Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism[1]

H. A. MARGOLIS[2] AND R. H. WARING
Department of Forest Science, College of Forestry, Oregon State University, Corvallis, OR, U.S.A. 97331

Received November 22, 1985[3]
Accepted April 18, 1986

MARGOLIS, H. A., and R. H. WARING. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Can. J. For. Res. **16**: 897–902.

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings at a western Oregon nursery were fertilized in October 1983 with ammonium nitrate and harvested for biochemical analyses on four dates over autumn and winter 1983–1984. Free amino acid and total nitrogen concentrations in the needles of fertilized seedlings showed a pronounced increase 1 month after fertilization. Free amino acid concentrations of fertilized seedlings decreased in needles during winter but remained stable in stems and fine roots. Just before budbreak in mid-March, free amino acid concentrations increased significantly in stems and fine roots. Total nitrogen concentrations increased 1 month after fertilization, remained stable throughout winter, and tended to decrease or remain stable just before budbreak. Starch and total nonstructural carbohydrate concentrations of needles and stems of fertilized seedlings were lower just before budbreak and sugar concentrations of fine roots of fertilized seedlings were lower when data from all harvests were combined. The reduction in carbohydrate reserves following fertilization probably reflects increased respiration associated with the synthesis and maintenance of higher levels of enzymes.

MARGOLIS, H. A., et R. H. WARING. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Can. J. For. Res. **16**: 897–902.

Des semis de sapin de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco) élevés dans une pépinière de l'ouest de l'Orégon ont été fertilisés en octobre 1983 avec du nitrate d'ammonium et furent prélevés en vue d'analyses biochimiques à quatre périodes successives durant l'automne et l'hiver 1983–1984. Les concentrations d'acides aminés libres et d'azote total dans les aiguilles des semis fertilisés ont augmenté de façon prononcée un mois après la fertilisation. Les concentrations d'acides aminés libres des semis fertilisés ont diminué dans les aiguilles durant l'hiver, mais demeurèrent stables dans les tiges et les radicelles. Peu avant le débourrage vers la mi-mars, les concentrations d'acides aminés libres ont augmenté de façon significative dans les tiges et les radicelles. Les concentrations d'azote total ont augmenté un mois aprés la fertilisation, sont demeurées stables durant l'hiver et ont eu tendance à diminuer ou à demeurer stables peu avant le débourrage. Les concentrations d'amidon et d'hydrates de carbone non structurés totaux des aiguilles et des tiges des semis fertilisés furent plus basses peu avant le débourrage, alors que les concentrations en sucre des radicelles des semis fertilisés étaient à leur plus bas niveau lorsque les données pour tous les semis étaient réunies. La diminution des réserves d'hydrates de carbone aprés la fertilisation peut être associée à une respiration accrue liée à la synthèse et au maintien de niveaux élevés d'enzymes.

[Traduit par la revue]

Introduction

Much of the fertilizer research in forestry has been of an empirical nature. A few studies, however, have examined the physiological aspects of fertilizer response in conifers (Brix and Ebell 1969; Brix 1971, 1981; Miller and Miller 1976; Fagerstrom and Lohm 1977; Brix and Mitchell 1980). To our knowledge, only two studies have substantially considered the biochemical nature of the tree's response (van den Driessche and Webber 1975, 1977).

Nitrogen plays a central role in plant metabolism, not only as a constituent of the chlorophyll molecule, but also as a key component of the enzymes necessary for photosynthetic, respiratory, and growth processes. 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 (FAAs) and nonstructural carbohydrates is considered important to the plant's ability to produce defensive phenolic compounds and thereby resist insect and disease attack (Mattson 1980; Bryant *et al.* 1983; Matson and Waring 1984; Waring *et al.* 1985).

Efficient use of nitrogen fertilizer in the nursery may be more advantageous for early seedling growth than inadvertently fertilizing competing vegetation along with young trees in the early stages of stand establishment. According to standard nursery practice in the Pacific Northwest, nitrogen is usually withheld from midsummer through winter to induce dormancy and protect seedlings from frost injury (Cleary *et al.* 1978). A few studies, however, seemed to indicate that applying nitrogen fertilizer to nursery seedlings in autumn did not injure them and could indeed induce a positive growth response (Anderson and Gessel 1966; Benzian *et al.* 1974). Yet these studies have mostly involved only relatively simple correlations of the fertilization treatment with height growth and survival. Because additional information is needed on how this sort of change in nutritional status affects seedling metabolism, we chose first to examine some aspects of the overwinter nitrogen and carbon metabolism of 2-0 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings fertilized in early autumn with ammonium nitrate (reported here) and then to assess the subsequent growth response of the fertilized seedlings after outplanting (Margolis and Waring 1986).

