THE EFFECTS OF GROWTH MEDIUM ACIDITY, EXOGENOUS GROWTH REGULATORS, AND NITROGEN FERTILIZER ON THE ACCELERATION OF FRASER FIR SEEDLING GROWTH

by

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(ABSTRACT)

Three factors were tested in an attempt to accelerate the growth of Abies fraseri seedlings grown in containers in a greenhouse: growth medium acidity, foliar application of growth regulators, and supply of nitrogen fertilizer.

Sphagnum peat growth medium was adjusted with 0 to 8 kg dolomitic limestone/m^3 compressed packaged peat to initial pH ranging from 3.9 to 6.7. Best growth (21.2 cm mean height at 19 months) was achieved with 1 kg/m^3 and 2 kg/m^3, with initial mean pH 4.2 and 4.5, respectively. Seedlings with 4 and 8 kg/m^3 grew more slowly (17.4 and 9.5 cm, respectively, in 19 months), and many were chlorotic, with poor root development.

Three growth regulators were sprayed on seedlings: benzylamino-purine (BAP), gibberellic acid (GA_3, and indolebutyric acid (IBA). BAP stimulated terminal bud activity, decreasing the periods of rest between active growth. BAP increased height and diameter of new shoot growth up to 19% and 32% respectively. BAP reduced root growth 22% when applied at the higher concentration (100 ppm). GA_3 had no main effect on either shoot or root growth. However, GA_3 did increase

shoot growth slightly in the absence of BAP. IBA increased root growth up to 26%. IBA had no significant effect on shoot growth.

Nitrogen fertilizer was supplied weekly in concentrations of 200, 400, and 600 ppm. At six months age there was no treatment effect on seedling growth. At nine months age 400 ppm N had produced 9% more shoot growth than either 200 or 600 ppm N. Nine-month-old seedlings with 400 ppm fertilizer averaged 8.9 cm in height.

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INTRODUCTION

Fraser fir (Abies fraseri (Pursh) Poir.) seedlings grow extremely slowly for several years. Brief periods of growth alternate with long periods of quiescence or dormancy, a growth pattern suited for survival of the rapid weather changes of Fraser fir's mountainous native habitat. Seedlings stay briefly in the succulent and vulnerable condition of active growth.

This mountain-top survival mechanism proves expensive and frustrating to growers, however. In a nursery bed, Fraser fir seedling growth before bud formation is both brief (two to four months) and short (two to four centimeters). Both the fixed growth (elongation of preformed needles) and the free growth of non-preformed needles and internodes (Jablanczy 1971a, Pollard and Logan 1976) are completed in this burst of growth. Nursery bed seedlings generally require three or four growing seasons to reach the height of seven to ten centimeters, the height required for out-planting (Barnett and Brissette 1986; Seiler 1985). Goodwin (1975) reported 4-year-old (2-2) transplants with an average height of 12.2 cm. Hinesley (1981) reported 5-year-old (3-2) nursery seedlings 30-50 cm in height.

Use of a greenhouse to extend the growing season, with control of environmental factors such as light duration and intensity, temperature, water, nutrient availability, carbon dioxide concentration, and supply of exogenous growth regulators (Hanover et al. 1976) can provide an accelerated growth program for container-grown seedlings (Tinus et al. 1974, Gulden and Barnett 1982). An accelerated growth program for Fraser fir might decrease the three year period before outplanting in lineout beds to twelve or eighteen months, while providing larger and more vigorous seedlings (Appendix 1).

If grown in a greenhouse with no interruption, however, Fraser fir soon develops abnormalities such as large terminal buds, loss of terminal dominance, terminal bud abortion, or misshaped leaders and branches. An accelerated growth program without interruptions in growth has not yet been developed for Fraser fir seedling production.

Given the apparent need for interruptions in growth, several requirements for an accelerated growth program for container- grown Fraser fir seedlings have been established. Warmth in winter (Hinesley 1981), interrupted by 4 to 6 weeks of spring and fall chilling to break dormancy (Hinesley 1982.), and increased duration and intensity of light (Hinesley 1982; Seiler and Kreh 1986), can enable two growth periods lasting most of the year. Initial trials have been made to establish the range of nitrogen requirements for accelerated growth (Black 1980, Seiler and Kreh 1986, Seiler unpublished data). Results of fertilizer trials that had taken place at high pH needed to be reviewed for applicability within acceptable pH ranges.

Other important growth factors yet to be established for Fraser fir were the optimum pH range in organic growth medium and the possible effects of exogenous growth regulators on Fraser fir's slow growth habit. Growth medium acidity has long been recognized as an important factor in plant growth (Leyton 1952), but the needs of Fraser fir had not yet been established. It was thought that investigation of the hormone balance of a plant with such prompt and persistent dormancy might prove especially useful, not only for the acceleration of the growth of Fraser fir, but also for understanding some of the puzzling effects of growth regulators.

This study is an attempt to more nearly approach the upper practical limits of Fraser fir seedling growth by establishing the desirable range of growth medium pH, testing foliar application of growth regulators for increasing the duration and degree of seedling growth, and adjusting the levels of nitrogen fertilizer. Growth increases are evaluated by measures of increased height, shoot weight, and root weight, as well as maintenance of active growth and terminal dominance. The specific objectives of this research are:

(1) to compare the effects of varied levels of organic growth medium acidity on Fraser fir seedling growth;

(2) to evaluate the effects of a cytokinin (benzylaminopurine), gibberellic acid, and an auxin (indolebutyric acid) on terminal bud activity and growth of Fraser fir seedlings; and

(3) to quantify the effects of nitrogen fertilizer on Fraser fir seedling growth.

LITERATURE REVIEW

Growth Medium Solution Acidity

Solution acidity of the medium surrounding plant roots affects all of the physiological systems of the plant directly or indirectly. Acidity influences the availability of various minerals in the solution (Lucas and Davis 1961; Pritchett 1979), as well as the ability of the plant to absorb available nutrients, both cations and anions (Epstein 1972). Hydrogen ion concentration activates or deactivates enzymes and membrane proteins, and thereby determines plant nutritional status and cellular activity (Poole 1978). Root growth including growth of root hairs is affected by pH, root growth being arrested at pH unacceptable to a given species (Moore 1974). Regulation of plant pH appears to require active H^+-ion transport across the plasma membrane and tonoplast (Sze 1984), a balance of carboxylating and decarboxylating reactions (Davies 1973), and differential uptake of NO_3^- or NH_4^+ (Raven and Smith 1976). Plant pH regulation requires expenditure of energy (Raven 1985), and greater amounts of energy are presumably required to the extent that soil solution pH varies from optimum pH for the plant. Acidity of growth medium also affects soil micro-organism populations, both pathogenic and beneficial.

Fraser fir growers have used various pH levels in organic growth medium. No analysis of pH effects has been found. Black (1980) amended organic medium of peat and vermiculite with approximately 2.9

kg of dolomitic limestone per cubic meter to adjust acidity to a pH of approximately 5.5. Hinesley (1981) amended a growth medium of 4 Canadian peat : 2 vermiculite : 1 coarse sand : 1 perlite : 1 composted pine bark (by volume) with 2.4 kg of dolomitic limestone per m^3, without reporting the resulting pH. Hinesley (1982) reported good results with a 1:1 mix of peat and vermiculite, with no mention of limestone amendments. For some years the Virginia Tech Department of Forestry has grown Fraser fir seedlings in a commercial horticultural potting mixture of peat and vermiculite modified with dolomitic limestone to an advertised pH of 5.5. Actual pH had not been determined prior to this study.

Various general pH recommendations are given for growing conifers in organic media. Tinus and McDonald (1979) recommend a target pH of 5.0 to 6.0 for container-grown conifer seedlings in organic growth medium. Barnett and Brisette (1986) suggest that sphagnum peat provides a favorably low pH, and state that the optimum growth for most conifer seedlings occurs in the range of 5.0 to 6.0 in mineral soil, or slightly lower in organic growth medium.

Benzian (1966) had found a more acidic soil generally preferable in conifer nurseries in Great Britain. While finding that a specific and generally narrow range was suitable for individual conifer species, she found this range was usually somewhere between 4.5 and 5.0. Her studies found that conifers were often stunted at higher pH. It is generally recommended that plants may be grown at a lower pH in organic medium than in mineral soil (Lucas and Davis 1961).

