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Drought and shade interact to cause fine-root mortality in Douglas-fir seedlings

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Summary Both desiccation and depleted carbohydrate reserves have been suggested as causes of fine-root ($\leq$ 2 mm in diameter) mortality in trees. In this study, Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings were subjected to four combinations of shading and watering to determine whether shading increases drought-induced root mortality and, if so, whether this effect is due to reduced levels of carbohydrate reserves or increased susceptibility to desiccation. Two correlated measures of root mortality (counting root tips and weighing roots) showed that significantly more fine roots died only when seedlings were both shaded and unwatered. Concentrations of suberin, a compound synthesized by plant roots to control desiccation, were unaffected by any combination of shading and watering; however, carbohydrate reserves were nearly exhausted in the shaded and unwatered treatment – the treatment with highest root mortality. Water stress may have increased root mortality indirectly by increasing root temperature and maintenance respiration and by inhibiting photosynthate transport to the root system, but massive die-off in response to drought was apparent only when starch and sugar reserves were nearly depleted. Drought cannot be considered directly responsible for death of fine roots. Instead, a root's ability to continue to respire, which in turn depends on the status of its starch and sugar reserves, seems to be the primary physiological control of fine-root mortality.

Introduction

Recent studies attempting to account for all biomass produced annually by trees have demonstrated the importance of the smallest elements of the root system, the fine roots[1,9,11]. Although fine roots may constitute less than 1% of the biomass of a mature tree, they account for as much as two-thirds of annual production[9].

The maintenance requirements of fine roots are not exceptionally high relative to other tissues[1]; rather, their high photosynthate requirement is a result of frequent replacement, or turnover. Fine roots may be replaced 2 to 3 times per year[11,25,31] or perhaps even more often[12,24], the turnover rate varying significantly with site conditions[25,31]. Because fine-root turnover is metabolically so costly for the tree, determining the physiological controls underlying differences in turnover rate would contribute greatly to our understanding of forest productivity.

I have approached the question of root turnover by concentrating on

the causes of root death. The most frequently suggested cause is drought[5,26,31]. Deans[5] observed that fine roots of Sitka spruce [*Picea sitchensis* (Bong.) Carr.] in Scotland began to die whenever soil water potentials fell below −0.02 MPa. Santantonio[31] reported increasing rates of fine-root turnover in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] stands on progressively drier sites in the Oregon Cascade Mountains. Yet fine roots have been known to survive and even grow in soil at water potentials well below the conventional wilting point (−1.5 MPa)[17,34]. Consequently, factors other than soil water potential must contribute to root mortality.

Carbohydrate depletion in roots also has been suggested as a possible cause of fine-root death[19,26,28]. Root starch and sugar concentrations have been examined in relation to root production in Sitka spruce forests in Scotland[7] and in Scots pine (*Pinus sylvestris* L.) stands in Sweden[6], but no clear patterns were identified. Nonetheless, carbohydrate depletion is implicated as a possible cause of root death in studies showing increased root mortality following defoliation[27].

Fine-root turnover might result from an interaction between drought and carbohydrate status. Reduced carbohydrate supply could limit suberization, the process whereby a layer of water-repellent material (suberin) is deposited within the root tissues. Suberization has been shown to reduce water loss from plant roots[29] and tubers[15], and suberization rates have been observed to increase in dry soil[17,19], which may represent one of a plant's mechanisms for surviving drought[17,35]. Due to its chemical structure, however, suberin would be metabolically expensive for the plant to synthesize[21]. Because synthesis of metabolically expensive compounds is often curtailed when carbohydrate status is low[18], I hypothesized that shading might curtail suberization, increasing susceptibility of fine roots to subsequent drought, and thereby increasing fine-root turnover. To evaluate these hypotheses, I subjected seedlings to differing combinations of water and light and evaluated subsequent root mortality, degree of suberization, and amount of stored carbohydrate in the fine roots.

Materials and methods

Seedling treatments

Two-year-old Douglas-fir seedlings grown from seed collected on the east side of the Oregon Coast Range at approximately 300 m elevation were obtained from the D L Phipps state nursery at Elkton, Oregon, in February 1982. The seedlings showed low rates of mycorrhizal infection, but some *Thelephora terrestris* (Ehrh.) Fr. was present.

