

Food Reserves and Seasonal Growth Of Douglas-Fir Seedlings

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Abstract. Seasonal growth of tops and roots and concomitant trends in food reserves were observed biweekly on *Pseudotsuga menziesii* (Mirb.) Franco seedlings of two seed sources growing in a Pacific Northwest forest nursery. A general pattern of alternating root, diameter, and shoot growth was found. Rapid root growth did not coincide with rapid shoot elongation, but preceded and followed it. Increased root activity was strongly correlated with lowered reducing sugar concentrations in seedling roots of the faster growing source. Sucrose and raffinose increased markedly during early winter and were apparently converted to starch in spring prior to growth. Concentrations of reducing sugars, crude fat, and protein nitrogen changed little with season. Implications of this information on timing of lifting, storage, and transplanting of seedlings are discussed.

STAGE of seedling development should govern timing of many nursery operations. For lack of information on seasonal fluctuations in root growth and food reserves, nurserymen have relied largely on seedling tops as indicators of development. Better knowledge of the total developmental pattern might suggest modification of nursery operations to maximize outplanting survival and growth of the seedlings produced.

Seasonal relationships were determined between food reserves and growth of roots and tops of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings at the U.S. Forest Service's Wind River Nursery near Carson, Washington. Single sources from two climatic provinces were studied: one from the dry east slope of the Cascade Range in Washington (Lake Wenatchee, 2,000-foot elevation, Wenatchee National Forest) and one from the moist west slope of the Oregon Cascades (Salmon-Salt Creek drainage, 3,000 to 4,000 feet, Willamette National Forest).

Growth of conifers is sustained from current photosynthate at certain seasons, but stored food reserves are also important, especially for spring shoot elongation (Allen 1964, Kozlowski and Winget

1964). Concentration changes, revealed by staining techniques, have been judged by some workers as evidence that starch converts to fats in conifers as temperatures decrease in autumn (David *et al.* 1959). From histochemical studies, Sinnott (1918) classed Douglas-fir as a fat-storing tree during midwinter. However, Clements' (1938) chemical analyses indicated that in winter Douglas-fir needles contained only about one-third greater concentration of crude fat than in summer, whereas sugars increased greatly from December through February. Worley (1937) showed that this increase was due to nonreducing rather than to reducing sugars. More recently, Winjum (1963) reported little midwinter change

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of reducing sugars in Douglas-fir seedling tops, but a severalfold increase in nonreducing sugars. Starch concentration rose in early spring.

Information on timing of conifer root growth has been reviewed recently (Romberger 1963). Studies provide evidence that roots may elongate during any month of the year and that changes in elongation rate coincide with environmental changes (Kramer and Kozlowski 1960). While periodicity in growth of individual roots must be distinguished from that of the root system, a common pattern reported is a burst of growth in the spring, a mid-summer low, and a renewed active period in the fall (Wilcox 1962). Root tips of seedling and sapling Douglas-fir trees, observed by Ross¹ in western Washington, were least active in late January and early February but, judging by external appearance, many did not become dormant. Root activity increased markedly several weeks before buds broke in the spring. Stein² also found active root tips in all seasons when lifting 1- and 2-year-old Douglas-fir seedlings from three different localities in southwestern Oregon.

Growth of Douglas-fir stems has been more commonly measured for large trees than for seedlings. Time of bud break in the spring depends on weather conditions and elevation but varies markedly in a single location, depending on both seed origin and individual variation between young Douglas-firs (Morris *et al.* 1957). Except for lammas growth, annual shoot elongation usually is completed between April and August in trees more than 1 year old, but stem diameter growth may continue for a longer period. Dimock (1964) observed apparent radial growth

¹ Ross, C. R. Root development of western conifers. 63 pp. 1932. (Unpublished master's thesis, College of Forestry, Univ. of Wash., Seattle.)

² Stein, W. I. Comparative juvenile growth of five western conifers. 194 pp. 1963. (Unpublished Ph.D. thesis, School of Forestry, Yale Univ., New Haven, Conn.)

several weeks before shoot elongation on pole-size Douglas-firs near Puget Sound. During 5 years' observations of 65-year-old Douglas-firs, Reukema (1965) found that diameter increase began in early April to May and in some years continued October or later.

