Lifting Date Influences the Ability of Auxins to Promote Root Regeneration of Colorado Spruce[1]

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Abstract

The inability of older conifer seedlings to regenerate new roots after bare-root transplanting can lead to plant death. Lifting date and auxin treatments were evaluated for their effects on root regeneration of Colorado spruce (*Picea pungens* Engelm.). Roots of five-year-old plants were treated monthly during fall and spring with 1000, 5000, or 10,000 mg/L of indole-3-butyric acid (IBA), 5000 mg/L of phenyl indole-3-thiolobutyrate (P-ITB), or 100% ethanol (control). After treatment, plants were grown in a greenhouse for 30 days before total number, length and dry weight of new roots were determined. Spruces treated with 1000 and 5000 mg/L of IBA in November had the greatest new root weight (1.4 g) and number (5000), respectively, per plant compared to 0.1 g and 200 roots per plant for controls. Plants dug in May produced the least number, weight and length of new roots for all treatments. Plants treated with P-ITB produced consistently greater numbers of new roots over the first three lifting dates than did controls. Absolute ethanol appeared to inhibit root regeneration. Data indicate that lifting date affected the ability of auxin to stimulate root regeneration on bare-root Colorado spruce.

Index words: bare root, ethanol toxicity, IBA, indole-3-butyric acid, P-ITB, phenyl indole-3-thiolobutyrate, *Picea pungens*

Introduction

Severe root loss during digging and transplanting causes plant stress. Even when approved nursery practices are followed, as little as 5% of the root system may remain on a transplanted tree (23, 25). This acute root loss greatly reduces the absorbing surface of the root system, decreases root/shoot ratio and is a common cause of death (9). Plants capable of developing new roots after transplanting are most likely to survive because they can avoid water stress (8). To compound the transplanting problem, root regeneration becomes more difficult as plants age (22).

Important factors affecting seedling root regeneration are its physiological condition and the time of year. Generally, conifer seedlings lifted during the middle of winter form more new roots than those lifted during fall or spring (14). In contrast, *Picea glauca* (Moench) Voss and *Picea mariana* (Mill.) BSP in Canada lifted in fall or spring formed more roots than those lifted during winter (14). Root regeneration capacity of some conifers declines sharply as shoots begin to elongate in the spring (1, 18).

Auxins can promote root formation on cuttings (6) and seedlings (7). IBA (indole-3-butyric acid), a synthetic auxin, promotes root formation on rooted cuttings of *Magnolia* × *soulangiana* Soul.-Bod. (11) and *Pinus contorta* Dougl. seedlings (16), whereas root numbers on *Picea jezoensis* (Siebold and Zucc.) Carriere. seedlings are unaffected by auxins (3). Another synthetic auxin, P-ITB (phenyl indole-3-thiolobutyrate), is an aryl ester of IBA and promotes adventitious root regeneration on *Quercus rubra* L. seedlings (19, 20). An inherent problem with high auxin concentrations used for promoting root formation is the high alcohol concentrations needed to dissolve the growth regulator.

In this study, lifting date and auxin treatments were evaluated for their effects on root regeneration of 3-2 Colorado spruce. Five-year-old plants were used in these experiments since older seedlings are generally harder to transplant and are less responsive to the root promoting effects of auxins than younger seedlings (7). P-ITB was included in this study since it promotes adventitious root initiation on jack pine cuttings (4, 5) but has yet to be tested on older conifer seedlings. Two concentrations of ethanol were also used during the spring months of this study to determine if the solvent was toxic to Colorado spruce roots.