In March 1982, 16 seedlings planted into 2-liter cardboard milk cartons containing washed river sand were placed under clear plastic frames to exclude rain. Within a randomized block design, half the seedlings (8) were covered with shade cloth, reducing light levels by 95%;

this shade treatment served as the blocking variable. Seedlings were watered about every 5 days for 5 months; then, as a drought treatment, water was withheld from half the seedlings (4) in both the unshaded and the shaded block. No mineral nutrients were provided during the course of the experiment in order to favor root growth[14]. Seedlings were harvested 22 days after drought treatment was begun, when measurements of leaf conductance with a null-balance porometer[3] showed that stomata were barely opening and foliage had begun to wilt.

Harvested seedlings were divided into roots and shoots at the point midway between the first branch and the first lateral root to compensate for differences in planting depth. The root systems were carefully washed and all fine roots (≤ 2 mm in diameter) separated from coarse roots (> 2 mm in diameter). Fine roots were cut into segments no longer than 10 cm and laid in a numbered tray; root segments were then selected at random and visually inspected for signs of mortality. Darkened, wrinkled roots were considered dead. If there was doubt about whether a root was dead, it was broken and the vascular cylinder examined; discoloration of the vascular cylinder was assumed to indicate that the root was dead[31]. Live and dead roots in subsamples of at least 1000 root tips per seedling (individual root tips on a single mycorrhizal short root were considered separate tips) were counted and also weighed; the subsamples represented an average of 13.9% (SE = 1.6%) of the total fine-root weight of each seedling. The live- and dead-root subsamples, as well as the remaining fine roots, coarse roots, foliage, and stems were separated and dried in a forced-draft oven at 70°C. Dried samples were weighed and ground to pass a 40-mesh sieve in preparation for chemical analysis.

Chemical analysis

Suberin concentrations were determined with the acid-detergent method of Goering and Van Soest[8], which was developed for analyzing lignin and cutin. Because the various parts of the suberin polymer resemble either lignin or cutin[16], this procedure was expected to give a good estimate of suberin concentration of roots. Ash concentrations of the residue were determined by loss on ignition[8].

Before carbohydrate analysis, samples were extracted with 100% acetone to remove chlorophyll and other pigments (W.D. Loomis, Oregon State University, Corvallis, personal communication). Sugars were then extracted with hot 80% ethanol 3 to 4 times, until the extract was colorless; this procedure is similar to the Soxhlet extraction[2]. The ethanol extract was cleared first with 100 mg insoluble polyvinylpyrrolidone and then with 100 μl saturated lead acetate solution[30]. Sugar concentrations were determined colorimetrically by the anthrone reaction[10,36]. Labile polysaccharides were extracted from the ethanol-extracted residue with 35% perchloric acid[10] on an orbital shaker for 16 h. Carbohydrate concentration was measured colorimetrically with anthrone[10].

Starch-extraction check

Because studies using starch analysis procedures similar to those used here have been criticized because carbohydrates other than starch have been extracted, an experiment was conducted to determine the amounts of starch and of carbohydrates other than starch in the perchlorate extracts. This section describes that experiment and explains how starch concentrations were calculated from perchlorate-extractable carbohydrate concentrations.

Samples were obtained from an experiment in which seedling shoots were maintained in darkness, causing the seedlings to die. Three samples each were taken from shoots, coarse roots, and fine roots at the beginning of the experiment and three each at the end, following more than 2 months of darkness. Samples were dried, ground, and extracted with 80% ethanol as described earlier, and then connected to a percolation system that dripped 35% perchloric acid through them for 18 h. The extract was immediately diluted to minimize hydrolysis of the starch. Hexose concentration of the extract was determined by the anthrone reaction[36]. Starch was then precipitated out of the extract with the iodine-precipitation method of McCready[20]; the precipitate was redissolved, and hexose concentration of the solution determined. To verify that the iodine precipitate was indeed starch, duplicate samples of the washed iodine precipitate were incubated with amyloglucosidase, a starch-degrading enzyme[32]. At the end of the incubation, the iodine precipitation procedure was repeated and hexose concentration

of the precipitate again determined. Starch concentrations of the samples from the beginning and end of the experiment were compared at each step of the analysis with simple t-tests[33].

