

AN ABSTRACT OF THE THESIS OF

Hank A. Margolis for the degree of Doctor of Philosophy
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Title: Carbon and Nitrogen Allocation Patterns of 2-0
Douglas Fir Seedlings Following Nitrogen Fertilization in
the Autumn

Abstract approved:

Signature redacted for privacy.

Richard H. Waring

Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seedlings at a nursery in western Oregon were fertilized with nitrogen in October. Free amino acid (FAA) and total nitrogen concentrations in needles, stems and fine roots were followed from before fertilization until just prior to budbreak the following spring. Before budbreak in mid-March, the FAA and total nitrogen concentrations in the fertilized seedlings were significantly higher than the unfertilized seedlings.

Fertilized seedlings showed significant depletion of non-structural carbohydrates (NSC) relative to the unfertilized seedlings. The reduction in carbohydrate reserves following fertilization probably reflects increased respiration associated with the synthesis and maintenance of higher levels of enzymes.

The seedlings were lifted from the nursery bed and planted in a split plot design. The main treatment was the presence or lack of grass competition. Within each main plot, the previously fertilized and unfertilized seedlings were planted. Sucrose was applied to the soil around each seedling to limit the availability of nitrogen to tree roots.

On the average, the fertilized seedlings broke bud ten days earlier than the unfertilized seedlings and produced more growth aboveground. The earlier budbreak was responsible for initial differences in growth response. Later harvests showed that fertilized seedlings also exhibited higher relative growth rates.

Seedlings growing in the grass plots had predawn water potentials of -1.5 MPa by early August. By September 3, the unfertilized seedlings growing with grass showed significantly more predawn water stress than any of the other three treatments.

Although the fertilized seedlings had higher FAA and total nitrogen concentrations than unfertilized seedlings when they were planted, by the end of one growing season the FAA and total nitrogen concentrations had equalized. However, the fertilized seedlings contained more FAA and nitrogen because of their greater size. Grass competition affected both seedling nitrogen and non-structural carbohydrate chemistry.

After one growing season, the fertilized seedlings showed a 3 cm increase in height increment; a 29% increase in the number of stem units on the terminal leader; a 44% increase in aboveground growth; a 25% increase in total seedling leaf area; a 23% increase in relative growth rate; and a 14% increase in production per unit nitrogen.

Carbon and Nitrogen Allocation Patterns
of 2-0 Douglas-Fir Seedlings Following
Nitrogen Fertilization in the Autumn

by

Hank A. Margolis

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At the end comes the acknowledgements, curiously placed at the beginning of an Oregon State University dissertation. Its the final step of my nearly three year journey from the wide-eyed astonishment of the newly returned Peace Corps Volunteer to the meditative satisfaction of seeing this doctoral program, at long last, completed. I have learned a great deal during this time. Although taking classes and reading papers has been useful, the interaction with the wonderful people that inhabit this university and its surrounding area has been, for me, the high point of the whole experience.

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CARBON AND NITROGEN ALLOCATION PATTERNS OF 2-Ø DOUGLAS-FIR
SEEDLINGS FOLLOWING NITROGEN FERTILIZATION IN THE AUTUMN

INTRODUCTION

The nutritional status of forest trees affects their growth, susceptibility to insects and disease, and the rate of litter decomposition. For these reasons, nutritional research merits considerable interest. There has been a recent emphasis toward understanding the cycling of nutrients among ecosystem compartments. In comparison, however, little emphasis has been directed toward the cycling and transformation of mineral nutrients within individual trees. A more complete knowledge of the nutrient cycling within forest trees will likely be necessary before we can fully understand and predict the response of forest vegetation to fertilization.

Much of the work with the mineral nutrition of forest trees is confounded by the dilution of nutrient concentrations by growth. A recent technique developed in Sweden, however, makes it possible to grow plants for several months at constant growth rates and with stable nutrient concentrations (Ingestad and Lund 1979). Another approach is altering the nutritional status of forest trees after they have become dormant in late summer. Until the following spring, nutrient concentrations are

unaffected by height growth.

Standard nursery practice often involves withholding nitrogen from mid-summer until the following spring to induce dormancy and protect seedlings from frost injury (Cleary et al., 1978). Consequently, nurserymen have been reluctant to add nitrogen to seedlings after budset. A few studies have been conducted, however, where seedlings fertilized with nitrogen in autumn were evaluated for subsequent growth.

Shoulders (1959) applied 168 kg and 337 kg of nitrogen per hectare to 1-0 longleaf (Pinus palustris), loblolly, and shortleaf pine (Pinus echinata) seedlings in December. Seedlings were lifted and outplanted in February. Shortleaf pine broke bud before lifting and had poor survival. Slash and longleaf pine showed increased height growth with fertilization and no reduction in survival. Gilmore et al (1959) also attempted to improve the survival of loblolly pine by late season nitrogen fertilization but his experiment demonstrated inconclusive results. Ursic (1956), on the other hand, found decreased survival and no height growth response in a similar experiment with loblolly pine.

Anderson and Gessel (1966) were first to report that the survival of outplanted Douglas-fir improved following late season fertilization with 56 kg/ha of nitrogen. A 7% significant increase in survival and a 4.1 cm significant

increase in average annual height growth was reported for the fertilized seedlings. Thompson (1983) conducted a 3x3 factorial experiment with three levels of nitrogen and three levels of potassium applied to 2-0 Douglas-fir seedlings in October. She found that the fall application of nitrogen increased frost hardiness, bud height, root growth potential and advanced budbreak. As in many of the other early experiments with fertilizers, however, no analysis of foliar nutrients or carbohydrate status was made.

Benzian et al (1974) in Great Britain were among the first to analyze how tissue chemistry was altered following late season applications of nitrogen and potassium. They found that the increased nitrogen supply resulted in earlier budbreak for Sitka spruce (Picea sitchensis), lodgepole pine (Pinus contorta), western hemlock (Tsuga heterophylla) and Norway spruce (Picea abies) seedlings. However, only Sitka spruce seedlings actually increased growth.

In general, field performance by nursery seedlings receiving late season nitrogen fertilization has not proven beneficial with pines in the southeastern U.S.; has been inconclusive with a variety of conifers in Great Britain; and has given favorable results with Douglas-fir in the Pacific Northwest. However, most of these studies have involved only relatively simple correlations with

height growth and survival. Additional information was needed on how the nutritional status affected the metabolism and productivity of the seedlings.

Consequently, the experiment in Chapter I describes some of the changes in nitrogen and carbohydrate chemistry of 2-0 Douglas-fir seedlings in the months following an autumn application of nitrogen fertilizer. Chapter II examines the field performance of these seedlings in relatively water stressed and unstressed environments.

Passioura (1979) suggests that good biological research should explore the mechanisms underlying a given phenomenon and explain the significance of the phenomenon to some higher level of organization. The intent of this research was to link the phenomenon of altered nitrogen nutrition in autumn with both changes in seedling biochemistry and the performance of the seedlings in plantations. Thus I have attempted to weave a path from the biochemical level of organization, through the physiology of the whole plant, to ultimately show significance at the level of plantation silviculture and management.

CHAPTER I

OVERWINTER METABOLISM OF 2-Ø DOUGLAS-FIR

FERTILIZED WITH NITROGEN

IN EARLY OCTOBER

by

Hank Margolis

ABSTRACT

Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seedlings at a nursery in western Oregon were fertilized in October with ammonium nitrate. Free amino acid (FAA) concentrations in the needles of the fertilized seedlings showed a pronounced increase one month after fertilization, followed by a decrease throughout the winter. Prior to budbreak in mid-March, FAA concentrations in the needles of the control seedlings increased significantly. The FAA levels in the needles of the fertilized seedlings also tended to increase before budbreak but the difference was not statistically significant.

Stems and fine roots also showed significant increases in FAA concentrations after the October fertilization. Just before budbreak in mid-March, the FAA concentrations in the stems and fine roots of the fertilized seedlings were significantly higher than the unfertilized seedlings by 74% and 18%, respectively.

Total nitrogen increased in needles, stems and fine roots in the month after fertilization by 21%, 74%, and 61%, respectively. Just prior to budbreak, the nitrogen concentration decreased.

Needles and stems of fertilized seedlings showed lower available polysaccharide and total non-structural

carbohydrate (NSC) concentrations in mid-March just prior to budbreak. Fine roots of fertilized seedlings showed lower sugar concentrations throughout the winter and trends towards lower available polysaccharide and total NSC in the month following fertilization. The reduction in carbohydrate reserves following fertilization probably reflects increased respiration associated with the synthesis and maintenance of higher levels of enzymes.

INTRODUCTION

Forest scientists have become aware of the need to understand and predict the response of forest vegetation to the input of fertilizers. Nitrogen is among the most frequently limiting nutrients in temperate forests and the application of nitrogen fertilizer often results in a significant increase in growth (Tamm 1964, Gessel and Atkinson 1979, Heilman 1979, Miller and Tarrant 1983). As fertilizer has become more costly, however, efficient use has become an increasing concern (Bengtson 1979, Bengtson 1981).

Most of the research with fertilizers has been very empirical (cf. Pritchett and Gooding 1975, Shumway and Atkinson 1977, Miller and Fight 1979). Several studies with conifers, however, have examined the physiological basis for fertilizer response (Brix and Ebell 1969, Brix 1971, 1981, Fagerstrom and Lohm 1977, Miller and Miller 1976, Brix and Mitchell 1980). However, only a few of these studies have given substantial consideration to the biochemical nature of the tree's response (Van den Driessche and Webber 1975, 1977).

Nitrogen plays a central role in the metabolism of plants. Not only is it a constituent of the chlorophyll molecule, but large quantities of nitrogen are needed to build the enzymes necessary for photosynthetic, respiratory and growth processes within a plant. The

balance between nitrogen and carbon availability is also important in determining the relative allocation of dry matter production between roots and shoots (Loomis 1954, Ingestad and Lund 1979, Chapin 1980, Reynolds and Thornley 1982). Furthermore, the balance between free amino acids and non-structural carbohydrates is considered important in the ability of a plant to produce defensive phenolic compounds and thereby maintain resistance to insect and disease attack (Bryant et al. 1983, Mattson 1980, Matson and Waring 1984, Larsson et al. 1985, Waring et al. 1985).

Along with the nutrition of entire forest stands, some research has been directed at the nutrition of nursery stock in the months preceding lifting and outplanting (Van den Driessche 1971, 1977, 1979, 1980a, 1980b, 1982, Van den Driessche and Dangerfield 1975). Efficient use of fertilizer in the nursery might be more advantageous for early seedling growth than fertilizing both young trees and competing vegetation in the early stages of stand establishment. In standard nursery practice in the Pacific Northwest, however, nitrogen is withheld from mid-summer through the winter in order to induce dormancy and protect seedlings from frost injury (Cleary et al., 1978).

In general, field performance by nursery seedlings receiving late season nitrogen fertilization has not proven beneficial with pines in the southeastern U.S.

(Shoulders 1959, Gilmore et al. 1959, Ursic 1956); has been inconclusive with a variety of conifers in Great Britain (Benzian et al. 1974); and has given favorable results with Douglas-fir in the Pacific Northwest (Anderson and Gessel 1966, Thompson 1983). However, most of these studies have involved only relatively simple correlation with height growth and survival. Additional information was needed on how this change in nutritional status affects the metabolism of the seedlings.

Consequently, I chose to examine some aspects of the nitrogen and carbon metabolism of 2-0 Douglas-fir seedlings after an autumn application of nitrogen fertilizer until just before budbreak the following spring. Douglas-fir, growing in a maritime climate, is known to be metabolically active in the winter months and conducts considerable amounts of photosynthesis (Emmingham and Waring, 1977). Therefore, certain changes in nitrogen and non-structural carbohydrates were expected.

Following an application of ammonium nitrate fertilizer in early October, a pulse of free amino acids (FAA) was anticipated. Furthermore, I predicted that this pulse of FAA would decline throughout the winter as the FAA were incorporated into working enzymes.

In regards to non-structural carbohydrates, one of two alternate hypotheses were expected to be true:

1. If the increased nitrogen resulted in greater rates of net photosynthesis throughout the winter, then sugar and starch concentrations were expected to increase.
2. If, during the winter, the increased nitrogen resulted in substantial increases in the rates of maintenance respiration over total photosynthesis, then sugar and starch concentrations were expected to decrease.

MATERIALS AND METHODS

A nursery bed at International Paper Company's Western Forest Research Center in Lebanon, Oregon (44.5°N latitude, 123°W longitude) was used in this experiment. The bed was planted with Douglas-fir seed in early May, 1982 at a density of 258 per square meter. Seed from zone 262, which includes the Willamette Valley in and around Corvallis, was used. The soil was of the Newburg series, a fine textured sandy loam consisting of 60% sand, 30% silt and 10% clay. Ammonium nitrate fertilizer was applied to the entire nursery bed in 1983 according to the schedule shown in Table I.1.

The nursery was watered as needed to maintain the soil at or near field capacity during the 1982 and 1983 growing seasons. Because of the wet summer in 1983,

Table I.1 Cultural Treatments Applied to Nursery Bed

<u>Date</u>	<u>Treatment</u>
May, 1982	Nursery bed seeded
March 16, 1983	Ammonium nitrate applied at 51 kg/ha to whole nursesey bed
April 19, 1983	Ammonium nitrate applied at 28 kg/ha to whole nursesey bed
May 4, 1983	Ammonium nitrate applied at 25 kg/ha to whole nursesey bed
May 15, 1983	Ammonium nitrate applied at 25 kg/ha to whole nursesey bed
May 30, 1983	Ammonium nitrate applied at 27 kg/ha to whole nursesey bed
July 18, 1983	Top pruned to 30 cm ¹
August 20, 1983	Experimental plots thinned to a uniform density
October 1, 1983	First application of 56 kg/ha of ammonium nitrate
October 7, 1983	Root pruned to a depth of 20 cm
October 11, 1983	Second application of 56 kg/ha of ammonium nitrate
February 2, 1984	Seedlings lifted

-
1. Only about 75% of the nursery bed was top pruned. Two blocks were in parts of the nursery bed that had not been top pruned.

little irrigation was necessary. Water stress cycles were begun in mid-August of both 1982 and 1983 to induce dormancy. The nursery soil was allowed to dry until the seedlings reached a predawn plant moisture stress of -1.0 MPa. The nursery was then irrigated back to field capacity. About 75% of the nursery bed was top pruned on July 18, 1983 to an approximate height of 30 centimeters.

In late August, 1983, I established sixteen 1.2 by 1.2 meter plots within the nursery bed. There was at least a 1.2 meter buffer between the plots. The plots were thinned so that seedling density was more uniform.

The sixteen plots were divided into eight blocks with two plots per block. Two of the blocks were in parts of the nursery bed that had not been top pruned in July. On October 2, 1983, one of the plots in each block was randomly selected and dissolved ammonium nitrate was applied uniformly at a rate of 56 kg of nitrogen per hectare. A similar amount of water was applied to the control plots. This procedure was repeated on October 11 so that a total of 112 kg per hectare of nitrogen was applied. On October 7, the nursery bed was undercut according to standard nursery practice to about 20 centimeters.

