

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

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**Abstract.** Seven concentrations of IBA and seven concentrations of NAA plus a nonauxin control were tested over three growth stages to determine their effectiveness in promoting adventitious root formation on stem cuttings taken from 3- and 4-year-old stock plants of Fraser fir [*Abies fraseri* (Pursh) Poir.]. Cuttings were prepared 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, the number of primary roots and total root length varied in similar patterns across concentration, 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. Chemical names used: indole-3-butyric acid (IBA); 1-naphthaleneacetic acid (NAA).

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 its pleasing 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 the introduced 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 preservation and archival purposes as well as for 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 taken 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 taken 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 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 without artificial chilling failed to root or break bud (Miller, 1982). However, the chilling requirements for rooting this type of cutting are met more quickly than those for budbreak. Visible terminal bud activity was negligible for cuttings artificially chilled at 4 °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).

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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, therefore, no optimum auxin concentration has been reported for softwood stem cuttings. Therefore, the present study evaluated a wider range of NAA and IBA concentrations than previous studies for rooting all three types of cuttings. 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 seedlings were placed immediately into seedling bags, transported to Raleigh, N.C., on ice, and stored at 4 °C. For the first two rooting experiments, cuttings harvested on 17 Mar. 2001 (hardwood) and 29 June 2001 (softwood) were inserted into the rooting medium the day following collection. Semi-hardwood cuttings were removed 17 Oct. 2001 and cold-stored for 4 weeks before insertion into the rooting medium 13 Nov. 2001. Cuttings were taken from the primary axes of the seedlings to avoid the plagiotropic growth habit often present in cuttings from secondary or tertiary axes.

IBA and NAA were tested for their ability to stimulate adventitious rooting of cuttings of 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 stored at 4 °C in opaque bottles and used within 2 d of preparation.

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, 2002). Treated cuttings were air-dried for at least 15 min before being inserted to a depth of 3 cm into individual 10 cm<sup>3</sup> Ray Leach SC-1- Super Cells (Steuwe and Son, Inc., Corvallis, Ore.) containing a rooting medium of 3 horticultural perlite : 2 peat (v:v).

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 rate of mist application increased; however, mist application was less frequent during the night. Flow rate per mist cycle for this study was held constant over the 4-month rooting period at 0.05 L·m<sup>-2</sup>. 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 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. 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, 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 -1.0 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<sup>2</sup> value is reported for each dependent variable.

Despite employing the most appropriate data transformations and including squared terms for concentration, the least-squares re-

gression 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, 1999). The double exponential model (Rawlings et al., 1998), modified by two constants (A and B), was used:

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

where, e = base of natural logarithm, θ<sub>1</sub>, θ<sub>2</sub>, 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

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

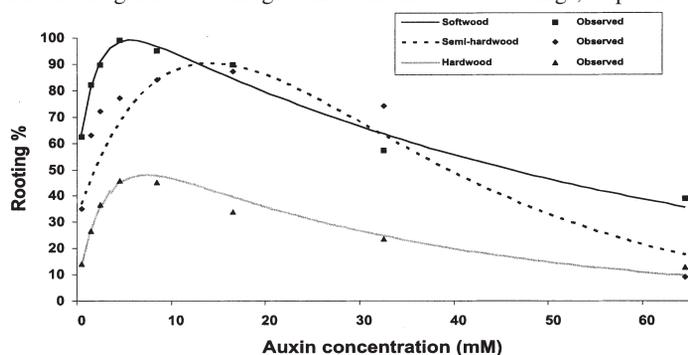
	Rooting (%)	Mortality (%)	Number of primary roots	Total root length	Root symmetry	Root angle
Y Transformation	Arcsine	Arcsine				
	Square root	Square root	None	Log	None	None
X Transformation	Square root	Log	Log	Log	Log	None
Growth stage	*	*	*	*	*	NS
Rep (Growth stage)	NS	*	NS	*	*	NS
Auxin	NS	NS	NS	NS	NS	NS
Growth stage × Auxin	NS	NS	NS	NS	NS	NS
Concn <sup>2</sup>	*	*	*	*	*	NS
C <sup>2</sup>	*	*	*	*	*	NS
Concn × Growth stage	*	*	*	NS	NS	NS
C <sup>2</sup> × Growth stage	*	*	*	*	*	NS
Concn × Auxin	NS	NS	*	*	NS	NS
C <sup>2</sup> × Auxin	NS	NS	*	*	NS	NS
Concn × Growth stage × Auxin	NS	NS	NS	NS	NS	NS
C <sup>2</sup> × Growth stage × Auxin	NS	NS	NS	NS	NS	NS

<sup>2</sup>Concn = auxin concentration, C<sup>2</sup> = auxin concentration squared.

