Vegetative Propagation
of 10-Year-Old Blue Spruce by Stem Cuttings[1]

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Abstract— Techniques for vegetative propagation of 10-year-old blue spruce (*Picea pungens* Engelm.) by stem cuttings were investigated. Month of collection of the cuttings and application of rooting hormones were examined. In addition, cutting position, cutting length and caliper were examined in relation to rooting response. December was the best collection date for rooting and root production. No hormone and 2500 ppm indole-butyric acid resulted in the highest rooting response. Hormone level and collection date interacted on root fresh weight. Shorter cuttings and cuttings from the lower two-thirds of the tree were more likely to root.

INTRODUCTION

Blue spruce (*Picea pungens* Engelm.) is used throughout the United States, primarily as an ornamental but also as a Christmas tree. The blue spruce's attractive natural form and broad ecological adaptiveness have made it a valuable ornamental. The natural range of blue spruce extends from southern Idaho to southern New Mexico. Hanover (1975) identified New Mexico sources as among the best for color development and rapid growth. Because distinct geographic ecotypes exhibit variations in form color and growth rates, which do not always breed true to type, the ability to vegetatively propagate superior trees would be advantageous in a tree improvement program.

Variable success has been reported in attempts to vegetatively propagate blue spruce. Hanover (1975) successfully rooted 85% of cuttings from seedlings 30 to 60 cm. tall. Rooting success of cuttings from 1-year seedlings varied from a low of 10% to a high of 80% (Struve, 1982). Thimann and DeLisle (1939, 1942) achieved 80% rooting success with cuttings taken in April from trees 10 to 20 years old. However, they had less success in November and no rooting in other months. Cultivars of blue spruce appear to root more reliably when cuttings are taken in January than in the summer months (Iseli and Howse, 1981). These differences may be the result of different growing environments and tree age. The consensus among growers appears to be, cuttings should be taken in late winter or early spring, and treated with rooting hormones to for maximum rooting response.

Seasonal variation in rooting response is a major factor in vegetative propagation. Season obviously plays a role in physiological conditioning of the stock plant, which in turn, affects the rooting response of the cutting. Lanphear and Meahl (1963) found root-forming capacity of cuttings from two evergreen species was seasonal, peaking in late fall and winter. This relationship could not be altered by the application of an exogenous root-promoting auxin. Norway spruce rooted best when cuttings were taken in April and May, just before or during budbreak (Girouard, 1975). The second best rooting was obtained from cuttings taken in October to November, when bud dormancy was not yet complete.

Unlike some species with preformed root initials, rooting in conifers requires the synthesis of root primordia. Endogenous factors are known to play a role in root primordia formation as well as root initiation and development. Smith and Thorpe (1975) identified two stages when the presence of auxin is essential in root initiation and development. The first stage is marked by the initial events leading to meristematic locus formation, and the second by events immediately preceding meristemoid development. A commonly accepted practice in vegetative propagation, particularly with the more difficult-to-root species such as conifers, involves the application of root-promoting hormones to compensate for a possible lack of endogenous levels of auxins.

In addition to differences in rooting ability described above, within tree differences are also seen. A phenomenon associated with aging is that tissues found at different locations on the same tree differ in juvenility. Paradoxically, tissues at the top of the tree are vegetatively mature, but are the youngest tissues in chronological age. Conversely, the oldest tissues found near the base of the tree tend to be more juvenile (Kester, 1976). Rootability has been related to crown position effects occurring in juvenile seedlings as well as sexually mature trees. Phillion and Mitchell (1984) found cuttings from the lower two-thirds of 15-month conifer seedlings rooted somewhat better than those harvested from higher positions, regardless of clone. However, the effect of

crown position on rootability was most pronounced among clones yielding rootable cuttings exclusively from the lower one-third of the crown. It was speculated, because this clone was the tallest, it might be less juvenile and the drop in rooting the result of this difference in height, and perhaps greater maturity.