Systematic determination of desirable pH for Fraser fir growth in mineral soil is apparently not available as a reliable guideline, however. Several recommendations are given in manuals for growing Fraser fir in mineral soil, without an indication of how the recommendations were developed. A Forest Service bulletin (Anonymous 1958) states that the soils best suited for this species are acid (about pH 3.5 to 5.5), and that planting on old pastures that have been limed in the past five years should be avoided unless the soil is determined to be acid by testing it. Huxter and Shelton (1983) state that experience indicates that soil with high organic matter content and pH 4.9 to 5.5 is excellent. In the Forest Service's Manager's Handbook for Balsam Fir, Johnston (1986) states that the closely related balsam fir (*Abies balsamea* (L.) Mill.) is most common on cool, wet to mesic sites with strongly to moderately acid soils with pH 5.1 to 6.0. The basis for this assertion was not made clear, and a recommendation for favorable pH range was not given.

In conjunction with fertilizer trials on young balsam fir, Bruns (1973) applied lime as CaO at low and high levels (0.45 lbs. and 1.05 lbs. per tree, respectively) on ten stands in northern New Hampshire and Vermont. Trees receiving lime in either amount had significantly less leader growth than control trees. Because Bruns did not report on either pre- or post-treatment soil pH, applicability of his results is limited. However, his results indicate the possibility of negative effects of lime amendments to young fir trees.

Timmer, Stone and Embree (1977) applied dolomitic limestone at the rate of 2.27 kg per young balsam fir tree growing in Nova Scotia, in mineral soil with high organic matter content, at pH 3.7. They found no significant effects on tree growth over a two year period. However, lime was applied only to trees receiving no fertilizer treatments, and the combined effect of lime treatment and application of nitrogen, which was found to be the limiting growth factor in their studies, was not tested. This study is another indication, however, that liming may not be required for fir tree growth.

Earlier research had reported beneficial effects from liming balsam fir in mineral soil. Jablanczy (1971b) states that balsam fir seedlings in the Maritime Provinces can grow in soil with acidity below pH 4.0 but also above pH 8.0. He reports optimum growth at pH around 5.5, but concludes that the desired rate of lime application for the conditions considered is not known. His results are not consistent with those of Timmer, Stone and Embree (1977) and Bruns (1973).

Liming has been found to retard height growth in other conifers. Shortleaf pine (Pinus echinata Mill.) showed a nearly linear decrease in height with an increase in the amount of lime. With growth measured over a five year period (Gilmore 1972). Amidon et al.(1982) studied the pH effects of commercial potting mixes on longleaf pine (Pinus palustris Mill.). Seedling growth was decreased and seedling mortality due to disease increased at the higher pH of these mixes. Barnett and Brissette (1986) generalize from this and other studies that commercial horticultural potting mixes are unsuitable for conifers unless the pH has been specially reduced to levels more nearly optimum for conifers. They suggest that the appropriate pH range for container grown conifers in organic potting medium should be 5.0 to 6.0. Brix and von den Driesche (1974) suggest that conifer rooting medium pH should usually be adjusted somewhat lower, in the range of 4.5 to 5.0. Combination of the general conifer recommendations of Barnett and Brissette (1986) with those of Brix and von den Driessch (1974) gives an acceptable pH range of 4.5 to 6.0.

In contrast to these broad general suggestions for pH ranges for conifer growth, Leyton (1952), working with Sitka spruce (*Picea sitchensis* (Bong.)Carr.), demonstrated that the optimum pH range for conifer seedling growth may be rather narrow. It therefore seemed that it might be important to determine whether the pH range for Fraser fir is broad or narrow, and, especially if it is narrow, what the required pH range for Fraser fir is.

Fraser fir's native soils are very acidic. Cain (1931) found the ridgetop soils in the Great Smoky Mountains had average pH of 2.9 to 3.6. Liu (1971) states that the types of soils on which Fraser fir grows generally range from pH 4.0 to 5.5. The source of this information was not apparent, but may have been derived from general soil type maps. Wolfe (1956, as cited in Bailey 1985) provides a soil profile of the spruce-fir ecosystem, indicating that the pH at the surface of mineral soil is about 4.0, ranging to 4.5 in the upper 20 cm of soil, which is the maximum exploited root zone for seedlings.

Fraser fir, a native of environments with acidic soils, might have adaptations permitting growth in highly acidic organic growth medium. The present study tests the hypothesis that Fraser fir seedlings in organic growth medium grow equally rapidly at the low pH of their native environment and in physiologically more neutral solutions versus the alternate hypothesis that they grow most rapidly in a narrow range of pH.

Application of Growth Regulators

The slow early growth of Fraser fir seedlings results from prompt bud formation and cessation of budbreak. After germination and an initial burst of growth, few if any growth flushes occur prior to winter chilling in the natural environment. In the outdoor nursery bed as well, after an initial period of growth, seedlings require a chilling period before resuming normal growth. With the provision of extra light, heat, and fertilizer in a greenhouse, in an accelerated growth system, recurrent flushes of growth may continue, sometimes for several months. However, continued cycles of growth without chilling are eventually followed by loss of apical dominance, failure of internodes to elongate, and inhibition of bud break or development of various growth abnormalities (Hinesley 1982). Inhibition of budbreak is decreased by long photoperiod combined with supplimentary heat and nutrients but dormancy, once established, appears to be satisfactorily broken only by chilling (Hinesley 1982). Menser et al. (1987) found that subalpine fir (<u>Abies</u> <u>lasiocarpa</u> (Hook.) Nutt.) also required an induction period of short days and cold temperatures to stimulate renewed flushing of seedlings after several months of accelerated growth.

Several exogenous growth regulators, including cytokinins, gibberellins, and ethylene, have been observed to break dormancy in various species, but since different exogenous hormones break dormancy of different species, the effectiveness of type and concentration of growth regulator depends on the species (Wareing and Phillips 1981, Powell 1987). Little (1984) found that foliar spray of BAP promoted lammas growth in 5- and 6-year-old balsam fir. In planning the present study, it was reasoned that a growth regulator which would promote lammas growth in a closely related species might also promote budbreak in germinant Fraser fir seedlings. Little and Loach (1975) had previously found that foliar application of GA_3 accelerated the start of leader elongation in 4- and 5-year-old balsam fir seedlings. Since both cytokinins and gibberellins appear to be involved in natural promotion of bud burst (Powell 1987) and had been found to promote bud break in related species, the present study made use of foliar sprays of cytokinin and gibberellin to explore promotion of bud break in Fraser fir.

Like budbreak, internodal elongation varies greatly between Fraser fir seedlings, regardless of nutrient regime or other environmental input: some seedlings appear to have an internally regulated ability to elongate more than others, and a given seedling's rate of growth may vary considerably from year to year, fluctuating in comparison to other seedlings in similar environmental conditions. The question therefore arose whether the exogenous supply of growth regulators might lead to increases in stem elongation. Both auxins and gibberellins had been found to promote internodal elongation in various species (Thimann 1977, Wareing and Phillips 1981). Little and Loach (1975) had found that foliar spray of GA_3 increased elongation of terminal and lateral shoots and needles in 4- and 5-year-old balsam fir seedlings. Therefore, both a gibberellin and an auxin were tested for stem elongation.

A third effect of growth regulators considered was the possible effect on root growth. Reallocation of plant resources to stems can be detrimental to root growth. For example, the root-weight to shoot-weight ratio was decreased by application of GA_3 to balsam fir even in relatively older (4- and 5-year-old) trees (Little and Loach (1975). Auxins have been found to promote initiation of lateral roots (Thimann 1936, Simpson 1986). Therefore it was decided to test an auxin for its effects on Fraser fir seedling root growth in the present study.

Most growth regulators have several naturally occurring and synthetic forms, some more stable, more active, or more effective in entering plant tissue than others. Naturally occurring cytokinins have frequently been found less stable and therefore less effective in exogenous application than a variety of synthetic cytokinins, of which benzylaminopurine (BAP, also named benzyladenine or BA) has found the widest use. Of the gibberellins, gibberellic acid (GA_3) has been found in many conifers (Pharis and Kuo 1977) and is often effective in exogenous applications. Both BAP and GA_3 are available commercially, and were therefore selected for initial trials. Of the auxins, the naturally occuring indole-3-acetic acid (IAA) has been found too unstable for positive effects in many circumstances, so the more stable indole-3-butyric acid (IBA) was used.

