). Significant interactions were detected between auxin and concentration, and between auxin and concentration squared (Table 1). Regardless of the auxin used, predicted total root length increased with increases in auxin concentration (Fig. 3). When IBA was applied, predicted total root lengths increased slightly from 266 mm in the nonauxin treated cuttings to 429 mm at 12 mM. When NAA was applied, predicted total root lengths dramatically increased from 266 mm in the nonauxin control to 614 mm at 12 mM.

Root Symmetry. The main effects of replication, concentration and concentration squared were significant (Table 1). Across all other treatments, root symmetry increased with increasing concentrations from 19% in the nonauxin treated cuttings to 33% at 12 mM (Fig. 4).

Experiment 2

Effect of Stumping Height and Crown Position

The stumping treatment means for primary needle length, rooting percentage and total root length are presented in Table 2. In general, as the severity of the stumping treatment increased, the length of the primary needle, rooting percentage and total root length significantly increased. All stumping treatments produced cuttings that generated longer total root lengths than those from nonstumped trees. Results from the step-wise discriminant analysis to classify rooted and nonrooted cuttings based on their crown position and primary needle lengths for each stumping treatment are also presented in Table 2. When compared to the position traits, primary needle length consistently explained more of the variation, 8.1%, 33.8%, 11.3%, and 4.8% for trees stumped to ¼, ½, ¾ height and the nonstumped control, respectively.

For the crown position variables, the effect of height (z) consistently explained more of the variation in rooting for the stumped stock plants. The partial squared canonical correlations for height were 4.63, 0.93, and 2.76 when stock plants were stumped to ¼, ½, and ¾ original height, respectively (Table 2). In the nonstumped stock plants, distance from the main stem was the most influential position variable.

Primary Needle Length

Percent rooting and total root length were the only two traits assessed that had a significant relationship with primary needle length. Predicted rooting percentage continued to increase with increases in primary needle length from 0% at 0.3 cm to 70% at 4 cm (Fig. 5). Predicted total root lengths increased with increases in primary needle length from 0 mm when no primary needle was present to 309 mm when the primary needle length was 2.8 cm, before decreasing slightly with increases in primary needle length.

Discussion

Effects of Stumping and Crown Position. In the present study using stumping, as in previous research in pine species using hedging, positive effects on rooting were observed (e.g. Bolstad and Libby, 1982; Cooney, 1999; Fielding, 1954; Libby et al., 1972; Menzies, 1986). Stumping or hedging stock plants produced shoots with different morphological and physiological characteristics than those of nonhedged mature material (Hartmann et al., 2002). Rooting percentage increased significantly and mortality decreased significantly, as the severity of the stumping treatments increased. Libby et al. (1972) also found that cuttings collected from hedged stock plants of radiata pine produced significantly more primary roots per rooted cutting than cuttings collected from non-hedged material. Their findings suggest that cuttings collected closer to the base of the stock plant might have the potential to root at higher frequencies and to form a greater number of adventitious roots due to greater juvenility.

The crown position from which the cutting was collected correlated highly with rooting percentage for cuttings collected from some individual 5-year-old Virginia pine trees stumped to various heights. In one case, the amount of variation explained within a single tree was as high as 77%; however, the large amount of tree-to-tree variation reduced the overall relationship within each stumping height treatment. After primary needle length, the height from which the cutting was collected was found to be the next most important variable, except in the nonstumped controls. In the stumped stock plants, as the distance from the base of the tree increased, the rooting percentage decreased. The fact that a cutting’s above-ground distance did not affect rooting in the nonstumped controls was surprising. Previous research has shown that maturation increases as the distance up the tree and away from the main stem increases (Zobel and Talbert, 1984); therefore, it would seem likely that height (z) would be an important factor in the nonstumped trees. However, rooting percentages were very low in all cuttings from these trees, making it more difficult to account for any variation that might be present. The distance from the main stem was more important in the nonstumped controls.

For rooting percentage, primary needle length consistently explained more of the variation, relative to position, regardless of stumping height (Table 2). Primary needle length, a characteristic associated with juvenility in pine species (Frampton, 1987; Haines et al., 1992; Cooney, 1999; Masri, 2000), was found to be significantly affected by stumping height and was positively correlated to rooting percentage and total root length (Fig. 5). Primary needle length generally increased with increases in the severity of the stumping treatment; however, no significant differences were detected between stock plants stumped to ¼ and ½ original height (Table 2). Subsequently, rooting percentage and total root length increased as the length of the primary needle increased (Fig. 5). These data strongly suggest that primary needle length may be used as an indicator of juvenility and subsequent rooting ability in Virginia pine.

Auxin Type and Concentration Effects. The type of auxin, IBA or NAA, significantly affected every rooting trait assessed, except root symmetry and root angle. Relative to IBA, NAA increased rooting percentage, number of primary roots and total root length for stem cuttings collected from stumped trees of Virginia pine. The application of NAA has also been shown to significantly increase root production in hypocotyl cuttings of eastern white pine (*Pinus strobus* L.) (Goldfarb et al., 1998). In another study, Diaz-Sala et al. (1996) suggest that NAA was more effective than IBA in adventitious root induction in hypocotyl cuttings of loblolly pine because it is metabolized more slowly. Moreover, in sugar pine (*Pinus lambertiana* Dougl.) it was determined that free auxins, not their metabolites, are essential for adventitious root formation (Greenwood et. al, 1974). On the other hand, when cuttings were collected from nonstumped control trees, no significant differences in rooting percentage, (*P* =

0.923), were detected for auxin type. This finding supports previous research in Virginia pine, in which auxin type did not significantly affect summer collected cuttings from nonstumped trees of the same age (Rosier, 2003 Chapter 3). The lack of response to auxin type in the mature cuttings collected from the nonstumped controls may be related to a loss of competence in responding to auxin, especially NAA. These findings suggest that maturation may affect endogenous auxin production, the recognition and metabolism of exogenously applied auxin, or both.

Auxin concentration significantly affected every rooting trait assessed except root angle (Tables 1 and 2). The inclusion of a wide range of auxin rates provided clear descriptions of the relationships. Generally, rooting percentage increased and mortality decreased with increases in auxin concentration, up to an optimum concentration, before higher concentrations of auxin detrimentally affected both rooting traits (Figs. 1 and 2). The number of primary roots and total root lengths continued to increase with increases in auxin concentration (Fig. 3). Depending on the auxin type, cuttings either showed little change (IBA) or steadily increased with increases in concentration (NAA). Regardless of the stumping height or type of auxin, primary root production and total root lengths were positively related and exhibited similar patterns as the concentration of auxin increased. Root symmetry, not significantly affected by auxin type, also steadily increased with increases in auxin concentration and paralleled primary root production. This may be expected since a greater number of primary roots increase the likelihood that symmetry will occur.

Higher concentrations of auxin, especially NAA, affected the origin of root initiation on the stem. In cuttings treated with 0 to 4 mM auxin, root primordia appeared to emerge from the callus formed below the cut end of the stem, although this was not determined microscopically. This phenomenon only occurred in cuttings collected from trees that had been stumped, especially the trees that had been stumped to ½ and ¼ original heights. At concentrations > 4 mM NAA and > 8 mM IBA, the bases of the cuttings collected from stumped trees appeared necrotic. Following tissue dieback, callus formation was slow to occur and formed just above the wounded tissue. Subsequent adventitious root formation consisted of numerous roots measuring a few millimeters in length that originated distally the callus. A similar response to auxin concentration has been reported for hypocotyl cuttings of eastern white pine (Goldfarb et al., 1998).

Higher concentrations of exogenously applied auxin not only affected the origin of root initiation on the stem, but also detrimentally affected rooting percentage and possibly led to increases in mortality, especially for cuttings collected from the most severe stumping treatments. When IBA was applied, regardless of the concentration, minimal basal tissue necrosis was observed and rooting percentages generally reached an optimum before decreasing slightly. However, when > 8 mM NAA was applied, the tissue at the base became necrotic leading to slower callus formation, and in several cases eventual mortality. This response may partially explain observed phenomena including: 1) why rooting percentage in NAA treated cuttings decreased rapidly above 6 mM, especially when the cuttings were collected from stumped stock plants and 2) why mortality increased with increases in auxin concentrations above 4 mM, regardless of auxin type or stumping treatment.