Materials and Methods

Five-year-old (3-2) Colorado spruce (*Picea pungens* Engelm.) were dug at a commercial nursery on October 9, and November 6, 1985 and March 28, April 25, and May 22, 1986. Lifting was discontinued during the winter due to frozen soil but resumed in March when the soil thawed. All plants were lifted from one uniform bed that had been divided into blocks, and each block was randomly assigned to a specified month. Trees were hand dug and roots were shaken free of soil before the root system was dipped in water and wrapped in moist burlap. Burlap bundles were placed in plastic bags, sealed and held at ambient temperatures while being transported. Trees were then held overnight in cold storage at 2°C (36°F). The following day, any remaining soil was washed from the roots and damaged plants were discarded. All plant roots were pruned to a length of 18 cm (7.1 in) from the root collar. New white roots were removed and ten plants randomly assigned to each treatment.

Roots of each plant were sprayed with either an auxin dissolved in absolute (100%) ethanol or with absolute ethanol alone (control) for a total of five treatments. Auxin treatments included IBA (Aldrich Chemical Company, Milwaukee, WI) at 1000, 5000, or 10,000 mg/L (4.9 mM, 24.6 mM, or 49.2 mM, respectively) and P-ITB at 5000 mg/L (16.9 mM). The P-ITB was supplied by Dr. Jack Gaines, South Dakota School of Mines and Technology, Rapid City,

[1]Received for publication March 7, 1989; in revised form June 5, 1989. Idaho Agricultural Experiment Station Research Paper No. 88955. We thank Plato Nursery, Bonners Ferry, ID, for their cooperation during this study, Dr. Jack Gaines for supplying the P-ITB and Dr. Bahman Shafii for his statistical advice.

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SD. Absolute ethanol was used as the solvent for the various treatments due to the inherent insolubility of P-ITB (personal communication from Dr. Jack Gaines) and the high concentrations of IBA. For the spring lifting dates, another treatment, 1000 mg/L of IBA in 20% ethanol (v/v), was added to the study. This treatment was used to test the effects of IBA on root regeneration while reducing any detrimental effects of the solvent. The 20% ethanol solution was selected as the lowest concentration which kept 1000 mg/L IBA in solution; lower concentrations of ethanol caused the IBA to precipitate.

Roots were sprayed to the point of runoff, and the plants were potted in #2 plastic pots with a 2:1 (by vol) mixture of Sunshine mix No. 1 (75% peat, 15% perlite, 10% vermiculite, Fisons Western Corp., Vancouver, B.C., Canada) and a coarse grade of horticultural perlite. Pots were placed on a greenhouse bench in a split-block experimental arrangement with treatment as the main plot, lifting date as the sub-plot and with 10 single tree replicates. The trees received a 16-hr photoperiod from high-pressure sodium lamps, and the plants were grown for 30 days at day/night temperatures of 27°/18°C (81°/64°F), resp. (12, 13). After the test period, the trees were moved to cold storage at 2°C (36°F) until the root system of each plant could be examined.

Plant roots were gently washed free of potting medium, and total number, length and dry weight of new roots determined and used as indicators of root growth potential (RGP, ref. 14). All new roots emerging from the epidermis of older roots were counted and placed in one of four length categories. These categories were 0 to 4 mm, 4 to 10 mm, 10 to 20 mm, and greater than 20 mm (0 to 0.16 in, 0.16 to 0.39 in, 0.39 to 0.79 in and greater than 0.79 in, resp.). Roots longer than 20 mm (0.79 in) were measured individually, whereas the length of roots in each category was calculated by multiplying the number of roots in the category times the mean length of that category [i.e., the 0 to 4 mm (0 to 0.16 in) category had a mean length of 2 mm (0.08 in)]. Total length of all new roots was determined by adding the lengths from each category. New and old roots as well as shoots were dried separately in an oven for 72 hr at 70°C (158°F) and weighed.

Data for root and shoot dry weights, root length and total numbers of roots were subjected to analysis of variance (Procedure GLM, ref. 15). Treatment means were compared by Fisher's LSD test ($p \leq 0.05$) if the interaction between treatment and month was significant. When testing for the effects of ethanol concentration on root regeneration, the interaction between month and treatment was not significant, and hence, the treatment means were calculated by combining months (March and April) and then separated by an LSD test ($p \leq 0.05$).