Methods

The study was begun in October 1962, after the seedlings' second summer in the nursery. Standard eight-row nursery beds of seedlings of Wenatchee and Willamette seed sources were portioned into plots 2 feet long and half-bed wide, and sampled during 19 and 14 months, respectively. At biweekly intervals, seedlings were dug from the center of three randomly chosen plots from each bed, leaving some seedlings near plot perimeters to minimize disturbance to adjacent plots. When a plot was dug, soil temperature was measured at 4-inch depth. Nearly all samples were taken in late morning.

Seedlings from each plot were sorted to retain the largest and least damaged and randomly allocated at the nursery into one of four 10-seedling groups. One group was randomly selected to provide five seedlings for subsequent determination of terminal bud size or length of new shoot above the bud scar, stem diameter 1 cm above the root collar, length of white root tips, and percent of roots with actively growing tips. Buds and stem diameters were measured to the nearest 0.1 mm with a binocular microscope. Proportion of active root tips was evaluated on a major lateral root from each seedling. Roots tipped with white, translucent tissue 0.1 mm or more in length were counted "active." Whether such roots tipped with only a few tenths of a millimeter of white tissue are actually "active" cannot be readily determined, but their proportion of all "active" tips remained relatively constant throughout the year; consequently, their inclusion did not change the trends.

Seedlings in the remaining three groups

were pooled at the nursery, washed free of soil, and severed at the root collar. Roots and tops were then dried immediately at 65°C. Dried material was ground to 40 mesh in a Wiley Mill and stored in sealed containers at -16°C until redried at 70°C just prior to analysis.

Tops and roots collected on each date from each plot were analyzed for sugars, starch, crude fat, and protein nitrogen. Sugars were extracted with 80-percent ethanol for 15 hours in micro-Soxhlet apparatus. Ethanol was then evaporated. Residual solutions were treated with neutral lead acetate, delead with sodium oxalate, decolorized with activated charcoal, and made to volume. Reducing sugars expressed as glucose were determined in one aliquot by iodometric titration (Somogyi 1945). A second aliquot was hydrolyzed with hydrochloric acid in a hot water bath (Association of Official Agricultural Chemists 1960) and total sugars determined colorimetrically with anthrone using glucose as a standard (Kahan 1953). A Klett colorimeter with number 62 filter was used. The ethanol-extracted residue was treated twice with 52-percent perchloric acid to extract starch (McCready *et al.* 1950). Glucose from the hydrolyzed starch was then measured colorimetrically with anthrone. Perchloric acid extraction of starch appeared satisfactorily specific. At various seasons, quantities of less than 10 mg per gram were detected, indicating that, at most, extraneous anthrone-reacting compounds were probably present in only small amounts.

Separation and identification of sugars was made by two-dimensional thin layer chromatography. Kieselguhr G was used as the stationary phase with developing solvents of n-butanol:pyridine:water (45:25:40 v/v) and ethyl acetate:n-propanol:water (65:23:12 v/v). Aniline diphenylamine phosphate reagent was used for color development of the unknowns and standards (Block *et al.* 1958).

From a separate portion of the original sample, crude fat was extracted with di-

ethyl ether for 15 hours in micro-Soxhlet apparatus. After ether was evaporated, the extract was dried over calcium chloride and weighed (Association of Official Agricultural Chemists 1960). Protein content was approximated as 6.25 times the organic nitrogen concentration determined by micro-Kjeldahl analysis of still another portion of the original sample (Association of Official Agricultural Chemists 1960).

Results³

Because weather influences seedling phenology and physiology, study results need examination within the context of prevailing weather. Climate at Wind River is typical for that of a narrow valley within the Cascade Range in Oregon or Washington. Heavy precipitation occurs in late fall, winter, and spring, while summers are usually dry with warm days and cool nights. In 1962, the first fall frost occurred October 5, six days before the first sampling. The last frost of the next spring occurred on April 29, followed by a 173-day frost-free period ending October 19. The frost-free season in 1963 was substantially longer than the 131-day average for this location. Mean temperatures from May through August averaged 58.6°F, only 1.2°F below the 40-year average. The nursery was free of snow cover for all but short periods during the winters of 1962-63 and 1963-64, rather atypical for Wind River where the average annual snowfall is 80.6 inches and where in only 7 out of 40 years were there less than 6 inches of snow in January (Steele 1952; U.S. Weather Bureau 1962, 1963, 1964).