While not presented here, a study investigating combinations of auxin types was conducted simultaneously with the current study (Rosier 2003 Appendix III). Eight combinations of three concentrations (0, 2, and 4 mM) of both IBA and NAA were tested in a factorial arrangement. Generally, these combinations produced negative effects on the rooting traits assessed relative to the use of a single auxin. Auxin was detrimental when 4 mM was applied in any combination. Future research should address combinations of auxin concentrations lower than those evaluated.

In conclusion, stem cuttings collected from 5-year-old Virginia pine Christmas trees rooted best when stock plants had been stumped to ¼ original height and were treated with 4 mM NAA. At this concentration, little mortality occurred and root production and root symmetry approached optimal levels. As the severity of the stumping treatments decreased, rooting percentages and primary root production generally decreased. In addition, primary needle length continued to increase with increases in stumping treatments; however, few differences were detected between stock plants stumped to ¼ and ½ original height. The positions from which the cuttings were collected had significant effects on the rooting percentage in numerous trees; however, due to the large tree-to-tree variation the overall relationship was weakl. In general, desirable rooting traits improved with the application of auxin and as the severity of the stumping treatment increased.

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Table 1. ANOVA for traits assessed in a rooting trial of Virginia pine stem cuttings from 4-year-old stock plants.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine Square Root	Square Root	Square Root	Square Root	None	None
X Transformation	None	Log	Log	None	Log	Log
Stumping	*	*	NS	NS	NS	NS
Replication	*	*	*	*	*	NS
Auxin	NS	NS	NS	*	NS	NS
Stumping x Auxin	NS	NS	NS	NS	NS	NS
Concn [z]	*	*	*	*	*	NS
C^2	*	*	*	NS	*	NS
Concn x Stumping	NS	NS	NS	NS	NS	NS
C^2 x Stumping	*	NS	NS	NS	NS	NS
Concn x Auxin	*	NS	*	*	NS	NS
C^2 x Auxin	*	NS	*	*	NS	NS
Concn x Stumping x Auxin	NS	NS	NS	NS	NS	NS
C^2 x Stumping x Auxin	NS	NS	NS	NS	NS	NS

[z] Concn=auxin concentration, C^2 =auxin concentration squared.

NS, * Nonsignificant or significant at $P < 0.05$.

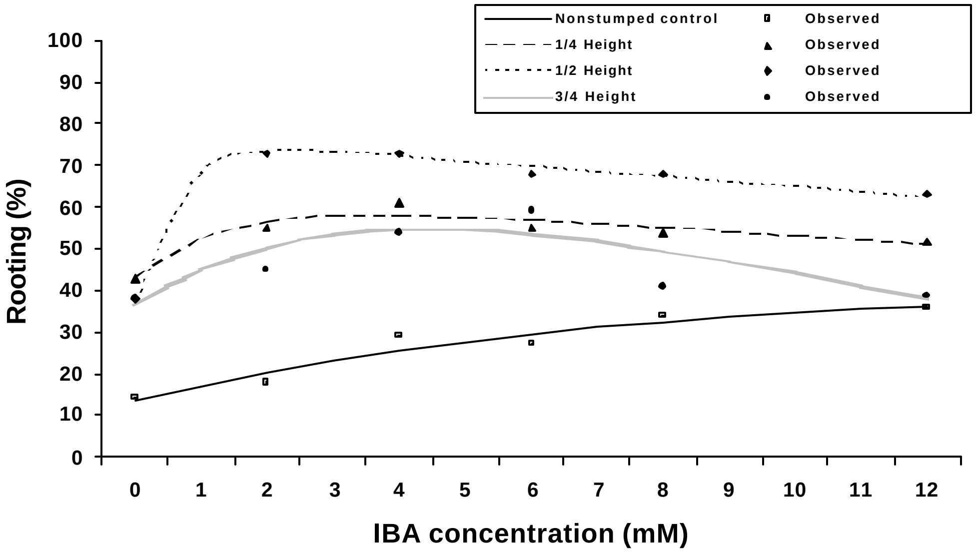
Table 2. Discriminant analysis results for crown position effects on rooting of stem cuttings of Virginia pine. x, y, and z standardized distances from the base of the donor tree. Means are also presented for primary needle length, rooting percentage, and total root lengths.

					Square Canonical Correlation	
Stumping treatments	Mean primary needle length (cm)	Mean rooting percentage	Mean total root length (mm)	Variables	Partial	Total
1/4 Height	3.44 A[Z]	69 A	303 A	PNL[Y]	8.07	14.41
				Z	4.63	
	(3.19 – 3.94)[X]	(30 – 100)	(175 – 519)	X^2	0.01	
				Y^2	1.38	
1/2 Height	3.24 A	63 AB	300 A	PNL	33.84	35.72
				Z	0.93	
	(2.88 – 3.42)	(22 – 94)	(207 – 540)	YZ	0.84	
				Z^2	1.11	
3/4 Height	2.31 B	57 B	275 A	PNL	11.26	15.93
				Z	2.76	
	(1.29 – 2.58)	(44 – 84)	(152 – 469)	X^2	1.82	
				XY	0.76	
Nonstumped Control	0.82 C	17 C	154 B	PNL	4.82	14.84
				Y^2	3.89	
	(0.69 – 0.99)	(2 – 34)	(55 – 306)	X^2	3.88	
				X	2.29	
				XY	0.89	

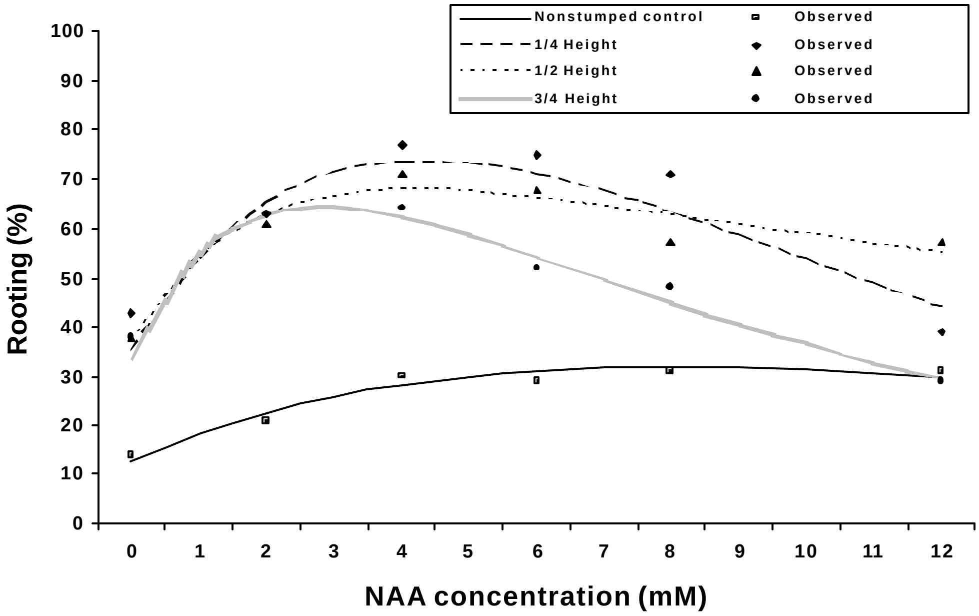
[Z] Values within columns followed by different uppercase letters are significantly different at $P<0.05$.

[Y] PNL = Primary needle length.

[X] Range of mean tree values.