Results and Discussion

Root regeneration of Colorado spruce varied significantly with lifting date. Length, number and dry weight of new roots (Figs. 1, 2 and 3) were greatest for plants dug in November and treated with 1000 or 5000 mg/L IBA. Root growth potential was moderate during October and March and was lowest during April and May. New root length, number and dry weight in May were not significantly greater than zero. Dry weights of shoots and old roots were similar over all months and between treatments (data not shown).

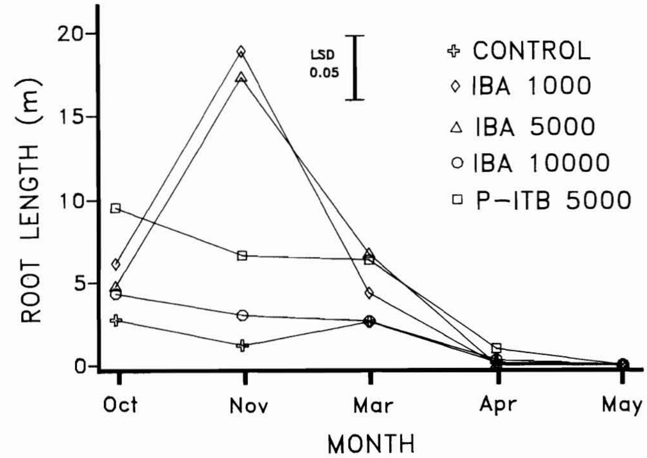

Fig. 1. Mean lengths of new roots on 3-2 Colorado spruce 30 days after treatment. Each month represents the digging dates used in this study. Absolute ethanol was used as the control treatment and the solvent for the auxin treatments. Data points are means of ten plants. Error bar indicates significant differences between means according to a protected Fisher's LSD test ($p < 0.05$).

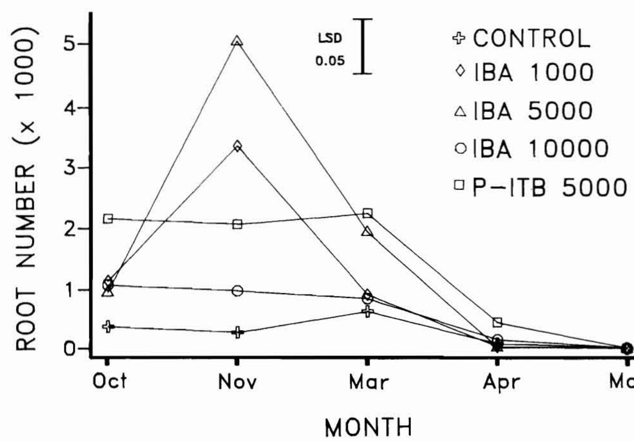

Fig. 2. Mean numbers of new roots on 3-2 Colorado spruce 30 days after treatment. Months, treatments and statistics are as described in Fig. 1.

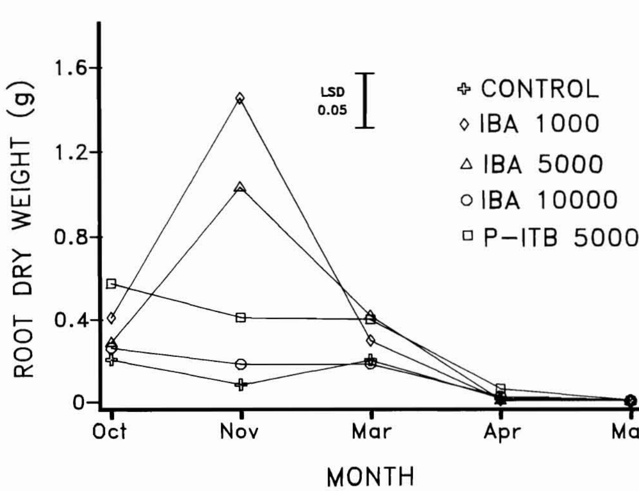

Fig. 3. Mean dry weights of new roots on 3-2 Colorado spruce 30 days after treatment. Months, treatments and statistics are as described in Fig. 1.