Seedlings were lifted on February 2, 1984 and placed in cold storage at 2°C . All the seedlings were outplanted near the nursery at the Peavy Arboretum outside

of Corvallis, Oregon by February 11.

Harvests were taken for laboratory analysis on October 1, November 1, February 1 and March 15 in the fall and winter of 1983-84. From each plot, one composite of five seedlings was placed in a plastic bag and immediately put in a cooler filled with dry ice. The seedlings were taken to Oregon State University and stored in a freezer at -40°C . I followed a sequential extraction and analysis procedure in determining free amino acids, sugars and available polysaccharides in the needles, stems and fine roots (see Appendix). The term available polysaccharide refers primarily to starch. However, recent work suggests that some polysaccharides other than starch may be utilized in the respiratory processes of Douglas-fir but at this point the data is tentative (Weiger Schaap, Dept of Forest Science, Oregon State University, personal communication). Until this issue is resolved, I chose to use the term "available polysaccharide" instead of "starch". Total nitrogen, phosphorus and nitrate were also measured using the procedures described in the Appendix.

Split plot analyses of variance were performed with time as the main variable and nitrogen fertilization as the secondary variable. The Statistical Package for the Social Sciences (SPSS) was used to calculate the analyses of variance and descriptive statistics (Nie et al 1975,

Hull and Nie 1981). Tukey's honestly significant difference procedure (Steel and Torrie, 1980) was used to compute least significant differences (LSD) at the 0.10 and 0.05 levels (Steel and Torrie 1980, Pearson and Hartley 1966).

RESULTS

A. Nitrogen

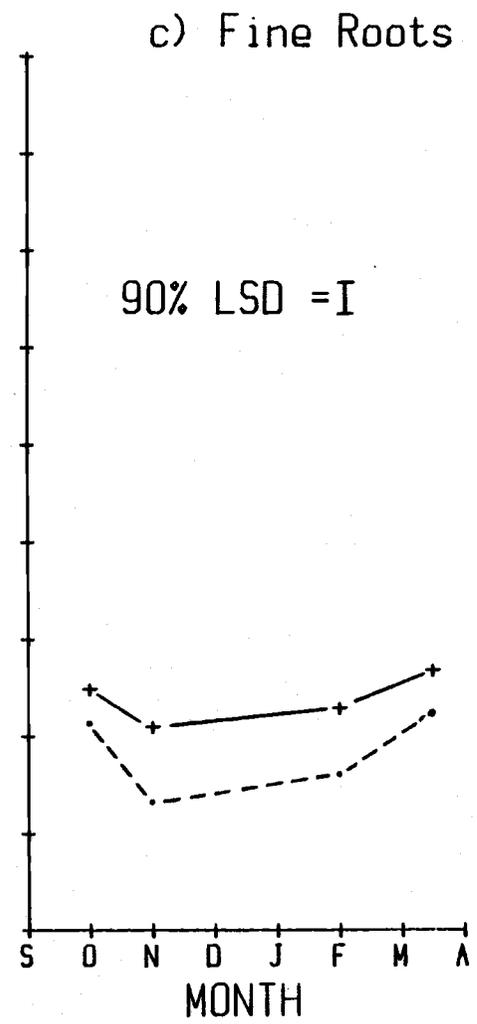
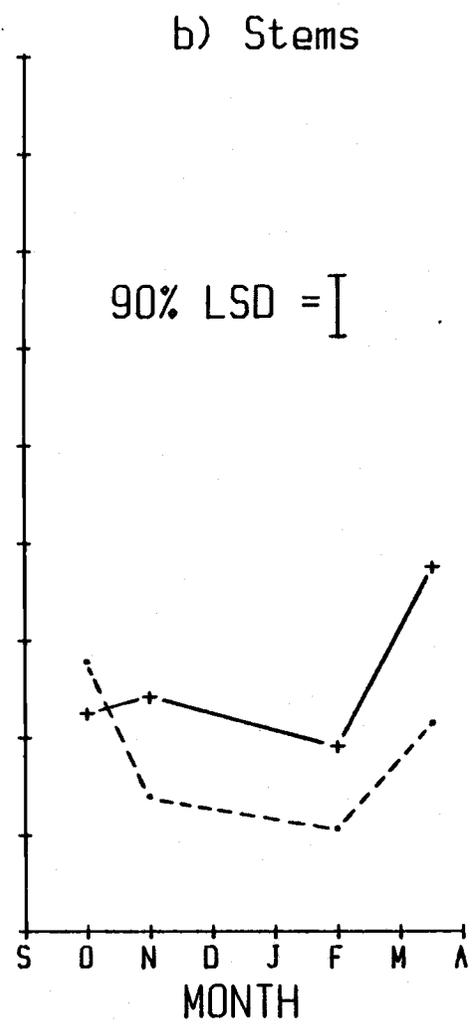
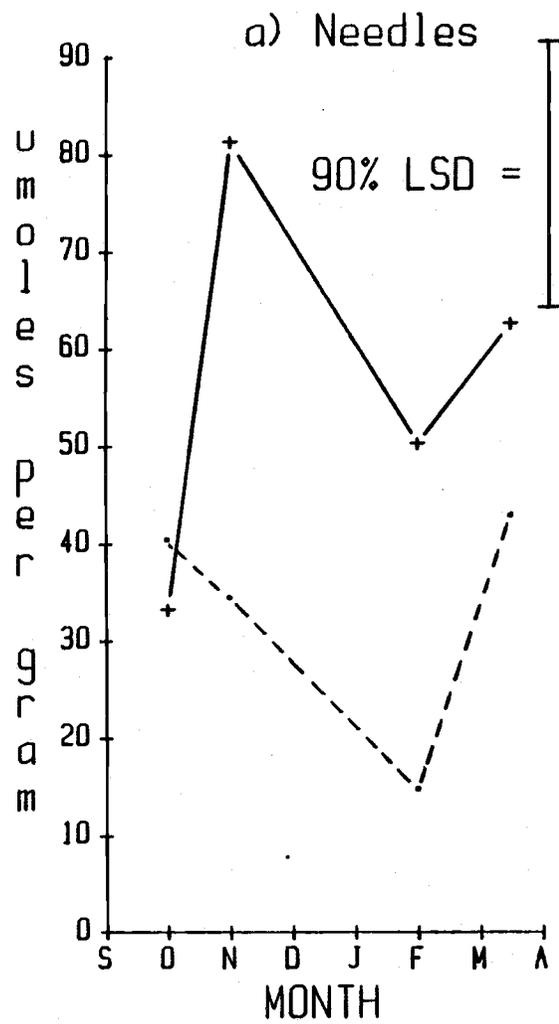
No nitrate was found in any plant part at any time. The analysis of variance for free amino acids (FAA) per gram dry weight showed significant interactions between time and fertilizer treatment for needles, stems and fine roots. These interactions were expected since the first harvest was taken before the fertilizer was applied. At later harvests, significant differences due to the added nitrogen were usually expressed.

Seedlings fertilized with ammonium nitrate fertilizer showed a large pulse of free amino acids (FAA) in the needles followed by a decrease throughout the winter (Figure I.1a). In the needles of the control seedlings, on the other hand, FAA concentrations showed a steady decrease throughout the fall and winter. Just before budbreak in mid-March, however, the needles of the control seedlings showed a significant increase in FAA concentrations. The fertilized seedlings also tended to increase FAA concentrations just prior to budbreak but

Fig I.1 Free amino acids (umoles per gram dry weight) from before the October fertilization until just before budbreak in mid-March for a) needles, b) stems and c) fine roots of nitrogen fertilized (+————+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 27.5, 6.6 and 3.6 umoles per gram dry weight for needles, stems and fine roots, respectively. (Figure on next page).

FIG I.1

FREE AMINO ACIDS



the increase was not statistically significant.

The stems, unlike the needles, did not show a large pulse of FAA in the month following fertilization (Figure I.1b). Rather the fertilized seedlings kept a constant concentration while the control seedlings showed a significant decrease. After November, the nitrogen treated seedlings kept significantly higher FAA levels and both groups of seedlings showed a similar increase in FAA just prior to budbreak.

The FAA concentrations in the fine roots did not vary as much with time as in the needles and stems (Figure I.1c). The control seedlings showed a small but significant decrease in FAA concentrations through the winter while the treated seedlings stayed relatively constant. This constant level in the fertilized seedlings, however, was significantly higher than the controls.

Total Kjeldahl nitrogen also showed significant interactions ($p < 0.001$) between time and the fertilizer treatment for needles, stems and fine roots (Figure I.2). The nitrogen concentrations were consistently higher in the fertilized seedlings. In the needles and fine roots there was a significant decrease in total nitrogen in mid-March just before budburst.

Fig 1.2 Total nitrogen (mg per gram dry weight) from before the October fertilization until just before budbreak in mid-March for a) needles, b) stems, and c) fine roots of nitrogen fertilized (+————+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 2.1, 1.3, and 1.3 mg per gram dry weight for needles, stems and fine roots, respectively. (Figure on next page).

FIG I.2

TOTAL NITROGEN

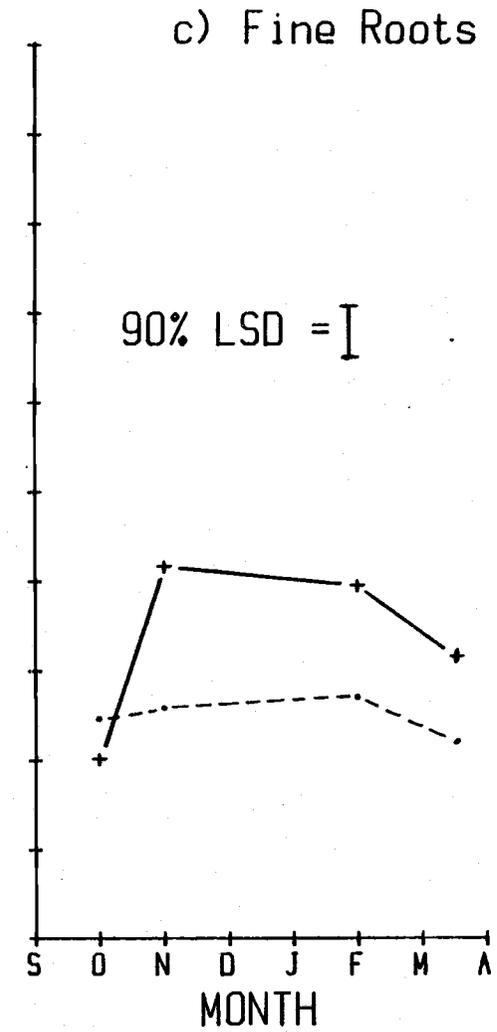
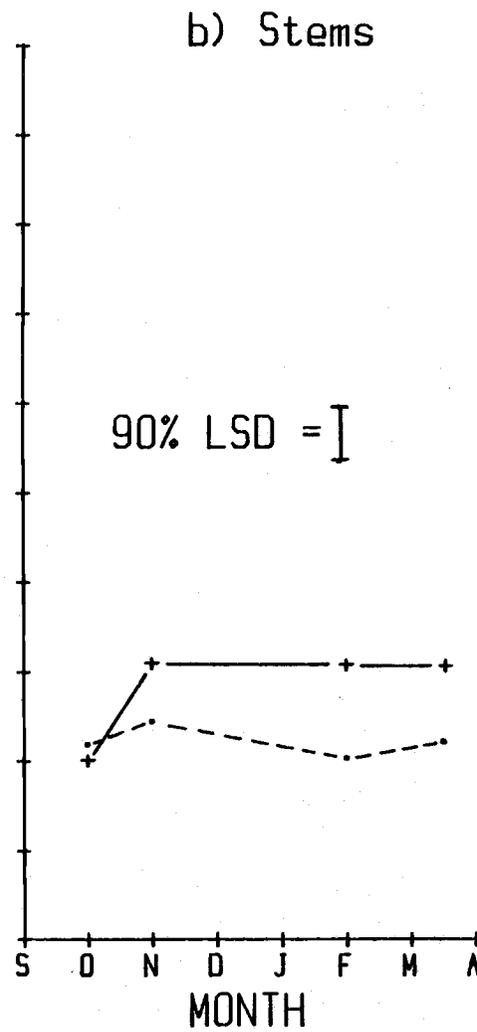
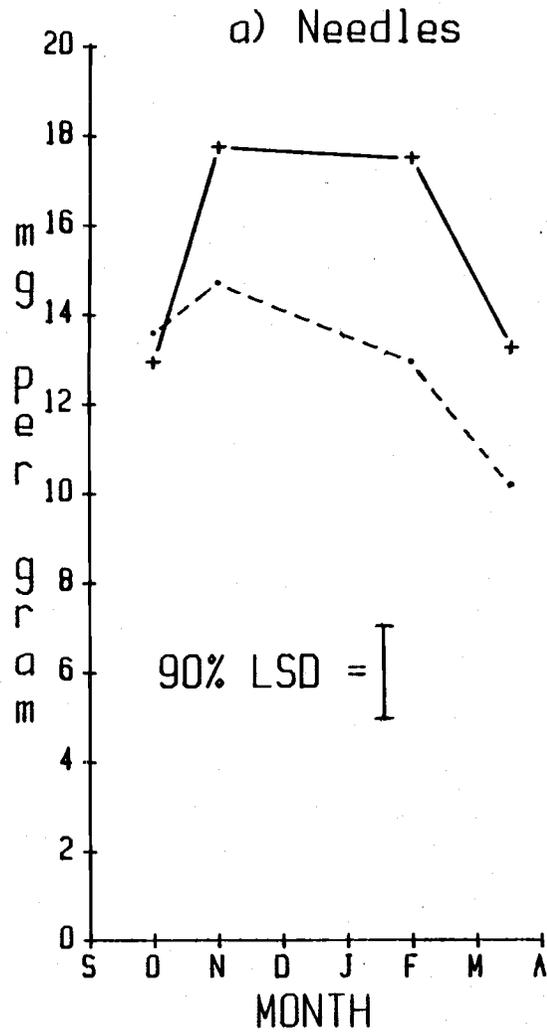


Figure I.3 shows the ratio of FAA to total nitrogen in the leaves, stems and fine roots expressed as umoles per milligram. This gives an index of the relative size of the FAA pool in relation to the total nitrogen pool. Except for the significant differences in the fertilized needles for two measurements following the initial fertilizer pulse, the application of ammonium nitrate did not significantly affect the ratio of FAA to total nitrogen. Over time, however, large differences in this ratio were observed in both fertilized and unfertilized seedlings. The ratio decreased over the autumn and winter and markedly increased just before budbreak (Figure I.3). Fine roots of the control seedlings had a lower ratio of FAA to total nitrogen at the beginning of the experiment despite the randomization and blocking during plot selection. However, the individual components of the ratio, the FAA and the total nitrogen, did not differ significantly at the beginning of the experiment.