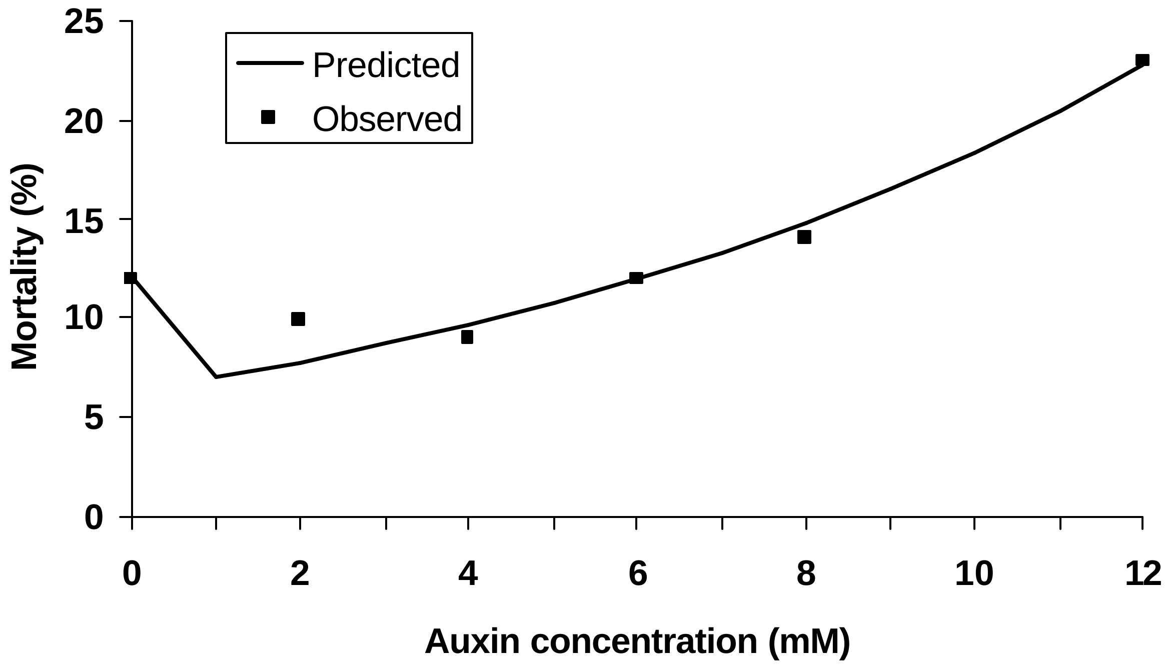


	q_1	q_2	**A**	**B**	***R^2***
Nonstumped control	0.0514	0.0505	1.1694	1.0028	0.93
Stumped to ¼ height	0.7075	0.0188	4.8664	3.0249	0.93
Stumped to ½ height	1.6039	0.0183	2.5159	1.9327	0.99
Stumped to ¾ height	0.1472	0.1464	0.9542	1.0019	0.86



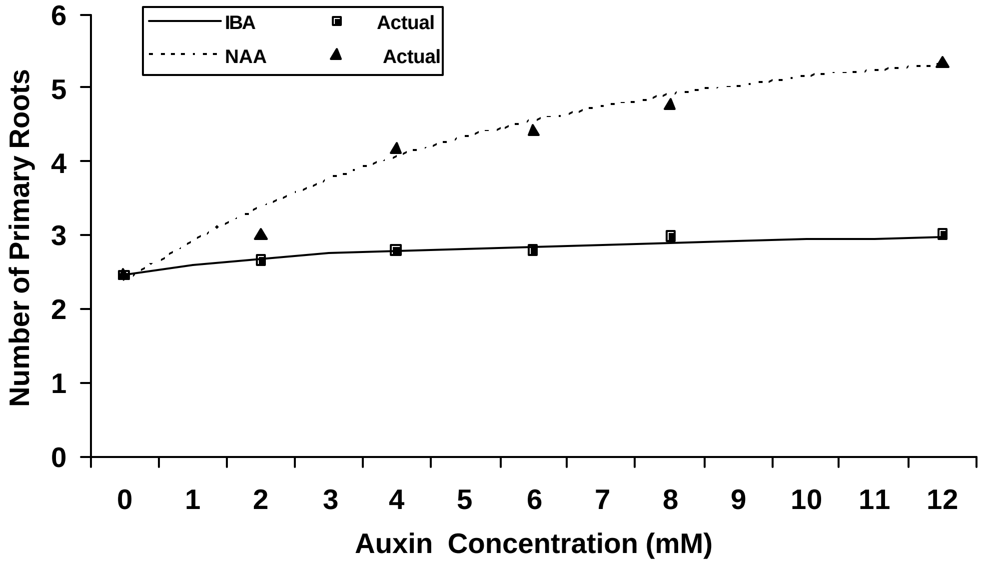
	q_1	q_2	**A**	**B**	***R^2***
Nonstumped control	0.1029	0.1036	1.3757	0.9988	0.90
Stumped to ¼ Height	0.1810	0.1805	0.6202	1.0006	0.90
Stumped to ½ Height	0.5610	0.0349	2.2954	1.8003	0.92
Stumped to ¾ Height	0.5521	0.1089	1.6537	1.4501	0.97

Fig. 1. The effect of stumping, auxin concentration, and IBA (upper) or NAA (lower) on rooting percentage of Virginia pine stem cuttings taken in 2001. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model (see text for equation).

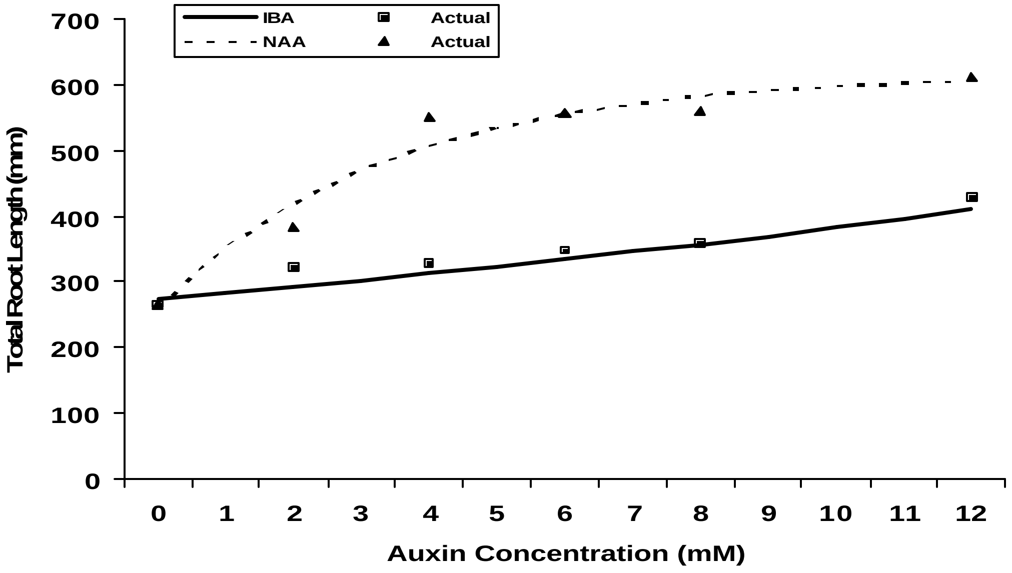


	q_1	q_2	**A**	**B**	***R^2***
Predicted	-0.1070	7.2731	-0.2303	-0.9149	0.96

Fig. 2. The effect of auxin concentration on percent mortality of Virginia pine stem cuttings taken in 2001. Values are averaged over auxin type and stumping treatments. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model (see text for equation).