[1]Paper No. 2067, Forest Research Laboratory, Oregon State University, Corvallis, OR. The mention of trade names or commercial products does not constitute recommendation or endorsement by the authors, Oregon State University, or the Canadian Journal of Forest Research.

[2]Present address: Departement des sciences forestières, Faculté de foresterie et géodesie, Université Laval, Ste-Foy (Qué.), Canada G1K 7P4.

[3]Revised manuscript received March 26, 1986.

Printed in Canada

Materials and methods

Treatments

A nursery bed at the International Paper Company's Western Forest Research Center in Lebanon, OR (44.5° N, 123° W), was planted with Douglas-fir seed in early May 1982 at a density of 258 seeds/m^2. Seed was from zone 262, which includes the Willamette Valley in and around Corvallis, OR. The soil is of the Newburg series, a fine-textured sandy loam consisting of 60% sand, 30% silt, and 10% clay.

The nursery was watered to maintain the soil at or near field capacity during the 1982 and 1983 growing seasons. Because of the wet summer in 1983, little irrigation was necessary. Water stress cycles, begun in mid-August of both 1982 and 1983 to induce dormancy, allowed nursery soil to dry until seedlings reached a predawn plant moisture stress of −1.0 MPa. At that point, soil was irrigated again to field capacity. Ammonium nitrate fertilizer was applied to the entire nursery bed in 1983 on five dates at four rates: March 16, 51 kg/ha; April 19, 28 kg/ha; May 4, and 15, 25 kg/ha; May 30, 27 kg/ha. About 75% of the seedlings were top pruned on July 18, 1983, to an approximate height of 30 cm.

In late August 1983, we established sixteen 1.2 × 1.2 m plots, each separated by at least a 1.2-m buffer, within the nursery bed. The plots were thinned so that seedling density was more uniform. The 16 plots were divided into eight blocks of 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 and 11, 1983, dissolved ammonium nitrate was applied at a rate of 56 kg N/ha to one randomly selected plot per block; the remaining plot served as an unfertilized control. On October 7, in between the two fertilizer applications, seedlings were undercut according to standard nursery practice to about 20 cm.

Seedlings were lifted on February 2, 1984, placed in cold storage at 2°C, and planted outside Corvallis, OR, by February 11.

Analyses

Seedlings were harvested for laboratory analysis on October 1 (before autumn fertilization), November 1, February 1, and March 15 (after outplanting) over the autumn and winter of 1983–1984. The integrity of the nursery experimental design was maintained in the mid-March harvest. From each plot, one composite of five whole seedlings was placed in a plastic bag, immediately put in a cooler filled with dry ice, and transported to the laboratory, where seedlings were stored in a freezer at −40°C.

FAAs, sugars, and starch in the needles, stems, and fine roots (<2 mm in diameter) were extracted and analyzed sequentially. FAAs and sugars were extracted with 80% ethanol. After being treated with insoluble polyvinylpyrrolidone to remove phenolics, the ethanol was evaporated. Samples were then filtered through Cellite® and brought to 100 mL volume. FAAs were estimated colorimetrically with ninhydrin (Moore and Stein 1954). Ethanol-soluble amides may also react with ninhydrin and result in an overestimation of actual FAAs. The remaining extract was cleared with lead acetate and sodium oxalate (Sanderson and Perera 1966). Sugars were determined colorimetrically by the anthrone reaction (Yemm and Willis 1954; Hansen and Moller 1975).

Labile polysaccharides were extracted from the ethanol-extracted residue with 35% perchloric acid on a rotary shaker for 24 h and were determined colorimetrically by the anthrone reaction (Hansen and Moller 1975). The perchlorate-extractable polysaccharides contain starch plus small amounts of other "background" carbohydrates. The amount of these "background" carbohydrates was determined by placing seedlings ready to break bud in a warm, moist, and completely dark environment for 12 weeks, during which time etiolated shoots developed and starch reserves were depleted. At this point, extraction of the old leaves, stems, and fine roots with 35% perchloric acid provided an estimate of the amount of background carbohydrate (28, 27, and 23 mg/g for needles, stems, and fine roots respectively). Subtracting the "background" carbohydrate from the extractable polysaccharide gave an estimate of the amount of starch in the sample (Marshall 1984; Marshall and Waring 1985).