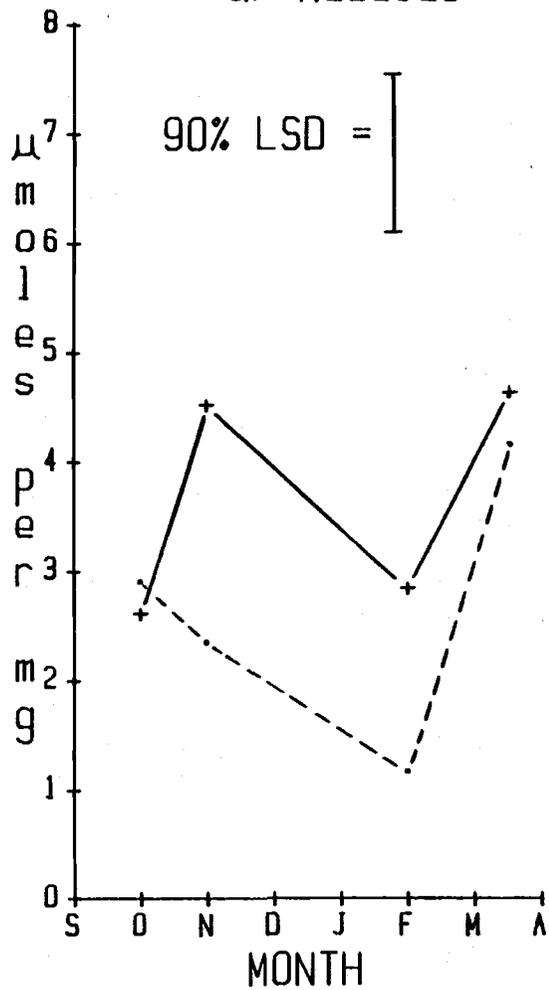
B. Non-Structural Carbohydrates (NSC)

Needle sugar concentration showed no fertilizer effect (Figure I.4a). A significant effect over time was found, attributable to a marked increase in sugar concentration when the February harvest occurred. The decrease in needle sugar concentration from February until mid-March corresponds to an even larger increase in

Fig I.3 Ratio of free amino acids to total nitrogen (umoles per mg) from before the October fertilization until just before budbreak in mid-March for a) needles, b) stems, and c) fine roots of nitrogen fertilized (+————+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 1.5, 1.7, and 1.1 mg per gram dry weight for needles, stems and fine roots, respectively. (Figure on next page).

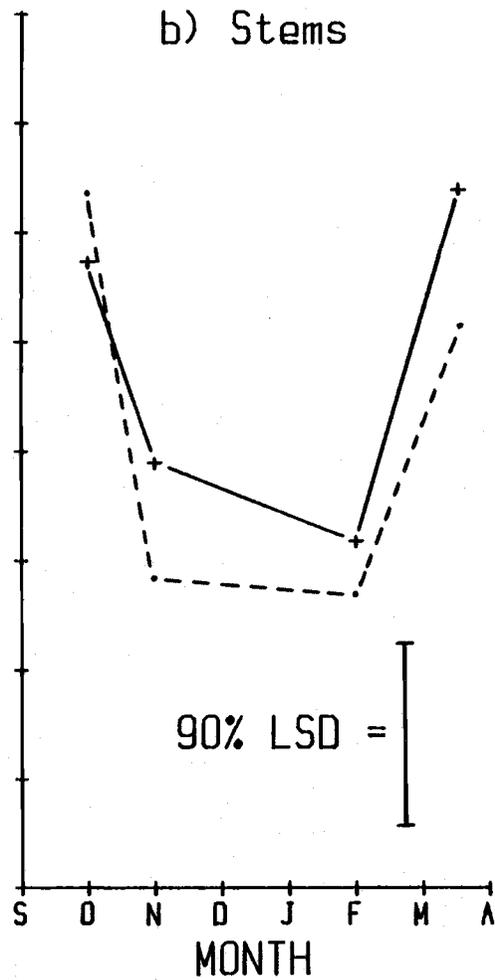
FIG 1.3

a) Needles



RATIO OF FAA TO TOTAL NITROGEN

b) Stems



c) Fine Roots

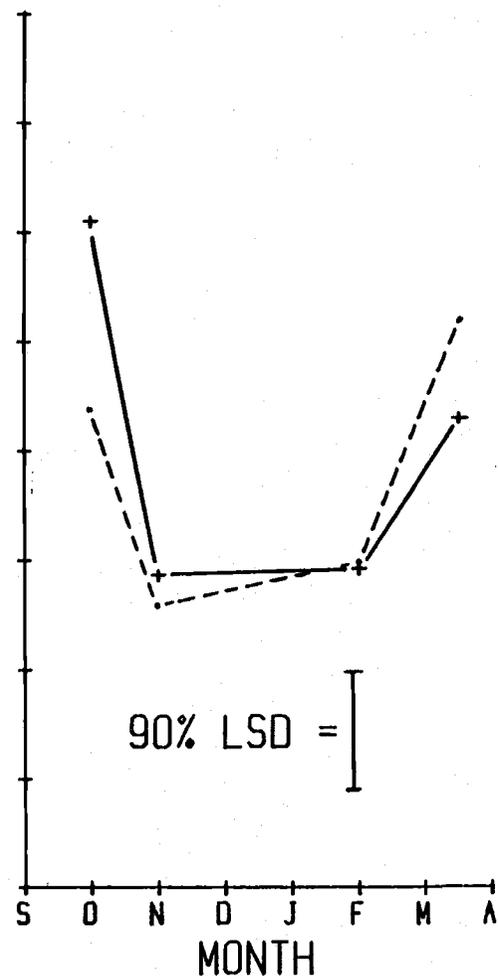
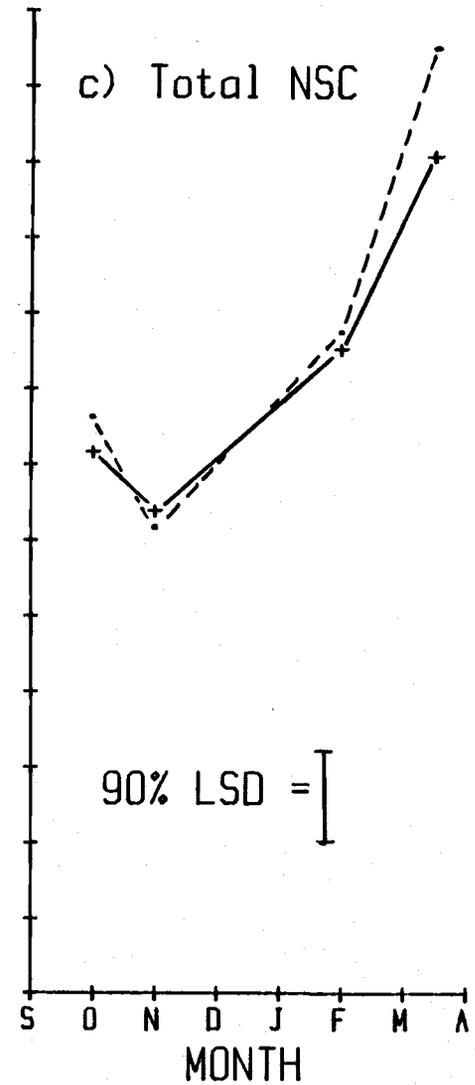
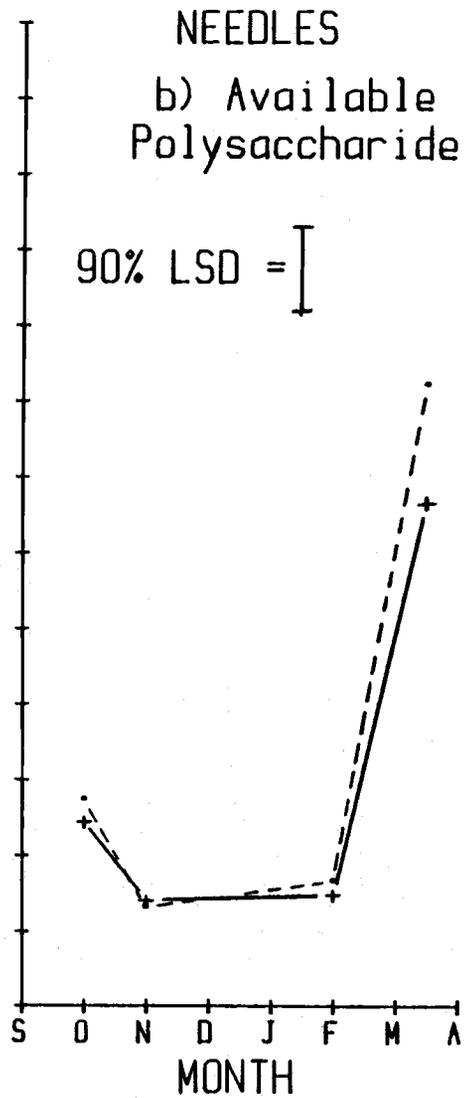
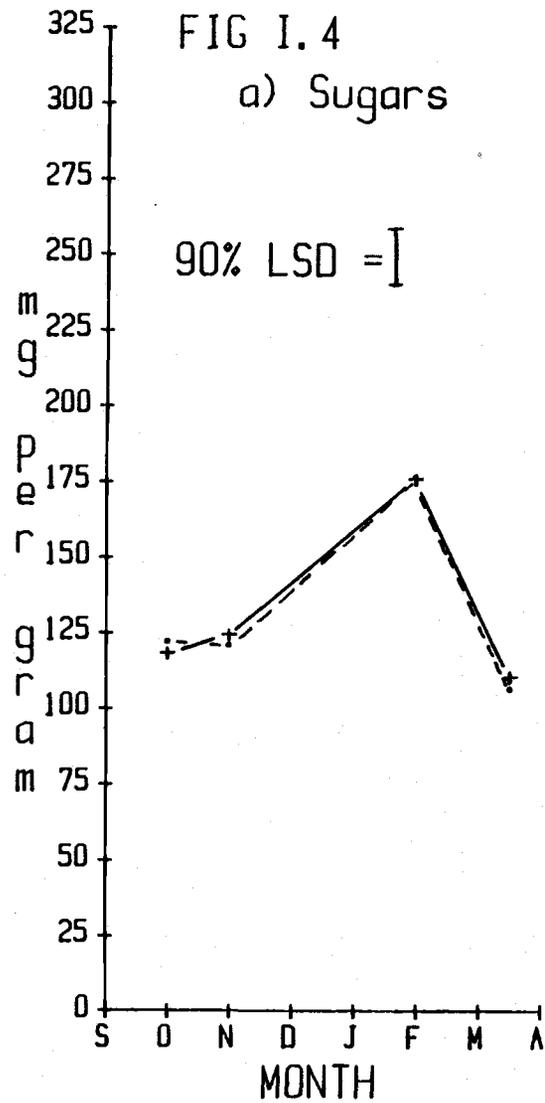


Fig. I.4 Concentrations (mg per gram dry weight) of a) sugars, b) available polysaccharide, and c) total non-structural carbohydrate (NSC) from before the October fertilization until just before budbreak in mid-March in the needles of nitrogen fertilized (+————+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Available polysaccharide refers primarily to starch but also includes any other polysaccharide that is extracted by perchloric acid and might be utilized as a respiratory substrate by Douglas-fir (see pages 14 and 101). Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 19.1, 30.0, and 33.3 mg per gram dry weight for sugars, available polysaccharide and total non-structural carbohydrate, respectively. (Figure on next page).



available polysaccharide concentration at that time (Figure I.4b). Thus there appears to be considerable conversion of sugar to available polysaccharides in the weeks preceding budbreak. Especially important in Figure I.4b is the 20 mg per gram lower available polysaccharide concentrations in the fertilized seedlings in mid-March. Similarly, total NSC concentrations (sugar + available polysaccharides) show an even larger decrease of 36 mg/gram in the fertilized seedlings just before budbreak (Figure I.4c).

Stems show similar patterns to needles in terms of NSC concentrations (Figures I.5). No effect of fertilizer on sugar concentrations was detected (Figure I.5a). However, like the needles, stems of fertilized seedlings had less available polysaccharide (18 mg/gram) and total NSC (17 mg/gram) concentrations in mid-March than unfertilized seedlings (Figures I.5b and I.5c).

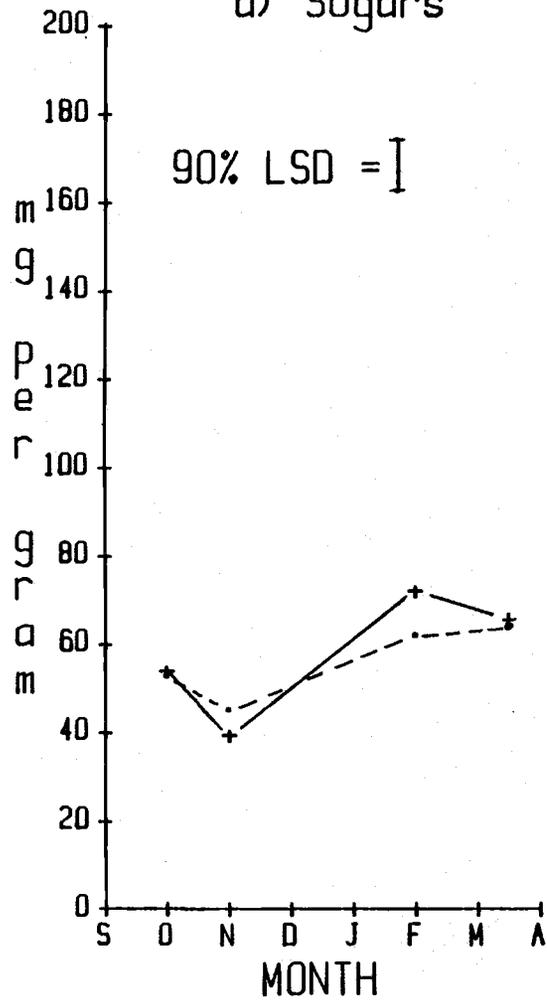
Fine roots showed different patterns than either needles or stems. When all four harvest dates were combined, sugars averaged over 8 mg/gram less ($p < 0.01$) in the fertilized than in the unfertilized seedlings (Figure I.6). The sugar concentrations did not vary significantly with time. The available polysaccharide and total NSC concentrations both showed a tendency towards lower values in the fertilized trees in November but the differences were not statistically significant (Figures I.6).

Fig 1.5 Concentrations (mg per gram dry weight) of a) sugars, b) available polysaccharide, and c) total non-structural carbohydrate (NSC) from before the October fertilization until just before budbreak in mid-March in the stems of nitrogen fertilized (+-----+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Available polysaccharide refers primarily to starch but also includes any other polysaccharide that is extracted by perchloric acid and might be utilized as a respiratory substrate by Douglas-fir (see pages 14 and 101). Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 12.4, 15.2, and 17.8 mg per gram dry weight for sugars, available polysaccharide and total non-structural carbohydrate, respectively. (Figure on next page).