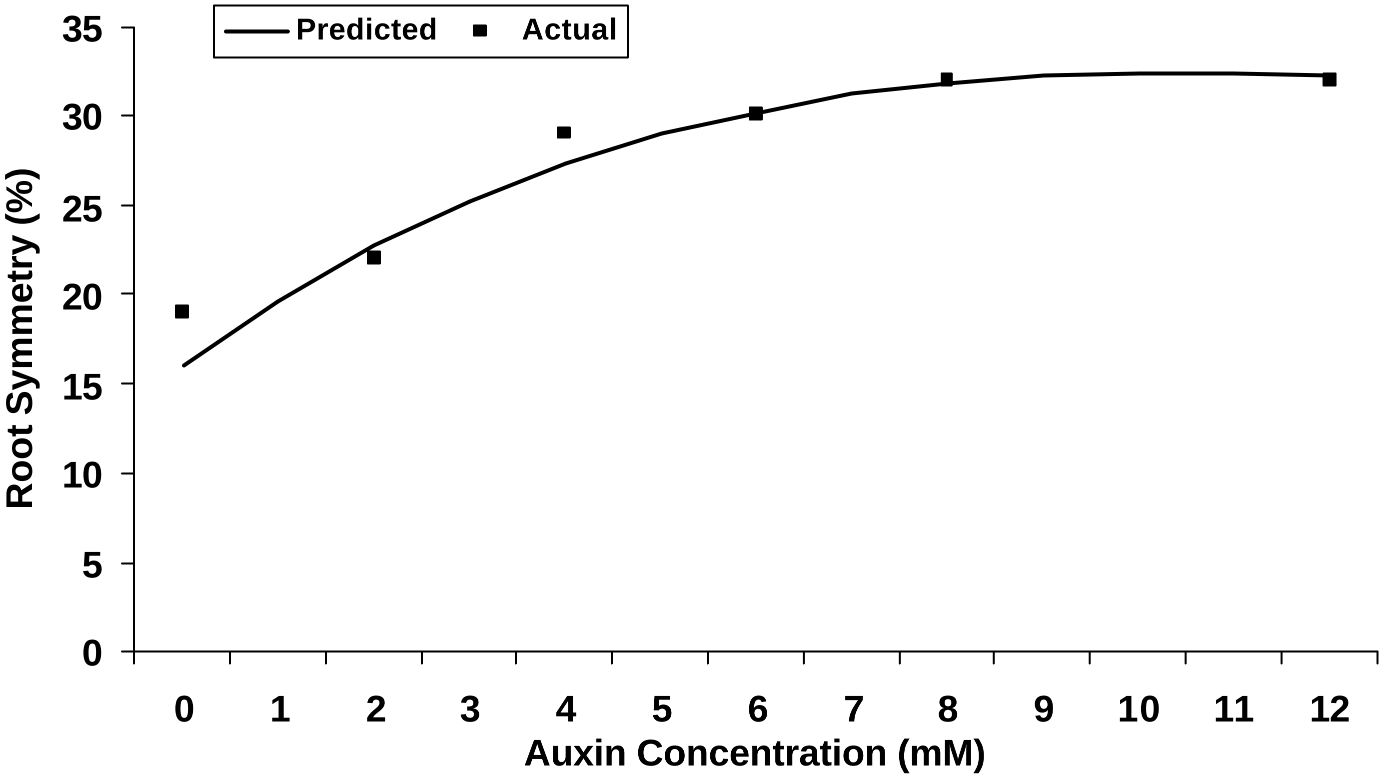


	q_1	q_2	**A**	**B**	$\mathbf{R^2}$
IBA	0.4768	-0.00595	3.2350	9.0919	0.98
NAA	0.1887	-0.00298	0.3234	1.7858	0.94



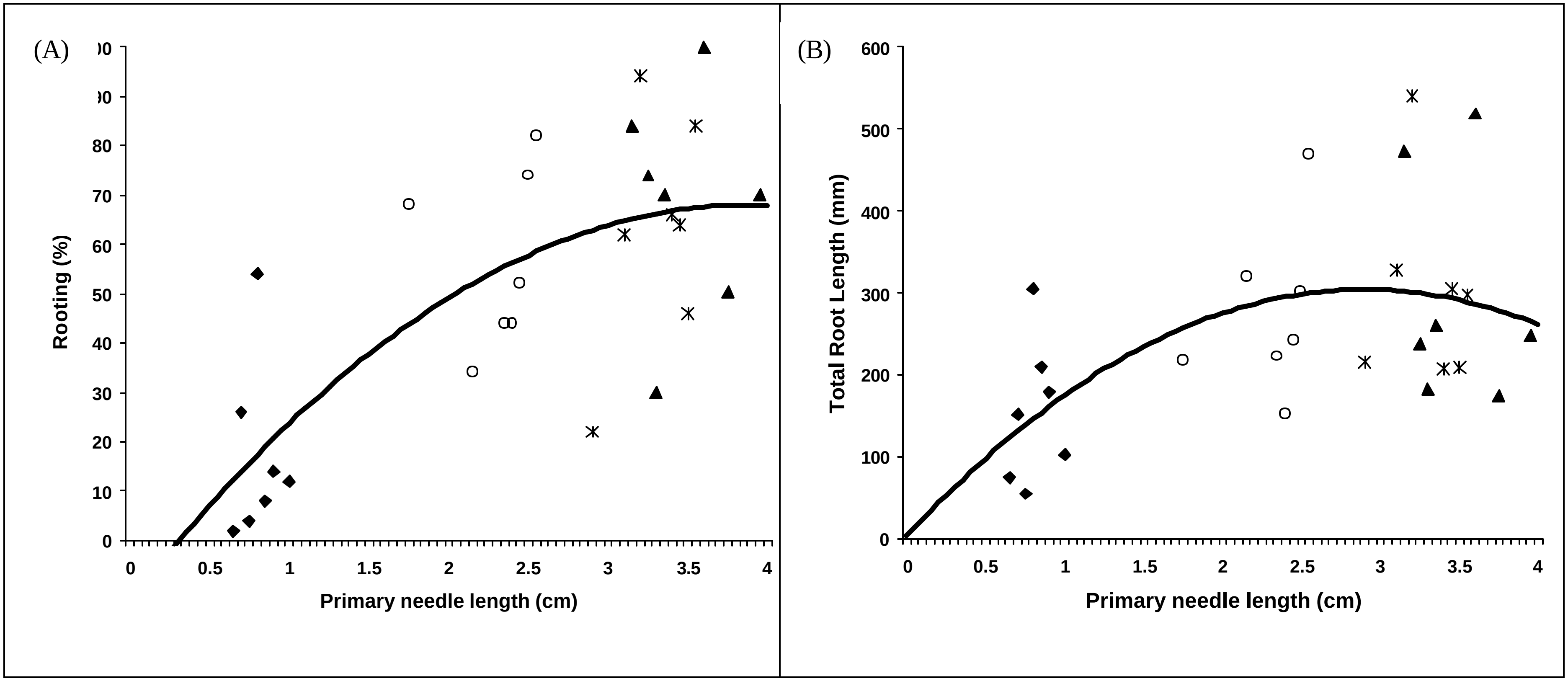
	q_1	q_2	**A**	**B**	R^2
IBA	-.0340	-.0339	4.9809	5.00	0.90
NAA	0.2969	0.000072	0.00279	1.7215	0.97

Fig. 3. The effect of auxin type, and concentration on the number of primary roots (upper) and total root length (lower) of Virginia pine stem cuttings taken in 2001. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model (See text for equation).



	q_1	q_2	**A**	**B**	***R^2***
Predicted	0.0728	0.0738	1.4103	0.9969	0.93

Fig. 4. The effect of auxin concentration on root symmetry of Virginia pine stem cuttings. Root symmetry is averaged over all stumping heights, and auxin concentration responses are averaged over IBA and NAA. R^2 values and estimated regression coefficients (θ_1, θ_2, A, B) are presented for the best model (see text for equation).



	Y-Intercept	Mean Needle Length	Mean Needle Length Squared	R^2
Rooting (%)	-0.1235	0.4143	-0.0534	0.52
Total root length	3.951	207.1	-35.71	0.28

Fig. 5. Relationship between primary needle length and (A) rooting percentage (B) and total root length for cuttings collected from 5-year-old Virginia pine trees subjected to four stumping treatments: where ? = nonstumped control, ° = stumped to ¾ original height, * = stumped to ½ original height and ? = stumped to ¼ original height.

Chapter 6

Wounding and Auxin Treatments Influence Rooting of Stem Cuttings of Fraser Fir

Abstract. Post-severance wounding and auxin treatments were examined for their effects on stimulating rooting of hardwood stem cuttings from 4-year-old Fraser fir (*Abies fraseri* [Pursh] Poir.) seedlings. Auxin treatments increased the rooting percentages and the number of primary roots. Wounding did not affect rooting percentage or number of primary roots; however it significantly increased mortality. Mean root length was not significantly affected by any auxin or wounding treatment.