Over the course of the experiment, perchlorate-extractable carbohydrate fell sharply and iodine-precipitable carbohydrate decreased almost to zero. Incubation with amyloglucosidase hydrolyzed 93.1 ± 1.3% of the iodine precipitate from the shoots and 97.1 ± 0.6% from the roots, indicating that the vast majority of that precipitate was starch. However, concentrations of the carbohydrate not precipitable by iodine did not change significantly during the experiment; therefore, a constant amount, 40 mg/g dry wt, could be subtracted from the perchlorate-extractable carbohydrate to obtain starch concentrations. The data presented in the results section of this experiment are corrected for this unidentified carbohydrate.

Statistcal analysis

Treatment effects and interactions were determined with analysis of variance and means of treatment combinations statistically compared at $P = 0.95$ with Student-Newman-Keuls test[33]. One seedling was excluded from the analysis of root mortality following changes in experimental procedures, and one tree was excluded from the starch and sugar analyses because the sample was lost.

Results

Total fine-root biomass (live and dead roots combined; ash-corrected dry wt) was greatest for the unshaded, watered treatment combination, and differences among the other three treatment combinations were not significant (Fig. 1).

The analysis of variance for dead-root biomass showed no significant treatment effects; nonetheless, when dead-root biomass was normalized by dividing it by total fine-root biomass, a significant interaction between shading and watering was found. The analysis of variance for root-tip mortality revealed significant effects for both shading and watering but no significant interaction. Both measures of mortality, which correlated fairly closely with one another (Fig. 2), showed a significant increase in fine-root mortality only when seedlings were both shaded and unwatered (Fig. 3).

When live and dead roots were combined for analysis, suberin concentrations did not differ significantly among any of the treatment combinations. However, starch concentrations were significantly higher when seedlings were unshaded and watered, and sugar concentrations were significantly higher when seedlings were unshaded independent of watering (Fig. 4). When live and dead roots were analyzed separately, suberin concentrations were significantly higher in the dead (43.7 ± 1.2%) than in the live (32.7 ± 0.7%) roots, possibly because suberin is resistant to microbial decomposition[16] and would be concentrated as dead roots decompose. These differences in suberin concentration remained significant when the analyses were corrected for differences in starch concentration. Plant material was insufficient for replicating the chemical analyses of the live and dead root subsamples;

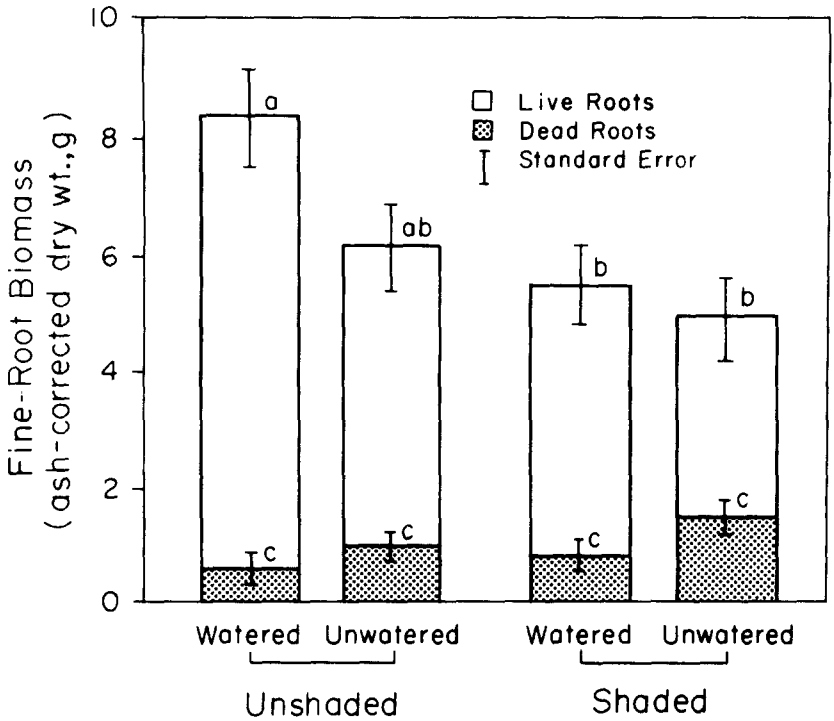

Fig. 1. Fine-root biomass for the four treatment combinations. Means with the same letter do not differ significantly, according to the Student-Newman-Keuls test[33], at $P = 0.95$.