The objectives of this study encompassed several aspects, most of which involved techniques of propagating blue spruce. The primary objective was to determine if rooting success of cuttings differed among several collection dates. Secondary objectives included quantifying the effects of rooting hormone and their effect on root initiation and development. Tertiary factors of interest were the effect of cutting position, initial cutting length and basal stem caliper on rooting response.

MATERIALS AND METHODS

Stock plants were selected from a blue spruce provenance study planted in 1978, at the Mora Research Center, Mora, New Mexico. All trees were unsheared and the plot was thinned to a 1.7 m by 2 m spacing the previous year. No fertilizer had been applied during the previous growing season, but had been uniformly applied in 1985.

Because of the number of cuttings available from each tree, six collection dates were selected in an attempt to identify optimum collection date. Previous work conducted elsewhere (Hanover 1975) indicated blue spruce roots best from late winter to early spring. Collection dates were selected to permit harvest at 4-week intervals beginning in December 1986. Cuttings were harvested: December 20-21, 1986; January 17, 1987; February 14, 1987; March 13, 1987; April 19, 1987; and May 8, 1987.

Indolebutyric acid (IBA), a synthetic auxin, was used to determine if rooting potential could be altered by treatment with exogenous auxins. Three hormone levels were selected along with a control (no hormone). The four treatments were control, 2500 ppm IBA, 5000 ppm IBA and 10,000 ppm IBA.

Each tree was randomly assigned to one of the four hormone levels initially. The study required 12 cuttings from each tree. Cuttings harvested from each tree at the assigned intervals received the same hormone treatment throughout the course of the study to eliminate tree-to-tree variation. Two cuttings were taken from each tree at each collection time. For each collection, 240 cuttings were stuck, with 60 cuttings for each hormone level. A total of 1440 cuttings were used in the study.

Cuttings were harvested in the same manner each collection. Entire primary lateral shoots with terminal buds were harvested. Cuttings included only the growth produced the previous growing season. Tree height was recorded for each tree before cuttings were taken. As each cutting was taken, the vertical distance between the ground and the point of stem severence was recorded (cutting height). In the laboratory, the basal end of the cuttings were recut at a 45° angle, and old wood, if present, was removed. Initial cutting length and basal stem caliper were measured and recorded after recutting.

Cutting lengths varied from 3.7 cm to 12.5 cm; all cuttings longer than 12.5 cm were cut at 12.5 cm. Needles were not stripped from the base of the cuttings.

Cuttings were then treated with a 5-second quick dip of the stem basal 2 cm in the preselected hormone treatment. The three hormone treatments containing indolebutyric acid (IBA) were dissolved in 50% isopropyl alcohol. Control cuttings were dipped in a 50% alcohol solution. After drying for 10 minutes; the basal portion of the cuttings were dipped in a 1:1 Captan fungicide/talc mixture. Treated cuttings were immediately stuck in 160 cm^3 polyethylene containers (Ray Leach tubes) containing a 1:1 vermiculite/ perlite mix (v/v), to a depth of approximately 2.5 cm.

Cuttings were then placed on a propagation bench, which was a mist bench system with bottom heat. The mist bench is a modification of the wet tent system designed by Whitcomb et al. (1982). Bottom heat was provided by a Biotherm® heating system at 20°C.

Relative humidity was kept high by the using two independent systems to apply moisture to the bench. A 100% polyester fabric draped over a pitched metal frame attached to the top of the bench provided the enclosure for maintaining relative humidity. The polyester fabric allowed air to circulate through the tent walls while keeping the humidity high. An automated track-mounted boom located 1.0 m above the bench was controlled by an automatic clock timer. The boom contained nine fan-type nozzles to provide uniform mist to the cuttings. Speeds and frequency were adjusted as needed to maintain humidity 65% +/- 10%. In addition, a fog system above the tent was controlled by an evaporative leaf moisture meter. The fog system kept the tent wet and helped maintain the humidity in the propagation bench. Cuttings were fertilized with Hoaglands complete nutrient solution applied with a hand applicator. Cuttings were fertilized three times a week to compensate for the effects of leaching from the mist applications.