Basal wounding is beneficial for rooting stem cuttings of several species, especially when cuttings are collected from older material (Hartmann et al., 2002). Following wounding, callus formation and subsequent root development typically occur along the margins of the wound. MacKenzie et al. (1986) suggest that wounded tissue increases cell division, which eventually gives rise to the formation of root primordia. Hartmann et al. (2002) also suggest that, in addition to stimulated cell division, wounding allows for natural accumulation of auxins and carbohydrates in and around the wounded area. Wounding also allows for an increase in respiration rate, water uptake and absorption of exogenously applied auxin, all of which are beneficial to root primordia initiation and subsequent survival of the cutting (Hartmann et al., 2002).

In Fraser fir (*Abies fraseri* [Pursh] Poir.), previous research demonstrates that, when used in combination with auxin, basal wounding can improve rooting, the number of roots produced per cutting and total root length (Blazich and Hinesley, 1994; Hinesley and Blazich, 1980; 1981; 1984). The effect of wounding has, however, proven to be variable. In one study with hardwood cuttings collected from 5-, 12- and 22-year-old material, Hinesley and Blazich (1980) found no significant differences, at any age, in rooting percentage, number of primary roots produced or mean root length between cuttings that were treated by wounding and given 25 mM IBA auxin treatment and cuttings only treated with auxin. Although not significant, Hinseley and Blazich (1980) found that, for cuttings collected from 5-year-old trees, non-wounded and auxin treated cuttings tended to root less frequently than cuttings wounded and treated with auxin. In contrast, the non-wounded cuttings produced a greater number of roots than wounded cuttings and the overall mean root lengths were greater (Hinesley and Blazich, 1980). In a follow-up study, Hinesley and Blazich (1981) found a significantly lower rooting percentage for non-wounded cuttings treated with auxin compared to cuttings that were wounded using four vertical slits and treated with auxin. However, no significant differences were detected for number of roots per cutting or mean root lengths. In a similar study that focused on the effects of disbudding, rooting percentage was found to be significantly affected by wounding treatments. Using cuttings with lateral buds intact, non-wounded cuttings treated with auxin rooted more frequently when compared to cuttings that were wounded and treated with auxin (Hinseley and Blazich, 1981).

Wounding is a time-consuming process, but as previously mentioned, the rooting performance of Fraser fir cuttings sometimes improves when wounding is used in combination with auxin treatments, especially in older material (Hinesley and Blazich, 1980; 1981). Another problem is the increased possibility of mortality. A wound provides an infection site for disease organisms and insects. The effects of wounding on mortality have not been reported for Fraser fir cuttings, but problems have been noted in preliminary research where mortality increased in wounded cuttings, regardless of auxin application (data not presented).

The objective of the current experiment was to determine the effects of several post-severance treatments, including wounding and auxin application, on the rooting ability of hardwood Fraser fir cuttings collected from juvenile, four-year-old seedlings.

Materials and Methods

Hardwood Fraser fir cuttings were collected from dormant seedling tops harvested at the North Carolina Division of Forest Resources' Linville River Nursery located near Crossnore, N.C. Fraser fir stock plants were obtained as bare root 4-0 seedlings on 27

Feb., 2001. They were immediately placed into seedling bags, transported to Raleigh, N.C. on ice, and stored at 4^{o}C. Cuttings were severed from the primary axes of the seedlings to avoid the plagiotropic growth habitat common to cuttings collected from secondary or tertiary axes.

The four treatments evaluated in this study were: 1) a control (no wounding or auxin application), 2) wounded with no auxin, 3) 8 mM application of IBA and 4) wounded with 8 mM application of IBA. The 8 mM concentration of IBA was made by dissolving the IBA powder into 70% isopropyl alcohol and then diluting with deionized water to make a 50% isopropyl alcohol/water solution. Solutions were then stored at 4 ^{o}C in the dark within opaque bottles and used the day following preparation.

Cuttings were set the day following collection in a propagation greenhouse located at the Horticulture Field Laboratory on the North Carolina State University campus, Raleigh. Before setting, the cuttings were re-cut to a length of 9 cm and auxin was applied for 3 seconds to the basal 1.5 cm for treatments 3) and 4). Cuttings for the treatments 1) and 2) were dipped into a 50% isopropyl alcohol/dionized water solution. For treatments 2) and 4), cuttings were wounded by applying 4 equidistant vertical cuts around the base of cuttings using a razor blade. The cuts were 2 cm in length, parallel to the long axis of the cutting, and deep enough to separate the outer tissues of the cutting, i.e., a light wound. When cuttings were treated with both wounding and IBA, the wound was made first. Needles were not removed before or after the auxin had been administered. The auxin treated cuttings were air-dried for a minimum of 15 minutes before being set into the rooting medium to a depth of 3 cm. The medium was a mixture of horticulture perlite and peat moss (3:2 v/v).

During the rooting period, the cuttings were under intermittent mist using the Grower Junior™ (McConkey Co., Sumner, Wa.) overhead boom. Frequency of mist application was controlled using relative humidity and time of day. As humidity in the rooting greenhouse decreased, the frequency of mist application increased; however, mist application was less frequent during the night decreasing from an average of one mist cycle every 6 min during the day to an average of one mist cycle every 60 min at night. Flow rate per mist cycle was held consist over the four-month rooting period at 0.045 $L \cdot m^{-2}$. The average day/night temperatures in the greenhouse were 26.1 ± 2/20.6 ± 2 ^{o}C, respectively.

The experimental design was a randomized complete block. There were 18 blocks with 8 cuttings per treatment per block for a total of 576 cuttings. The entire study was surrounded by a border row. Rooting data were collected following 16 weeks in the greenhouse, including rooting percentage, percent mortality, number of primary roots per cutting, and mean root length per cutting. A cutting was considered rooted if a minimum of one root ≥ 1 mm in length was present.

Analyses of variance were conducted using the General Linear Model (GLM) procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Replication, IBA, wounding treatment, and IBA x wounding treatment were sources of variation. Several transformations were explored including the square root, the arcsine of the square root, and a range of transformations using the Box and Cox (1964) procedure that varied λ from -1.00 to +1.00 in increments of 0.25. The transformation, or no transformation, yielding the smallest differences among treatment variances was selected for each dependent variable.

After investigating transformation options, the arcsine-square root was selected to transform both rooting percentage and percent mortality, the log was selected to transform total root length and no transformation was needed for number of primary roots. In this study, the log was used to transform the independent variable of every trait except rooting percentage, which required a transformation using the square root.

Results

The main effect of IBA was significant for both rooting percentage and number of primary roots; however, there were no significant differences detected among treatments for mean root length (Table 1). The means for each rooting trait and treatment combinations are presented in Table 2. When examining the main effects of IBA, the highest rooting percentage (58%) and the greatest number of primary roots (9.7) were induced when an 8 mM concentration of IBA was applied to the bases of the cuttings. The rooting percentage and number of primary roots were much lower when cuttings were not treated with an auxin solution; 35% and 8.3, respectively. The mean root length over all treatments was 29.6 mm.

Wounding significantly increased mortality when applied to hardwood Fraser fir stem cuttings (Table 1). The percent morality was much lower in non-wounded cuttings (6%) than in wounded cuttings (20%).

Discussion

For these hardwood cuttings from four-year-old Fraser fir seedlings, the rooting percentage significantly increased when the cuttings were treated with IBA. Although not significant, rooting percentage tended to be lower when cuttings were wounded and treated with IBA versus non-wounded auxin treated cuttings. It was noted that extensive swelling and callus formation occurred around the bases of auxin-treated cuttings and along wounds; however, roots usually emerged from the cutting bases and rarely from the wounds. The results for the non-wounded cuttings in this study were similar to those in a concurrent study that was using similar material (Rosier, 2003 Chapter 2).