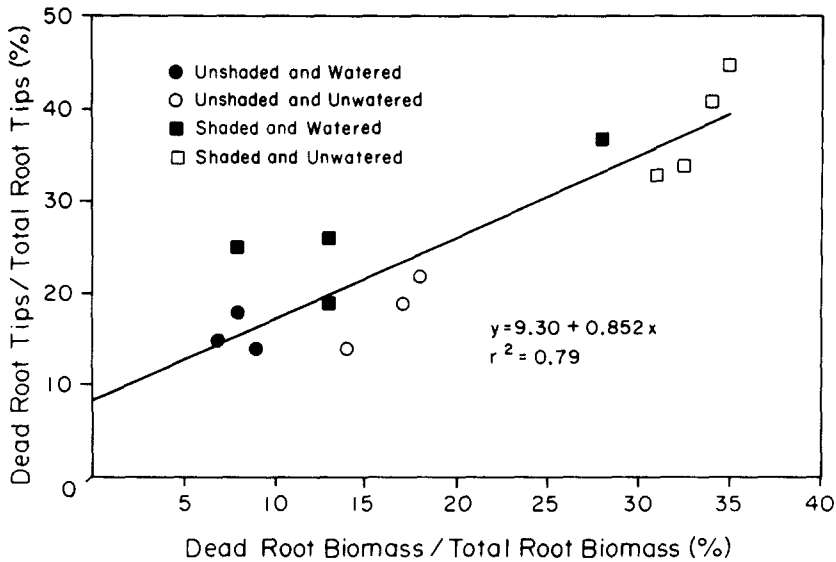

Fig. 2. Correlation between root-tip mortality and root mortality by weight, the two measures used to assess fine-root mortality.

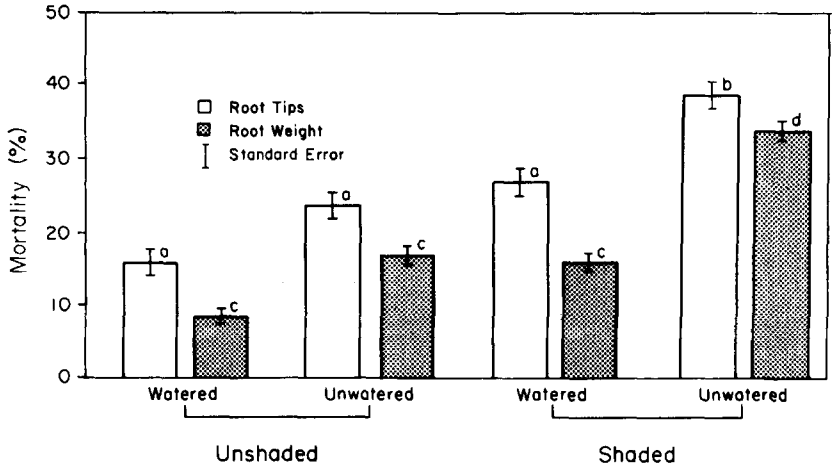

Fig. 3. Fine-root mortality, determined with the two measures of root mortality, for all four treatment combinations. Means with the same letter do not differ significantly at $P = 0.95$.