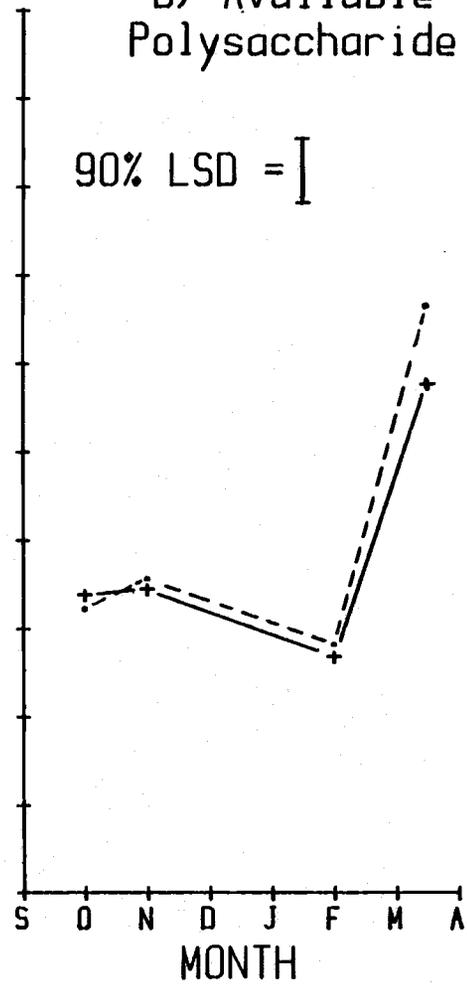
FIG 1.5

STEMS

a) Sugars



b) Available Polysaccharide



c) Total NSC

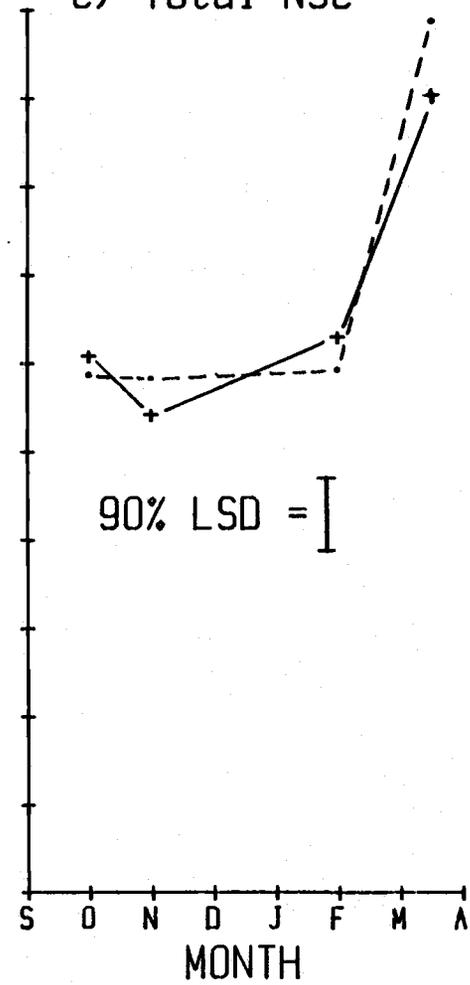
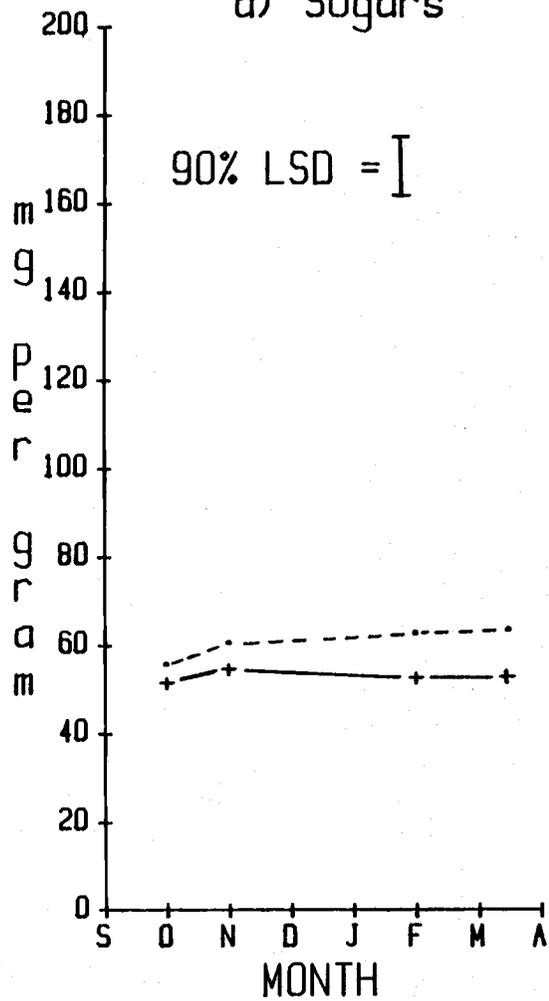


Fig I.6 Concentrations (mg per gram dry weight) of a) sugars, b) available polysaccharide, and c) total non-structural carbohydrate (NSC) from before the October fertilization until just before budbreak in mid-March in the fine roots of nitrogen fertilized (+-----+) and unfertilized (•-----•) 2-0 Douglas-fir seedlings. Available polysaccharide refers primarily to starch but also includes any other polysaccharide that is extracted by perchloric acid and might be utilized as a respiratory substrate by Douglas-fir (see pages 14 and 101). Each point is the mean of eight replications. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie, 1980). 90% LSD = 13.6, 18.6, and 28.0 mg per gram dry weight for sugars, available polysaccharide and total non-structural carbohydrate, respectively. (Figure on next page).

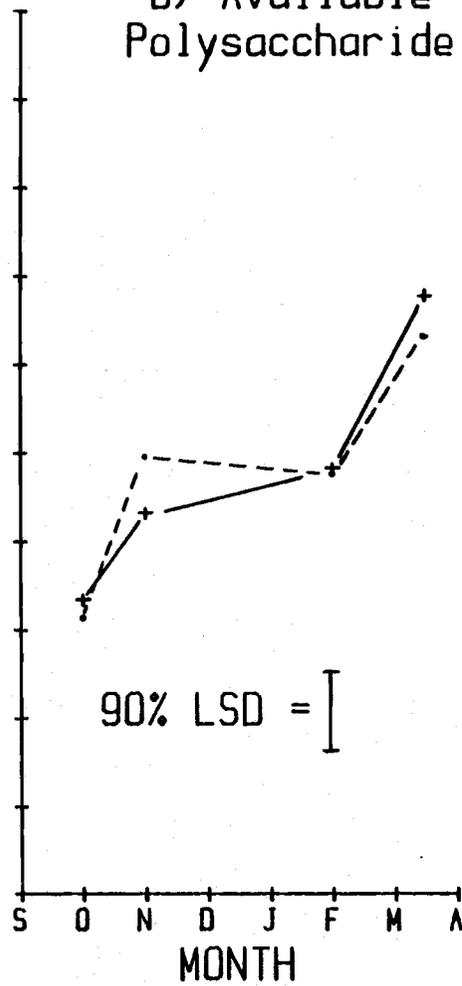
FIG I.6

a) Sugars



FINE ROOTS

b) Available Polysaccharide



c) Total NSC

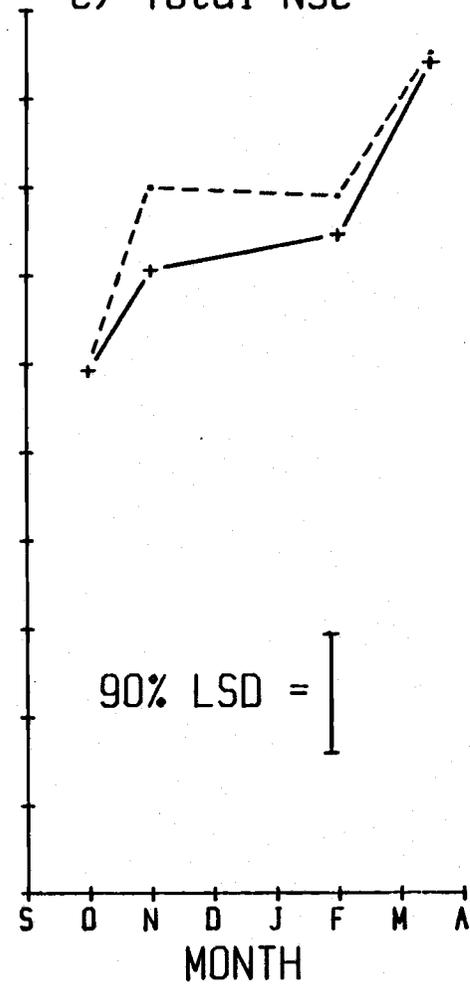


Table I.2 shows that nitrogen to phosphorus ratios pooled over time were significantly increased in the needles, stems and fine roots of the fertilized seedlings.

Figure I.7 presents a summary of the differences in biochemistry of the fertilized 2-0 Douglas-fir seedlings relative to the unfertilized seedlings as a result of nitrogen fertilization in the autumn.

DISCUSSION

The nearly 2.5 fold increase in FAA concentration in the needles one month after application of ammonium nitrate suggests that considerable amounts of the fertilizer were metabolized and transported to photosynthetically active parts of the seedlings (Figure I.1a). The fact that no nitrate could be found in any plant part suggests that the Douglas-fir seedlings were able to fully metabolize all the nitrate they absorbed. Whereas nitrate reductase activity has been found in Douglas-fir in British Columbia (Krajina et al, 1973) and Utah (Bigg and Daniels, 1978), both these regions have calcareous soils. In one study in Oregon, Douglas-fir was found lacking in nitrate reductase activity (Li et al, 1972). The Douglas-fir seedlings in this study appeared to have no trouble assimilating the nitrate and ammonium within 30 days of application. Whether this occurred in the fine roots as suggested by Pate (1980) or nitrogen

Table I.2 Nitrogen to phosphorus ratios for needles, stems and fine roots of fertilized and unfertilized Douglas-fir seedlings in a nursery bed. Fertilized seedlings received 112 kg per hectare of nitrogen in early October. Each value is the mean (+-s.e.) for 16 plots pooled over two harvest periods (November and March). Asterisks indicate significant differences (* = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$).

	<u>Control</u>	<u>Fertilized</u>	<u>$\Delta\%$</u>
Needles	11.2 (0.56)	13.5 (0.31)***	+21%
Stems	7.3 (0.85)	9.1 (0.42)*	+25%
Fine Roots	5.5 (0.37)	8.3 (0.39)***	+51%

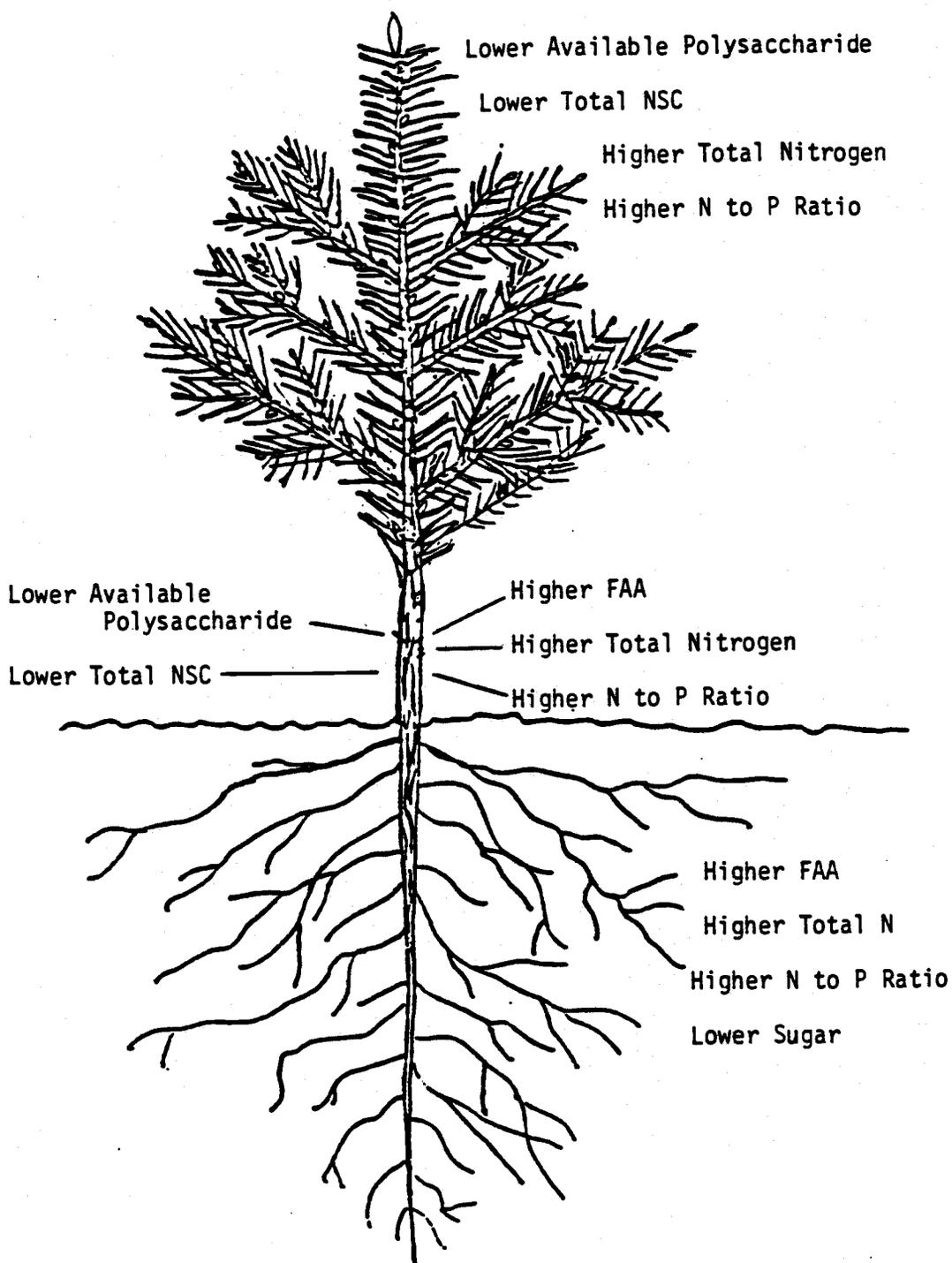


Fig I.7 Summary of the nitrogen and non-structural carbohydrate chemistry of the needles, stems and fine roots of a 2-0 Douglas-fir seedling fertilized with nitrogen in early October relative to an unfertilized seedling.

assimilation enzymes were induced in the needles (Smirnoff et al., 1984) was not determined.

The assimilation of substantial amounts of inorganic nitrogen into free amino acids (FAA) by November was followed by a marked decline through the winter months as the FAA were presumably metabolized into protein or other polymeric molecules. Durzan (1968) found that protein synthesis in unfertilized white spruce (Picea glauca) became quite active following the first fall frost. The needles of both the fertilized and control seedlings in my study showed similar declines in FAA while total nitrogen remained constant. These FAA were probably incorporated into working enzymes since the needles of Douglas-fir are known to be metabolically active during the mild Oregon winters.