Supply of Nitrogen Fertilizer

Apart from the provision of acceptable amounts of light, air, water and growing temperatures, there may be no single factor which affects the growth of seedlings as much as nitrogen nutrition. Nitrogen affects all seedling growth systems. Cellular activities of plants are dependent on nitrogen in numerous ways: genetic material, enzymes, other proteins, the systems of energy transfer, chlorophyll and growth regulators all require nitrogen (Goodwin and Mercer 1983, Lehninger 1975).

Nitrogen is frequently the factor limiting plant growth. Increased growth resulting from added nitrogen may be nearly linear where nitrogen is in short supply. Above a certain level of availability, additional nitrogen is luxury consumption, of limited or no benefit to plant growth. Supplied above the level of luxury consumption, nitrogen becomes toxic to the plant.

Due to the small volume of medium in which they grow, container-grown seedlings must be provided a steady supply of nutrients throughout the growing season. The amount of nitrogen needed depends on the physiology of the species, the plant's rate and stage of growth, and environmental conditions including growth medium cation exchange capacity and water-holding capacity, as well as water quality at a given site (Brix and van den Driesche 1974, Tinus and McDonald 1979).

Nutrients may be supplied as slow-release fertilizers or as granular top dressing in the containers. Most tree growers use soluble fertilizers added to irrigation water or sprayed over the seedlings (Barnett and Brissett 1986). Required concentration of fertilizers varies with the frequency of application. Liquid fertilizer applications may occur as often as plants are irrigated, or as seldom as once a week (Brix and van den Driessche 1974).

Successful acceleration of Fraser fir seedling growth would be indicated by increased seedling height and root and shoot dry weight, without toxic effects. This may occur through an increase in either the extent or duration of growth.

In studying the effects of nitrogen on Fraser fir seedling growth, Black (1980) supplied 0, 150, or 300 ppm nitrogen as $(NH_4)_2SO_4$ or $NaNO_3$) and, due to the small amount of fertilizer supplied (2 ml per week) achieved a linear height increase in each series (with greater increase from NH_4^+ than from NO_3^-). From these results, there is no reason to believe that Black had applied enough nitrogen to maximize seedling growth.

Given in amounts which would saturate the rooting medium, the concentration of nitrogen required to mazimize growth could be either lower or higher than those used by Black (1980). Barnett and

Brissette (1986) recommend that initial nitrogen concentration for growing southern pines in containers should be in the broad range of 125-625 ppm, until more specific information is available.

Holding phosphorus and potassium constant at non-limiting concentrations (87 ppm phosphorus and 166 ppm potassium) Seiler and Kreh (1986) found that 10 ml of 200 or 308 ppm nitrogen per seedling per week produced more growth in 36 weeks than nitrogen applied at higher rates. However, a heavily limed commercial horticultural potting mix was used, and high pH must be considered a possible factor in limiting seedling ability to utilize higher nutrient concentrations. The present study attempts to determine the nitrogen concentration at which Fraser fir seedling growth is maximized, as well as the concentration at which luxury consumption or toxic levels of nitrogen are reached, when seedlings are grown in a suitably acidic medium.

ADJUSTMENT OF GROWING MEDIUM ACIDITY OF CONTAINER GROWN FRASER FIR

Introduction

In November, 1986, a group of 14-month-old Fraser fir seedlings grown in commercial horticultural potting mix were growing slowly and appeared in puzzlingly poor health. Most were chlorotic. Budbreak after chilling was slow, sporadic, and lacking accustomed vigor. Many seedlings had lost terminal dominance or suffered terminal bud abortion. Seedlings appeared diseased and mortality was high. In exploring the reasons for the seedlings' lack of health and vigor, the acidity of the growth medium was one factor considered. The hypothesis was raised that Fraser fir, native of southern Appalachian mountaintops with soil of granitic parent material, might be adapted to growth at a lower pH.

The potting mix being used, a combination of sphagnum peat, vermiculite, and perlite, was labeled at pH 5.5, but was later found to have a pH of 6.5, which persisted for over a year. Telephone communication with a company representative confirmed our guess that the potting mix had been amended with about 8 kg dolomitic limestone per cubic meter.

Growing medium acidity has long been recognized as important to successful conifer growth (Leyton 1952). The suitable pH ranges of conifer species are often narrow (Benzian 1966). Nevertheless, pH often goes unreported in nursery studies, and is sometimes ignored by researchers and growers alike. In some nurseries, commercial horticultural potting mixes are used interchangeably with peat for growing conifers. However, the change in pH from 3.9 (new sphagnum peat) to 6.5 (potting mix) is a vast environmental change for the plant, even though invisible and not always convenient to measure.

In a search of the literature, no pH recommendations for Fraser fir (*Abies fraseri* (Pursh.)Poir.)) were found, for either organic media or mineral soil. Recommendations of pH for closely related balsam fir (*Abies balsamea* (Linn.)Mill) are few, contradictory, and apparently limited to seedlings grown in mineral soils. However, a clue to possible pH requirements was found in Goodwin's (1975) report that the best growth of Fraser fir after 48 weeks, 5.8 cm average height, occured in sphagnum peat and vermiculite compared to an average height of 4.1 cm in Pro-Mix B, a commercial horticultural potting mix of sphagnum peat and vermiculite with supplimental nutrients and limestone. While pH was not reported in Goodwin's study, the added limestone in Pro-Mix B was the only obvious factor which might be suspected as a possible cause of decreased growth.

Excess liming has been implicated in stunted conifer seedlings damaged by pathogenic fungi in mineral soil (Benzian 1966, Gilmore 1972). Pawuk (1981) found that shortleaf pine (*Pinus echinata* Mill.) seedlings grew better in a 50:50 peat: vermiculite mixture at pH 5.4 than in a similar mixture amended to pH 6.4. Longleaf pine (*Pinus palustris* Mill.) seedlings grew equally rapidly at either pH, but seedlings grown at the higher pH suffered more mortality due to pathogens.

The purpose of this study is to compare the effects of five dolomitic limestone amendments to an acidic organic growth medium, sphagnum peat, on the growth of Fraser fir seedlings, and thereby to determine the acceptable pH range for Fraser fir seedlings grown in organic medium. The study tests the hypothesis that Fraser fir seedlings in organic growth medium grow equally rapidly at the low pH of their native environment and in physiologically more neutral solutions versus the alternate hypothesis that they grow most rapidly in a narrow range of pH.

Materials and Methods

Canadian sphagnum peat was amended with 0, 1, 2, 4, and 8 kg dolomitic limestone per cubic meter compressed packaged peat, as suggested by Chrustic and Wright (1983), placed in 8 inch root-trainer books (Spencer-Lemaire Industries, Ltd., Edmonton, Canada), and thoroughly watered. Two hundred forty 2-month-old seedlings were transplanted from a commercial horticultural potting mix into the five limestone treatments in root-trainer books in each of 6 trays.

Seedlings were grown in a greenhouse with photoperiod extended to sixteen hours by the provision of supplimental sodium vapor lighting (approximately 150 $\mu M/m^2s$ photosynthetic photon flux density at the level of the seedlings). The greenhouse was heated to a minimum of 19° C, and ventilated when temperatures exceeded 22° C. Summer temperatures in the greenhouse occasionally exceeded 40° C. Seedlings were watered as needed to maintain sufficient moisture for continuous growth: once or twice daily on hot sunny days, as little as once in three days in cloudy, cool weather. Ten ml liquid fertilizer was provided to each seedling weekly during the growing season, at the rate of 200 ppm nitrogen, 87 ppm phosphorus, and 166 ppm potassium, supplied as 20-20-20.

At eleven and sixteen months age, supplimentary light and nutrients were withheld for one month. Growth was then interrupted for two six-week chilling periods in a room refrigerated to 3° C, after which seedlings were returned to the greenhouse growing conditions described above. Thus, seedlings had completed three periods of growth by the end of the experiment in May, 1988, when they were 20 months old.

Growth medium acidity was measured by a displacement method as described by Wright (1987). Seedling heights were measured to the nearest millimeter at the completion of each growth period. Seedlings were severed at the root collar at harvest, and dried at 60° C to constant weight, for determination of root and shoot weights.