Cuttings were removed from the bench after 20 weeks. Treatment blocks were removed individually, and all measurements were made within 72 hours of removal from the bench. If cuttings were removed in advance of evaluation, they were placed in a walk-in refrigerated cooler at 4°C. All cuttings were destructively sampled.

For each cutting the following attributes and measurements were recorded:
Cutting condition; dead, alive, callused or rooted; shoot elongation; shoot measurements; shoot length, shoot caliper, and shoot green weight;
root measurements; primary root number, primary root length, secondary root number (total), secondary root length (sum), tertiary root number (total), tertiary root length (sum) and total root fresh weight (primary and associated root total).

Primary roots were defined as originating from the cut end of the cutting, or callus tissue (if present). After evaluation, cuttings and roots were oven-dried at 65°C +/- 3°C for 60 hours.

The experimental design was a split-plot design. The whole plot treatment design was a 3 (source) X 4 (hormone) factorial. Collection date was the split factor. Cutting height to tree height ratio, final cutting length,

basal stem caliper and cutting fresh weight were used as covariates. Statistical analyses were done using analysis of variance techniques (GLM, SAS Institute, 1985). Range tests were done using the Student-Newman-Keuls test. Discrete data were analyzed using categorical model analysis (chi-square tests) and logistic regression. For analysis, the total number of primary roots was used for each rooted cutting. Root length and root fresh weight were totaled to give a single value for each cutting. Height ratio was a variable created by taking the ratio of cutting height over the total tree height.

RESULTS

Rooting response

After restricting cutting condition classification to only two categories, rooted and not rooted, a categorical model analysis (SAS Institute, 1985) was used to test for significant effects. Overall rooting was low, with 107 cuttings out of 1440 rooted (7%). Collection date and hormone level showed significant effects on rooting response. Collection date was significant with a χ^2 value of 6.26 (probability < 0.0001). By collection date, rooting was highest in December at 15%, followed by 13% in February. There was a drop in rooting in January, and overall rooting declined steadily after February (fig. 1). Hormone level was significant at the 10% level ($\chi^2 = 6.26$; probability = 0.0996). Best treatments were the control and 2500 ppm IBA with rooting percentages of 9% and 11%. Rooting success dropped at the higher levels of IBA (fig. 2).

Logistic regression was used to analyze rooting response in relation to height ratio, initial cutting length, initial caliper and initial fresh weight. The natural logarithm of the ratio of the probability of not rooting over the probability of rooting (called the logit response) was used as a response, and the slope and intercept of the
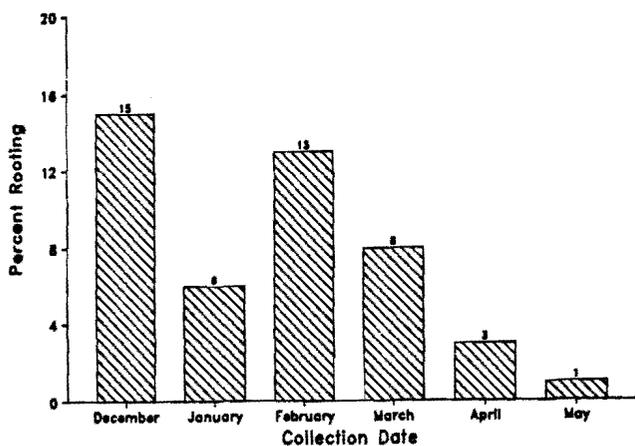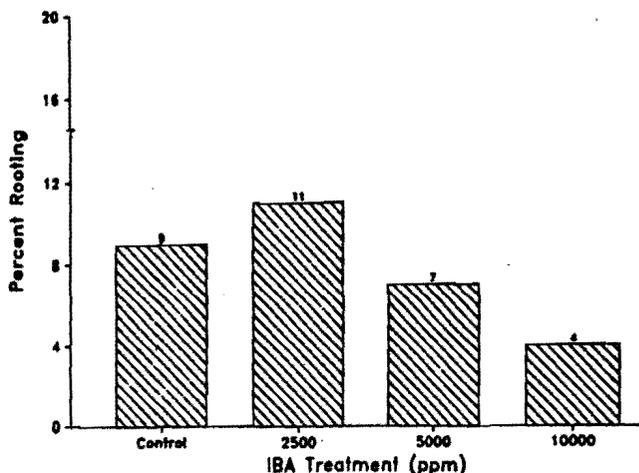
PERCENT ROOTING BY IBA TREATMENT