Total nitrogen was measured colorimetrically after an acid digest (Technicon Industrial Systems 1975, 1976), but a $Se/CuSO_4$ rather than a HgO catalyst was used. Nitrate was measured with a distilled water extraction followed by colorimetric analysis (Technicon Industrial Systems 1973).

Data were subjected to analyses of variance for a split-plot design; time was the main variable and nitrogen fertilization 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). For multiple comparisons, Tukey's honestly significant difference test (Steel and Torrie 1980) was used to compute least significant differences (LSDs) at the 90 and 95% levels (Pearson and Hartley 1966; Steel and Torrie 1980).

Results

Nitrogen

No nitrate was found in any plant part at any time. As expected, the analysis of variance for FAAs per gram dry weight showed significant interactions between time and fertilizer treatment for needles ($p < 0.002$), stems ($p < 0.001$), and fine roots ($p < 0.06$).

FAA concentrations in the needles of fertilized seedlings showed a large increase the month after fertilization, which decreased throughout winter and then tended to increase, though not significantly, just before budbreak (Fig. 1A). In contrast, FAA concentrations in the needles of control seedlings steadily declined throughout autumn and winter but increased significantly just before budbreak.

Unlike in the needles, FAA concentrations in the stems of fertilized seedlings remained constant the month after fertilization, although those of control seedlings significantly decreased (Fig. 1A). After November, the stems of fertilized seedlings maintained significantly higher FAA concentrations than did those of the controls and both groups of seedlings showed an increase in FAA just before budbreak.

FAA concentrations in the fine roots did not vary as much with time as those in the needles and stems (Fig. 1A). Concentrations in control seedlings showed a small but significant decrease over winter, whereas concentrations in fertilized seedlings, while significantly higher than those of the controls, remained relatively constant. As in stems, both groups of seedlings showed an increase in FAA in fine roots just before budbreak.

The analysis of variance for total Kjeldahl nitrogen also showed significant interactions ($p < 0.001$) between time and fertilizer treatment for needles, stems, and fine roots (Fig. 1B). Nitrogen concentrations were consistently higher in fertilized than control seedlings. In the needles and fine roots, total nitrogen significantly decreased in mid-March just before budbreak.

Applying ammonium nitrate did not significantly affect the ratio of FAA to total nitrogen, except for the November harvest in needles of fertilized seedlings (Fig. 1C). Over time, however, large differences in this ratio were observed in both fertilized and control seedlings; the ratio decreased over autumn and winter and markedly increased just before budbreak (Fig. 1C). Fine roots of the control seedlings had a lower FAA : total nitrogen ratio at the beginning of the experiment despite the randomization and blocking during plot selection. Neither fine root FAA concentration nor total nitrogen alone, however, differed significantly at the beginning of the experiment.

Nonstructural carbohydrates

Needle sugar concentration showed no direct fertilizer effect (Fig. 2A). However, sugars increased significantly at the time of