The spring decline in root regeneration may have been related to shoot activity during this period since April plants were dug just after the buds started to expand; the buds on the May plants had already expanded 1 to 6 cm (0.4 to 2.4 in) when they were dug. This pattern of moderate RGP on Colorado spruce during late fall and early spring and poor root regeneration during active shoot growth has been documented for other conifers such as *Pseudotsuga menziesii* (Mirb.) Franco (17, 18) and *Pinus taeda* L. (1). The April and May reductions in root growth of Colorado spruce may have been due to the competition between roots and newly expanded shoots for carbohydrates within the plants (14).

Concentrations of 1000 and 5000 mg/L IBA increased RGP on Colorado spruce although their effects varied with lifting date (Figs. 1, 2 and 3). Plants treated with 1000 and 5000 mg/L of IBA had similar lengths of new roots in November, yet these values were at least 2.6 times greater than the next best auxin treatment (P-ITB) which was also significantly greater than controls that month (Fig. 1). In addition, plants lifted in November and treated with 1000 mg/L of IBA produced the greatest number of new root dry weight (Fig. 3), whereas those treated with 5000 mg/L of IBA produced the greatest number of new roots (Fig. 2). Root growth potential of plants treated with 10,000 mg/L of IBA was similar to that of controls throughout the study.

Although 1000 and 5000 mg/L of IBA and P-ITB generally promoted RGP during October, November and March, these treatments were ineffective during April and May (Figs. 1, 2 and 3). Therefore, it appears that exogenously applied auxins had less influence over RGP than did other endogenous factors during this stage of plant development. Shoot phenology (1) and carbohydrate content in roots during active shoot growth (14, 24) have been reported to exert strong control over RGP of plants.

Based on new root weight and length, 1000 mg/L of IBA appeared to promote the greatest RGP on Colorado spruce in November compared to the higher auxin concentrations tested. Plants treated with 1000 mg/L of IBA also lacked callus or swollen tissues in their root systems. Both of these characteristics indicate that plant tissues have received too much auxin (6), and these symptoms were found on roots of plants treated with P-ITB and the two higher IBA concentrations.

Plants treated with P-ITB produced 5.9, 7.8 and 3.6 times more new roots than controls in October, November and March, respectively (Fig. 2). However, these plants had a significantly lower RGP in November than those treated with the lower IBA concentrations. In contrast, Struve and Rhodus (20) reported that 1-0 red oak seedlings treated with equivalent concentrations of IBA and P-ITB had similar numbers of new roots, and Struve and Arnold (19) reported that P-ITB increased the number of new roots formed on 3-0 red oak seedlings compared to plants treated with an equivalent concentration of IBA. Since 5000 mg/L of P-ITB caused callusing and swelling of root tissues on Colorado spruce, a lower concentration of this compound may be more effective for promoting RGP of this species.

Absolute ethanol apparently affected RGP of Colorado spruce in March and April (Table 1). We observed darkened roots and root damage on October and November plants regardless of the treatment applied. However, Mullin (10) reported blackening of white spruce roots was associated with their exposure during transplanting without additional root treatments.

Table 1. Mean lengths, numbers and dry weights of new roots on 3-2 Colorado spruce 30 days after lifting in March and April.

Treatment	Root length (m)	Root number	Root dry weight (g)
100% ethanol	1.38 a[z y]	338 a	0.10 a
1000 mg/L IBA in 100% ethanol	2.18 a	453 a	0.15 a
1000 mg/L IBA in 20% ethanol	5.32 b	1684 b	0.34 b

[z]Each value is a mean of twenty plants, ten dug in March and ten dug in April.

[y]Means within columns followed by different letters are significantly different ($p < 0.05$).