A randomized complete block design was used in this experiment, with six blocks. Size-dependent variables were transformed logarithmically before regression analysis, in order to provide more uniformity of variance between small and large seedlings.

Results and Discussion

The dolomitic limestone treatments caused an initial range of pH from 3.9 to 6.8 (Table 1). With all limestone treatments, growth medium became more acidic during the course of the experiment. However, the 8 kg limestone/m^3 peat treatment maintained a pH of 6.4 even after 17 months (Table 1), comparable to the commercial potting mix (data not shown). By the time of harvest, the most heavily limed peat (8 kg/m^3) had decomposed considerably. Leachate was dark, with many particles of peat. The leachate of the three lowest lime amendments (0,1, and 2 kg/m^3) remained transparent yellow at the end of the experiment.

Within the first growth cycle, after 8 months in the amended peat growth media, two trends became apparent. First, the addition of a small amount of limestone was beneficial to seedling growth. Second and much more important, the addition of large amounts of lime was detrimental to seedling growth (Table 2). By the end of the first growth cycle, heights of seedlings growing in the 8 kg/m^3 treatment (pH 6.4 to 6.9) were 21% shorter than any others, and 24% shorter than those with 1 kg/m^3 (pH 3.5 to 4.2) (Table 2).

During the following growth cycle, the heavily limed seedlings were slow to break bud and became chlorotic. It was during the second growth cycle that seedlings in peat with 1 kg/m^3 distinguished themselves, growing an average 8.4 cm compared to 6.9 cm of any other treatments, and compared to 3.0 cm of the heavily limed seedlings (Table 2). The superiority of the 1 kg/m^3 seedlings over seedlings

Table 1. Mean pH* of sphagnum peat amended with dolomitic limestone.

Lime added	Months since planting in Sphagnum peat		
kg/m^3	3 months	8 months	17 months
	(pH)		
0	3.9 **	3.9 **	3.4 **
1	4.2	4.1	3.5
2	4.5	4.4	3.7
4	5.0	5.0	4.3
8	6.7	6.9	6.4

*Mean pH per treatment = -log[sum of antilogs(-rep pH)]/n.

**Treatment showed significant linear effects (p = .0001).

Table 2. Effects of dolomitic limestone on heights of container-grown Fraser Fir seedlings.

Lime added	Seedling Age			
	5 months	10 months	15 months	19 months
kg/m^3	Height (cm)			
0	2.2 ns*	4.3 **	11.0 **	19.5 **
1	2.5	4.5	12.9	21.8
2	2.4	4.2	11.2	20.5
4	2.6	4.1	10.2	17.4
8	2.2	3.4	6.4	9.5

* Treatment not significant at alpha = .05 level.

** Treatment showed quadratic effects (p = .0001).

with more lime is consistent with Pawuk's (1981) results for shortleaf and longleaf pine. The high pH was not beneficial to Fraser fir growth. The advantage of 1 kg/m^3 over 0 kg/m^3 and over 2 kg/m^3 conforms with Leyton's (1952) determination that the optimum pH range for conifer growth may be rather narrow. The addition of small quantities of Mg^{2+} and Ca^{2+} may also have been beneficial.

At the time of harvest, after 17 months in the amended growth medium, differences were slight between seedlings in 1 kg/m^3 peat and those with 2 kg/m^3, in any of the parameters measured (Table 3). Shoots of seedlings grown in the two heavily limed treatments (initial pH 5.0 and 6.7, respectively) were significantly shorter (20.2% and 56.4%, respectively) and lighter (30.8% and 75.8%, respectively) than the seedlings grown in 1 kg/m^3. Thus, the pH requirements of Fraser fir seedlings do not fall within the general pH range for conifer seedlings in organic media (pH 5.0 to 6.0) by Tinus and McDonald (1979) and Barnett and Brissette (1986). Fraser fir seedlings appear to be adapted to more acidic growth medium than most conifers.

Root growth also varied with lime treatment (Table 3). Seedlings in the three lowest lime treatments had root systems of golden-brown color, with white unsuberized laterals 1 to 3 cm in length. The most heavily limed seedlings had thin black roots with 1 to 2 mm unsuberized tips at the end of short (1-3 mm) blackened laterals (unreported data). Roots of the 4 kg/m^3 treatment were also blackened, and many had stunted unsuberized tips, like those of the 8 kg/m^3 seedlings. The effects of high pH on Fraser fir roots appeared

Table 3. Effects of dolomitic limestone amendments of sphagnum peat medium on the growth of 19-month-old Fraser fir seedlings.

Lime added	Height	Root weight	Shoot weight	Root:Shoot ratio
kg/m^3	(cm)	(g)	(g)	(g:g)
0	19.5 **	.443 **	1.863 **	.24 *
1	21.8	.488	2.165	.23
2	20.5	.489	1.949	.26
4	17.4	.398	1.498	.27
8	9.5	.165	0.523	.33

* Treatment showed significant linear effects (p = .0001).

** Treatment showed significant quadratic effects (p = .0001).

similar to those described by Leyton (1952) for Sitka spruce at pH 7. Fraser fir seedlings in the present experiment showed none of the discoloration, abnormal thickening or stunting of Leyton's Sitka spruce rootlets at pH 3 to 4, indicating that Fraser fir may tolerate greater acidity.

Increased Fraser fir growth at lower pH could result in part from Fraser fir's preference for NH_4^+ over NO_3^-. Chrustic and Wright (1983), using 0, 1, 2, 4 and 8 kg dolomitic limestone amendments per m^3 pine bark growth medium, found a higher concentration of NH_4^+ available in the leachate of more acidic medium. Black (1980) found Fraser fir to grow best using NH_4^+ as a nitrogen source.

The increase in growth medium acidity during the 17 months of the experiment (Table 1) did not appear to have an adverse affect on seedling growth. Even in the most acidic medium, the Fraser fir seedlings appeared to thrive. However, the benefit of 1 kg/m^3 liming, especially for the youngest seedlings, suggests the possible advantage of continued lime applications during growth. Extremely light lime applications would be consistent with the recommendation of Brix and van den Driesch (1974) that pH should be regulated during the growing period because of the acidity changes caused by fertilizer and leaching of the rooting medium. The possible benefit of continued light liming is further suggested by the growth advantage of the 2 kg/m^3 seedlings in the final growth cycle. Seedlings in the 2 kg/m^3 treatment averaged 9.3 cm growth in the last growth period, compared to 8.9 cm for seedlings in the 1 kg/m^3 treatment (Table 2). In this last period of growth, however, the greatest differences were still between seedlings grown in 0, 1, or 2 kg/m^3 (final pH 3.4-3.7) and those grown with 4 or 8 kg/m^3 (final pH 4.3 and 6.4).

Conclusion

Best growth of Fraser fir seedlings occured in sphagnum peat amended with 1 or 2 kg $lime/m^3$ peat (initial pH 4.2 or 4.5). Slower height growth of the 8 kg/m^3 seedlings was apparent in the first growth cycle, at 10 months age (Table 2). Decreased growth of the 4 kg/m^3 seedlings was slower to become evident, but was clear by the conclusion of the study. Fraser fir seedlings grow best within a fairly narrow range of pH, which is considerably more acidic than is generally recommended for conifers.

Significance to the Nursery Industry

Assessment of desirable pH range is quicker and easier than many growth factors often investigated for improving plant growth, such as CO_2 enrichment, growth regulator applications, strength of illumination required, tissue culture techniques. Growth medium pH is important enough to plant growth that it influences the effects of other environmental conditions and may overwhelm other treatment effects. Therefore, a species' pH range should be one of the first growth requirements established.

General recommendations for pH do not necessarily apply to a given species. Some of the pH ranges suggested for conifers result in slow growth and unhealthy seedlings, when used for growing Fraser fir.

It is worth the effort to test pH of a potting mix before use. Indications of pH on the packages of commercial horticultural potting mixes may vary widely enough from pH of the contents to damage or kill seedlings.

Vegetative reproduction is often attempted in a rooting medium buffered to pH 5.7 to 5.8, which is not within the acceptable pH range for Fraser fir and other species. Acidity requirements of a species may also need to be established for vegetative reproduction.