Figure 2.--Percent rooting by hormone treatment of blue spruce cuttings. Based on 360 cuttings/treatment. $\chi^2 = 4.65$, PR < 0.0979.

line representing the relationship were calculated. Only height ratio and initial cutting length had detectable effects on rooting response. The relation between rooting and height ratio was seen when the height ratio data was transformed into categorical data (table 1). Vertical position of the cutting on the tree did influence rooting success. The chi-square test of independence showed rooting and cutting height are not independent factors. Cuttings from the lower third of the tree had rooting rates that exceeded the expected value.

There was a significant relation between initial cutting length and rooting ($\chi^2 = 54.95$, probability < 0.0001). This relationship was positive and had a slope of 0.337, so as cutting length increased the probability of rooting decreased (fig. 3). Probabilities of rooting were generated using this relationship. For the shortest cutting, 3.7 cm, the estimated probability of rooting was 40%. The probability of rooting dropped significantly to 3% as cutting length increased to a maximum of 12.5 cm.

PERCENT ROOTING BY COLLECTION DATE

Figure 1.--Percent rooting of blue spruce cuttings by collection date. Based on 240 cuttings/collection date. $\chi^2 = 6.26$, PR < 0.0001.

Table 1.--Frequency of rooting by height ratio. Height ratio = cutting height/tree height.

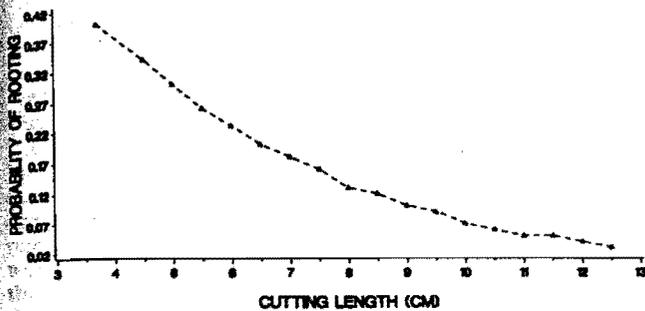| | Height ratio | | |
	0 - 0.33	0.34 - 0.66	0.67 - 1.00
No rooting			
Observed	29	696	608
Expected	35	702	596
Rooting			
Observed	9	62	36
Expected	3	56	48

Figure 3.--Logistic regression of probability of rooting and initial cutting length (cm). LN $(P0/P3) = -0.8317 + 0.3369 * (X)$. $P0$ = Probability of not rooting, $P3$ = Probability of rooting, X = initial cutting length.

Root analysis

For the analysis of root data, three variables were used as indicators of root quality. Root fresh weight, number of primary roots and root length were analyzed. Because of low rooting response for the April and May collections, those dates were omitted from further analyses of root data. Root data were analyzed using GLM (SAS Institute, 1985). Due to the small number of rooted cuttings, normality assumptions were not met and, while significant affects may be determined, p values may not be accurate.

Root fresh weight

Differences in root fresh weight were detected among collection dates. Root fresh weight was analyzed using the Student-Newman-Keuls test. December cuttings showed greater mean root weight than the other collection dates, which did not differ significantly. December cuttings had a mean root fresh weight of 0.124 g. The mean fresh weight for cuttings taken in March was 0.055 g, and 0.053 g for cuttings taken in February. January cuttings had the lowest root fresh weight with a mean of 0.028 g (table 2). Collection dates were significantly different with respect to root dry weight, which followed the same pattern as fresh weight. There were no significant interactions between root fresh weights by hormone level.