To check for potential solvent problems, roots on four atypical plants not used in the lifting experiments were sprayed with distilled water and handled in the same manner as the other plants in October and November. Root systems of these plants appeared similar to each other, and their new root growth averaged 1880 roots, 15.4 m (50.5 ft) total length and 1.0 g dry weight per plant for both months. This amount of new root growth appeared greater than that of plants receiving any other treatments during October, but root growth of spruces treated with the two lower IBA concentrations in November was comparable or better than that of the water-treated plants. Although the new root growth on water-treated plants cannot be statistically compared to that of plants receiving other treatments, this information emphasizes the apparent toxicity of absolute ethanol. Root growth on water-treated plants also serves as a benchmark of natural root regeneration inherent to the experimental plant material. In contrast with the fall observations, root regeneration of plants lifted during March, April and May and treated with water appeared similar to that of plants receiving other treatments in the spring.

To further test the effects of ethanol concentration on RGP, a treatment of 1000 mg/L of IBA in 20% ethanol was added to the experiment for the spring lifting dates. Root growth potential of plants treated with IBA in 20% ethanol was compared against RGP of spruce treated with absolute ethanol in the presence or absence of 1000 mg/L of IBA. May data for RGP was excluded from this analysis since their values were not significantly greater than zero. Plants treated with IBA in 20% ethanol had at least 2.4, 3.7 and 2.3 times the amount of new root length, number and dry weight, respectively, than those treated with absolute ethanol alone or combined with IBA (Table 1). Only roots 0 to 4 mm (0 to 0.16 in) long were significantly affected by absolute ethanol alone or combined with IBA (data not shown). Plants treated with IBA in 20% ethanol had over four times as many roots 0 to 4 mm (0 to 0.16 in) long (average of 1566 roots per plant) than those treated with IBA in 100% ethanol or absolute ethanol alone (average of 338 and 295 roots per plant, respectively).

Consequently, plant establishment may be hindered by absolute ethanol since the number of small roots drastically decreased which in turn decreased root absorbing area (8). Delayed establishment can lead to plant mortality (8, 14).

As an example, most 3-2 Colorado spruce that were dug bare root in May and treated with absolute ethanol with or without 1000 mg/L of IBA died (100% and 80%, respectively) within 6 weeks of field planting (21). These results contrast sharply with the outplanting response of spruces whose roots were treated with water or 1000 mg/L of IBA in 20% ethanol since almost all of them (100% and 90%, respectively) survived field planting.

The Colorado spruce plants used in this study maintained the ability to produce new roots; although being five years old, they were older than most seedlings used in studies of this type. The mean number of new roots formed on each plant (averaged from October through March and over treatments) was 1208 (Fig. 2), indicating these five-year-old spruces were capable of regenerating their root systems after transplanting. Similarly, Fuchigami and Moeller (3) found four- and six-year-old Colorado spruces that were dug as bare-root plants readily formed new roots.

Significance to the Nursery Industry

Lifting date affects the ability of auxin to enhance root regeneration of 3-2 Colorado spruce. Auxins can enhance root regeneration of spruce lifted bare root in the fall, but their benefits were minimal when applied during spring transplanting. Nursery trials involving the use of auxins to promote root regeneration of Colorado spruce should include the lowest possible concentration of ethanol to limit its phytotoxicity. Alternative solvents such as isopropyl alcohol or dimethyl sulfoxide can be tried or the potassium salt of IBA may be used. Based on the results of our study, the amount of ethanol used in auxin treatments could be reduced since the IBA or P-ITB concentration should be less than 5000 mg/L to promote root regeneration of this species.

(*Ed. note*: This paper reports the results of research only, and does not imply registration of a pesticide [auxin] under amended FIFRA. Before using any of the products mentioned in this research paper, be certain of their registration by appropriate state and/or federal authorities.)

Literature Cited

1. DeWald, L.E. and P.P. Feret. 1987. Changes in loblolly pine root growth potential from September to April. Can. J. For. Res. 17:635–643.