EFFECTS OF THREE EXOGENOUS GROWTH REGULATORS

ON FRASER FIR SEEDLING GROWTH

Introduction

Fraser fir seedlings are known for their slow growth habit, in which brief periods of growth with slight shoot elongation are interrupted by long periods of bud-set and quiescence or dormancy. Attempts to by-pass the chilling requirement by continued warmth, nutrients and lighting have resulted in morphological abnormalities, stunting, and terminal bud abortion or loss of terminal dominance, with a resulting loss of symmetry (Hinesley 1982). A desirable program for acceleration of Fraser fir growth would provide for continued terminal meristem activity, with recurrent, normal flushes of growth, increased shoot elongation, and adequate root growth to support the large shoot.

In preliminary investigations, foliar sprays of benzylaminopurine (BAP) were found to stimulate budbreak in young seedlings and when applied in concentrations of 125 to 1000 ppm to reduce internode elongation and damage some of the seedlings. Little (1984) sprayed 5- and 6-year-old balsam fir transplants with 600 ppm BAP repeatedly during the growing season, with the result of late season bud-swell and lammas growth, inhibited elongation, some damage to seedlings and increased production of lateral buds. Low concentration and frequent application of BAP were therefore selected for this experiment to stimulate cell division and bud-break while minimizing damage to seedlings.

Little and Loach (1975) sprayed 1000 ppm GA_3 on 4-year-old balsam fir seedlings just before budswell, and continued applications until after leader elongation ceased. Leaders treated with GA_3 began elongation sooner and elongated more than controls. Root drench treatments with GA_3 had similar effects in the same study. Both spray and root drenching treatments decreased root weight. Our preliminary investigations with 5-month-old Fraser fir seedlings sprayed with 1000 ppm GA_3 indicated that GA_3 at that concentration inhibited rather than stimulated budbreak in the young seedlings. GA_3 at lower concentrations appeared to increase elongation. Therefore, 100 ppm concentration of GA_3 was selected for this experiment, in an attempt to increase shoot elongation without inhibiting budbreak.

In balsam fir, the synthetic auxin IBA has been found to contribute to cambial meristem activity (Little 1981, Zagórska-Marek and Little 1986). Hinesley and Blazich (1980) found that dipping the base of Fraser fir stem cuttings in IBA increased the number of roots initiated and the mean length of roots produced by the cuttings in rooting medium. It was therefore hypothesized that IBA might increase stem thickening and root growth. In addition, since auxins are involved in shoot elongation in other species, it was thought that IBA might have a beneficial effect on Fraser fir seedling height.

The objectives of the study reported here were to provide for uninterrupted growth of Fraser fir seedlings and to increase shoot and root growth, while growing vigorous seedlings with normal form, by foliar application of three growth regulators, BAP, GA_3, and IBA.

Materials and Methods

Fraser fir seeds collected from Mount Rogers, Virginia, were stratified by soaking in water followed by two month's storage at 3^{o} C to assure uniform germination. Seeds were then germinated in Canadian sphagnum peat amended with 1.5 kg dolomitic limestone per m^3 compressed packaged peat with an average growth medium solution pH of 4.3. In six weeks, four hundred eighty seedlings were transplanted into 8 inch (150 cm^3) Ray Leach seedling tubes (Ray Leech Nurseries, Canby, Oregon) containing well-watered sphagnum peat with the same dolomitic limestone amendment. Seedlings were grown in a greenhouse which was heated to 20^{o} C and ventilated at temperatures above 22^{o} C. Summer temperature occasionally exceeded 40^{o} C. Supplimentary lighting (about 150 $\mu M/m^2$ s photosynthetic photon flux density at the level of the seedlings) extended photoperiod to 16 hours per day. Ten ml fertilizer solution per seedling was applied weekly in the concentration of 200 ppm nitrogen, 87 ppm phosphorus, and 166 ppm potassium, supplied as 20-20-20. Water was provided as needed to prevent stress.

After 18 weeks of growth, growth regulators were applied in 10 biweekly foliar sprays to the point of runoff. Hormone treatments were continued until just before harvest of 38-week-old seedlings. The three growth regulators applied were benzylaminopurine (BAP) (0, 50, and 100 ppm), gibberellic acid (GA_3) (0 and 100ppm), and indolebutyric acid (IBA) (0, 12.5, 25, and 50 ppm). Growth regulators were dissolved in water with 0.1% Tween 80 to facilitate solution and to serve as a surfactant. Earlier investigations had found no effect of the surfactant on Fraser fir seedling growth. The three growth regulators were sprayed in random sequence. Spray was allowed to dry on the seedlings for at least 24 hours before subsequent watering.

During the last 10 weeks of the experiment, seedlings were evaluated biweekly for active growth of the terminal leader. Determination of active growth was made on the basis of presence of new pale green needles and absence of a formed bud. Evaluation of the presence of terminal dominance was made on the basis of the terminal shoot's active growth or larger size compared to that of lateral shoots. Shoots bent in other than perfectly vertical direction were counted as drooped. Seedlings were also observed to develop purple needles during the experiment. Seedlings developing purple needles were marked and counted. Seedling heights were measured to the nearest mm, from the growth medium surface to the top of the formed bud or growing stem. Stem diameter was measured by caliper at both root collar and the middle of the last flush of growth. Stems of representative seedlings were hand sectioned for microscopic inspection. Seedlings were cut at the root collar and roots and shoots oven dried at 60^{o} C to a constant weight.

The experiment had a randomized complete block design with all concentrations of each of the three growth regulators factorially crossed, for a total of 24 treatment combinations in all 5 blocks.

The mean response of four-seedling plots was used in the analysis. Size-dependent variables were transformed logarithmically before analysis of variance, in order to provide more uniformity of variance between small and large seedlings. Counts (numbers of seedlings growing actively, with terminal dominance, with leader droop, and with purple needles) were transformed with arc sine transformations prior to analysis of variance. Where effects were significant at an alpha=0.05 level, means were separated using Fisher's Least Significant Difference (LSD) procedure.

Results and Discussion

Of the three growth regulators used, BAP had the most visible effects on seedling growth, and proved most significant upon statistical analysis. Seedlings treated with BAP broke bud more often and continued to produce new needles longer than seedlings without BAP (Table 4). Some BAP-treated seedlings had recurrent flushes of growth, leaving no bud-scale scars on the stem between flushes. The long periods of quiescence typical of Fraser fir seedlings were generally avoided. One hundred ppm BAP treatments maintained well over 60% active growth throughout the rest of the experimental period, and showed no sign of decreasing effect at the conclusion of the experiment. Fifty ppm BAP treatments also increased active growth during most of the experiment (Table 4). The increase in active growth of 5- to 9-month-old seedlings was consistent with results of preliminary investigations on 6-month-old Fraser seedlings breaking

Table 4. Effects of BAP on maintenance of active growth of Fraser fir seedlings.*

BAP	Measurement Date					
ppm	3/15	4/1	4/20	5/4	5/19	5/28
	%					
0	14.4 a**	20.5 a	44.4 a	53.0 a	29.4 a	31.9 a
50	43.8 b	45.6 b	52.5 a	56.3 a	46.9 b	58.8 b
100	61.9 c	67.5 c	75.0 b	79.4 b	80.0 c	86.3 c

*Seeds planted 8/31/87. Foliar sprays began 1/15/88.

**Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

bud after chilling (data not reported). Increased bud activity in Fraser fir is also consistent with the results of Little's (1984) study of closely related balsam fir at an older age (5 and 6 years).

BAP had several positive effects on seedling growth. Compared with controls, 100 ppm and 50 ppm BAP treatments increased seedling height 19.2% and 8.8%, increased stem diameter in the middle of the last flush of growth 32.4% and 8.3%, and increased shoot weight 56.8% and 32.3%, respectively (Table 5).

Although BAP increased the stem diameter of new growth, BAP treatments did not increase stem diameter at the root collar (Table 5). Thus, in seedlings treated with 100 ppm BAP, the mean stem diameter in the middle of the last flush of growth was 54.3% greater than the stem diameter at root collar. The thick upper stems stimulated by BAP were comparable in diameter and bark scales to 2-year-old leaders of 4- and 5-year-old seedlings, but remained succulent at the time of harvest.

Increase in diameter of newly developed stem results from an increase in apical meristem size and/or activity, while an increase in width of older stem would result from an increase in cambial meristem activity (Esau 1977. Fahn 1982). BAP treatments thickened that part of the stem which was produced during growth regulator treatment, but not portions of the stem produced earlier, as measured at root collar (Table 5). Thus, it appears that BAP stimulated apical meristem activity but did not affect cambial meristem activity enough to change stem diameter at root collar. Microscopic inspection of typical thick

Table 5. Effects of foliar application of benzylaminopurine (BAP) on Fraser fir seedling growth.