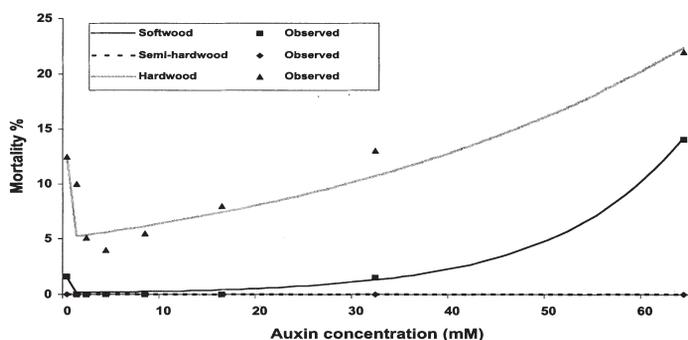
<sup>NS</sup>, \*Nonsignificant or significant at  $P < 0.05$ .

primary root production (Table 1). A significant interaction was 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. Regard-



	$\theta_1$	$\theta_2$	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



	$\theta_1$	$\theta_2$	A	B	$R^2$
Softwood	-0.0756	56.4883	-1.1963	-12.9914	0.99
Semi-hardwood <sup>z</sup>	-	-	-	-	-
Hardwood	-0.0230	22.4097	-0.0200	-1.435	0.88

<sup>z</sup>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 ( $\theta_1$ ,  $\theta_2$ , A, and B) are presented for the best model for each cutting type (see text for equation).

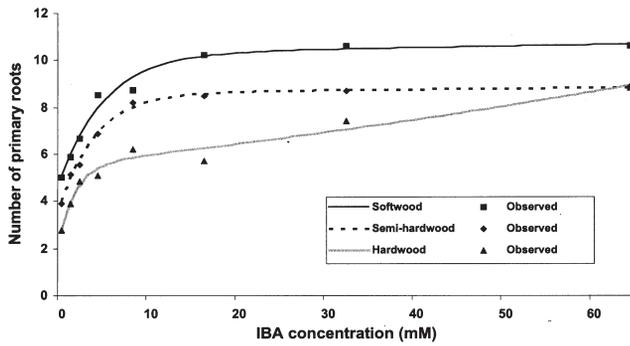
less of growth stage, primary root production increased rapidly at lower auxin concentrations and began to level off between 8 mM and 16 mM, increasing slightly to 64 mM. When hardwood cuttings were treated with NAA, the predicted number of primary roots increased from the control (3.1) to 8 mM (6.1) and then increased slightly with increases in NAA concentration up to the predicted optima 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.

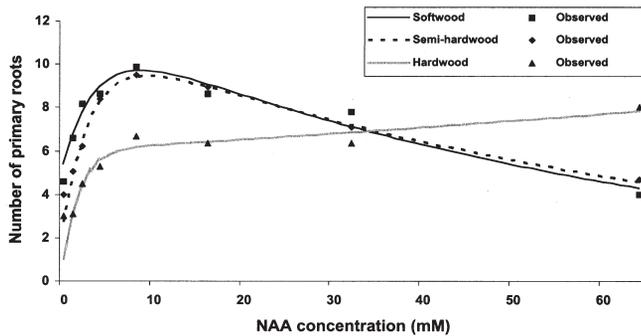
**Root symmetry.** Root symmetry was significantly affected by growth stage, replication within growth stage, concentration, concentration squared, and by the interaction between concentration squared and growth stage (Table 1). Because no significant differences were detected for auxin type and its 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;



	$\theta_1$	$\theta_2$	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



	$\theta_1$	$\theta_2$	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 ( $\theta_1$ ,  $\theta_2$ , A, and B) are presented for best model for each cutting type (see text for equation).

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 cutting 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 were diverted from root formation as shown 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 crown 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.