Data obtained from biweekly sampling

³ Since the two seedbeds sampled were 1,300 feet apart in the nursery and Wenatchee stock grew at a 30-foot lower elevation, comparisons of seedlings from the two seed sources may be confounded by bed location. Physical and chemical soil characteristics of the two areas were rather similar when routinely sampled in 1963.

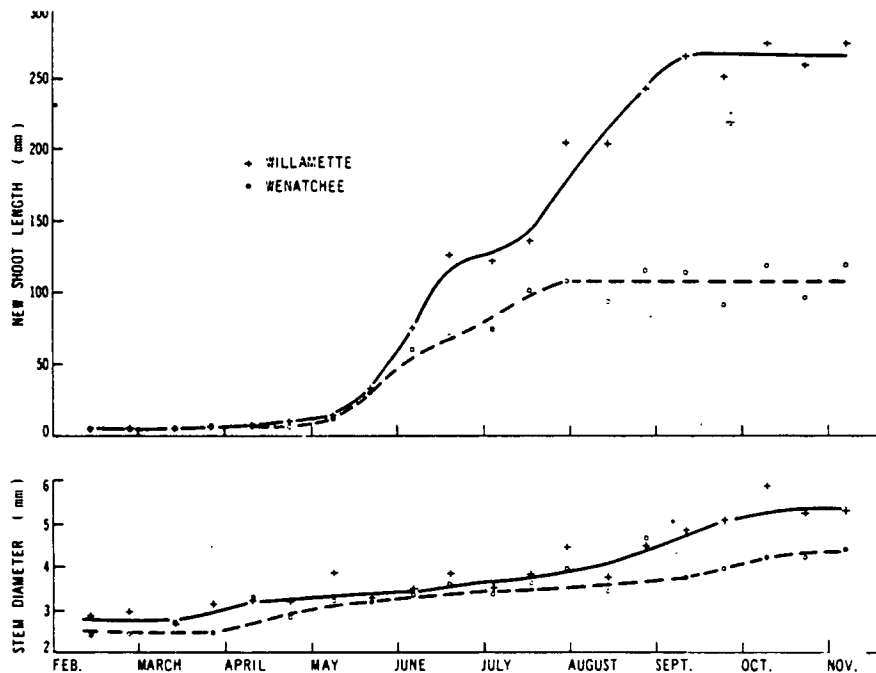


FIGURE 1. Gain in stem diameter and new shoot length during 1963 for Douglas-fir seedlings of Willamette and Wenatchee seed sources.

of seedlings are presented in Figures 1 to 4. Because of destructive sampling, repeat observations could not be made on the same seedlings. Hence, growth and food reserve data do not always show a smooth progression from one sampling date to the next. Freehand curves have been used to bring out the general trends which were reinforced by statistical comparisons and computed curves as needed. Growth rate data used in Figures 2 and 3 were read from the curves in Figure 1 and plotted at the midpoint of the observation interval. Individual plotted points in all figures represent the average of the three samples from one seed source.

Phenology. Bud break, the most conspicuous feature of spring growth, occurred about mid-April 1963 on Wenatchee seedlings and slightly later on those of Willamette source. Terminal shoots elongated rapidly until early June (Wenatchee) or mid-June (Willamette), then growth rate slowed (Figs. 1, 2, and

3). Most Willamette seedlings had produced a second flush by mid-July, and rapid terminal elongation resumed (Fig. 3). Wenatchee seedlings also produced a secondary peak of shoot growth in July (Fig. 2), but this was apparently due to varying rates of elongation since a second flush occurred only in late August on a few trees. Elongation largely ceased by early August for Wenatchee seedlings and by mid-September for Willamette seedlings. Terminals of Willamette seedlings grew both faster and for a longer period than terminals of Wenatchee seedlings (Fig. 1).