2. Dirr, M.A. 1981. Rooting compounds and their use in plant propagation. Proc. Intern. Plant Prop. Soc. 31:472–479.

3. Fuchigami, L.H. and F.W. Moeller. 1978. Root regeneration of evergreen plants. Proc. Intern. Plant Prop. Soc. 28:39–49.

4. Haissig, B.E. 1979. Influence of aryl esters of indole-3-acetic and indole-3-butyric acids on adventitious root primordium initiation and development. Physiol. Plant. 47:29–33.

5. Haissig, B.E. 1983. N-phenyl indolyl-3-butyramide and phenyl indole-3-thiolobutyrate enhance adventitious root primordium development. Physiol. Plant. 57:435–440.

6. Hartmann, H.T. and D.E. Kester. 1983. Plant Propagation. Fourth Ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.

7. Kling, G.J. 1984. Root regeneration techniques. Proc. Intern. Plant Prop. Soc. 34:618–627.

8. Kozlowski, T.T. and W.J. Davies. 1975. Control of water balance in transplanted trees. J. Arboriculture. 1:1–10.

9. Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, New York.

10. Mullin, R.E. 1963. Planting check in spruce. For. Chron. 39:252–259.

11. Perkins, L.M. and G.J. Kling. 1987. Root regeneration in *Magnolia* × *soulangiana* and *Magnolia* × 'Betty' in response to auxin applications. HortScience. 22:889–891.

12. Ritchie, G.A. 1984. Assessing seedling quality. p. 243–259 *In:* M.L. Duryea and T.D. Landis (Editors). Forest Nursery Manual: Production of Bareroot Seedlings. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Netherlands.

13. Ritchie, G.A. 1985. Root growth potential: principles, procedures and predictive ability. p. 93–105 *In:* M.L. Duryea (Editor). Evaluating Seedling Quality: Principles, Procedures and Predictive Abilities of Major Tests. Oregon State University, Corvallis, OR.

14. Ritchie, G.A. and J.R. Dunlap. 1980. Root growth potential: its development and expression in forest tree seedlings. N.Z. J. For. Sci. 10:218–248.

15. SAS Institute, Inc. 1985. SAS/STAT guide for personal computers—Version 6. SAS Institute, Inc., Cary, NC.

16. Selby, C. and D.A. Seaby. 1982. The effect of auxins on *Pinus contorta* seedling root development. For. 55:125–135.

17. Stone, E.C. and J.L. Jenkinson. 1971. Physiological grading of ponderosa pine nursery stock. J. For. 69:31–33.

18. Stone, E.C. and G.H. Schubert. 1959. Root regeneration by ponderosa pine seedlings lifted at different times of year. For. Sci. 5:322–332.

19. Struve, D.K. and M.A. Arnold. 1986. Aryl esters of indolebutyric acid increase root regeneration in 3-0 red oak seedlings. Can. J. For. Res. 16:673–675.

20. Struve, D.K. and W.T. Rhodus. 1988. Phenyl indole-3-thiolobutyrate increases growth of transplanted 1-0 red oak. Can. J. For. Res. 18:131–134.

21. Tripepi, R.R. and J.E. Carter. 1989. Growth and quality of Colorado spruce transplanted during the spring growth flush. J. Environ. Hort. 7:____.

22. Watson, G.W. 1985. Tree size affects root regeneration and top growth after transplanting. J. Arboriculture. 11:37–40.

23. Watson, G.W. and E.B. Himelick. 1982. Root distribution of nursery trees and its relationship to transplanting success. J. Arboriculture. 8:225–229.

24. Watson, G.W. and E.B. Himelick. 1982. Seasonal variation in root regeneration of transplanted trees. J. Arboriculture. 8:305–310.

25. Watson, G.W. and T.D. Sydnor. 1987. The effect of root pruning on the root system of nursery trees. J. Arboriculture. 13:126–130.