The increased FAA concentrations in mid-March are probably the result of protein breakdown associated with the requirement for large amounts of readily transportable FAA to be fluxed into the new growth following budbreak. Total nitrogen in needles decreased at this time as the nitrogen was probably retranslocated to areas of high growth potential (Figure I.2). Durzan (1968) found a similar pattern in white spruce. In this study, the fertilized Douglas-fir seedlings were able to mobilize considerably more nitrogen as FAA than the control seedlings. Fagerstrom's (1977) model to explain the

growth response of conifers to nitrogen fertilization suggests that the size of the mobile nitrogen pool (ie. free amino acids) relative to needle biomass could determine the rate of production of new needle biomass. On this basis, the elevated mid-March FAA concentrations of the fertilized Douglas-fir in this experiment would indicate greater needle production should be expected in the coming growing season (see Chapter 2).

Like van den Driessche and Webber (1975), this study showed a much greater percent increase in FAA relative to total nitrogen in needles (Figure I.1a, I.2a). In November, total nitrogen had been increased from 14.7 mg/gram in the unfertilized seedlings to 17.8 mg/gram in the fertilized seedlings (Figure I.2a). Brix (1981) found that 17.4 mg/gram was optimum for photosynthesis of Douglas-fir under high light. However, no increase in sugars or available polysaccharides was found in the fertilized seedlings used in this experiment. Either sunlight during the Oregon winter was insufficient to permit utilization of all available enzymes in photosynthesis or maintenance respiration rates increased above any gains in photosynthesis.

Unlike the needles, the stems and fine roots showed greater increases in total nitrogen concentration than FAA (Figures I.1, I.2, I.3). The rain in September followed by the clear days in October appeared to result in

considerable transpiration and mass flow of FAA to the foliage. Other than the increase in FAA in the stems prior to budbreak, FAA levels did not fluctuate dramatically in the stems and fine roots. The controls showed slight decreases in these tissues in the first month probably due to increased transpiration, while the fertilized seedlings, with a greater nitrogen source, were able to maintain constant levels. Root amino acid levels have been suggested as a sensitive indicator of plant nitrogen status (van den Driessche and Webber, 1976). While this may be true for certain individual amino acids like arginine, this study shows much more dramatic increases in needles for total FAA when the treatment was a single, highly mobile fertilizer application in early autumn.

The ratio of umoles of FAA to mg of total nitrogen (Figure I.3) gives an index of the relative size of the FAA pool relative to total nitrogen. It was not possible with the technique used to estimate the actual amount of nitrogen in FAA since any given amino acid may have from one to four nitrogen atoms. Figure I.3 shows that there are considerable changes in the ratio of FAA per mg of total nitrogen over time with a particularly large increase in all tissues in mid-March before budbreak. Furthermore, the needles show a significant difference in the ratio due to fertilization. Increases in FAA

concentrations due to water stress (Chen et al. 1964, Stewart and Larher 1980) and temperature (van den Driessche and Webber 1975) have been reported. Although the percentage of the FAA making up the total nitrogen pool may be small (around 1% to 4%), it is possible that a 50% or 100% increase may have considerable biological significance. Consequently, I believe it may not be a good practice to infer FAA concentrations from total nitrogen data alone.

Shoots of Douglas-fir normally cease growth in mid-summer. Following this, during the month of October, Douglas-fir goes through a phase of its life cycle referred to as "dormancy deepening" (Lavender and Cleary, 1974). During this period, metabolic changes occur that increase frost hardiness and bring Douglas-fir to a truly dormant state (ie. shoots will not elongate even when conditions are favorable). Also during this period, the bud primordia are developing. The nitrogen pulse induced by fertilization in the autumn has been shown to increase the number of needle primordia and the degree of cold hardiness in Douglas-fir (Thompson, 1983).

However, the benefits of this type of fertilization have a cost. In this study, the increase in nitrogen concentration resulted in a significant depletion of non-structural carbohydrates (NSC) reserves. The fine roots showed significantly lower sugars and trends towards

lower available polysaccharides soon after fertilization. This may be the result of the carbon costs of nitrate and ammonium assimilation as well as the increased respiratory costs needed to maintain the newly formed enzymes.

Stems and needles, however, do not show significantly lower NSC until just prior to budbreak. In stems and needles, it is the available polysaccharide levels, not the free sugars, that are depleted by the additional nitrogen. Since this does not occur until mid-March, the carbon cost of ammonium and nitrate assimilation is probably not the reason for the carbohydrate depletion. Rather, it is likely that the warmer March temperatures increase maintenance respiration rates to levels where the costs of maintaining the additional enzymes levels result in significant decreases in available polysaccharides. Furthermore, since the date of budbreak is significantly advanced in the fertilized trees (see Chapter II), they may be more actively synthesizing the enzymes required for growth than the control seedlings in mid-March. This could also result in higher respiration rates.

Therefore, it is important that seedlings receiving an application of nitrogen fertilizer in the autumn have adequate NSC reserves to maintain the increased enzyme levels as well as the demands for growth in the spring. Nursery or storage practices that result in seedlings with lower NSC reserves may have poor field survival and growth

(Marshall 1985, Ronco 1973, Ericsson et al. 1983).

Non-structural carbohydrate depletion is a possible reason for the negative or inconclusive results sometimes reported for seedlings receiving nitrogen fertilizer in the autumn.

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CHAPTER II

FIELD PERFORMANCE OF 2-Ø DOUGLAS-FIR SEEDLINGS
AFTER AUTUMN FERTILIZATION WITH NITROGEN

by

Hank Margolis

ABSTRACT

After fertilization with ammonium nitrate in the October before lifting, 2-Ø Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seedlings were planted in a split plot design. One half of each planting block was seeded with grass to induce a water stress in the Douglas-fir seedlings during the summer drought. Within each main plot, the previously fertilized and unfertilized seedlings were planted. Sucrose was applied to the soil around each seedling to limit the availability of nitrogen to tree roots.

The fertilized seedlings broke bud ten days earlier on the average than the unfertilized seedlings and produced more growth aboveground. The earlier budbreak was responsible for initial differences in growth response. Later harvests showed that fertilized seedlings also exhibited higher relative growth rates.

Seedlings growing in the grass plots had predawn water potentials of -1.5 MPa by early August. By September 3, the unfertilized seedlings growing with grass showed significantly more predawn water stress than any of the other three treatments.

Although the fertilized seedlings had higher free amino acid (FAA) and total nitrogen concentrations than unfertilized seedlings when they were planted

(see Chapter I), by the end of one growing season the FAA and total nitrogen concentrations had equalized. However, the fertilized seedlings contained more FAA and nitrogen because of their greater size.

Grass competition affected both seedling nitrogen and carbohydrate chemistry. Seedlings growing with grass competition had 15% higher FAA concentrations in new growth; 21% lower N:P ratios in the old leaves; and 46% higher FAA to total N ratios in stems. The fine roots of seedlings growing with grass competition showed similar alterations in their nitrogen chemistry.

The grass treatment resulted in higher sugar, lower available polysaccharide and lower total non-structural carbohydrate (NSC) concentrations in new growth, old leaves and stems. Fine roots, on the other hand, had higher sugar, lower available polysaccharide and higher total NSC concentrations.

After the end of one growing season, the fertilized seedlings showed a 3 cm or 37% increase in height increment, a 29% increase in the number of stem units on the terminal leader; a 44% increase in aboveground growth; a 25% increase in total seedling leaf area; a 23% increase in relative growth rate; and a 14% increase in production per unit nitrogen.

INTRODUCTION

Fertilizing tree seedlings in the early stages of stand establishment can be inefficient since it may result in a growth response from competing vegetation. Because of intense competition on crop trees in the early stages of stand establishment, there has been much interest in developing cultural procedures to grow nursery seedlings of high physiological vigor that are capable of rapid early growth (Duryea and Landis 1984, Wakely 1954). In the Pacific Northwest, however, standard nursery practice includes withholding water and nitrogen in mid-summer to induce dormancy and protect seedlings from frost injury (Cleary et al. 1978). For this reason, late season nitrogen applications have been avoided.

In general, field performance by nursery seedlings receiving late season nitrogen fertilization has not proven beneficial with pines in the southeastern U.S. (Shoulders 1959, Gilmore et al. 1959, Ursic 1956); has been inconclusive with a variety of conifers in Great Britain (Benzian et al. 1974); and has given favorable results with Douglas-fir in the Pacific Northwest (Anderson and Gessel 1966, Thompson 1983). However, most of these experiments have only involved relatively simple correlations with height growth. To my knowledge, few if any studies have explored the mechanisms behind the

observed growth response. By altering the environment in a controlled but severe manner and measuring certain changes in biochemistry and growth, I tried to obtain some additional insight.

Consequently, I chose to outplant 2-0 Douglas-fir seedlings whose internal nutrition had been altered by an October application of ammonium nitrate fertilizer in the nursery. One half of each planting block was seeded with a heavy grass cover in an effort to induce water stress during the summer drought, a procedure used by others (Hedrick and Keniston 1966, Newton 1967, Larson and Schubert 1969, Preest 1977, Eissenstat and Mitchell 1983). This combination of treatments allowed study of the interaction between seedling nutrition and water stress.

Specifically, I wished to test the following hypotheses:

1. The fertilized seedlings would break bud earlier and have greater growth than unfertilized seedlings.
2. Both earlier budbreak and a greater relative growth rate would be important in the growth response of fertilized seedlings.

3. Grass would cause greater water stress in the fertilized seedlings than in the unfertilized.
4. Although the fertilized seedlings were planted with elevated nitrogen and free amino acid concentrations, the concentrations would equalize after one growing season.
5. Non-structural carbohydrate (NSC) concentrations would show a significant interaction between the grass and fertilizer treatments. With grass competition, sugar concentrations were expected to increase while available polysaccharide and total NSC concentrations were expected to decrease. However, the decrease in available polysaccharide and total NSC concentrations was predicted to be greater in the fertilized seedlings because they should have been affected more adversely by the summer drought.
6. The shoot growth per unit of nitrogen would be the same for the fertilized and unfertilized seedlings.

MATERIALS AND METHODS

The fertilized and unfertilized Douglas-fir seedlings from the nursery bed described in Chapter I were lifted on February 2, 1984 and placed in cold storage at 2°C. All the seedlings were outplanted at an elevation 110 meters above sea level at the Peavy Arboretum near Corvallis, Oregon (44° 30'N latitude, 123° 15'W longitude) by February 11, 1984. This area has considerable rainfall throughout the autumn, winter and spring but typically has a substantial dry period during the summer months (Franklin and Dyrness, 1973). The planting site was a gently sloping south-facing aspect on a deep, well-drained Jory series clay (U.S. Soil Conservation Service, 1975). The surface soil was about 50 cm deep, friable and had a high clay content.

Nearly 900 seedlings were carefully planted with a shovel in a split plot design in three blocks. The main treatment was the presence or lack of grass competition. This was intended to place the seedlings under different degrees of water stress during the summer months. The entire area, about 0.2 hectare, had been disked and planted with annual rye grass (Lolium multiflorum L.) the previous fall. In March, when the grass was already several centimeters tall, one half of each of the three blocks was randomly selected to have the grass removed. On these plots, atrazine was applied at 3.4 kg per hectare

on March 2, 1984. On March 16, an additional 1.3 kg per hectare was applied. This resulted in nearly total removal of the rye grass cover. In mid-July, any grass that had become established since the atrazine treatment, was spot sprayed with glyphosate. Grass on the untreated plots grew about 0.75 meter tall. The grass did not seem to significantly shade the tree seedlings and the major effect of the grass appeared to be the induction of a significant summer water stress.

Within each main plot, previously fertilized and unfertilized seedlings were randomly planted in pairs. The fertilized seedlings had been treated in the nursery bed with ammonium nitrate fertilizer. Two applications of 168 kg per hectare each were applied on October 1 and October 11, 1983. Thus the fertilized and unfertilized treatments comprised the subplots (3 blocks x 2 grass treatments x 2 fertilizer treatments = 12 subplots).

On March 9, 1984 about 29 grams of sucrose was placed in a 30 cm radius around each seedling. This was the equivalent of about 1000 kg per hectare. On March 21, another application of sucrose was applied in a similar manner. The sucrose provides a readily available carbon substrate to the soil microbes. The rapid growth of the microbe population results in the immobilization of much of the nitrogen in microbial biomass. Thus, a condition of nitrogen stress is created (Johnson and Edwards 1979,

Waring 1982). This was done to maximize the chance of detecting a response of the seedlings to the nursery fertilization treatment.

To determine the date of budbreak, a survey was conducted every two days between March 21 and May 9, 1984. A seedling was considered to have broken bud if two buds on the upper half of the seedling had new leaves extending out of the bud. On May 17 and 18, black-tailed deer (Odocoileus hemionus columbianus Richardson) caused considerable damage to seedlings by browsing the terminal leaders. On May 19, a small amount of BGR Big Game Repellent was put on every terminal leader. No additional deer damage was observed. All browsed seedlings were removed from the experiment. Of the remaining seedlings, less than 2% died during the first growing season.

Five seedlings were removed from each of the 12 subplots on both May 15 and June 15, 1984. The dry weights of new aboveground growth, old leaves, and stems were determined and standard growth analyses were performed (Evans, 1972).

On July 11, August 2, August 18 and September 3, predawn water potentials were measured on 3 seedlings per subplot (36 seedlings in total) using a pressure chamber (Scholander et al 1964, Ritchie and Hinkley 1975). Once a seedling was sampled, it was removed from the experiment. On August 7 and 8, diurnal water potentials were measured

approximately every three hours throughout a 24 hour period from 6:00 AM to 3:00 AM. Two seedlings per subplot or 24 seedlings in total were sampled at every time period.

On September 3, two composites of five seedlings each were taken from each subplot. From each of the 24 composites, samples of new aboveground growth, old leaves, stems and fine roots were analyzed for free amino acids, total Kjeldahl nitrogen, total phosphorus, sugars and available polysaccharides. A sequential extraction and analysis technique was used following procedures described in the Appendix. The term available polysaccharide refers primarily to starch. However, recent work suggests that some polysaccharides other than starch may be utilized in the respiratory processes of Douglas-fir but at this point the data is tentative (Weiger Schaap, personal communication). Until this issue is resolved, I chose to use the term "available polysaccharide" rather than "starch".

On September 4, the length of the terminal leader and total seedling height was measured on 300 seedlings (25 from each subplot). Only seedlings with an easily identifiable terminal leader were sampled. On September 5, the aboveground portions of these 300 seedlings were harvested and standard growth analyses were performed. The

dry weights of the new aboveground growth, the old needles and the stems were determined. The number of stem units (needles plus buds) on the terminal leader was also measured for each seedling.

A sample of leaves equivalent to about 10 cm^2 was collected and the area determined precisely with a Licor Model 3100 Area Meter. The leaves were then dried in a forced-air oven at 70°C and weighed. Specific leaf area was calculated by dividing leaf area by the dry weight.