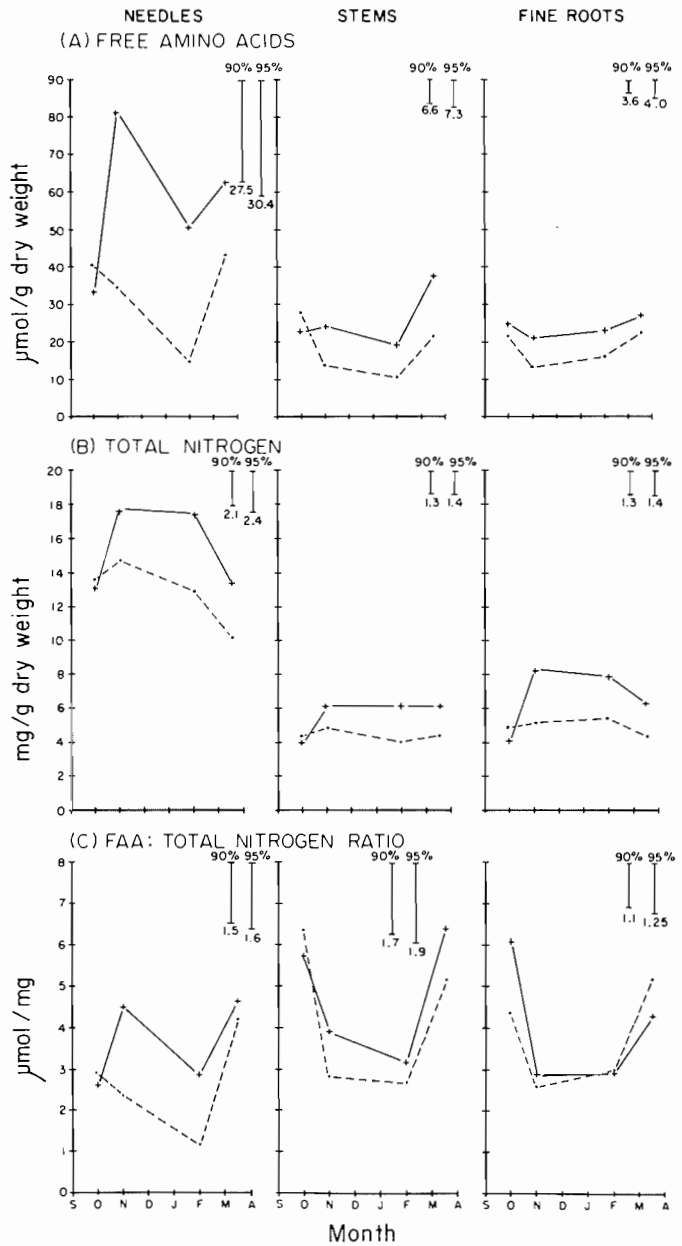

FIG. 1. (A) Free amino acids (FAAs), (B) total nitrogen, and (C) ratio of FAA to total nitrogen in nitrogen-fertilized (+—+) and unfertilized (control; ·····) 2-0 Douglas-fir seedlings from before fertilization in October 1983 until just before budbreak the following mid-March. Each point is the mean of eight replications. Tukey's honestly significant difference test was used to compute LSDs at the 90 and 95% levels (bars).

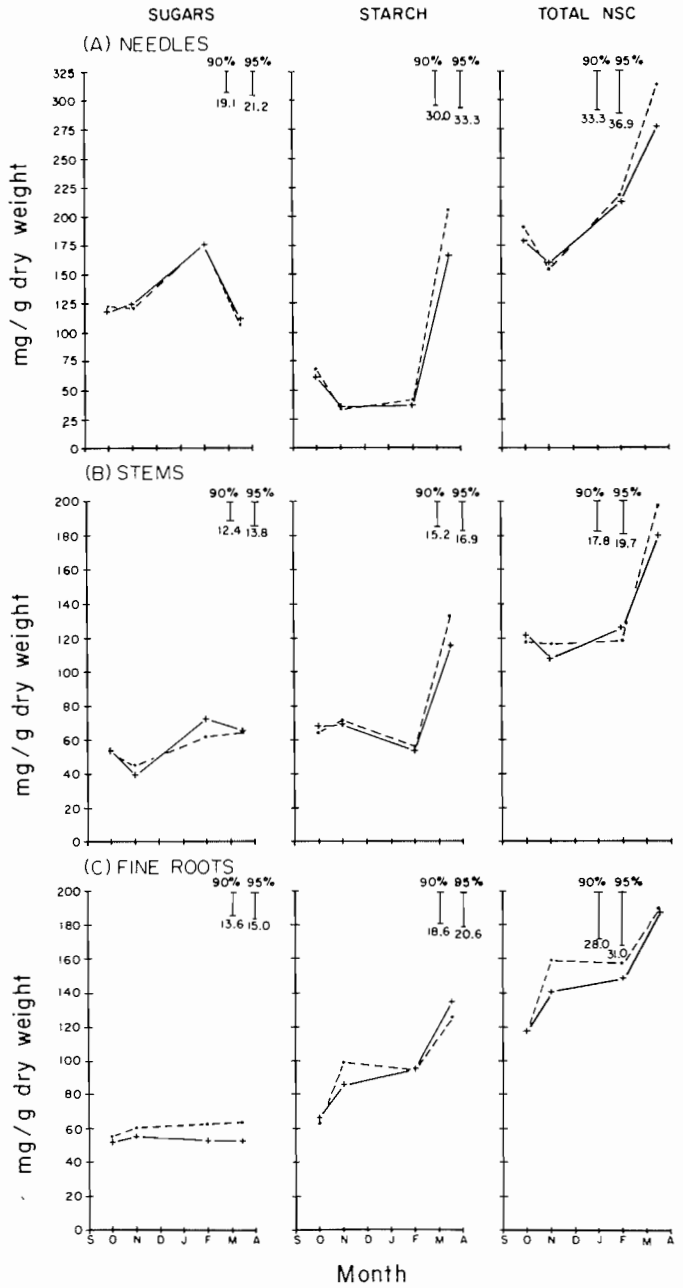

FIG. 2. Concentrations of sugars, starch, and total nonstructural carbohydrate (NSC) in the (A) needles, (B) stems, and (C) fine roots of nitrogen-fertilized (+—+) and unfertilized (control; ·····) 2-0 Douglas-fir seedlings from before fertilization in October 1983 until just before budbreak the following mid-March. Each point is the mean of eight replications. Tukey's honestly significant difference test was used to compute LSDs at the 90 and 95% levels (bars).