Differences were seen in root fresh weight in hormone by collection date (fig. 4). Examining the collection date by hormone interactions, the greatest root biomass production was the December sampling with the control treatment. Among December cuttings, biomass production decreased as hormone level increased. After December, IBA at the 2500 ppm level resulted in greater fresh weight production by treatment until March when 5000 ppm IBA resulted in a slightly higher fresh weight for the cuttings. Rooting among cuttings receiving the 5000 ppm level peaked in March, whereas the control and 2500 ppm IBA peaked in December. Rooting among cuttings that received 10,000 ppm IBA was low for all dates, but increased slightly in February.

Table 2.--Root fresh weight, number of primary roots and total root length by collection date. Numbers are averages of all 240 cuttings/collection date, including cuttings that did not root. Analyzed using Student-Newmans-Keuls test. Means in a column followed by the same letter are not significantly different. (α = 0.05)

Collection date	Root fresh weight (g)	No. primary roots	Root length (cm)
December	0.12454 A	0.6684 A	118.0 A
January	0.02805 B	0.1991 B	38.0 A
February	0.05257 B	0.3673 AB	24.0 A
March	0.05514 B	0.4615 AB	124.0 A

Primary root number

Differences in primary root number were seen among collection dates. Lack of normality was a problem in the analysis because of large numbers of missing values. Differences were seen between cuttings taken in December and January, but no differences were found among December, Feburary and March cuttings, or January, February and March cuttings (table 2). However, mean number of roots did drop for cuttings taken after December. The range test (Student-Newman-Keuls) did not indicate these in total number of primary roots by hormone level. This was probably the result of unequal sample sizes.

Height ratio was the only covariate with a significant effect on mean number of primary roots. The negative slope estimate of -1.61 indicates, as height ratio increased, number of primary roots decreased. Cuttings harvested from positions higher on the tree initiated fewer primary roots.
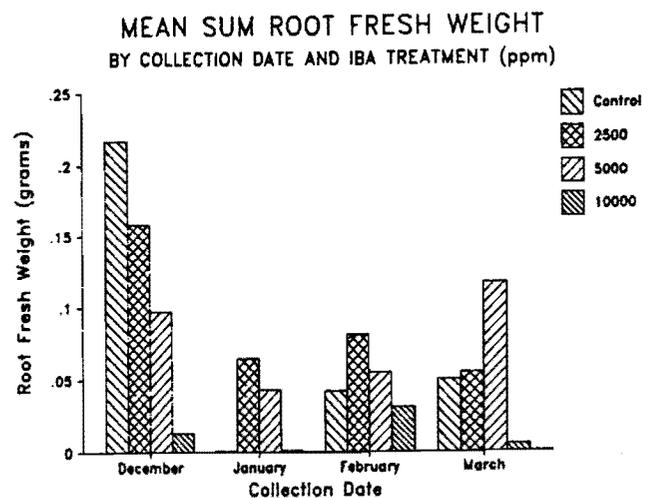
MEAN SUM ROOT FRESH WEIGHT
BY COLLECTION DATE AND IBA TREATMENT (ppm)

Figure 4.--Blue spruce root fresh weight (g) of all cuttings by collection date and hormone level (ppm IBA).

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Root length

Root length for each cutting was analyzed using GLM (SAS Institute, 1985). Differences were seen by collection date, and collection date by hormone level by source. However, the Student-Newman-Keuls range test indicated no significant differences for collection date. This again was probably the reslt of unequal sample sizes. However, examining the means, March cuttings had a mean length of 124 cm., while February cuttings showed a mean length of 24 cm. December cuttings showed a similar response to March cuttings with a mean of 118 cm. January cuttings had a mean root length of 38 cm (table 2). There were no significant differences in root length among hormone levels.

DISCUSSION

Collection date

Although overall rooting success was low, it is possible to root stem cuttings from 10-year-old blue spruce. Collection date was the single factor significant for almost every response variable analyzed.

The best collection date was December, followed by February. The drop in rooting of cuttings taken in January was not expected. The expected rooting percentage was somewhere between the 15% in December and the 13% in February. One explanation is that January was not a favorable time for root initiation in stem cuttings. Perhaps there was some difference among December, January and February which favored root initiation among cuttings taken in December and February but not in January.