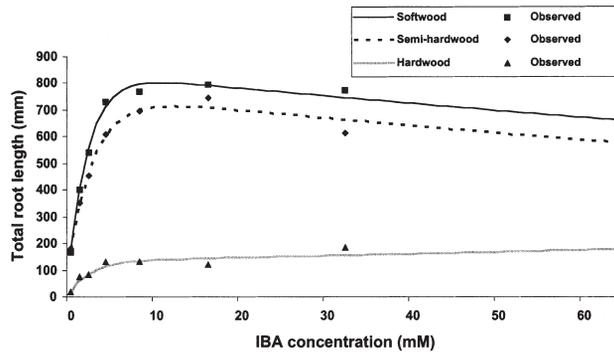
Hartmann et al. (2002) suggest that softwood cuttings may have higher metabolic activity and increased levels of endogenous auxin production than other types of cuttings. In the present investigation, softwood cuttings reached optimal rooting at a lower auxin concentration (5 mM) than the other two cutting 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 cuttings 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.

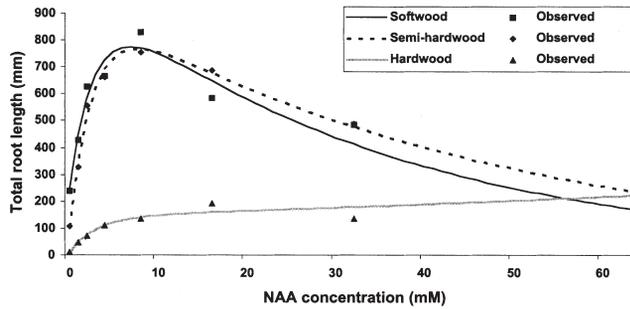
Hartmann et al. (2002) suggest that as the annual growing cycle progresses, endogenous auxin production begins to decrease and, therefore, higher levels of exogenous auxin are needed to promote optimal rooting. This is most apparent when the semi-hardwood cuttings are compared with the softwood cuttings, 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 (*Pinus taeda* L.) 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 (the inverse of percent mortality) 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 correlated

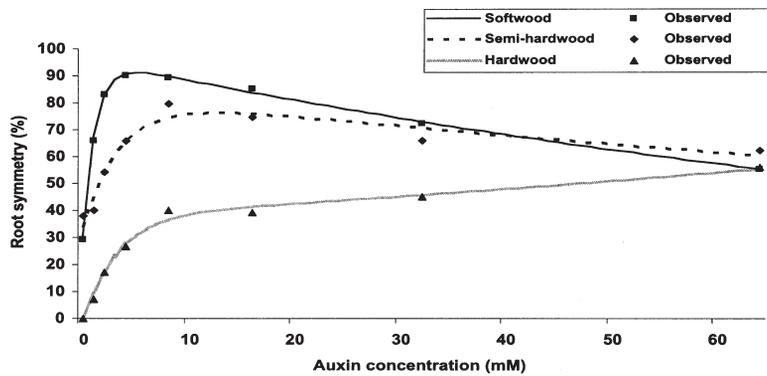


	$\theta_1$	$\theta_2$	A	B	$R^2$
Softwood	0.4340	0.00382	0.00149	1.2401	.98
Semi-hardwood	0.3424	0.00438	0.00174	1.3084	0.96
Hardwood	0.3850	-0.00443	0.00853	1.1612	0.88



	$\theta_1$	$\theta_2$	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 ( $\theta_1$ ,  $\theta_2$ , A, and B) are presented for the best model for each cutting type (see text for equation).



	$\theta_1$	$\theta_2$	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 concentrations are averaged over IBA and NAA.  $R^2$  values and estimated regression coefficients ( $\theta_1$ ,  $\theta_2$ , A, and B) are presented for the best model for each cutting type (see text for equation).

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 will 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  $\geq 16$  mM NAA or 32 mM IBA, the bases of hardwood cuttings 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 extended up 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, 2002). Four factorial combinations of two concentrations (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 herein.

In conclusion, stem cuttings taken 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.

#### Literature Cited

- Arnold, R.J., J.B. Jett, and S.E. Mckeand. 1994. Natural variation and genetic parameters in Fraser fir for growth and Christmas tree traits. *Can. J. For. Res.* 24:1480-1486.
- Blazich, F.A. and L.E. Hinesley. 1994. Propagation of Fraser fir. *J. Environ. Hort.* 12:112-117.
- Blazich, F.A. and L.E. Hinesley. 1995. Fraser fir: Two North Carolina researchers explain how to propagate this popular *Abies* species. *Amer. Nurseryman* 181:54-58, 60-65.