Split plot analyses of variance were performed with the grass competition as the main treatment and the nursery fertilization as the secondary treatment. For the seasonal predawn and diurnal water potential data, split-split plot analyses of variance were conducted with time as the main treatment, grass competition as the secondary treatment and the nursery fertilization as the tertiary treatment. The Statistical Package for the Social Sciences (SPSS) was used to calculate the analyses of variance and descriptive statistics (Nie et al 1975, Hull and Nie 1981). For multiple comparisons, Tukey's honestly significant difference procedure (Steel and Torrie, 1980) was used to compute the least significant differences (LSD) at the 0.10, 0.05 and 0.01 levels (Steel and Torrie 1980, Harter 1960). For single comparisons of two means, the t-test was used (Snedecor and Cochran 1980).

RESULTS

Figure II.1 shows the cumulative frequency of budbreak for both the fertilized and unfertilized seedlings during the growing season. Applying nitrogen fertilizer to nursery beds in early October resulted in significantly earlier budbreak. The mean date of budbreak shifted from April 16 for the unfertilized seedlings to April 7 for the fertilized. The date of 50% budbreak was April 15 and April 5 for the unfertilized and fertilized seedlings, respectively.

The analyses of variance indicated that all the variables measured before August showed only fertilizer effects. The effects of the grass treatment and interactions between the grass and fertilizer treatments were not apparent until after the summer drought had begun.

The fertilized seedlings not only broke bud earlier but had significantly greater amounts of new aboveground tissue on all three of the sampling dates; May 15, June 15, and September 5 (Table II.1). Similarly, the relative growth¹ of the fertilized seedlings was also greater on all three of the sampling dates.

1. Grams of new growth per aboveground weight in grams of the old seedling.

Fig II.1 Cumulative frequency of budbreak for nitrogen fertilized (+————+) and unfertilized (•-----•) Douglas-fir seedlings. Budbreak surveys were conducted every two days on 341 fertilized and 341 unfertilized seedlings. A seedling was considered to have broken bud if two buds on the upper half of the seedling had new leaves extending out of the bud. (Figure on next page).

FIG II.1 CUMULATIVE FREQUENCY OF BUDBREAK

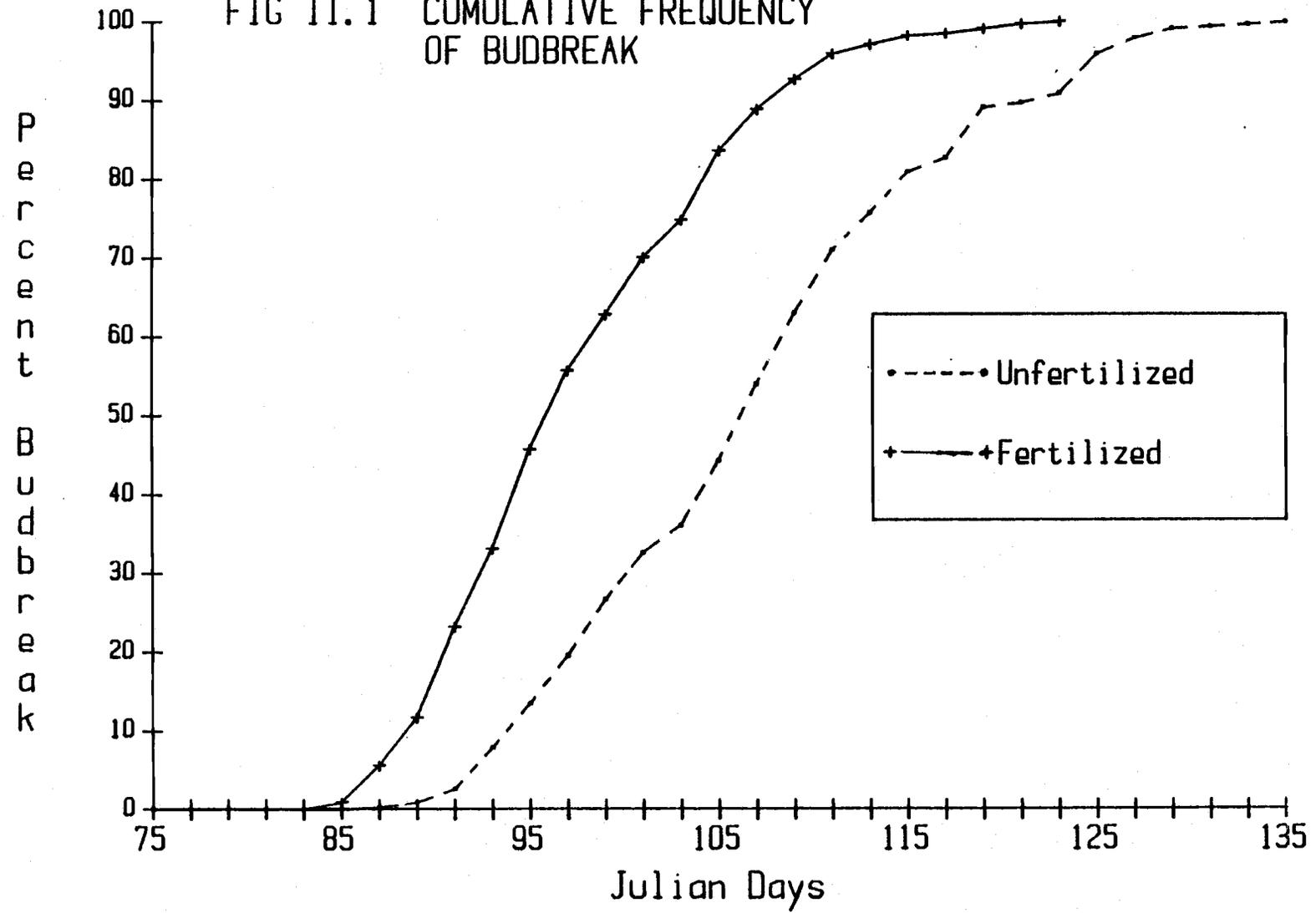


Table II.1

New aboveground growth, relative growth, relative growth rate, and net assimilation rate of fertilized and unfertilized Douglas-fir seedlings on May 15, June 15 and September 5. Each value is the mean (\pm s.e.) for 6 subplots. May 15 and June 15 data contained 5 seedlings per subplot. September 5 contained 25 seedlings per subplot. The interactions between the fertilizer and grass treatments were not significant so the grass treatments were pooled. Asterisks indicate significant differences between treatments (n.s = not significant, ** = $p < 0.05$, *** = $p < 0.01$).

<u>NEW ABOVEGROUND GROWTH</u>			
(grams)	<u>Unfertilized</u>	<u>Fertilized</u>	Δ %
May 15	0.8 (0.1)	1.3 (0.1)***	+63%
June 15	3.1 (0.5)	5.0 (0.6)**	+61%
September 5	4.5 (0.3)	6.5 (0.4)***	+44%
<u>RELATIVE GROWTH</u>			
(gram/gram)			
May 15	0.08 (0.01)	0.12 (0.01)***	+50%
June 15	0.25 (0.02)	0.39 (0.03)***	+56%
September 5	0.42 (0.01)	0.55 (0.02)***	+31%
<u>RELATIVE GROWTH RATE</u>			
(mg/gram/day)			
May 15	3.3 (0.4)	3.5 (0.2)	ns
June 15	4.4 (0.4)	6.0 (0.4)**	+36%
September 5	3.0 (0.1)	3.7 (0.1)***	+23%
<u>NET ASSIMILATION RATE</u>			
(mg/gram needles/day)			
May 15	9.5 (1.5)	10.3 (0.9)	ns
June 15	12.4 (1.5)	17.9 (1.6)**	+44%

The relative growth rates² showed no significant difference between fertilized and unfertilized seedlings on the May 15 harvest. On the June 15 and September 5 harvests, however, the fertilized seedlings did have higher relative growth rates. Net assimilation rate³ showed a pattern similar to that for relative growth rate (Table II.1).

When the amount of new growth was compared with the date of budbreak for each of the sampling dates, the regression coefficient of determination (R^2) decreased from 0.51, to 0.13, to 0.09 for the May 15, June 15 and September 5 harvests, respectively.

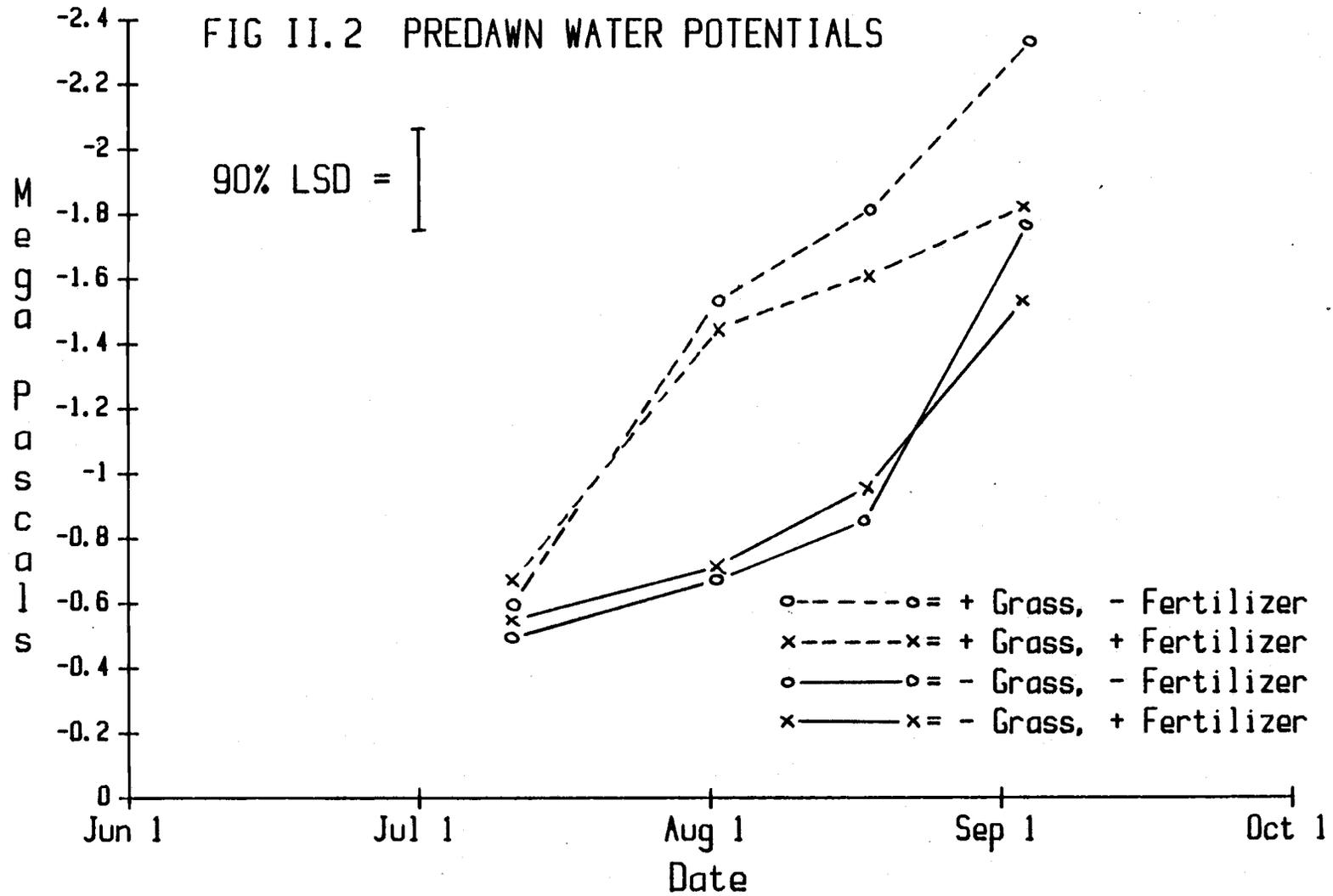
The deer damage, although not a planned part of this experiment, showed an interesting trend. Out of the fifty-five seedlings that had been browsed on May 17 and May 18, forty-three (78%) were seedlings from the fertilizer treatment. Of the twelve unfertilized seedlings that had browse damage, seven (13%) had been growing adjacent to fertilized seedlings that had been damaged by deer.

Grass competition did not significantly affect predawn water potential until sometime between mid-July

-
2. Mg new growth per aboveground weight in grams of the old seedling per day.
 3. Mg new growth per gram of old leaves per day.

Fig II.2 Predawn water potentials (MPa) of nursery fertilized and unfertilized Douglas-fir seedlings growing with and without grass competition in the first year after outplanting. (With Grass, Without Fertilizer = •-----• ; With Grass, With Fertilizer = X-----X; Without Grass, Without Fertilizer = •————• ; Without Grass, With Fertilizer = X————X). Each point is the mean of measurements on nine seedlings. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie 1980). 90% LSD = 0.31 MPa. (Figure on next page).

FIG II.2 PREDAWN WATER POTENTIALS



and early August (Figure II.2). The month of June had a record high 110 mm of rain. From June 29 to September 6, however, a severe summer drought occurred. During this nine week period, the only measurable rain fell on the evening of July 25. While the grass treatment clearly caused a significant amount of water stress, the fertilized and unfertilized seedlings had similar predawn values until the final September 3 measurement. On this date, the unfertilized seedlings increased their predawn water stress significantly over the fertilized seedlings when both were growing with grass competition.

At both 9:00 AM and 12:00 PM on the August 7-8 diurnal water stress curve (Figure II.3), the unfertilized trees with grass competition showed more stress ($p < 0.1$) than fertilized seedlings growing without grass competition. The other treatments were not significantly different from each other in the morning and none of the treatments differed in the afternoon. After sundown, however, the seedlings growing without grass recovered quickly from the daytime water stress while seedlings growing with grass recovered more slowly.

The final harvest of the seedlings occurred on September 5, one day before the first fall rain. The results for the nitrogen chemistry of new growth, old leaves, stems and fine roots are shown in Table II.2. To assure that analyses expressed on the basis of dry weight

Fig II.3 Diurnal water potentials (MPa) for August 7-8, 1984 for nursery fertilized and unfertilized Douglas-fir seedlings growing with and without grass competition in the first year after outplanting. (With Grass, Without Fertilizer = •-----• ; With Grass, With Fertilizer = X-----X; Without Grass, Without Fertilizer= •-----• ; Without Grass, With Fertilizer = X-----X). Each point is the mean of measurements on nine seedlings. The 95% least significant difference (LSD) bar was calculated according to Tukey's honestly significant difference procedure (Steele and Torrie 1980). 90% LSD = 0.45 MPa. (Figure on next page).