One trait not discussed in previous research is mortality. In this study, wounding significantly increased mortality. The current findings support the idea that wounding may be detrimental when rooting juvenile orthotropic hardwood Fraser fir cuttings. On the other hand, Hinesley and Blazich (1980) showed that wounding increased the rooting frequency in cuttings collected from older material.

In conclusion, there were no significant advantages to wounding these cuttings. The highest overall rooting percentage and greatest number of primary roots occurred when auxin was applied to the base of a non-wounded cutting. The overall percent mortality was greater in wounded cuttings with no significant differences detected for mean root length.

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SAS® Institute, Inc. 1999. SAS®/STAT User's Guide. Version 6, 4th Ed. Vol. 1 and 2. Cary, NC.

Table 1. ANOVA for a Fraser fir rooting trial examining the effects of wounding and IBA after severance.

	Rooting (%)	Mortality (%)	Number of primary roots	Mean root length (mm)
Replication	NS [Z]	NS	NS	NS
IBA	*	NS	*	NS
Wounded	NS	*	NS	NS
IBA*Wounded	NS	NS	NS	NS

[Z] * = (Probability>F) ≤0.05, NS = (Probability >F) >0.05

Table 2. Mean values for a Fraser fir rooting trial examining the effects of several postseverance treatments.

Treatment	Rooting (%)	Mortality (%)	Number of primary roots	Mean root length (mm)
Control	33	7	8.1	21.8
Wounded	37	22	8.7	31.1
IBA	63	5	9.8	32.8
IBA + Wounded	52	18	9.5	32.5

APPENDIX I: Supplement to Chapter 2

Factorial Study Examining the Effects of Growth Stage, Auxin Type, and Concentration on Rooting of Stem Cuttings of Fraser Fir

This study was conducted simultaneously with the study reported in Chapter 2, which focused on applications of IBA and NAA individually in concentrations that ranged from 0-64 mM. Time of collection and insertion into the rooting medium, environmental conditions during rooting, and rooting traits assessed were exactly the same as in the Chapter 2 study. The objective of this study was to examine eight factorial combinations of IBA and NAA (4 mM IBA/0 mM NAA, 16 mM IBA/0 mM NAA, 0 mM IBA/4 mM NAA, 0 mM IBA/16 mM NAA, 4 mM IBA/4 mM NAA, 4 mM IBA/16 mM NAA, 16 mM IBA/4 mM NAA, 16 mM IBA/16 mM NAA) and a solvent control.

Materials and Methods

For each concentration, the auxin was dissolved into a 50% isopropyl alcohol/ deionized water solution. The prepared auxin solutions were then stored at 4 ^{o}C in opaque bottles and used within 2 d.

The experimental design was a randomized complete block. There were eight blocks of eight cuttings per treatment within each block for a total of 320 cuttings. This entire study was mixed in the main study reported in Chapter 2.

Analyses of variances (ANOVAs) were conducted using the General Linear Models procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Growth stage, replication within growth stage, IBA concentration, NAA concentration, IBA x growth stage, NAA x growth stage, IBA x NAA, and IBA x NAA x growth stage were used as the sources of variation in the analysis of variance. All other aspects of the data analyses followed the methods described in Chapter 2.

Results & Discussion

ANOVAs and transformations of the independent and dependent variables for all rooting traits are presented in Tables 1. In the following discussion, IBA and NAA treatment combinations were compared to equivalent IBA or NAA treatments (8 or 32 mM) from the larger study reported in Chapter 2.

Rooting percentage. The main effects of growth stage, IBA, and NAA were found to significantly affect rooting percentage (Table 1). A significant interaction was detected between NAA and growth stage as well as between IBA and NAA (Table 1). Across all IBA and NAA treatments, the overall rooting percentages were 70% for semi-hardwood cuttings, 36% for hardwood cuttings, and 72% for softwood cuttings. Overall rooting percentages were 60% for the control treatments, 58% and 60% for 4 and 16 mM IBA, and 63% and 55% for the 4 and 16 mM NAA, respectively. The combination of IBA and NAA (Table 2) almost always produced a negative interaction when compared to an individual auxin type of an equal concentration. For example, the combination of 4 mM IBA and 4 mM NAA rooted less frequently (52%) than an equivalent concentration of 8 mM IBA (79%) or NAA (77%). In contrast, the combination of 16 mM IBA and 16 mM NAA was found to have a lower rooting percentage (47%) than the equivalent concentration of 32 mM IBA (65%), but a higher rooting percentage than the equivalent concentration of 32 mM NAA (38%). The highest rooting percentage of any combination (61%) occurred when cuttings were treated with 16 mM IBA and 4 mM NAA; however, this was less than any treatment of either IBA or NAA alone.

Percent Mortality. Only the main effect of growth stage significantly affected percentage mortality (Table 1). Across all IBA and NAA treatments, the greatest mortality (8%) occurred in hardwood cuttings, followed by softwood cuttings (2%). No mortality occurred in the semi-hardwood cuttings.

Number of Primary Roots. The main effects of growth stage, IBA, and NAA significantly influenced the number of primary roots (Table 1). The interactions between NAA and growth stage, IBA and NAA, and the three-way interaction between IBA, NAA, and growth stage were also significant (Table 1). Across all IBA and NAA treatments, the greatest mean number of primary roots was produced in softwood cuttings (8.2). The number of primary roots decreased slightly in the semi-hardwood cuttings (7.6) and was lowest for hardwood cuttings (5.1). For both IBA and NAA, the number of primary roots increased with increases in auxin concentration, ranging from 6.5 and 6.4 with no auxin treatment to 7.9 and 7.2 at 16 mM for IBA and NAA, respectively. The combinations of IBA and NAA, presented in Table 2, almost always resulted in a negative interaction for the two- and three-way interactions. The only exception was the combination between 16 mM IBA and 16 mM NAA (7.6), which resulted in slightly more primary roots than 32 mM NAA (7.2). The 16 mM IBA and 4 mM NAA treatment produced the greatest number of primary roots of any combination (7.8) and the fewest roots (6.1) resulted when 4 mM IBA and 4 mM NAA were used.

Total Root Length. The main effects of growth stage, IBA, and NAA, as well as the interaction between IBA and NAA significantly affected total root length (Table 1). Across all IBA and NAA treatments, total root lengths were 136, 658, and 598 mm for hardwood, softwood, and semi-hardwood cuttings, respectively. For the main effect of both IBA and NAA, total root length increased from the control (430 mm for IBA and 462 mm for NAA) to 4 mM (530 mm for IBA and 570 mm for NAA) and then decreased at 16 mM (520 for IBA and 364 mm for NAA). A negative interaction was found for all IBA by NAA combinations (Table 2) with the exception of 16 mM IBA and 16 mM NAA treatment (498 mm), which was greater than an equal concentration of 32 mM NAA (376 mm).

Root Symmetry. For root symmetry, the main effects of growth stage, replication within growth stage, and IBA, as well as the interaction between IBA and NAA were significant (Table 1). Across all IBA and NAA treatments, root symmetry was 67% for semi-dormant cuttings, 29% for dormant cuttings, and 76% for softwood cuttings. Across all growth stages and NAA treatments, root symmetry was 50% when no IBA was applied, 57% at 4 mM IBA and 64% at 16 mM IBA. A negative interaction occurred for all IBA by NAA combinations (Table 2) with the exception of the 16 mM IBA and 16 mM NAA treatment (62%), which resulted in approximately the same root symmetry as an equal concentration of 32 mM NAA (60%). The greatest root system symmetry for combination treatments occurred when 16 mM IBA was used in combination with 4 mM NAA (63%).

Root Angle. The main effect of IBA was significant for root angle (Table 1). Across all growth stages and NAA treatments, the percentages of cuttings with vertical roots was 74% when no IBA was applied, 80% for 4 mM IBA, and 75% for 16 mM IBA.

Conclusion

This study examined factorial combinations of two auxin types (IBA and NAA) and three concentrations (0, 4, and 16 mM). Generally, auxin combinations produced negative effects on the rooting traits assessed and auxin was detrimental when 16 mM was applied in any combination. Future research should address combinations of auxin concentrations lower than those evaluated herein.