Except for the drop in January, it appears the best time to take blue spruce stem cuttings is during winter months, after the chilling requirement has been met (as determined by budbreak in the greenhouse). Rooting dropped off among cuttings collected in the late winter to early spring months, which had been recommended by some researchers (Hanover 1975). These results differ from studies indicating Norway spruce roots well in April and May (Girouard, 1975). However, Fraser fir, as well as other conifer species, roots well in January and February (Hinesley and Blazich, 1984). Thimann and DeLisle (1942) found blue spruce rooted best in April, with some rooting in November. But Iseli and Howse (1981) have more consistent rooting with blue spruce in January.

Hormone level

The best treatment for rooting success was the control followed by 2500 ppm IBA. However, there was little difference between the control and IBA at the 2500 ppm level. In another study with blue spruce cuttings taken in March, there were no differences in rooting responses of cuttings from 10-year trees when treated with no hormone, 2500 ppm IBA or 5000 ppm IBA. Cuttings from 1-year seedlings however, rooted at higher levels, with 100% rooting when treated with 5000 ppm IBA.

Cuttings from 15-year old trees, on the other hand, had the highest rooting response (29%) when treated with 10,000 ppm IBA (Wagner unpublished data). Although the collection date by hormone treatment interaction was not significant in terms of rooting success, examination of the data indicates the control treatment in December and 2500 ppm IBA in February are the best treatment by collection date combinations. These results differ from other blue spruce studies indicating higher levels of auxins are necessary to promote rooting (Hanover, 1975; Struve, 1982)

Cutting characteristics

Shorter cuttings are more likely to root than are the longer cuttings. This may be, in part, be related to overall stock plant condition, which favors shoot extension to the detriment of rooting capacity. Farrar and Grace (1942) found some differences in rooting of Norway spruce. They found shorter cuttings may have higher success in rooting, but that shortening longer excised cuttings was of no benefit. Fraser fir, however, showed no effect of cutting length on rooting percentages, but longer cuttings tended to initiate more and longer roots (Miller et al., 1982). The question arises whether rooting success results from the growth form that involves short terminal shoots on the branches, a higher inherent rooting capacity, or a coincidental relationship with cutting length. Another possibility could be that shoot length is a function of crown position.

Cuttings from the lower portion of the tree were more likely to root. Phillion and Mitchell (1984) found similar differences, even in juvenile black spruce cuttings; cuttings from the lower two-thirds of the crown rooted better. There was no significant difference found between cutting stem caliper and cutting fresh weight and the probability of root intitiation in this study.

Root production

Several factors affected root quality. Collection date was significant for all root characteristics analyzed. As discussed above, rooting success was highest among cuttings taken in December, followed by February. December cuttings also showed the highest root biomass production, followed by March. Maximum number of primary roots was highest in December cuttings, followed by February, and root length was greatest in March cuttings, closely followed by cuttings taken in December. Overall, rooting percentages were relatively high for cuttings taken in February, but root quality was less optimal than in December. Considering all the factors of rooting and root quality, an overall recommendation would be to take blue spruce cuttings in December.

Root fresh weight was the only root characteristic to exhibit a significant collection date by hormone level interaction. Root fresh weight changed with hormone level as the season progressed. In December, the highest biomass production was seen with the control level. Cuttings made in January and February showed increased production with 2500 ppm IBA, and March cuttings showed the highest root fresh weight with 5000 ppm IBA.

Conclusions

In summary, the single factor that overrode every variable examined was collection date. Hormone treatment altered somewhat the inherent rooting capacity, but could not completely compensate for non-optimum collection date. Within a tree, cutting position and length do appear to influence rooting success. Overall, a general recommendation for rooting of 10-year-old blue spruce would be to take short cuttings from the lower portion of the tree in December with no hormone treatment. From the results, 10-year-old blue spruce does not appear to be easily mass propagated from field-grown stock plants. However, on a limited scale, such as propagation of superior trees for a breeding program, it would be possible to successfully propagate clones *en masse*, but perhaps with a narrowing of the genetic base.

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