- Box, G.E.P. and D.R. Cox. 1964. An analysis of transformations. *J. Royal Stat. Soc. (Series B)* 26:211–246.
- Diaz-Sala, C., K.W. Hutchison, B. Goldfarb, and M.S. Greenwood. 1996. Maturation-related loss in rooting competence of loblolly pine stem cuttings: The role of auxin transport, metabolism and tissue sensitivity. *Physiol. Plant.* 97:481–490.
- Frampton, J. 2002. North Carolina's Christmas tree genetics program. Proc. 26<sup>th</sup> Southern Forest Tree Improvement Conf., Athens, Ga., 26–29 June 2001.
- Frampton, L.J., B. Goldfarb, S.E. Surles, and C.C. Lambeth. 1999. Nursery rooting and growth of loblolly pine cuttings: Effects of rooting solution and full-sib family. *Southern J. Appl. For.* 23:108–115.
- Goldfarb, B., W.P. Hackett, G.R. Furnier, C.A. Mohn, and A. Plietzch. 1998. Adventitious root initiation in hypocotyl and epicotyl cuttings of eastern white pine (*Pinus strobus*) seedlings. *Physiol. Plant.* 102:513–522.
- Greenwood, M.S., A.C. Harlow, and H.D. Hodgson. 1974. The role of auxin metabolism in root meristem regeneration by *Pinus lambertiana* embryo cuttings. *Physiol. Plant.* 32:198–202.
- Harlow, W.M., E.S. Harrar, J.W. Hardin, and F.M. White. 1996. Textbook of dendrology. 8th ed. McGraw-Hill, New York.
- Hartmann, H.T., D.E. Kester, F.T. Davis, Jr., and R.L. Geneve. 2002. Hartmann and Kester's plant propagation: Principles and practices. 7th ed. Prentice Hall, Upper Saddle River, N.J.
- Hinesley, L.E. and F.A. Blazich. 1980. Vegetative propagation of *Abies fraseri* by stem cuttings. *HortScience* 15:96–97.
- Hinesley, L.E. and F.A. Blazich. 1981. Influence of postseverance treatments on the rooting capacity of Fraser fir stem cuttings. *Can. J. For. Res.* 11:316–323.
- Hinesley, L.E. and F.A. Blazich. 1984. Rooting Fraser fir stem cuttings. *J. Environ. Hort.* 2:23–26.
- Miller, N.F., L.E. Hinesley, and F.A. Blazich. 1982. Rooting of Fraser fir cuttings: Effects of postseverance chilling and photoperiod during rooting. *Can. J. For. Res.* 12:607–611.
- Powell, G.R. 1982. Shoot and bud development in balsam fir: Implications for pruning of Christmas trees. *Forestry Chron.* 58:168–172.
- Rawlings, J.O., S.G. Pantula, and D.A. Dickey. 1998. Applied regression analysis: A research tool. 2nd ed. Springer-Verlag, New York.
- Rosier, C.L. 2002. Factors affecting rooting of stem cuttings of Fraser fir (*Abies fraseri*) and Virginia pine (*Pinus virginiana*). MS Thesis, N.C. State Univ., Raleigh.
- SAS Institute, Inc. 1999. SAS/STAT user's guide. Version 6, 4<sup>th</sup> ed. vols. 1 and 2. SAS Inst., Cary, N.C.
- Wise, F.C. 1985. Development of systems for vegetative propagation of Fraser fir by stem cuttings. PhD Diss., N.C. State Univ., Raleigh.
- Wise, F.C., F.A. Blazich, and L.E. Hinesely. 1985a. Propagation of *Abies fraseri* by softwood stem cuttings. *Can. J. For. Res.* 15:1172–1176.
- Wise, F.C., F.A. Blazich, and L.E. Hinesely. 1985b. Propagation of *Abies fraseri* by semi-dormant hardwood stem cuttings. *HortScience* 20:1065–1067.
- Zobel, B. and J. Talbert. 1984. Applied forest tree improvement. Wiley, Prospect Heights, Ill.