Table 1. ANOVA for traits assessed in a rooting trial of Fraser fir stem cuttings collected from 3- and 4-year-old seedlings.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine Square Root	Arcsine Square Root	None	Log	None	None
X Transformation	Square Root	Log	Log	Log	Log	None
Growth stage	*	*	*	*	*	NS
Rep (Growth stage)	NS	NS	NS	NS	*	NS
IBA	*	NS	*	*	*	*
NAA	*	NS	*	*	NS	NS
IBA x Growth stage	NS	NS	NS	NS	NS	NS
NAA x Growth stage	*	NS	*	NS	NS	NS
IBA x NAA	*	NS	*	*	*	NS
IBA x NAA x Growth stage	NS	NS	*	NS	NS	NS

NS, * Nonsignificant or significant at $P < 0.05$, respectively.

Table 2. Rooting traits when combinations of three concentrations (0, 4, and 16 mM) of IBA and NAA were applied to Fraser fir stem cuttings, averaged over three growth stages.

	Rooting (%)				**Primary roots (#)**				**Total root length (mm)**				**Root symmetry (%)**			
	NAA Concn(mM)															
IBA Concn (mM)	**0**	**4**	**16**	**Overall**	**0**	**4**	**16**	**Overall**	**0**	**4**	**16**	**Overall**	**0**	**4**	**16**	**Overall**
0	37	72	68	**59**	4.5	8.0	8.4	**7.0**	287	564	577	**476**	28	72	69	**56**
4	70	52	51	**58**	7.3	6.1	6.6	**6.7**	573	436	491	**500**	68	50	50	**56**
16	72	61	47	**60**	8.8	7.8	7.6	**8.1**	654	467	498	**540**	77	63	62	**67**
Overall	**60**	**62**	**55**	*59*	**6.9**	**7.3**	**7.5**	**7.2**	**505**	**489**	**522**	*505*	**58**	**62**	**60**	*60*

APPENDIX II: Supplement to Chapter 3

Factorial Study Examining the Effects of Growth Stage, Auxin Type, and Concentration on Rooting of Virginia Pine Stem Cuttings

This study was conducted simultaneously with the study reported in Chapter 3, which focused on applications of IBA and NAA individually in concentrations that ranged from 0-64 mM. The time of collection and insertion into the rooting medium, environmental conditions during rooting and rooting traits assessed were exactly the same as in the Chapter 3 study. The objective of this study was to examine eight factorial combinations of IBA and NAA (4 mM IBA/0 mM NAA, 16 mM IBA/0 mM NAA, 0 mM IBA/4 mM NAA, 0 mM IBA/16 mM NAA, 4 mM IBA/4 mM NAA, 4 mM IBA/16 mM NAA, 16 mM IBA/4 mM NAA, 16 mM IBA/16 mM NAA) and a solvent control.

Materials and Methods

For each auxin concentration evaluated, the auxin was dissolved into a 50% isopropyl alcohol/ dionized water solution. The prepared auxin solutions were then stored at 4^{o} C in opaque bottles and used within 2 d.

The experimental design was a randomized complete block design and was integrated into the study described in Chapter 3. There were 8 blocks of 8 cuttings per treatment within each block for a total of 320 cuttings. The entire study was surrounded by a row of border cuttings.

Analyses of variance were conducted using the General Linear Model procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Growth stage, replication within growth stage, IBA concentration, NAA concentration, IBA x growth stage, NAA x growth stage, IBA x NAA and IBA x NAA x growth stage were used as the sources of variation in the analysis of variance. All other aspects of the data analyses followed the methods described in Chapter 3.

Results & Discussion

Analyses of variance and transformations of the independent and dependent variables for all rooting traits are presented in Table 1. There were no significant main effects or interactions for either root symmetry or root angle, therefore, no data are presented (Table 1). In the following discussion, IBA and NAA treatment combinations are compared equivalent IBA or NAA treatments (8 or 32 mM) from the larger study reported in Chapter 3.

Rooting Percentage. The main effects of NAA and its interaction with IBA were significant, but IBA main effects were not for rooting percentage (Table 1). Across growth stages and IBA treatments, rooting percentage was 20% when no NAA was applied, 22% when 4 mM NAA was applied and 16% when 16 mM NAA was applied. The combination of IBA and NAA always produced a negative response when compared to an individual auxin type of an equal concentration. For example, the rooting percentage for the combination of 4 mM IBA and 4 mM NAA (21%) was lower than 8 mM of either IBA (31%) or NAA (32%). The rooting percentage for the combination of 16 mM IBA and 16 mM NAA (9%) was also lower than 32 mM of either IBA (17%) or NAA (13%). The rooting percentage for the combination of 4 mM IBA and 16 mM NAA (9%) was lower than the rooting percentage for the combination of 16 mM IBA and 4 mM NAA (18%).

Percent Mortality. For percent mortality, the main effects of NAA concentration and growth stage were significant (Table 1). Across growth stages and IBA treatments, the highest percent mortality (21%) occurred at 16 mM NAA, followed by the control (16%), and the lowest mortality occurred with the application of 4 mM NAA (6%). Across NAA and IBA treatments, the greatest mortality occurred for semi-hardwood cuttings, 30% and 20%, set in 2000 and 2001, respectively. The lowest mortality occurred for hardwood (14%) and softwood cuttings (16%).

Primary Root Production. Primary root production was significantly affected by growth stage and the interaction between IBA and NAA (Table 1). For main effects of growth stage, the greatest (4.4) and fewest (3.6) numbers of primary roots were produced by semi-hardwood and softwood cuttings, respectively. IBA and NAA interacted negatively as shown by the combination of 4 mM IBA and 4 mM NAA (4.2) which was lower than an equivalent concentration of 8 mM IBA (4.7) or NAA (5.1). The combination of 16 mM IBA and 16 mM NAA produced the same number of roots (5.6) as the equivalent concentration of 32 mM NAA, but slightly more roots than the equivalent concentration of 32 mM IBA (5.4). The number of primary roots produced for the combination of 4 mM IBA and 16 mM NAA (4.0) was higher than the number of primary roots produced for the combination of 16 mM IBA and 4 mM NAA (3.6).

Total Root Length. The main effects of replication within growth stage and NAA along with the interaction between growth stage and IBA significantly affected total root length (Table 1). For the main effect of NAA, total root length increased with increases in auxin concentrations, ranging from 84 mm in the control to 287 mm at 16 mM. For the interaction between IBA and growth stage, the greatest total root lengths occurred in semi-hardwood cuttings set in 2001 (299 mm) and softwood cuttings (230 mm). The lowest total root lengths occurred for semi-hardwood cuttings set in 2000 (150) and the hardwood cuttings (85 mm).

Conclusion

This study examined combinations of two auxin types (IBA and NAA) and two concentrations (4 and 16 mM) in a factorial arrangement. In nearly every case, these combinations produced negative effects on the rooting traits assessed, and auxin was detrimental when 16 mM was applied in any combination. Future research should address combinations of auxin concentrations lower than those evaluated.

Table 1. ANOVA for traits assessed in a rooting trial of Virginia pine stem cuttings collected from 3- and 4-year-old stock plamts.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine-Square Root	Square Root	None	Square Root	Log	None
X Transformation	Log	None	Log	Log	Log	None
Growth stage	NS	*	*	NS	NS	NS
Rep (Growth stage)	NS	NS	NS	*	NS	NS
IBA	NS	NS	NS	NS	NS	NS
NAA	*	*	NS	*	NS	NS
IBA x Growth stage	NS	NS	NS	*	NS	NS
NAA x Growth stage	NS	NS	NS	NS	NS	NS
IBA x NAA	*	NS	*	NS	NS	NS
IBA x NAA x Growth stage	NS	NS	NS	NS	NS	NS

NS, * Nonsignificant or significant at $P < 0.05$, respectively.

Table 2. Rooting traits when combinations of three concentrations (0, 4, and 16 mM) of IBA and NAA were applied to Virginia pine stem cuttings, averaged across three growth stages.