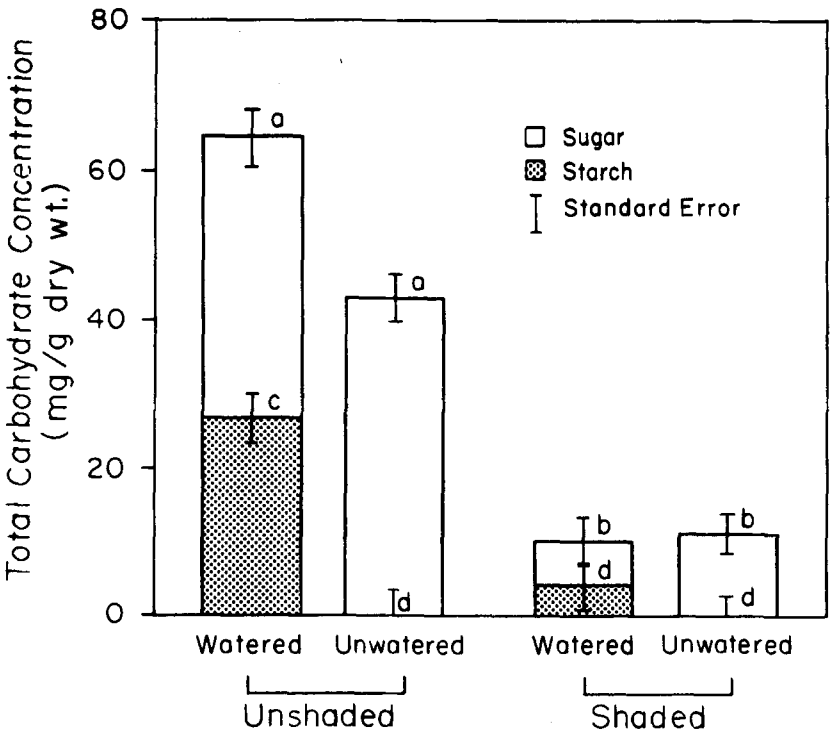

Fig. 4. Starch and sugar concentrations of live and dead fine roots combined. Means with the same letter do not differ significantly at $P = 0.95$.

however, the trends in the data resemble those revealed from the combined (live and dead) fine-root analysis presented in Figure 4.

Discussion

Estimating root mortality is problematical because classifying roots as live or dead is at present unavoidably subjective. It is not known how long after roots die the vascular cylinder becomes discolored. If discoloration proceeds slowly, roots that have died only recently would not be recognized as dead. Moreover, some roots might decay and fragment before mortality could be fully tallied. Either way, root mortality would be underestimated. In any case, if mortality was underestimated, it was probably underestimated to about the same extent in all treatments because the treatments were applied simultaneously.

Seedlings varied enough in size that differences in dead-root biomass among the treatment combinations could not be demonstrated (Fig. 1). Only when the root mortality data were normalized was a significant interaction between shading and watering found. Total fine-root biomass varied significantly among certain treatment combinations; however, the patterns bore no relationship to those of fine-root mortality regardless of how mortality was measured (Fig. 3).

The hypothesis that drought is the direct cause of root mortality was rejected because root mortality did not significantly increase when unshaded seedlings were not watered. Nonetheless, drought must at least be indirectly related to root mortality – both drought and shading were required to increase it. But this increased mortality apparently was not caused by different susceptibilities to water loss because suberin concentrations did not differ among the treatment combinations.

The indirect effects of drought were probably related to inability of the fine roots to meet maintenance requirements. Previous experiments have shown that starch is deposited primarily in new roots, with older roots gradually depleting their starch reserves in maintenance respiration (Marshall and Waring, unpub.). Although continued root growth might provide additional starch reserves to the fine roots, drought would cause stomatal closure, halting photosynthesis, root growth, and starch deposition, forcing the roots to depend solely upon their reserves to meet their maintenance requirements. Given that these reserves are not distributed evenly throughout the root system, a large percentage of roots would be expected to die during a prolonged drought.

In this study, starch and sugar concentrations were extremely low

in the shaded treatment cominations. Reserves of the shaded, unwatered seedlings (11 mg/g dry wt, Fig. 4) – those for which root mortality was greatest – would have been sufficient to meet maintenance requirements at 20°C (1.5 mg starch per day) for only about 1 week (Marshall and Waring, unpub.). In contrast, starch and sugar concentrations were much higher in the unshaded seedlings. The watered, unshaded seedlings had a 6-week supply of carbohydrate reserves in their fine roots and the unwatered, unshaded seedlings a 4-week supply at the end of the experiment.