the February harvest and then decreased until mid-March, when starch concentration substantially increased. This indicates considerable conversion of sugar to starch in the weeks preceding budbreak. Just before budbreak, starch concentration was 20 mg/g lower and total nonstructural carbohydrate (NSC; sugar + starch) concentration was 36 mg/g lower in the needles of fertilized seedlings than in those of control seedlings.

Stems had patterns similar to those of needles (Fig. 2B). Fertilization did not affect sugar concentrations. However, as in the needles, stems of fertilized seedlings had lower starch (18 mg/g) and total NSC (17 mg/g) concentrations in mid-March than did the controls.

Fine roots had patterns that differed from those of either needles or stems (Fig. 2C). When results from all four harvest dates were combined, sugar concentrations averaged over 8 mg/g less ($p < 0.01$) in fertilized than control seedlings and did not vary significantly with time. Starch and total NSC concentrations both tended to be lower in fertilized seedlings in November, but the differences between fertilized and control seedlings were not statistically significant (Fig. 2C).

Figure 3 summarizes the biochemical differences between autumn-fertilized and unfertilized 2-0 Douglas-fir seedlings in mid-March just before budbreak.

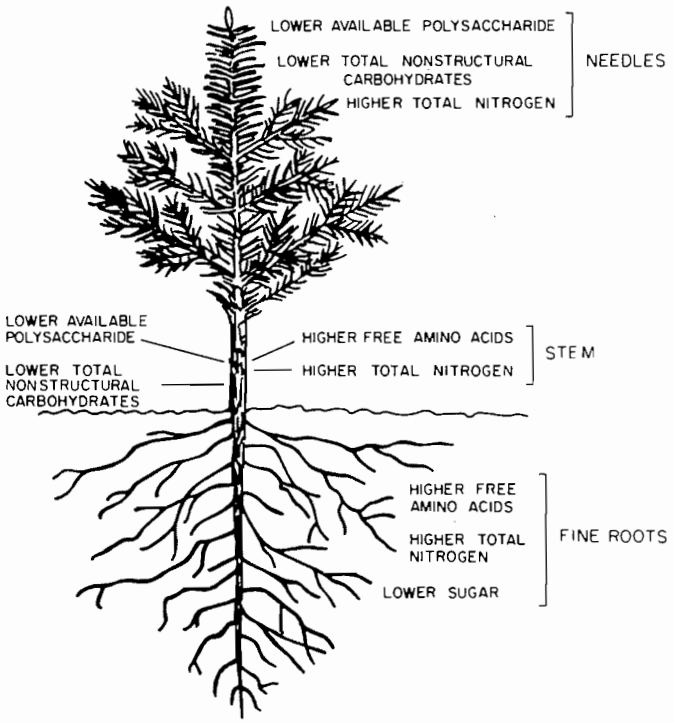

FIG. 3. Differences in concentrations of biochemical components in the needles, stems, and fine roots of 2-0 Douglas-fir seedlings fertilized with nitrogen in early October relative to unfertilized seedlings.

Discussion

Because Douglas-fir growing in a maritime climate may continue to be metabolically active in the winter months (Emmingham and Waring 1977), certain changes in nitrogen and NSC were expected. More specifically, two responses seemed likely: (*i*) if the higher nitrogen concentrations increased the net photosynthetic rate throughout winter, then sugar and starch concentrations should increase; (*ii*) if, on the other hand, the higher nitrogen concentrations substantially increased maintenance respiration compared with photosynthesis, then sugar and starch concentrations should decrease. We also anticipated an increase of FAA directly after autumn fertilization followed by a decline throughout winter as FAAs were incorporated into protein. Any root growth occurring during the fall and winter might also result in a decline in the FAA pool as proteins for the new tissue were synthesized.