BAP	Height	Root wt.	Shoot wt.	Root: shoot ratio	Stem diameter root collar	Stem diameter last flush	Active growth	Leader droop
ppm	(cm)	(g)	(g)	(g:g)	(mm)	(mm)	(%)	(%)
0	6.8 a*	.396 a	.659 a	.576 a	2.91 a	3.39 a	31.9 a	9.6 a
50	7.4 b	.448 a	.872 b	.497 b	3.03 a	3.67 b	60.0 b	26.7 b
100	8.1 c	.307 b	1.033 c	.320 c	2.91 a	4.49 c	86.3 c	42.9 c

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

BAP-treated stem sections and of the thinner stem sections of controls revealed that BAP-treated stem sections had more than twice the number of cortical cells per radial transect than stem sections of controls (data not reported). Since cortical cells are produced by the apical meristem, increased number of cortical cells indicates that there was an increase in extent, as well as duration, of apical meristem activity.

Some effects of BAP were of questionable benefit. BAP treatments increased the incidence of slightly drooping leaders. Of seedlings treated with 100 ppm BAP, 42.9% had drooping leaders, compared to 26.7% of seedlings with 50 ppm BAP and 9.6% of controls (Table 5). Drooping leaders had been observed in preliminary investigations with 1-year-old seedlings after chilling (data not reported). The long-term effects of leader droop on seedling form and strength are not yet known. Further investigation will also be required to evaluate the long-term effects of the thick stems produced by BAP treatment, to determine whether or not these unnaturally massive and succulent stems will provide the basis for solid older stems with normal strength, vigor, and appearance.

While BAP stimulated height growth, 100 ppm BAP had the opposite effect on root growth. Roots of 100 ppm BAP treated seedlings were 22.5% lighter than roots of controls. Seedlings treated with 50 ppm BAP had no difference in root weight from controls. Ratios of root weight to shoot-weight ratios decreased with BAP treatment. Total weight of seedlings in both BAP treatments was greater than total weight of control seedlings (Table 5).

Purple needles are a unique effect of BAP. Ordinarily not seen in Fraser fir, bright purple needles frequently appear in a flush of growth following BAP treatment. As the needles mature, normal dark green needle color develops. In this study, 91.9% of the seedlings treated with 100 ppm BAP and 73.8% of seedlings with 50 ppm BAP developed purple needles (Table 6). (Three BAP untreated seedlings (1.9%) also developed purple needles, probably a result of BAP contamination of the experimental environment.)

In treatments of 50 ppm BAP, GA_3 increased the number of seedlings with purple needles. With the stronger concentration of BAP, GA_3 had no effect on purple needles (Table 6). Temporarily purple needles might seem an irrelevant effect of growth regulator treatment; they were, however, the first indication that the growth regulators had entered seedlings and were having some effect. Therefore, the fact that GA_3 influenced the production of purple needles might suggest some undetected effect of GA_3 on seedling metabolism or growth.

GA_3 had no significant main effect on any of the measures of seedling growth (Table 7). An increase in height growth had been anticipated on the basis of trends seen in preliminary investigations and Little and Loach's (1975) effects with 4-year-old balsam fir. GA_3 did, however, have significant interactions with BAP, not only in the production of purple needles, but also in growth of stem diameter at root collar, maintenance of active growth and height growth (Table 6).

The interaction between BAP and GA_3 on stem diameter at root

Table 6. Interactions between BAP and GA_3 in Fraser fir seedling growth.

BAP	GA_3	Height	Stem diameter root collar	Purple needles	Active growth
ppm	ppm	(cm)	(mm)	(%)	(%)
0	0	6.4 a*	2.99 ab	2.5 a	38.8 ab
	100	7.2 b	2.83 a	1.3 a	25.0 a
50	0	7.2 b	2.97 ab	65.0 b	63.8 c
	100	7.7 bc	3.09 b	82.5 c	56.3 bc
100	0	8.2 c	2.80 a	93.8 d	87.5 d
	100	8.1 c	3.02 b	90.0 cd	85.0 d

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

Table 7. Effects of foliar application of gibberellic acid (GA_3) on Fraser fir seedling growth.

GA_3	Height	Root wt.	Shoot wt.	Root: shoot ratio	Stem diameter root collar	Stem diameter last flush	Active growth
ppm	(cm)	(g)	(g)	(g:g)	(mm)	(mm)	(%)
0	7.2 a*	.375 a	.867 a	.453 a	2.92 a	3.85 a	63.3 a
100	7.6 a	.393 a	.841 a	.476 a	2.98 a	3.85 a	55.4 a

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

collar (Table 6) is difficult to explain without further testing. Stem diameter at root collar is a measure which otherwise remained constant from treatment to treatment. The interaction between BAP and GA_3 on maintenance of active growth (Table 6) will also require further testing.

In the absence of BAP, GA_3 increased the height of seedlings somewhat (Table 6). Without BAP applications, GA_3 treated seedlings were comparable in height to seedlings treated with 50 ppm BAP, with or without GA_3 (Table 6). It seems worth exploring, at higher concentrations of GA_3, the possibility that GA_3's unexpected failure to stimulate height growth in this trial is due to interaction with BAP.

From the work of Little (1971) and Zagórska-Marek and Little (1986) on balsam fir, increased stem growth had been expected with the application of IBA. However, IBA had no significant effects on height, shoot weight, stem diameter, or maintenance of active growth at nine months (Table 8).

Foliar application of all concentrations of IBA increased root weight (Table 8). The possibility of increased root growth in Fraser fir seedlings had been suggested by Hinesley and Blazich's (1980) finding that IBA increased root growth in Fraser fir cuttings. In the present study, Fraser fir seedlings with IBA applied at the highest concentration, 50 ppm, had roots 26.5% heavier than those with no IBA applications (Table 8). Experimental conditions did not distinguish between effects of IBA entering seedlings through the needles, buds or

Table 8. Effects of foliar application of indolebutyric acid (IBA) on Fraser fir seedling growth.

IBA	Height	Root wt.	Shoot wt.	Root: Shoot ratio	Stem diameter root collar	Stem diameter last flush	Active growth
ppm	(cm)	(g)	(g)	(g:g)	(mm)	(mm)	(%)
0	7.4 a*	.336 a	.884 a	.419 a	2.85 a	3.99 a	64.2 a
12.5	7.3 a	.392 b	.863 a	.491 b	2.95 a	3.62 a	58.3 a
25	7.4 a	.382 b	.849 a	.459 ab	2.97 a	3.88 a	55.0 a
50	7.7 a	.425 b	.822 a	.489 b	3.03 a	3.89 a	60.0 a

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

stems, and IBA which may have reached the growth medium and thence the roots.

Since IBA applications from 12.5 to 50 ppm increased root growth without decreasing shoot growth, IBA appears to have the practical value of helping to maintain an adequate root system in conjunction with the BAP effect of greatly accelerating shoot growth.

Conclusion

Foliar application of both concentrations of the synthetic cytokinin benzylaminopurine (BAP) enabled 10-week-old Fraser fir seedlings to avoid the long periods of quiescence typical of the species, and to increase stem growth significantly by age of nine months, the duration of the experiment. Thus, BAP may enable the development of uninterrupted production programs for Fraser fir seedlings. Root growth was decreased by the more concentrated BAP treatment.

Foliar application of the synthetic auxin indolebutyric acid (IBA) increased root growth of Fraser fir seedlings, without decrease in shoot growth, and may also be useful in an accelerated growth program for Fraser fir seedlings.

Foliar application of gibberellic acid (GA_3) had no significant main effects on shoot or root growth of Fraser fir seedlings at this age.