Diameter increase started after mid-March and continued through October for both Willamette and Wenatchee seedlings (Figs. 1, 2, and 3). Periods of rapid increase preceded and followed the period of most rapid shoot elongation. Exact beginning and ending of diameter increase was difficult to detect because of the bi-weekly sampling interval and variation in

successive seedling samples. In both diameter and height, Willamette seedlings grew more (Fig. 1).

Activity of major lateral roots of both sources was concentrated mainly in two periods, although some white tips were found in every month of the year (Figs. 2 and 3). The first high-activity period began in late February to mid-March, peaked in late March, and declined to a low level by late April when bud break occurred. Root activity remained low until shoot elongation rate diminished in June, then rose during July and August to another high for Wenatchee seedlings and to medium levels for Willamette seedlings, declining again to low levels by mid-September. Percent of root tips active during spring peak periods was signifi-

cantly higher than for neighboring periods by the Tukey test (Snedecor 1956). A significant high-activity period for Wenatchee seedling roots also occurred in December 1962 and summer 1963.

Longer white root tips were produced during periods of high root activity than during low root activity. The percent of roots actively growing correlated positively with average length of the longest white root tip at $r = +0.61$ for Willamette and $+0.56$ for Wenatchee stock; both values are highly significant statistically.

Less than half the tips of lateral roots on sampled seedlings were active even in peak periods of root growth. Highest root activity for Wenatchee seedlings was observed in March 1964 and next highest in

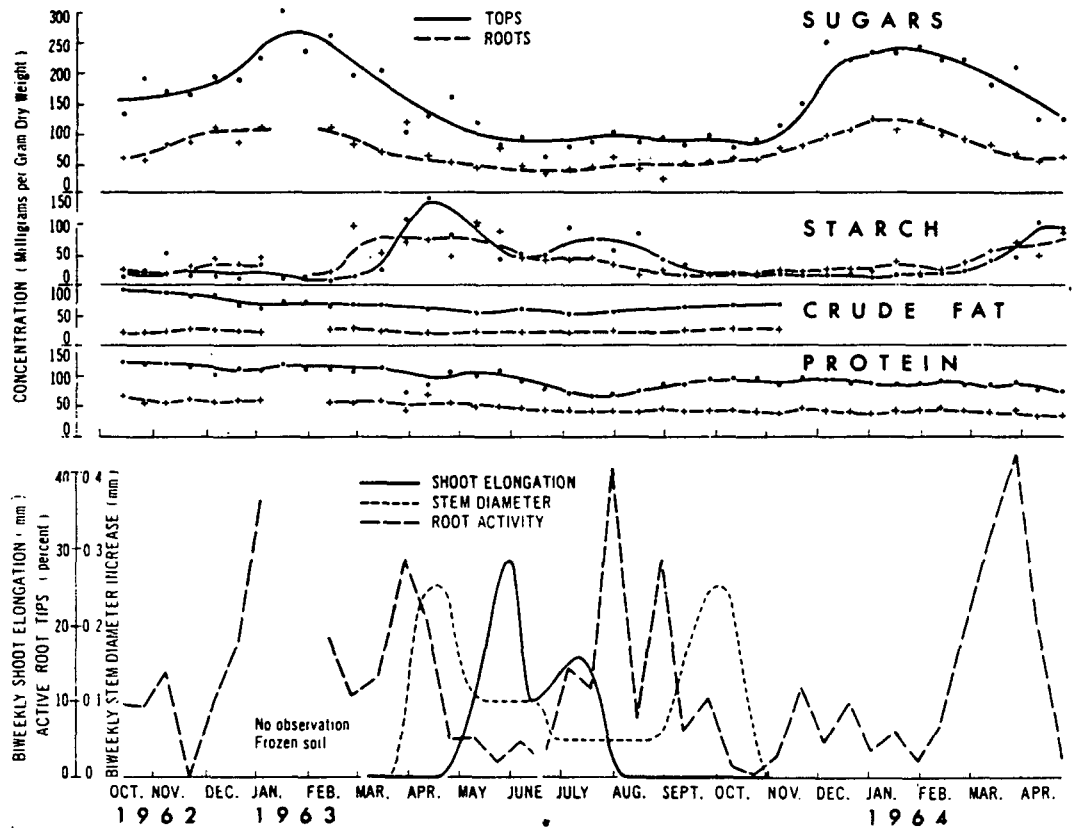


FIGURE 2. Seasonal trends in food reserves, root activity, and rate of diameter and shoot growth as revealed by biweekly sampling of Douglas-fir seedlings of Wenatchee source.