The nearly 2.5-fold increase in FAA concentration in the needles 1 month after ammonium nitrate application indicates that considerable amounts of the fertilizer were metabolized and transported to photosynthetically active parts of the seedlings (Fig. 1A). The fact that no nitrate could be found in any plant part suggests that fertilized seedlings were able to fully metabolize any nitrate they absorbed within 30 days; however, whether assimilation occurred in the fine roots (Pate 1980) or in the needles (Smirnoff *et al.* 1984) was not determined.

The marked decline in FAA concentrations in the needles of both fertilized and control seedlings through winter suggests their conversion into protein or other polymeric molecules. Durzan (1968) found that protein synthesis in unfertilized white spruce (*Picea glauca* (Moench) Voss) became quite active after the first fall frost. The increased FAA concentrations in mid-March are probably the result of protein breakdown associated with the large amounts of readily transportable FAA required for new growth following budbreak. Total nitrogen concentrations in needles, which had remained constant over winter, decreased at this time, reflecting probable retranslocation of nitrogen to areas of high growth potential (Fig. 1B). Durzan (1968) found a similar pattern in white spruce. In our study, the fertilized Douglas-fir seedlings were able to mobilize considerably more nitrogen as FAA than were the control seedlings. Fagerstrom and Lohm's (1977) model describing the growth response of conifers to nitrogen fertilization suggests that the size of the mobile nitrogen pool (i.e., FAA) relative to needle biomass could determine the production rate of new needle biomass. On this basis, the elevated mid-March FAA concentrations of our fertilized Douglas-fir would indicate that increased needle production should be expected in the coming growing season.

As in van den Driessche and Webber (1975), this study showed a greater percent increase in FAA than in total nitrogen in needles (Figs. 1A and 1B). Rain in September followed by clear days in October probably allowed considerable transpiration and mass flow of FAAs to foliage. In November, total nitrogen in the control seedlings was 14.7 mg/g; total nitrogen in the fertilized seedlings was 17.8 mg/g (Fig. 1B). Brix (1981) found that 17.4 mg/g was optimum for photosynthesis of Douglas-fir under high light (i.e., ~590 μE s^{-1} m^{-2} photosynthetically active radiation). However, sugar or starch concentrations did not increase in our fertilized seedlings. Irradiance during the Oregon winter is probably insufficient to permit utilization of all available enzymes (Emmingham and Waring 1977).

Unlike the needles, the stems and fine roots showed greater percent increases in total nitrogen concentration than in FAA concentration (Fig. 1). Other than the increase in stems before budbreak, FAA levels in these tissues did not fluctuate dramatically. The controls showed slight decreases in the 1st month, whereas the fertilized seedlings, with a greater nitrogen source, were able to maintain constant levels.

The FAA : total nitrogen ratio (Fig. 1C) gives an index of the relative size of the FAA pool. Although we could not estimate the actual amount of nitrogen in FAA with the technique used because any given amino acid may have from one to four nitrogen atoms, we found that the ratio changed considerably over time, with a particularly large increase in all tissues in mid-March before budbreak. Furthermore, fertilization significantly affected the ratio in needles. Increases in FAA concentrations as a result of water stress (Chen *et al.* 1964; Stewart and Larcher 1980) and temperature (van den Driessche and Webber 1975) have been reported. Although FAAs may represent only a small part (~1–4%) of the total nitrogen pool, a 50 or 100% increase may have considerable biological significance. Consequently, we believe it is dangerous to infer FAA concentrations from total nitrogen data alone.

The potential benefits of nitrogen fertilization in autumn apparently have a cost (Fig. 3). In this study, the increased nitrogen concentration resulted in significantly depleted NSC reserves. The fine roots had significantly lower sugar concentrations and tended towards lower starch concentrations 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 for maintaining newly formed enzymes. The greater root growth reported for Douglas-fir seedlings receiving late-season nitrogen fertilization (van den Driessche 1985) might also result in lower carbohydrate status.

Stems and needles, however, did not show significantly

lower NSC reserves until just before budbreak. In these tissues it was the starch concentrations, not the free sugars, that were depleted by the additional nitrogen. Because this depletion did not occur until mid-March, the carbon cost of ammonium and nitrate assimilation was probably not important. Rather, warmer March temperatures likely increased maintenance respiration rates to levels at which the costs of maintaining additional enzymes significantly decreased starch concentrations. Furthermore, since budbreak date is significantly advanced in fertilized trees (Thompson 1983; van den Driessche 1985; Margolis and Waring 1986), such trees may be more actively synthesizing enzymes required for growth than unfertilized seedlings in mid-March. Furthermore, cambial activity may have begun earlier in the fertilized seedlings. These could all result in higher respiration rates.