APPLICATION OF NITROGEN FERTILIZER FOR ACCELERATION OF FRASER FIR SEEDLING GROWTH

Introduction

In exploring the effects of nitrogen on accelerated growth of Fraser fir, Black (1980) supplied 3.4 ml of up to 300 ppm of nitrogen twice weekly to container-grown germinant seedlings in peat-vermiculite medium. The best growth in respect to height, needle number, and needle length occurred at the highest concentration of nitrogen, supplied as ammonium. This demonstrated that Fraser fir seedlings had a significant response to nitrogen fertilization, and, further, that the minimum amount required is at least 6.8 ml of 300 ppm nitrogen per week. Goodwin (1975) had found supplimental nutrition (12-6-6, 1 tablespoon per gallon applied five times monthly) almost doubled the size of Fraser fir seedlings at 48 weeks age from 2.3 to 4.1 cm, when compared to unfertilized controls. This nitrogen concentration is about 475 ppm. Goodwin's study also demonstrated Fraser fir's response to nitrogen, but did not determine the amount required.

Assuming that nitrogen is the limiting nutrient for Fraser fir in organic growth medium, a first approximation of Fraser fir nutrition requirements should be made while holding the other macronutrients, phosphorus or potassium, at constant, non-limiting levels. Holding phosphorus and potassium constant at 87 and 166 ppm, respectively, Seiler and Kreh (1986) found that 10 ml of 200 or 308 ppm nitrogen per seedling per week produced more growth in germinant seedlings through 36 weeks than nitrogen applied at higher rates.

Growth medium pH was not reported by Seiler and Kreh (1986), but a heavily limed commercial horticultural potting mix was used; therefore, high pH must be considered a possible factor in limiting seedling ability to utilize higher nutrient concentrations. The present study is an attempt to re-examine, using an acidic growth medium, effects on seedling growth of the nitrogen concentrations used by Seiler and Kreh (1986)

Promotion of bud break is an important mechanism for accelerating seedling growth. In many conifers of fixed growth habit, free growth or polycyclic growth occurs extensively during the first growth cycle before onset of dormancy, and decreases during the following seasons. As seedlings age, recurrent flushes decrease or cease, and growth becomes more fixed or determinant (Jablanczy 1971a). In Fraser fir in its native environment, germination and emergence of the primary root, cotyledons, and apical meristem is immediately followed by the formation of a few other needles in a single flush of growth (Liu 1971). It is hypothesized that with long photo-periods, a continuous supply of water, and maintenance of favorable growing temperatures, the concentration of supplimental nitrogen will affect the duration of active growth of germinant Fraser fir seedlings.

The nitrogen supplied by Seiler and Kreh (1986) was a mixture of ammonium, nitrate, and urea. Although the form of nitrogen supply may affect plant response, a combination of NO_3^- nitrogen and NH_4^+

nitrogen is generally found to be more effective for nursery purposes than either nitrogen source alone (Wright and Niemiera 1987). Therefore, a mixture is used again in the present research.

The purpose of this study is to examine the nitrogen requirements for container-grown Fraser fir seedlings within an acceptably acidic pH range, holding levels of potassium and phosphorus constant.

Methods and Materials

Seeds collected on Mount Rogers, Virginia, were stratified by soaking in water and chilling to 3^{o} C for 6 weeks prior to planting. Seeds were then planted in 4.5 inch root-trainer trays (Spencer-Lemaire Industries, Ltd., Edmonton, Canada) filled with sphagnum peat amended with 1.5 kg dolomitic limestone per cubic meter compressed packaged peat. This limestone amendment produced an initial mean growth medium pH of 4.3. Seedlings were grown in a greenhouse which was heated to 20^{o} C and ventilated at temperatures above 22^{o} C. Summer temperature occasionally exceeded 40^{o} C. Supplimentary lighting (about 150 $\mu M/m^2$ s photosynthetic photon flux density at the level of the seedlings) extended photoperiod to 16 hours per day. Water was provided as needed to prevent stress.

Ten ml fertilizer solution was supplied to each seedling weekly. Nitrogen was supplied as a mixture of ammonium, nitrate and urea, at 200, 400 and 600 ppm concentrations. Phosphorus and potassium were included in each fertilizer solution at 87 and 166 ppm respectively. Combinations of 21-7-7 and 20-20-20 fertilizer were used to keep phosphorus and potassium concentrations constant while varying the nitrogen concentrations.

Half of the seedlings were harvested after 6 months growth and half were harvested after 9 months. Heights were measured to the nearest mm from the surface of the growth medium to the top of the terminal bud or, if the seedling was actively growing, to the top of the growing leader. At the time of harvest, seedlings were severed at the root collar and dried at 60° C to constant weight, for determination of root and shoot dry weights. Mean growth for the 16 seedling treatment plots was analyzed following logarithmic transformation for uniformity of variability.

A randomized block design was used in this experiment. In both harvest groups, more seedlings had received 200 ppm and 400 ppm treatments than 600 ppm treatment. Therefore, General Linear Models procedure, a standard procedure for unbalanced data (SAS 1979) was used for analysis of variance. When treatment effects were significant at an alpha = .05 level, means were separated using Fisher's LSD procedure.

Results and Discussion

At the age of 6 months, there were no effects of fertilizer treatment on height, root weight, shoot weight, or root weight to shoot weight ratio (Table 9). After nine months, seedlings fertilized with 400 ppm nitrogen were taller than seedlings grown at either 200 or 600 ppm (Table 10). The 400 ppm seedlings were also

Table 9. Effects of three nitrogen fertilizer concentrations on size of Fraser fir seedlings after six months' growth.

N	Height	Root wt	Shoot wt	Root:shoot ratio
ppm	(cm)	(g)	(g)	(g:g)
200	3.8 a*	.047 a	.107 a	.485 a
400	3.9 a	.044 a	.109 a	.431 a
600	4.1 a	.045 a	.105 a	.472 a

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

Table 10. Effects of nitrogen fertilizer concentration on size of Fraser fir seedlings after nine months' growth.

N	Height	Root wt	Shoot wt	Root:shoot ratio	Active growth
ppm	(cm)	(g)	(g)	(g:g)	(%)
200	8.1 a*	.169 a	.521 a	.322 a	42.2 a
400	8.9 b	.184 a	.628 b	.298 b	46.9 a
600	8.2 a	.163 a	.551 ab	.289 b	38.2 a

*Within column, data followed by the same letter are not significantly different at 5% level (Fisher's LSD test).

heavier in shoots than the 200 ppm seedlings, though there was no difference in shoot weight between 400 ppm and 600 ppm seedlings.

The ability of seedlings in this experiment to successfully utilize more nitrogen than Fraser fir seedlings in the experiments of Seiler and Kreh (1986) may be a benefit of the more acidic growth medium in the present experiment. Growth medium pH 4.2 to 4.5 has been found significantly superior to pH 5.0 and above for Fraser fir seedling growth (Chapter 2).

There was no difference in root weight between fertilizer treatments. The 200 ppm fertilizer treatment produced the greatest root to shoot ratio. There was no difference between fertilizer treatments in the maintenance of active growth (Table 10). Although more detailed analysis would be required for definitive determination, it appears that the increase in height growth at 400 ppm N is a result of increase in extent but not duration of elongation.

Conclusions

Container grown Fraser fir seedlings in their first growth cycle benefit from being fertilized weekly with 400 ppm nitrogen as opposed to either 200 ppm or 600 ppm. Possible benefits of higher fertilizer concentration at later growth stages, as well as interactions between fertilizer treatments and the growth regulator treatments described earlier remain to be explored.

CONCLUSION

Three factors were examined to develop a program for the acceleration of Fraser fir seedling growth. Adjustment of growth medium pH, foliar application of growth regulators, and increasing the supply of nitrogen fertilizer all produced significant increases in rate of growth over previous methods. The importance of the gains obtained was not equal for each of the three factors investigated, however.

The most significant gains in seedling growth were obtained by adjusting growth medium acidity. Sphagnum peat growth medium was adjusted with 0 to 8 kg dolomitic limestone/m^3 compressed peat to initial pH ranging from 3.9 to 6.7. Best growth (21.2 cm mean height at 21 months) was achieved with 1 kg/m^3 and 2 kg/m^3, with initial mean pH 4.2 to 4.5. Seedlings with 4 and 8 kg/m^3 grew more slowly (17.4 and 9.5 cm in 19 months), and many were chlorotic, with stunted and blackened roots. Thus, the mean growth obtained with the best treatments was more than twice as rapid as that obtained with the worst treatment. Seedlings grown in acidic growth medium were much healthier and more vigorous.