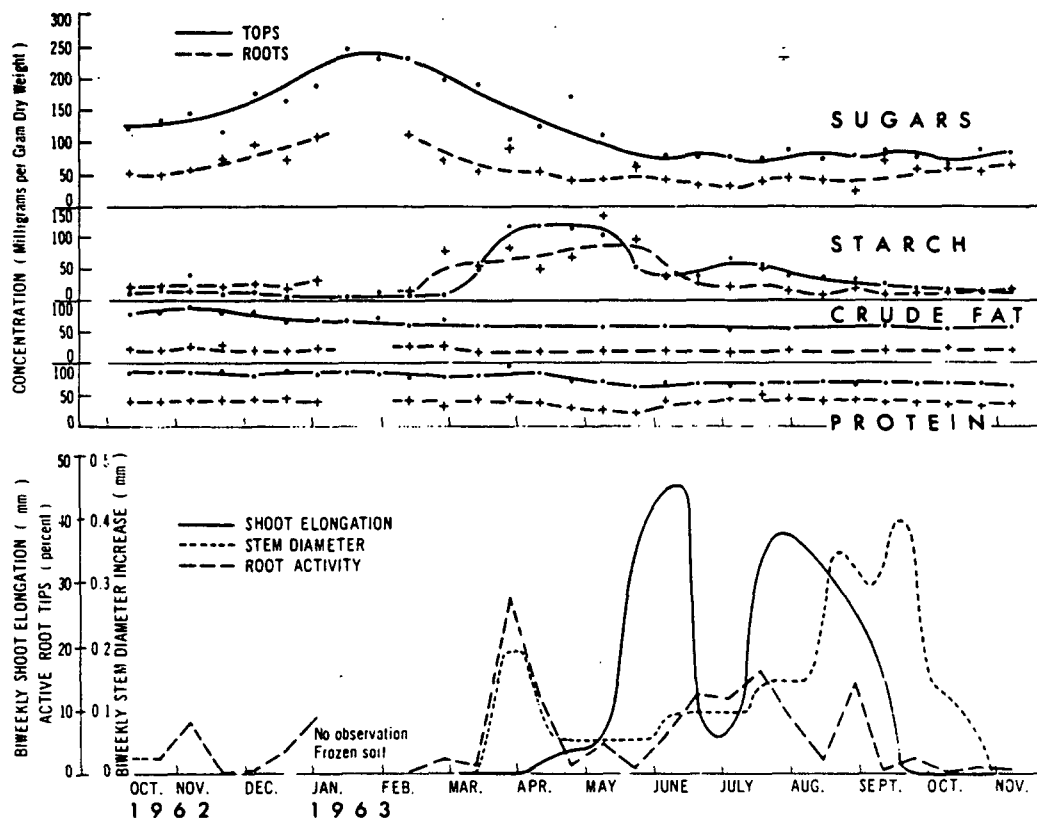


FIGURE 3. Seasonal trends in food reserves, root activity, and rate of diameter and shoot growth as revealed by biweekly sampling of Douglas-fir seedlings of Willamette source.

late July 1963, averaging 42 and 41 percent, respectively. Root activity for Willamette seedlings reached a high of 28 percent in March 1963. Root activity was nil on only a few sampling dates, but there were many dates when less than 10 percent of all root tips were active.

High soil temperature or low soil moisture is not likely to have curtailed root activity. Seedbeds received sprinkler irrigation regularly, and the summer of 1963 was much wetter than average. Except for September 11, the soil was visibly moist on each sampling date. Highest soil temperatures at 4-inch depth were found on September 25, 1963, and averaged 17°C for the six plots measured that day; the highest individual plot reading on that date was 19°C.