It is important that seedlings fertilized with nitrogen in the autumn have adequate NSC reserves to maintain the increased enzyme levels as well as to help meet demands for growth in the spring. Nursery or storage practices that cause NSC reserves in seedlings to decline may produce stock with poor field survival and growth (Ronco 1973; Ericsson *et al.* 1983; Marshall 1985). Late-season nitrogen fertilization may be ineffective if it interferes with dormancy induction or otherwise reduces the ability of seedlings to tolerate cold temperature. However, depleted NSC reserves may be another reason for the negative or inconclusive results sometimes reported for seedlings fertilized with nitrogen in autumn.

Acknowledgments

This research was partially supported by National Science Foundation grant DEB-8111015. Our thanks to Barbara Thompson and the staff of International Paper Company's Western Forest Research Center, Sigma Xi Grants-in-Aid of Research and the Oregon State University Computer Center for their generous assistance. Also, our thanks to the Weyerhaeuser Foundation for providing fellowship support for one year to the senior author.

ANDERSON, H. W., and S. P. GESSEL. 1966. Effects of nursery fertilization on outplanted Douglas-fir. J. For. **64**: 109–112.

BENZIAN, B., R. M. BROWN, and S. C. R. FREEMAN. 1974. Effect of late-season top-dressing of N (and K) applied to conifer transplants in the nursery on their survival and growth on British forest sites. Forestry, **47**(2): 153–184.

BRIX, H. 1971. Effects of nitrogen fertilization on photosynthesis and respiration in Douglas-fir. For. Sci. **17**: 407–414.

——— 1981. Effects of nitrogen fertilizer source and application rates on foliar nitrogen concentration, photosynthesis and growth of Douglas-fir. Can. J. For. Res. **11**: 775–780.

BRIX, H., and L. F. EBELL. 1969. Effects of nitrogen fertilization on growth, leaf area and photosynthetic rate in Douglas-fir. For. Sci. **15**: 189–196.

BRIX, H., and A. K. MITCHELL. 1980. Effects of thinning and nitrogen fertilization on xylem development in Douglas-fir. Can. J. For. Res. **10**: 121–128.

BRYANT, J. P., F. S. CHAPIN III, and D. R. KLEIN. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos, **40**: 357–368.

CHAPIN, F. S. 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. **11**: 233–260.

CHEN, D., B. KESSLER, and S. P. MONSELISE. 1964. Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress. Plant Physiol. **39**: 379–386.

CLEARY, B. D., R. D. GREAVES, and R. K. HERMANN. 1978. Regenerating Oregon's forests: a guide for the regeneration forester. Oregon State University Extension Service, Corvallis, OR.

DURZAN, D. J. 1968. Nitrogen metabolism of *Picea glauca*. I. Seasonal changes of free amino acids in buds, shoot apices, and leaves, and the metabolism of uniformly labelled ^{14}C-L-arginine by buds during the onset of dormancy. Can. J. Bot. **46**: 909–919.

EMMINGHAM, W. H., and R. H. WARING. 1977. An index of photosynthesis for comparing forest sites in western Oregon. Can. J. For. Res. **7**: 165–174.

ERICSSON, A., A. LINDGREN, and A. MATTSON. 1983. Effects of cold storage and planting date on subsequent growth, starch and nitrogen content in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) seedlings. Stud. For. Suec. No. 165.

FAGERSTROM, T., and U. LOHM. 1977. Growth in Scots pine (*Pinus sylvestris*): mechanism of response to nitrogen. Oecologia, **26**: 305–315.

HANSEN, J., and I. MOLLER. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal. Biochem. **68**: 87–94.

HULL, C. H., and N. H. NIE. 1981. SPSS update: new procedures and facilities for releases 7–9. McGraw-Hill, Inc., Hightstown, NJ.

INGESTAD, T., and A. LUND. 1979. Nitrogen stress in birch seedlings. I. Growth technique and growth. Physiol. Plant. **45**: 137–148.

LOOMIS, W. E. 1954. Growth correlation. *In* Growth and differentiation in plants. *Edited by* W. E. Loomis. Iowa State College Press, Ames, IA.

MARGOLIS, H. A., and R. H. WARING. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. II. Field performance. Can. J. For. Res. **16**. This issue.

MARSHALL, J. D. 1984. Physiological control of fine root turnover in Douglas-fir. Ph.D. thesis, College of Forestry, Oregon State University, Corvallis, OR.