The drought treatment also raised soil temperatures: midday soil temperatures averaged 7°C higher in the unwatered than in the watered containers near the end of the experiment. Such a rise in temperature would increase maintenance respiration 60%, further shortening the time for which a seedling could meet its maintenance requirements from its reserves.

If root mortality is strongly tied to level of carbohydrate reserves, roots might be expected to die even when soil moisture levels are high[5] if their supply of stored photosynthate is exhausted. Conversely, they might be able to survive periods of extreme drought[17,34] if they are sufficiently supplied with stored carbohydrate. Desiccation is apparently not an important cause of root mortality, perhaps because water losses from moist roots to drier soil are quite limited[22]. Electron micrographs of suberized barley (*Hordeum vulgare* L.) roots show that the endodermis contains many plasmodesmata, valve-like pores which allow water to flow inward along a water potential gradient but which close whenever that gradient favors water flow outward into the soil[4]. Douglas-fir roots have probably evolved similar mechanisms to impede root desiccation, including suberization.

Hermann[13] has discussed reduced survival of planted seedlings after root systems have been exposed to dry air. It must be remembered, however, that even at a soil water potential of −5.0 MPa (−50 bars), equilibrium relative humidity exceeds 96%[23]. Therefore, because evaporative demand is much lower in the soil than in the atmosphere, the results of studies in which roots have been exposed to air cannot be assumed to apply when root moisture level is varied within the soil.

I conclude that drought is not directly responsible for death of fine roots, but instead contributes to root mortality indirectly by inhibiting reserve deposition and allowing soil temperatures to rise. Massive die-off of roots in response to drought was apparent only when starch and sugar reserves were nearly depleted. The physiological control of fine-root mortality appears to be mainly related to a root's ability to continue to respire, which in turn depends upon its starch

and sugar reserves. This insight augments our understanding of the factors controlling carbohydrate allocation and should eventually increase our ability to predict the effects upon tree growth of altering the environment.

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References

1 Agren G I, Axelsson B, Flower-Ellis J G K, Linder S, Persson H, Staaf H and Troeng E 1980 Annual carbon budget for a young Scots pine. *In* Structural and Function of Northern Coniferous Forests – An Ecosystem Study. Ed. T Persson, Ecol. Bull. (Stockholm) 32, 307–314.

2 AOAC 1980 Official Methods of Analysis of the Association of Official Analytical Chemists (13th ed). Washington, D.C.

3 Beardsall N F, Jarvis P G and Davidson B 1972 A null-balance diffusion porometer suitable for use with leaves of many shapes. J. Appl. Ecol. 9, 677–690.

4 Clarkson D T and Robards A W 1975 The endomermis, its structural development and physiological role. *In* The Development and Function of Roots. Ed. J G Torrey and D T Clarkson, Academic Press, London, New York, San Francisco, pp 415–436.

5 Deans J D 1979 Fluctuations of the soil environment and fine root growth in a young Sitka spruce plantation. Plant and Soil 52, 195–208.

6 Ericsson A and Persson H 1980 Seasonal changes in starch reserves and growth of fine roots of 20-year-old Scots pines. *In* Structure and Function of Northern Coniferous Forests – An Ecosystem Study. Ed. T Persson, Ecol. Bull. (Stockholm) 32, 239–250.

7 Ford E D and Deans J D 1977 Growth of a Sitka spruce plantation: spatial distribution and seasonal fluctuations of lengths, weights, and carbohydrate concentrations of fine roots. Plant and Soil 47, 463–485.

8 Goering H K and Van Soest P J 1970 Forage fiber analysis. USDA Agric. Res. Serv. Agric. Handb. 379, Washington, D.C., 20 p.

9 Grier C C, Vogt K A, Keyes M R and Edmonds R L 1980 Biomass distributions and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. Can. J. For. Res. 11, 155–167.

10 Hansen J and Möller I 1975 Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal. Biochem. 68, 87–94.

11 Harris W F, Kinerson R S and Edwards N T 1978 Comparisons of belowground biomass of natural deciduous forest and loblolly pine plantations. Pedobiologia 7, 369–381.