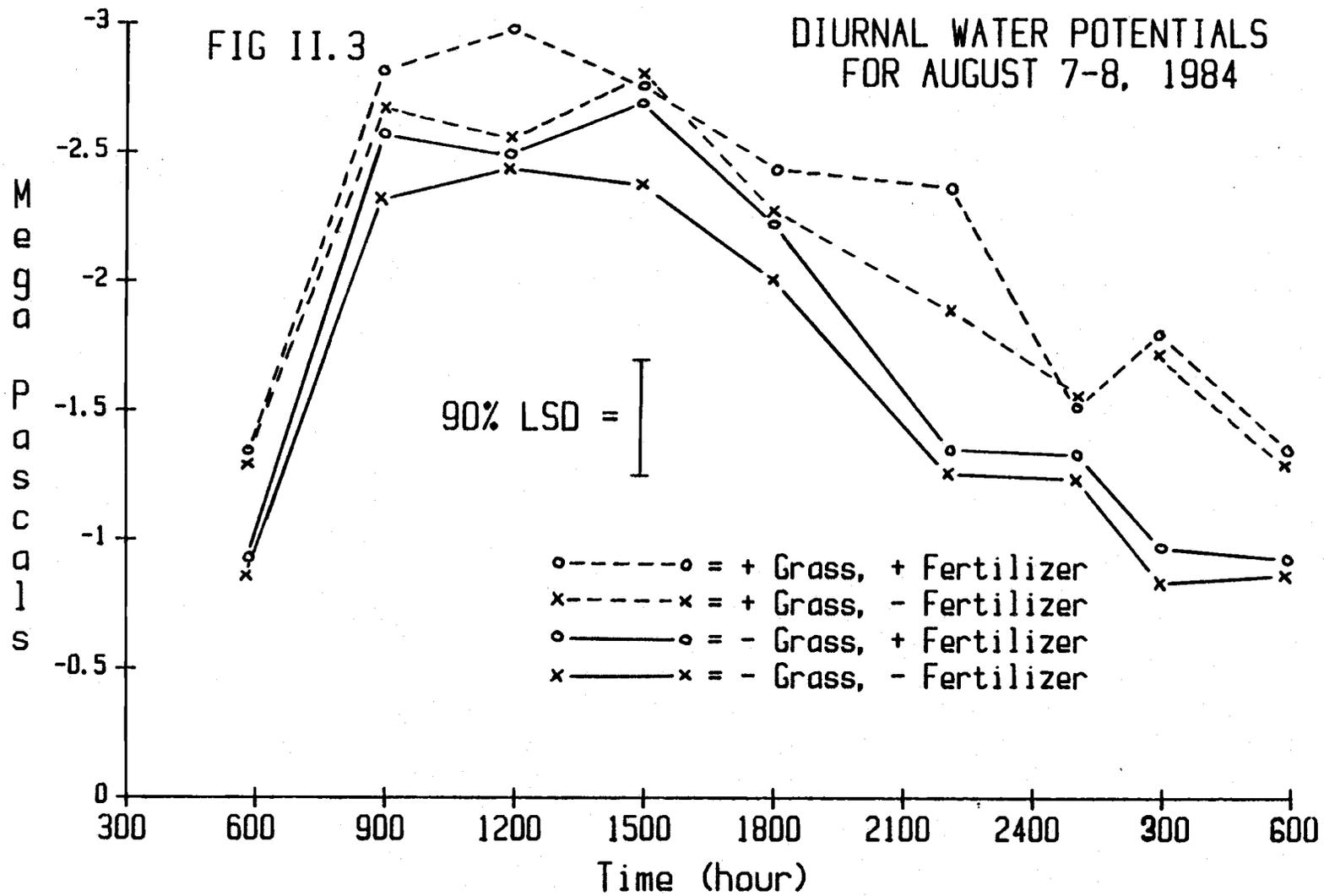


Table II.2

Nitrogen chemistry for new aboveground growth, old needles, stems and fine roots of Douglas-fir seedlings growing with and without grass competition in a split plot design. Each value is the mean (+s.e.) for 3 of the main plots. Each main plot value consists of four determinations done on four composites of five seedlings. The interactions between the fertilizer and grass treatments were not significant so the fertilizer treatments were pooled. Asterisks indicate significant differences between treatments (ns = not significant, * = $p < 0.1$, ** = $p < 0.05$). Abbreviations: FAA=Free Amino Acids, N=Nitrogen, P=Phosphorus.

<u>NEW ABOVEGROUND GROWTH</u>	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Free Amino Acids (umoles/dry gram)	26.4 (1.0)	30.4 (0.1)**	+15%
Total Nitrogen (mg/gram)	12.4 (1.0)	9.3 (0.3)	ns
Total N to Total P Ratio (mg/mg)	8.2 (0.6)	7.0 (0.3)	ns
Ratio of FAA to Total N (umoles/gram)	2.6 (0.2)	2.9 (0.2)	ns
 <u>OLD LEAVES</u>	 <u>Without Grass</u>	 <u>With Grass</u>	 <u>Δ %</u>
Free Amino Acids	26.4 (0.8)	27.7 (1.5)	ns
Total Nitrogen	10.4 (0.5)	9.1 (0.2)	ns
Total N to Total P Ratio	8.2 (0.7)	6.5 (0.4)*	-21%
Ratio of FAA to Total N	2.5 (0.1)	3.0 (0.2)	ns
 <u>STEMS</u>	 <u>Without Grass</u>	 <u>With Grass</u>	 <u>Δ %</u>
Free Amino Acids	17.2 (1.2)	19.5 (1.1)	ns
Total Nitrogen	2.7 (0.3)	2.2 (0.2)	ns
Total N to Total P Ratio	4.8 (0.2)	4.7 (0.3)	ns
Ratio of FAA to Total N	6.5 (0.7)	9.5 (0.8)**	+46%
 <u>FINE ROOTS</u>	 <u>Without Grass</u>	 <u>With Grass</u>	 <u>Δ %</u>
Free Amino Acids	25.0 (1.0)	35.0 (0.9)**	+40%
Total Nitrogen	7.5 (0.3)	7.2 (0.3)	ns
Total N to Total P Ratio	7.1 (0.2)	6.7 (0.1)**	-6%
Ratio of FAA to Total N	3.4 (0.2)	5.0 (0.3)*	+47%

were not confounded by differences in non-structural carbohydrate (NSC), I made a comparison and found that correcting for different NSC levels did not significantly affect the results. For this reason, the data are reported here simply on a dry weight basis. The analyses of variance showed that only the effects of the grass competition were significant. The effects of the fertilizer treatment and the interactions between the fertilizer and grass treatments were not significant. The grass treatment resulted in higher free amino acid (FAA) concentrations in the new growth, higher nitrogen to phosphorus ratios in the old leaves, and a higher ratio of FAA to total nitrogen ($\mu\text{moles/mg}$) in the stems. The fine roots of the seedlings grown with grass showed similar responses to those observed in the aboveground tissues (higher FAA concentrations, higher N to P ratio, and a higher FAA to total N ratio).

The analyses of variance for non-structural carbohydrates (NSC) showed significant grass effects (Table II.3). Old leaves showed a 8.1 mg/gram increase in sugar concentrations in the fertilized seedlings. None of the fertilizer and grass interactions were significant. The seedlings growing in the grass showed higher sugar, lower available polysaccharide, and lower total NSC (sugar + available polysaccharide) concentrations in new growth and old leaves than seedlings growing without grass (Table

Table II.3

Sugars, available polysaccharide and total non-structural carbohydrate (NSC) concentrations for new aboveground growth, old needles, stems and fine roots of Douglas-fir seedlings growing with and with out grass competition in a split plot design. Each value is the mean (+s.e.) for 3 of the main plots. Each main plot value consists of four determinations done on four composites of five seedlings. The interactions between the fertilizer and grass treatments were not significant so the fertilizer treatments were pooled. Asterisks indicate significant differences between treatments (ns = not significant, * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$).

<u>NEW ABOVEGROUND GROWTH</u>	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Sugars (mg/dry gram)	113.9 (1.5)	131.9 (5.3)*	+16%
Available Polysaccharide	56.0 (6.1)	18.5 (3.4)**	-67%
Total NSC	169.9 (6.2)	150.3 (4.5)**	-12%
<u>OLD LEAVES</u>	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Sugars	124.5 (2.6)	149.4 (3.8)**	+20%
Available Polysaccharide	41.6 (6.8)	0 (0.6)**	-100%
Total NSC	166.1 (6.6)	149.4 (3.8)***	-10%
<u>STEMS</u>	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Sugars	53.5 (2.3)	60.7 (3.1)*	+13%
Available Polysaccharide	23.3 (3.4)	8.1 (0.7)*	-65%
Total NSC	76.8 (5.2)	68.8 (3.0)	ns
<u>FINE ROOTS</u>	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Sugars	49.5 (2.5)	66.9 (4.2)***	+35%
Available Polysaccharide	15.6 (2.0)	7.1 (1.4)**	-54%
Total NSC	65.1 (3.9)	74.0 (5.1)*	+14%

II.3). The stems of grass grown seedlings also showed higher sugar and lower available polysaccharide concentrations than found in seedlings growing without grass competition. Total NSC in the stems, while tending to have lower values in the grass, were not statistically different than seedlings growing without grass. On the other hand, the fine roots of seedlings growing with grass competition had higher sugars, lower available polysaccharides and higher total NSC concentrations than the seedlings growing without grass competition (Table II.3).

Although the nitrogen and NSC concentration data showed primarily grass effects (Tables II.2 and II.3), the total seedling nitrogen and NSC contents showed both fertilizer effects and grass effects (Table II.4, II.5). Fertilized seedlings had greater FAA, total nitrogen, sugar and total NSC contents than unfertilized seedlings. However, available polysaccharide and total NSC contents showed a significant grass effect in addition to the fertilizer effect. Both were lower in the seedlings grown with grass competition.

Table II.6 presents a summary of the morphology and growth of seedlings harvested in September after one growing season in the field. The height of the terminal leaders was increased 3.0 centimeters or 37% by fertilization. Total height increased 6% while relative

Table II.4

Total content of free amino acids (FAA), total nitrogen, sugars, available polysaccharide, and total non-structural carbohydrate (NSC) in the aboveground portions of fertilized and unfertilized Douglas-fir seedlings growing in a split plot design. Each value is the mean (+s.e.) for 6 subplots. Each subplot consists of two determinations done on two composites of five seedlings. The interactions between the fertilizer and grass treatments were not significant so the grass treatments were pooled. Asterisks indicate significant differences between treatments (ns = not significant, * = $p < 0.10$, ** = $p < 0.05$).

	<u>Unfertilized</u>	<u>Fertilized</u>	<u>Δ %</u>
Free Amino Acids (umoles)	366.1 (12.6)	403.7 (30.9)**	+10%
Total Nitrogen (mg)	102.7 (10.8)	120.8 (14.7)**	+18%
Sugars (mg)	1462.7 (46.2)	1642.8 (79.9)*	+12%
Available Polysaccharide (mg)	350.7 (111.2)	438.4 (117.4)	ns
Total Non-Structural Carbohydrate (mg)	1813.4 (109.6)	2081.2 (134.8)*	+15%

Table II.5

Total content of free amino acids, total nitrogen, sugars, available polysaccharide and total non-structural carbohydrate in the aboveground portions of Douglas-fir seedlings growing with and with out grass competition in a split plot design. Each value is the mean (+-s.e.) for 3 of the main plots. Each main plot consists of four determinations done on four composites of five seedlings. The interactions between the grass and fertilizer treatments were not significant so the fertilizer treatments were pooled. Asterisks indicate significant differences between treatments (ns = not significant, * = $p < 0.10$, ** = $p < 0.05$).

	<u>Without Grass</u>	<u>With Grass</u>	<u>Δ %</u>
Free Amino Acids (umoles)	411.2 (25.7)	358.7 (17.9)	ns
Total Nitrogen (mg)	133.0 (12.9)	90.6 (4.2)	ns
Sugars (mg)	1542.4 (67.9)	1563.1 (84.3)	ns
Available Polysaccharide (mg)	634.6 (58.3)	154.4 (21.7)**	-76%
Total Non-Structural Carbohydrate (mg)	2177.1 (93.7)	1717.6 (86.4)**	-21%

Table II.6

Growth analysis and morphological characteristics of fertilized and unfertilized Douglas-fir seedlings harvested on September 5. Values, except those for budbreak, are the means (+s.e.) for 6 subplots with 25 seedlings per subplot. Budbreak values are based on measurements of 682 seedlings. The interactions between the fertilizer and grass treatments were not significant so the grass treatments were pooled. Asterisks indicate significant differences between treatments (ns = not significant, * = $p < 0.10$, ** = $p < 0.05$, *** = $p < 0.01$).

	<u>Unfertilized</u>	<u>Fertilized</u>	<u>Δ %</u>
Mean Budbreak	April 16	April 7***	
50% Budbreak	April 15	April 5	
Height Growth (cm)	8.2 (0.3)	11.2 (0.4)***	+37%
Relative Height Growth (cm/cm)	51.5 (1.1)	54.4 (0.7)***	+6%
Stem Units on Terminal Leader (#)	217 (6.1)	281 (10.9)***	+37%
Stem Units Per Length of Leader (#/cm)	29.2 (1.0)	25.2 (0.8)***	-14%
New Aboveground Growth (grams)	4.5 (0.3)	6.5 (0.4)***	+44%
Leaf Area of New Growth (sq cm)	362 (19)	522 (30)***	+44%
Leaf Area of Total Seedling (sq cm)	574 (16)	715 (30)***	+25%
Relative Growth (gram/gram)	0.42 (0.01)	0.55 (0.02)***	+31%
Relative Growth Rate (gram/gram/day)	2.97 (0.07)	3.65 (0.10)***	+23%
Nitrogen Productivity ¹	43.4 (2.7)	49.5 (3.1)***	+14%

1. Mg of new growth /mg of nitrogen in the total seedling previous November.

height growth increased 37%. The terminal leader had 29% more stem units but 14% fewer stem units per unit length of terminal leader. The amount of new aboveground growth, whether expressed on a dry weight or on a leaf area basis, was increased 44%. Total seedling leaf area increased 25%, relative growth increased 31% and relative growth rate increased 23%. The growth efficiency of nitrogen, expressed as mg of new growth produced by September per mg of total nitrogen in the seedling the previous November, increased 14% in the fertilized trees. None of these traits were significantly affected by the grass competition.

The specific leaf areas of seedlings growing with grass showed an 8% increase from 78 to 84 cm²/gram in new needles and a 12% increase from 58 to 65 cm²/gram in old needles. The fertilized seedlings growing with grass shed significantly more of their old needles (40%) than any of the other three treatments (grass/unfertilized=27%, no grass/fertilized=20%, no grass/unfertilized=18%).

DISCUSSION

The possibility of advancing budbreak ten days by altering the nitrogen nutrition of a seedlings has a number of practical implications. Defoliating insects, such as the gypsy moth (Lymantria dispar L.) appear to time the beginning of their spring feeding activity to

warming temperatures (Leonard, 1981). This usually coincides to the period when new vegetative growth is expanding and protective phenolic compounds have not yet formed in the new foliage. Advancing the phenology by ten days could give a tree the additional time necessary to form enough protective compounds to confer a significant amount of resistance to the defoliating insect. If the earlier budbreak resulted in frost damage, however, the potential advantages would be lost.

The ability to advance budbreak could also have applications in broadening seed transfer zones. A genotype from a northern seed zone might be made more adaptable to a warmer southern environment by continuing applications of nitrogen fertilizer in the autumn. Once again, the potential for frost damage due to the earlier budbreak would have to be given close consideration.