——— 1985. Carbohydrate status as a measure of seedling quality. *In* Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. *Edited by* M. Duryea. Forest Research Laboratory, Oregon State University, Corvallis, OR. pp. 49–58.

MARSHALL, J. D., and R. H. WARING. 1985. Predicting fine root production and turnover by monitoring root starch and soil temperature. Can. J. For. Res. **15**: 791–800.

MATSON, P., and R. H. WARING. 1984. Effect of nutrient and light limitation on mountain hemlock: susceptibility to laminated root rot. Ecology, **65**: 1517–1524.

MATTSON, W. H. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. **11**: 119–161.

MILLER, H. G., and J. D. MILLER. 1976. Effect of nitrogen supply on net primary production in Corsican pine. J. Appl. Ecol. **13**: 249–256.

MOORE, S., and W. H. STEIN. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. **211**: 907–926.

NIE, N. J., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, and D. H. BENT. 1975. SPSS: statistical package for the social sciences. 2nd ed. McGraw-Hill, Inc., Hightstown, NJ.

PATE, J. S. 1980. Transport and partitioning of nitrogenous solutes. Annu. Rev. Plant Physiol. **31**: 313–340.

PEARSON, E. S., and H. O. HARTLEY. 1966. Biometrika tables for statisticians. Vol. I. 3rd ed. Cambridge University Press, Cambridge, U.K.

REYNOLDS, J. F., and J. H. M. THORNLEY. 1982. A shoot:root partitioning model. Ann. Bot. (London), **49**: 587–597.

RONCO, F. 1973. Food reserves of Engelmann spruce planting stock. For. Sci. **19**: 213–219.

SANDERSON, G. W., and B. P. M. PERERA. 1966. Removal of polyphenolic compounds interfering with carbohydrate determinations in plant extracts with an insoluble polyphenol absorbent. Analyst (London), **91**: 335–336.

SMIRNOFF, N., P. TODD, and G. R. STEWART. 1984. The occurrence of nitrate reduction in the leaves of woody plants. Ann. Bot. (London), **54**: 363–374.

STEEL, R. D. G., and J. H. TORRIE. 1980. Principles and procedures of statistics. A biometrical approach. 2nd ed. McGraw-Hill, Inc., Hightstown, NJ.

STEWART, G. R., and F. LARCHER. 1980. Accumulation of amino acids and related compounds in relation to environmental stress. *In* The biochemistry of plants: a comprehensive treatise. Vol 5. Amino acids and their derivatives. *Edited by* P. K. Stumpf and E. E. Conn. Academic Press, New York. pp. 609–635.

TECHNICON INDUSTRIAL SYSTEMS. 1973. Nitrate and nitrite in water and wastewater. Industrial Method No. 100-70W. Technicon Instruments Corp., Tarrytown, NJ.

——— 1975. Digestion and sample preparation for the analysis of total Kjeldahl nitrogen and/or total phosphorus in food and agricultural products using the Technicon BD-20 block digestor. Industrial Method No. 369-75A/A. Technicon Instruments Corp., Tarrytown, NJ.

——— 1976. Individual or simultaneous determination of nitrogen and/or phosphorus in BD acid digests. Industrial Method No. 334-74A/A. Technicon Instruments Corp., Tarrytown, NJ.

THOMPSON, B. 1983. Why fall fertilize? *In* Proceedings of the Western Nurseryman's Conference, August 10–12, 1982, Medford, OR. Southern Oregon State College, Ashland, OR.

VAN DEN DRIESSCHE, R. 1985. Late-season fertilization, mineral nutrient reserves, and retranslocation in planted Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. For. Sci. **31**: 485–496.

VAN DEN DRIESSCHE, R., and J. E. WEBBER. 1975. Total and soluble nitrogen in Douglas-fir in relation to plant nitrogen status. Can. J. For. Res. **5**: 580–585.

——— 1977. Variation in total and soluble nitrogen concentrations in response to fertilization of Douglas-fir. For. Sci. **23**: 134–142.

WARING, R. H., A. J. S. MCDONALD, S. LARSSON, T. ERICSSON, A. WIREN, E. ARWIDSSON, A. ERICSSON, and T. LOHAMMAR. 1985. Differences in chemical composition of plants grown at constant growth rates with stable mineral nutrition. Oecologia, **66**: 157–160.

YEMM, E. W., and A. J. WILLIS. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. **57**: 508–514.