Root activity decreased at low soil temperatures, but the lowest temperature for white root tips to be present could not be pinpointed. At low temperatures, a month or more may be required for a white tip to become suberized.

Minimum temperature needed for active root growth appeared to vary by seed source and season. Presence or absence of a December root-activity peak for Wenatchee seedlings seemed to depend on soil temperature exceeding 4° or 5°C. Soil temperatures at the 4-inch depth were 4.5° to 5.5°C when many roots were active in December 1962, but 1° to 3.5°C in 1963 when activity was low. However, spring root growth may start at even lower temperatures, since Wenatchee seedling roots had started active growth

when soil averaged 2°C on February 28, 1964.

Food reserves. Beginning about early November, total sugars in seedling tops increased markedly to a January peak nearly three times the summer concentration (Figs. 2 and 3). Sugar concentration decreased at a fairly constant rate thereafter to a low level by late May. Trends in tops and roots were similar in timing and relative concentration change. Sugar concentration in tops correlated with concentration in roots, with $r = +0.61$ for Willamette seedlings and $+0.74$ for Wenatchee seedlings; both coefficients are highly significant statistically. Most of the time, sugar concentration in tops was about double that in roots. Concentration in tops was similar to that found in needles by Worley (1937) and Clements (1938).

Four sugars were detected chromatographically: glucose, fructose, sucrose, and raffinose. Sugar buildup was primarily due to the two nonreducing sugars, sucrose and raffinose. Some sucrose was found in all seasons, but raffinose was barely detectable in summer. Throughout the year, the reducing sugars, glucose and fructose together, averaged about 50 and 30 mg per gram of dry tissue weight in tops and roots, respectively. Concentration of reducing sugars did increase briefly during a particularly cold period in January 1963.

Though no marked seasonal pattern was apparent for reducing sugars, for Willamette seedlings an inverse relationship was observed between concentration in roots and percent of active root tips (Fig. 4). After logarithmic transformation, root activity and concentration data of February to November 1963 correlated linearly with a highly significant coefficient, $r = -0.78$. The point furthest from the computed curve in Figure 4 represents the April 4 observation when spring root activity was declining. It seems possible that root tips were still white then, and were counted as active when reducing sugar content already reflected a change in

root growth. If this point is omitted, the equation changes slightly and $r = -0.88$. During the same February-to-November period, reducing sugar concentration in roots of Wenatchee seedlings was not significantly related to root activity.

Starch in tops remained low in autumn and early winter, but increased rapidly beginning in March (Figs. 2 and 3). A peak was reached by mid-April, followed closely by a rapid decline. Subsequently, concentrations increased moderately during June and July.

Starch concentrations in roots began increasing about mid-February, somewhat earlier than in tops. The increase to a broad peak in March, April, and May was followed by a decrease toward the former low level starting in late May. Starch concentration in roots significantly exceeded that in tops in late February and early March. Tops exceeded roots in starch concentration during April and again in July and August, when concentration increased in tops but not in roots.

The crude fat fraction in tops, which included compounds such as plant pigments in addition to actual reserves, ranged from 5.1 to 9.1 percent during the year (Figs. 2 and 3). The range in roots was from 1.8 to 3.1 percent. No marked seasonal pattern was evident in roots, but concentration in tops showed a gradual downward trend in late winter and spring. A slight increase from summer concentrations was found during fall 1963 for Wenatchee seedlings tops. Other lipids (e.g., waxes, phospholipids, carotenoids, etc.) present in the ether extract might mask small fluctuations in seedling fat reserves.

Protein nitrogen fluctuated little through the seasons (Figs. 2 and 3). A gradual decline in nitrogen concentration, apparently accelerated during spring growth, occurred during the study. For example, percent of nitrogen in tops of Wenatchee seedlings began near 1.9 and decreased below 1.4 by the second spring. Nitrogen percent in roots also declined from about

1.0 to 0.6. Lack of periodic nitrogen fertilization during the study, plus increasing dilution with older, woody tissue, may explain the observed decline.

Discussion

Food reserves and growth. Quantities of food reserve components and timing of their fluctuations differed little between seedlings of the two seed sources. Thus, results will be discussed for both sources together.