	Rooting (%)				**Primary Roots (#)**			
	NAA Concn (mM)				**NAA Concn (mM)**			
IBA Concn (mM)	**0**	**4**	**16**	**Overall**	**0**	**4**	**16**	**Overall**
0	10	26	21	**19**	2.3	4.0	5.1	**3.8**
4	27	21	19	**22**	4.2	4.2	4.0	**4.1**
16	24	18	9	**17**	4.5	3.6	5.6	**4.6**
Overall	**20**	**22**	**16**	***19***	**3.7**	**3.9**	**4.9**	***4.2***

APPENDIX III: Supplement to Chapter 5

Factorial Study Examining the Effects of Stumping Height, Crown Position, Auxin Type and Concentration on Rooting of Stem Cuttings of Virginia Pine

This study was conducted simultaneously with the study reported in Chapter 5, which focused on applications of IBA and NAA individually in concentrations that ranged from 0-12 mM. The stumping treatments, time of collection, and insertion into the rooting medium, environmental conditions during rooting, and rooting traits assessed were the same as in the Chapter 5 study. The objective of this study was to examine eight combinations of IBA and NAA (2 mM IBA/0 mM NAA, 4 mM IBA/0 mM NAA, 0 mM IBA/2 mM NAA, 0 mM IBA/4 mM NAA, 2 mM IBA/2 mM NAA, 4 mM IBA/2 mM NAA, 2 mM IBA/4 mM NAA, 4 mM IBA/4 mM NAA) and a solvent control arranged in a factorial arrangement.

Materials and Methods

For each auxin concentration evaluated, the auxin was dissolved into a 50% isopropyl alcohol/ deionized water solution. The prepared auxin solutions were then stored at 4^o C in opaque bottles and used within 2 d.

The experimental design for this study was a randomized complete block. There were seven blocks with eight cuttings per treatment per block for a total of 1120 cuttings. The entire study was incorporated into the main study reported in Chapter 5.

Analyses of variance were conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System version 8.1 (SAS Institute, Inc., 1999). Stumping, replication, IBA concentration, NAA concentration, IBA x stumping, NAA x stumping, IBA x NAA and IBA x NAA x stumping served as sources of variation. All other aspects of the data analyses followed the methods described in Chapter 5.

Results & Discussion

ANOVAs and transformations of the independent and dependent variables for all rooting traits are presented in Table 1. Since no significant differences were noted for percent mortality or root angle, these data are not presented. In the following discussion, IBA and NAA treatment combinations are compared to equivalent IBA or NAA treatments (6 or 8 mM) from the larger study reported in Chapter 5.

Rooting Percentage. The main effects of stumping, replication, IBA, NAA as well as the interaction between IBA and NAA were significant (Table 1). Rooting percentages increased as the severity of the stumping treatment increased: 23%, 50%, 66% and 72% for the control, stumped to ¾, ½ and ¼ original height, respectively. For the main effect of IBA, rooting percentage increased with increases in auxin concentration from 48% in the control to 54% at 4 mM IBA (Table 2). For the main effect of NAA, rooting percentage increased from 45% in the control to 57% at 2 mM before decreasing to 54% at 4 mM NAA (Table 2). In general, the combination of IBA and NAA produced a positive interaction the majority of the time. For example, rooting percentages were greater when the combination of 4 mM IBA and 4 mM NAA (51%) was compared to 8 mM IBA (49%), however, this combination rooted less frequently than 8 mM NAA (53%). The combination of 2 mM IBA and 2 mM NAA rooted more frequently (61%) than 4 mM IBA (54%) or the same as 4 mM NAA (61%). The combination of 2 mM IBA and 4 mM NAA rooted less frequently (49%) than the combination of 4 mM IBA and 2 mM NAA (58%). The combination of 4 mM IBA and 2 mM NAA rooted more frequently than 6 mM IBA (52%); however, it was not significantly greater than 6 mM NAA (55%).

Number of Primary Roots. The main effects of replication, IBA and NAA, as well as the interaction between IBA and NAA were significant (Table 1). For the main effect of IBA, the number of primary roots produced increased from 3.4 in the nonauxin treated cuttings to 4.1 at 2 mM IBA before decreasing to 3.9 when 4 mM IBA was applied (Table 2). For the main effect of NAA, root production increased with increases in auxin concentration from 2.7 in the control to 5.0 at 4 mM NAA (Table 2). The combination of IBA and NAA produced both positive and negative interactions. Across all stumping and auxin treatments, the number of primary roots produced for the combination of 2 mM IBA and 2 mM NAA (4.3) was greater than an equal concentration of 4 mM IBA (2.8), but less than an equal concentration of 4 mM NAA (4.7). Primary root production in the combination of 4 mM IBA and 4 mM NAA (5.1) was less than an equal concentration of 8 mM of NAA (5.3) but greater than 8 mM IBA (2.9). The combination of 2 mM IBA and 4 mM NAA produced the greatest number of roots (5.3) when compared to all other treatments in this factorial study. The combination of 4 mM IBA and 2 mM NAA (3.9) produced fewer roots than 6 mM NAA (4.8), but more than 6 mM IBA (3.0).

Total Root Length. The main effects of replication, IBA and NAA were significantly affected total root length (Table 1). When IBA was applied, total root lengths increased with increases in auxin concentration from 344 mm in the nonauxin treated cuttings to 492 mm at 2 mM, and increased further to 601 mm at 4 mM. For NAA treated cuttings, the greatest total root length (570 mm) occurred in the nonauxin treated cuttings. At the remaining concentrations, total root lengths were 515 and 480 mm for 2 and 4 mM, respectively.

Root Symmetry. The main effects of replication and NAA significantly influenced root symmetry (Table 1). Root symmetry increased with increasing levels of NAA, ranging from 19% in the nonauxin treated cuttings to 45% at 4 mM.

Conclusion

This study examined factorial combinations of two auxin types (IBA and NAA) and three concentrations (0, 2, and 4 mM). For rooting percentage, the combination of IBA and NAA always induced a higher percentage than the nonauxin control and in a few situations was comparable to an equivalent concentration of a single auxin type. The combination of IBA and NAA had both positive and negative effects on primary root production. The combination of IBA and NAA produced fewer roots than NAA alone; however, generally produced more roots than IBA alone. The interaction of IBA and NAA did not significantly affect either total root length or root symmetry. Total root length was longest at 4 mM IBA and shortest at 4 mM NAA. Root symmetry was the greatest following an application of 4 mM NAA and was not significantly affected by IBA.

Table 1. ANOVA for traits assessed in a rooting trial of Virginia pine stem cuttings collected from 4-year-old stock plants.

	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine Square Root	Square Root	Square Root	Square Root	None	None
X Tranformation	None	Log	Log	None	Log	Log
Stumping	*	NS	NS	NS	NS	NS
Replication	*	NS	*	*	*	NS
IBA	*	NS	*	*	NS	NS
NAA	*	NS	*	*	*	NS
IBA x Stumping	NS	NS	NS	NS	NS	NS
NAA x Stumping	NS	NS	NS	NS	NS	NS
IBA x NAA	*	NS	*	NS	NS	NS
IBA x NAA x Stumping	NS	NS	NS	NS	NS	NS

*, NA Nonsignificant and significant at *P* ≤0.05, respectively.

Table 2**.** Rooting traits when three concentrations (0, 2, and 4 mM) of IBA and NAA were applied to Virginia pine stem cuttings, averaged across all stumping heights.

	Rooting (%)				**Primary roots (#)**			
	NAA Concn (mM)				**NAA Concn (mM)**			
IBA Concn (mM)	**0**	**2**	**4**	**Overall**	**0**	**2**	**4**	**Overall**
0	32	52	61	**48**	2.6	3.2	4.7	**3.4**
2	48	61	49	**53**	2.6	4.3	5.3	**4.1**
4	54	58	51	**54**	2.8	3.9	5.1	**3.9**
Overall	**45**	**57**	**54**	***52***	**2.7**	**3.8**	**5.0**	***3.8***