Another important implication involves the "greenhouse effect." Rising atmospheric CO₂ concentrations caused by the dramatic increases in both the combustion of fossil fuels and global deforestation are predicted to result in a climatic warming trend. A 1-5°C rise in winter temperatures by the year 2050 has been postulated (Schneider 1975, Baes et al. 1977, Chan et al. 1980). This could result in large areas of Douglas fir being unable to meet their winter chilling requirement. Trees that have not met their full chilling

requirement have delayed budbreak (Lavender, 1981). It is possible, therefore, that nitrogen fertilization in the autumn may partially compensate for inadequate winter chilling. Consequently, it may be possible to partially offset some of the effects of rising global temperatures on forest vegetation. It is also possible that nitrogen fertilization in the autumn might lower a tree's chilling requirement. This hypothesis merits further study.

It is clear that the improved nitrogen nutrition advanced budbreak and increased growth. By sampling at three times throughout the summer, I was able to distinguish to what extent growth was influenced by early budbreak and by increased relative growth rate. On May 15, no difference in relative growth rates could be discerned. Therefore, the greater amount of aboveground growth in the fertilized seedlings for the first few weeks after budbreak was due to the earlier budbreak. On May 15, 51% of the variation in the amount of new aboveground growth could be explained by the date of budbreak. By June 15 and throughout the remainder of the growing season, a difference in relative growth rates was primarily responsible for the greater growth of the fertilized seedlings. By June 15, only 13% of the variation in new aboveground growth could be explained by the date of budbreak. On the other hand, the relative growth rate was 36% higher and net assimilation rate was

44% higher in the fertilized seedlings.

The carbon to nitrogen ratio in foliage is thought to influence the relative allocation of resources between the roots and shoots (Lainson and Thornley 1982, Reynolds and Thornley 1982, Chapin 1980, Novoa and Loomis 1981). Consequently, increasing the nitrogen concentrations in the Douglas-fir seedlings in this experiment probably shifted the relative allocation of dry matter production away from the roots to the stem and leaves (Table II.1). Thompson (1983) showed greater potential root growth in fall fertilized seedlings, but whether a greater fraction of carbon was allocated to shoots was not reported. Further work is warranted on how autumn nitrogen fertilization changes patterns of carbon allocation in Douglas-fir.

A shift in carbon allocation away from roots toward shoots could increase susceptibility to drought by fertilized seedlings. The predawn water potentials in Figure II.2, however, show that the fertilized Douglas-fir seedlings did not have significantly greater predawn water stress than the unfertilized seedlings. In fact, the September 3 sample indicated that the fertilized seedlings growing with grass competition may have been somewhat less stressed. Conceivably, a larger and more established root system gave fertilized seedlings a greater ability to exploit the soil for water. The diurnal water stress

pattern (Figure II.3) also indicates a tendency for the fertilized seedlings to be under less water stress than the unfertilized. High variability, however, prevents any firm conclusions.

It is important to keep in mind that although the seedlings had different internal nitrogen concentrations, the soil in which they were planted did not differ in fertility. This is distinctly different from the more common type of experiment in which seedlings are grown in both fertilized and unfertilized soils.

Although the fertilized seedlings had greater nitrogen concentrations at lifting, I expected nitrogen concentrations to equalize after one growing season if the growth of the fertilized seedlings was enhanced. This is precisely what occurred (Table II.2). Whereas the total nitrogen and free amino acid contents of the fertilized seedlings were greater in September (Table II.4), the concentrations did not differ from the unfertilized seedlings. Increased foliage on the fertilized seedlings (Table II.5) will allow increased absorption of light in subsequent years and cumulative improvements in growth.

It should be noted that the 30% increase in the foliar nitrogen just before budbreak corresponded to the 31% increase in relative growth measured in September and the 29% increase in the number of stem units on the terminal leader (Table II.5).

I thought that many of the variables measured would show interactions between the grass and fertilizer treatments and was surprised to discover that, by and large, this did not occur. The nitrogen and carbon chemistry, when expressed on a concentration basis, almost exclusively showed simple grass effects. There was some evidence to suggest that the grass competed with the seedlings for nitrogen (e.g. the significantly lower nitrogen to phosphorus ratios in the old leaves and fine roots). The grass also altered the nitrogen chemistry by competing for water. This is suggested by the increase in the size of the free amino acid pool and the ratio of free amino acids to total nitrogen (Table II.2).

As expected, the grass competition resulted in higher sugar and lower available polysaccharide concentrations. This agrees with previous work with sugar maple (Parker, 1970), black oak (Parker and Patton 1975) and cotton (Eaton and Eargle, 1948). The conversion of starch to sugar under drought stress is often associated with increased activity of amylases (Vaadia et al. 1966). It is interesting, however, that while the grass did cause significant decreases in total non-structural carbohydrates (NSC), the differences did not exceed 20 mg/gram. The seedlings growing with grass competition, while experiencing significant water stress for a large part of the summer, were not in danger of dying from

carbohydrate starvation. Consistent with the expected shift of carbon resources from shoots to roots with water stress, the fine roots of the seedlings growing in the grass had greater NSC concentrations.

It is clear from Table II.6 that the fertilized seedlings showed a greater aboveground growth response than the unfertilized seedlings whether grown with grass competition or not. Most of the traits measured showed only fertilizer effects. The grass treatment, while definitely affecting the seedling's water relations and carbohydrate status, did not appear to affect the seedling's height increment, dry weight gain, leaf area, stem units or relative growth rate. Eissenstat and Mitchell (1983), on the other hand, found that Douglas-fir in Idaho had reduced shoot and height growth in areas seeded with grass and clover. Of all the traits measured, only the old needle biomass and leaf area showed a significant interaction between the grass and fertilizer treatments. The fertilized seedlings in the grass shed significantly more old needles than any of the other three treatments.

The increase in nitrogen productivity⁴ indicates

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4. Mg of new aboveground growth in September per mg of nitrogen in the entire seedling the previous November.

that the fertilized seedlings were able to use their nitrogen more efficiently. However, because nitrogen productivity increased only 14%, while the amount of new aboveground growth increased 44%, the greater amount of nitrogen available to the fertilized seedling was more important than the efficiency with which it was used.

Increasing the nitrogen concentration of 2-0 Douglas-fir in the October before lifting resulted in a considerably greater growth response the year after outplanting. However, this was feasible only because serious carbohydrate depletion from increased winter respiration rates and frost damage from early budbreak were avoided. Furthermore, since the site had been treated with sucrose to decrease nitrogen availability these results probably approach the maximum differences that can be expected for seedlings with nitrogen values similar to those in this experiment. On planting sites with greater nitrogen availability, a less dramatic treatment effect would probably occur. Of course, this experiment was done with Douglas-fir seedlings from a single Willamette Valley seed zone. Whether or not Douglas-fir from other seed zones would respond in a similar manner merits further research.

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APPENDIX

APPENDIX

SAMPLE PREPARATION AND LABORATORY PROCEDURES

HARVESTING AND PREPARATION OF SAMPLES

Harvests were taken for laboratory analyses on October 1 and November 1, 1983; and February 1, March 15, and September 5, 1984. Composites consisting of five seedlings each were put in plastic bags and immediately placed in a cooler filled with dry ice. The seedlings were taken to Oregon State University and stored in a freezer at -40°C .

To prepare the samples for analysis, the roots of the seedlings were thoroughly washed to remove all soil. For each composite, needles were randomly stripped from the seedlings. One sample of approximately one gram and another sample of approximately four grams were weighed on a Mettler H80 balance. Next, sections of the stems of the seedlings in each composite were randomly cut. One sample of approximately one gram and another sample of approximately six grams were weighed. Fine roots of less than 2 mm in diameter were randomly cut from the seedlings in each composite and one sample of approximately 1.5 grams and another sample of approximately six grams were weighed. The smaller sample for each of the three tissue types was returned to the freezer for later analysis of free amino acids (FAA), sugars, and available

polysaccharide. The larger sample was oven-dried in a forced draft oven at 70°C for 72 hours to determine the moisture content and for later Kjeldahl analysis.

LABORATORY PROCEDURE

A sequential extraction and analysis procedure was developed with the help of Professor Te May Ching, Dept of Crop Science and Professor David Loomis, Dept of Biochemistry, Oregon State University.

1. The samples were extracted in 80% ethanol using a Polytron tissue homogenizer. Only needle tissue extracts required centrifugation and this was done in 300 ml plastic bottles on a Servall Superspeed rotary centrifuge at 6000 rpm for 30 minutes. All three types of tissue extracts were filtered through washed Whatman No. 5, 5.5 cm filter paper using a Buchner funnel and filter flask.
2. The residue on the filter paper was immediately removed and placed in a 125 ml Erlenmeyer flask for later analysis of available polysaccharides. The Erlenmeyer flask was covered and placed in the refrigerator.
3. The filtrate in the filter flask was then poured into a 250 ml beaker and 100 mg of insoluble polyvinylpyrro-

lidone (PVPP) was added to the filtrate to remove phenolic compounds that could potentially interfere with the spectrophotometric analyses (Sanderson and Perera 1966). The filtrate was then evaporated under a hood until the ethanol had disappeared. The concentrated filtrate next was filtered through a washed Whatman No. 1, 12.0 cm filter paper into a 100 ml volumetric flask and brought to volume. This extract was to be used for the determination of FAA and sugars.

4. Thirty-five ml of 35% perchloric acid was added to the residue in the 125 ml Erhlemeyer flask and placed on a rotary shaker for 24 hours. Cold perchloric acid will extract the available polysaccharides from the residue plus a certain amount of unavailable polysaccharide, probably pectic materials (Hansen and Moller 1975, Marshall 1983, Marshall and Waring 1985).

5. The perchloric acid extract was filtered through washed Whatman No. 1, 12.0 cm filter paper into a 100 ml volumetric flask and brought to volume. This extract was used for the determination of available polysaccharides.

6. The ethanol extracts were used to determine free amino acids (FAA) by a method modified from Moore and Stein

(1954) and Te May Ching (pers. comm.). FAA can only be determined accurately on wet tissue.

6-A. The following reagents are required:

- i) 4N sodium acetate buffer at pH 5.5. (Mix 35 ml H_2O with 32.8 grams anhydrous sodium acetate. Adjust to pH 5.5 with glacial acetic acid. Bring to 100 ml volume.)
- ii) Potassium cyanide at 0.01 M (0.065 grams in 100 ml solution).
- iii) Methylcellosolve (ethylene-glycol- monomethyl- ether)
- iv) Ninhydrin (1,2,3-Indantrione monohydrate) 3.75 grams in methylcellosolve, bring to 100 ml volume.
- v) 60% ethanol
- vi) Leucine

6-B. The Moore-Stein reagent was made by mixing 100 ml of reagent (i) above with 9.3 ml of reagent (ii) and 123 ml of reagent (iii).

6-C. One gram of Celite (diatomaceous earth) was dispersed in distilled H_2O and poured into a Buchner funnel so that the filter paper was evenly coated. The funnel was transferred to a new filter flask and about

20 ml of the ethanol extract was filtered through the Celite. This filtration helps clarify the sample by removing high molecular weight lipids and proteins.

6-D. Two ml of each Celite filtered extract was pipeted into a test tube.

6-E. 0.5 ml of the Moore-Stein buffer reagent described in 6-B above was added to each of the test tubes.

6-F. 0.5 ml of the ninhydrin reagent was added to each of the test tubes and the test tubes were loosely covered with bottle caps.

6-G. The test tubes were heated in boiling water for 20 minutes.

6-H. The test tubes were cooled for five minutes and then 5 ml of 60% ethanol was added. Each test tube was mixed on a vortex mixer.

6-I. The samples were read for absorption at 570 nm on a Beckman Acta III spectrophotometer using leucine standards from 0 to 50 ppm.

7. 100 ul of saturated lead acetate was added to the remaining 80 ml of the ethanol extract. After 20 minutes, 500 ul of saturated sodium oxalate was added and the flasks were placed in the refrigerator overnight. The lead acetate and sodium oxalate remove charged molecules from the solution thus decreasing interference of the sugars with the reagents used in the assay.

8. Sugars were determined colorimetrically by the anthrone reaction (Yemm and Willis, 1954).

8-A. The anthrone solution was made by slowly adding 200 ml of concentrated sulfuric acid to 500 ml of H₂O. After the mixture had cooled, 1.146 grams of anthrone was added and allowed to mix overnight.

8-B. 600 ul of the lead acetate/sodium oxalate cleared extract was added to each test tube.

8-C. 5 ml of the anthrone solution was then added to the test tubes and briefly mixed on a vortex mixer.

8-D. The test tubes were placed in boiling water for 12 minutes and then allowed to cool.

8-E. Samples were read for absorption at 625 nm on a Beckman Acta III spectrophotometer using glucose standards ranging from 0 to 160 ppm.

9. Extractable polysaccharides were determined colorimetrically from the perchloric acid extract as described in 8 above. The standards in this assay must contain the same amount of perchloric acid as the sample extracts.

10. A small percentage of the polysaccharides extracted by the perchloric acid are not available to the seedlings as fuel for respiratory processes (Hansen and Moller 1975, Marshall 1983). To determine the amount of unavailable polysaccharide extracted, Douglas-fir seedlings were placed in a warm, dark room just before they broke bud and kept well watered for 12 weeks. The seedlings broke bud and grew etiolated shoots. When the seedlings were nearly dead and their carbohydrate reserves had presumably been depleted, the seedlings were harvested and extractable polysaccharides were determined as described previously. This baseline amount was assumed to be the unavailable polysaccharide extracted from the tissue. By subtracting this baseline value from the values measured for each of the samples, the amount of available polysaccharides was determined. Baseline values for needles, stems and fine

roots were 25.8, 26.9 and 22.7 mg/gram dry weight, respectively.

11. The oven-dried samples were weighed and the moisture contents were calculated. The moisture contents were used to adjust the FAA, sugar, and available polysaccharide analyses from a fresh weight to a dry weight basis. The dried samples were then ground through a 40 mesh screen on a Wiley mill. Total organic nitrogen plus ammonium was determined with a semimicro-Kjeldahl digestion followed by ammonium analysis on a Technicon Autoanalyzer (Technicon Industrial Systems, 1975). A Se/CuSO_4 rather than a HgO catalyst was used. Total phosphorus could also be measured from the same samples. To do this, the reaction of orthophosphate, molybdate ion, and antimony ion is followed by a reduction with ascorbic acid under acidic pH (Technicon Industrial Systems, 1976).

12. Nitrate was analyzed using a distilled water extraction of between 2 and 3 grams of wet tissue. The extracts were kept frozen until the analysis was conducted. Analysis was done on a Technicon Autoanalyzer. Nitrate was converted to nitrite by a copper-cadmium reduction. The nitrite was then reacted with sulfanilamide under acidic conditions to form a

diazo compound. This compound then reacted with N-1-naphthyl-ethylenediamine to form a reddish dye that was analyzed colorimetrically (Technicon Industrial Systems, 1973).

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