In some respects the gains obtained by application of growth regulators were even more impressive than those resulting from lowering pH. One of the great difficulties in growing Fraser fir seedlings has been the seedlings' requirement for periods of rest and chilling in order to continue normal and vigorous growth. The requirement of interruptions in growth has made production of Fraser fir seedlings slow and expensive. Foliar application of benzylaminopurine (BAP) stimulated terminal bud activity, greatly decreasing the periods of rest between active growth. Eighty-six percent of the 9-month-old seedlings treated with BAP were still breaking bud and growing actively at the conclusion of the experiment. There was no indication of decreasing terminal meristem activity at that time. As a result, BAP greatly increased height and diameter of new shoot growth, up to 19% and 32%, respectively. While BAP treatment also decreased root growth, another growth regulator applied, indolebutyric acid (IBA) increased root growth significantly. Thus, it appears that a combination of exogenous growth regulators, applied by foliar spray, may permit Fraser fir to be grown in a greenhouse continuously until ready for outplanting.

Nitrogen fertilizer trials also produced significant improvement in Fraser fir production procedures. Nitrogen fertilizer was supplied at the rate of 10 ml per seedling per week, in concentrations of 200, 400, and 600 ppm. At nine months age 400 ppm nitrogen had resulted in 9% greater seedling height than either 200 or 600 ppm nitrogen. Nine-month-old seedlings with 400 ppm fertilizer averaged 8.9 cm in height.

Two of the factors examined, adjustment of pH and of nitrogen supply, are essential, and must be considered in any successful seedling production program. The growth increases derived from adjusting pH were much greater than the benefits from increasing nitrogen fertilizer concentration. The range of acceptable nitrogen concentration appears to be wide. While growing most rapidly with a supply of 400 ppm nitrogen fertilizer, seedlings thrived over the wide range of fertilizer applied from 200 to 600 ppm.

Acceptable pH range for Fraser fir is much narrower than the acceptable range of nitrogen fertilizer supply. At the highest pH tested, 6.5, the effects of improper pH were most severe: growth was almost arrested, all seedlings were unhealthy, and many died. Seedlings were also damaged by pH 5.0. Successful adjustment of pH, on the other hand, seemed almost to assure successful seedling growth. At initial pH of 4.2 and 4.5, seedlings all appeared healthy, and attained a mean height of 21 cm in a year and a half. Establishment of the sometimes narrow range of acceptable growth medium acidity is essential for the grower.

While adjustment of pH and of nitrogen supply are basic requirements for seedling production, application of growth regulators is a much more radical treatment. Application of growth regulators, though supplied externally, is in some respect a step beyond management of the seedlings' environmental conditions of temperature, duration and intensity of light, water and nutrient supply, CO_2 concentration of the air, and growth medium acidity. While increases in growth rate achieved with application of growth regulators were impressive, the potential for unknown problems should be regarded as equally impressive. Application of growth regulators should be considered experimental and unpredictable at this time.

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APPENDIX. A PROGRAM FOR ACCELERATING FRASER FIR SEEDLING GROWTH

The following program for accelerated growth of containerized Fraser fir seedlings has been developed and is now used in the Forestry Department greenhouse at Virginia Tech, Blacksburg, Virginia.

Seeds are soaked in water overnight and stratified for six weeks at 3°C to promote rapid and uniform germination. Canadian sphagnum peat moss is amended with one and a half kilograms of dolomitic limestone per cubic meter of compressed packaged peat, to provide a soil solution pH between 4.2 and 4.5. Plastic containers 10-15 centimeters deep are filled with approximately 150 cubic centimeters of peat. Root-trainer containers (Spencer-Lemaire Industries, Ltd., Edmonton, Canada. Spencer 1974) are used except in growth regulator experiments, in which ease of seedling separation was require for foliar spray applications. For these experiments, seedlings are grown in individual plastic Ray Leach Brand seedling tubes (Ray Leech Nurseries, Canby, Oregon. Allison 1974).

In March or April, seeds are sown on top of peat and covered loosely with one cm. peat. From the time of germination through August, seedlings are grown in the greenhouse with supplimentary illumination (150 $\mu M/m^2 \cdot sec$ photosynthetic photon flux density) in the evenings to simulate 16 hour days. The greenhouse is ventilated when the temperature exceeds 22° C. Temperatures occasionally exceed 45° C with no apparent ill effects on seedlings. Seedlings are supplied weekly with 10 ml of 400 ppm nitrogen fertilizer (0.5 g 20-20-20 plus

1.43 g 21-7-7 per liter water). BAP and IBA are applied in biweekly foliar sprays in ongoing growth regulator trials. Fertilizer and growth regulators are discontinued August 15.

On September 1, artificial lighting is discontinued and the growth medium is thoroughly flushed with water to remove excess nutrients. On October 1, seedlings are subjected to natural chilling for one month to six weeks, depending on the weather. Night-time heat may be provided occasionally to protect the seedlings from cold damage. During the first five days of chilling seedlings are protected from temperatures below freezing. After they have gained winter cold hardiness, the roots continue to be protected from temperatures below -6° C.

Between November 1 and 15, heat, fertilizer and supplimentary lighting are resumed, until February 1. Seedlings are grown with no fertilizer or supplimental lighting through the month of February. During the month of March they are again subjected to natural chilling. A summary of this accelerated growth program for Fraser fir seedlings is displayed in Table 1A.

As discussed by Hay and Keegan (1982) physiological conditions of seedlings at outplanting may be more important than size. Until further experimentation, whatever acceleration procedures are used should be concluded with 4 weeks of short days and four to six weeks of chilling to prepare seedlings of planting out. Chilled seedlings need several days of gradual acclimation to sunlight to prevent sunscald on exposure to full sun.

Table 1A. Proposed Accelerated Growth Program for Fraser Fir Seedlings.

January	Stratify seeds at least 6 weeks at 3 ^{o}C.
March	Plant seeds in 150 cm^3 containers in sphagnum peat amended with 1 1/2 kg dolomitic limestone/m^3 compressed packaged peat (42g/ft^3).
April-Aug	Grow in greenhouse with 16 hours illumination, 400 ppm nitrogen (10 ml/week), with phosphorus and potassium in non-limiting amounts.
June-Aug	Spray BAP and IBA biweekly.
Sept 1	Discontinue supplimentary illumination, growth regulators, and fertilizer. Flush with water.
Oct	Natural chilling. Protect from excessive cold.
Nov-Jan	Resume fertilizer, 16 hour illumination.
Feb 1	Discontinue supplimentary lighting and fertilizer, flush with water.
March	Natural chilling.
April	Plant out in a line-out bed.

Success of seedling growth under accelerated conditions is not assured. As Harris (1982) has pointed out, acceleration of the biological process also means accelerated possibilities for disaster. In our experience, high pH assures a gradual disaster, while rapid exposure to sunlight causes rapid disaster. High temperatures are often considered fatal to Fraser fir seedlings. This has not been our experience. While the occasional high temperatures in the greenhouse (to 45° C) were not intended but the result of thermostat failures, burnt-out fan motors, and climate, and certainly did not fall within the temperature optimum range suggested by Hinesley (1981), high temperature did not appear to harm the seedlings. Fraser fir seedlings appear far more flexible to high temperature than to the high pH of commercial horticultural potting mixes, or to sudden exposure to full sunlight after dark chilling periods.

It should be noted that this program for accelerated growth of Fraser fir seedlings has not yet been evaluated for success in outplanting.

VITA

James Alfred Bryan was born in St. Louis, Missouri, in January, 1941, and grew up in Massachusetts and Missouri. He studied literature and languages at Oberlin College, Oberlin, Ohio, and received his B.A. in 1963. After agricultural work, travel, teaching German, French and Mathematics, and other adventures of the 1960's, he received his M.A. in Comparative Literature from Washington University in St. Louis, Missouri, in 1969.

He then began a fifteen-year career in special education, working with learning disabled children and emotionally disturbed adolescents and adults. From 1974 to 1985 he was Director of the School for Adolescents at Western State Hospital in Staunton, Virginia, ad therapeutic education program based on the value of learning as a method of treatment of psychiatric disorders.

During the last three years as his work as a school director, he began taking science courses at night school and during vacations to prepare for a change of career. In 1985 he returned to school full-time, at Virginia Tech in Blacksburg, Virginia. He took one year of undergraduate courses in forestry and related sciences and began graduate studies in tree physiology in 1986. After completion of his M.S. degree in forestry at Virginia Tech, he is continuing his studies at Yale University, specializing in tropical silviculture.