12 Head G C 1973 Shedding of roots. *In* Shedding of Plant Parts. Ed. T T Kozlowski, Academic Press, New York and London, pp 237–293.

13 Hermann R K 1964 Effects of prolonged exposure of roots on survival of 2-0 Douglas-fir seedlings. J. For. 62, 401–403.

14 Ingestad T 1979 Nitrogen stress in birch seedlings II. N, K, P, Ca, and Mg nutrition. Physiol. Plant. 45, 149–157.

15 Kolatukuddy P E and Dean B B 1974 Structure, gas chromatographic measurement, and function of suberin synthesized by potato tuber slices. Plant Physiol. 54, 116–121.

16 Kolatukuddy P E 1980 Biopolyester membranes of plants: cutin and suberin. Science 208, 990–1000.

17 Leshem B 1965 The annual activity of intermediary roots of the Aleppo pine. For. Sci. 11, 291–298.

18 Loomis W E 1953 Growth correlation. *In* Growth and Differentiation in Plants. Ed. W E Loomis, Iowa State College Press, pp 197–217.
19 Lyr G and Hoffman G 1967 Growth rates and growth periodicity of tree roots. *In* International Review of Forestry Research. Vol. 2. Eds. J A Romberger and P Mikola, Academic Press, New York, pp 181–236.
20 McCready R M 1970 Starch and dextrin. *In* Methods in Food Analysis. Ed. M A Joslyn, Academic Press, pp 541–563.
21 McDermitt D K and Loomis R S 1981 Elemental composition of biomass in relation to energy content, growth efficiency, and growth yield. Ann. Bot. 48, 275–290.
22 Molz F J and Peterson C M 1976 Water transport from roots to soil. Agron. J. 68, 901–904.
23 Nobel P S 1974 Introduction to Biophysical Plant Physiology. W H Freemand and Co., San Francisco.
24 Persson H 1978 Root dynamics in a young Scots pine stand in central Sweden. Oikos 30, 508–519.
25 Persson H 1979 Fine-root production, mortality and decomposition in forest ecosystems. Vegetation 41, 101–109.
26 Persson H 1980 Death and replacement of fine roots in a mature Scots pine stand. *In* Structure and Function of Northern Coniferous Forests – An Ecosystem Study. Ed. T. Persson, Ecol. Bull. (Stockholm) 32, 251–260.
27 Redmond D R 1959 Mortality of rootlets in balsam fir defoliated by the spruce budwork. For. Sci. 5, 64–69.
28 Reynolds E R C 1970 Root distribution and the cause of its spatial variability in *Pseudotsuga taxifolia* (Poir.) Britt. Plant and Soil 32, 501–307.
29 Robards A W, Clarkson D T and Sanderson J 1979 Structure and permeability of the epiderman/hypodermal layers of the sand sedge (*Carex arenaria* L.). Protoplasma 101, 331–347.
30 Sanderson G W and Perera B P M 1966 Removal of polyphenolic compounds interfering with carbohydrate determinations in plant extracts with an insoluble polyphenol adsorbent. Analyst 91, 335–536.
31 Santantonio D 1982 Production and turnover of fine roots of mature Douglas-fir in relation to site. Ph. D. Thesis, Oregon State University, Corvallis.
32 Smith D 1979 Removing and analyzing total nonstructural carbohydrates from plant tissue. Univ. Wis., Coll. Agric. Life Sci., Res. Pap. 41.
33 Steel R and Torrie J 1980 Principles and Procedures of Statistics – A Biometrical Approach (2nd ed.). McGraw-Hill, New York.
34 Teskey R O and Hinckley T M 1981 Influence of temperature and water potential on root growth of white oak. Physiol. Plant. 52, 363–369.
35 Wilcox H E 1964 Xylem in roots of *Pinus resinosa* Ait. in relation to heterorhizy and growth activity. *In* The Formation of Wood in Forest Trees. Ed. M H Zimmerman, Academic Press, New York, pp 459–478.
36 Yemm E W and Willis A J 1954 The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514.