Sugars and starch fluctuate considerably by season, constitute a substantial percentage of plant dry weight, and thus appear to be the principal class of food reserves. Little seasonal variation was found in crude fat or protein concentration, and neither appears to be quantitatively important as a food reserve in the sense that it accumulates to support future growth. Peaks in crude fat concentration were 77 and 48 percent greater than seasonal lows for tops and roots, respectively. In contrast, peaks in sugar concentration were 367 percent (tops) and 455 percent (roots) greater than the lows. Fats have higher energy content per unit weight than sugars, but not sufficient to match the energy represented by buildup in sugar reserves.

In late October, as diameter growth stops and root activity slows, sugar reserves begin to increase gradually. Rate of increase accelerates as autumn progresses, perhaps because cool temperatures reduce respiration. Maximum concentrations coincide with coldest weather. The midwinter high and the virtual summer disappearance of raffinose observed here for Douglas-fir has been previously reported for *Picea pungens* Engelm. (Neish 1958).

Late winter decrease in sugar concentration coincided with a buildup in starch. Accumulation of starch prior to active growth may represent more than simply a temperature-related sugar conversion. Average maximum daily air temperature for the 3 days preceding each collection did not rise but fluctuated between 10°C and

15°C from February to mid-May 1963, evidence against increasing daily temperatures being the cause of sugar-starch interconversion. Moreover, starch increased earlier in roots than in tops despite cooler soil temperatures. Timing of this increase is consistent with the earlier onset of new root growth. Like the Douglas-fir in our study, growth flushes of broadleaf evergreens are preceded by starch accumulation (Jones and Steinacker 1951). Some possible energy relations in the biochemical processes involved have been postulated by Eifert and Eifert (1963).

Starch concentration in tops reached a maximum in early April and then declined rapidly with bud break and subsequent shoot growth. This decrease suggests that current photosynthesis did not meet metabolic demands during spring growth. Neuwirth (1959) has reported that respiration may exceed photosynthesis of Douglas-fir at bud break.

Use of sugar and starch for shoot growth could account for their decrease in tops after bud break. Less obvious, however, is the fate of sugar that disappeared from tops before bud break and a month before any signs of starch buildup. Some may have been used in increased respiration, and some may have contributed to starch accumulation in roots prior to the March surge of root activity. Also, starch concentrations in roots did not decrease during spring root growth, suggesting a steady-state situation maintained by sugars from the tops.

Glucose plays a key biochemical role in both assimilation and respiration; the inverse relationship found between reducing sugar concentration and root activity (Fig. 4) is evidence of this role. Reducing sugars were lowest for Willamette seedlings when roots were most active. Slower growth of Wenatchee seedlings may have allowed equilibrium sugar concentrations to be more closely maintained and possibly explains the lack of correlation between their root activity and reducing sugar concentration.

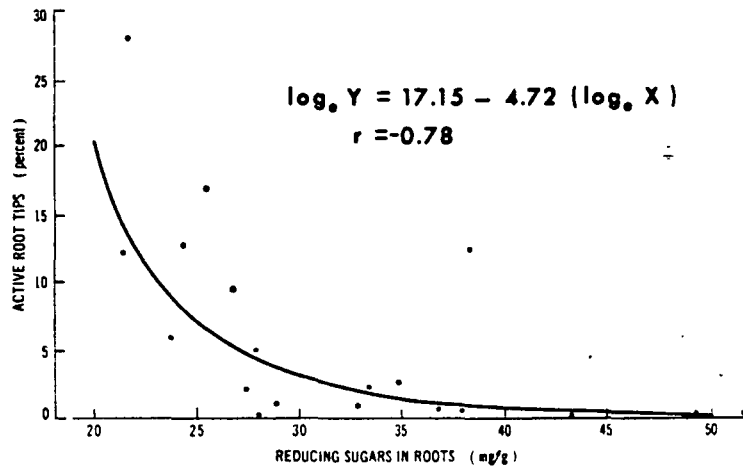


FIGURE 4. Root activity correlated inversely with reducing sugar content from February to November 1963 for Douglas-fir seedling roots of Willamette source.

Role of hemicellulose as a food reserve was not measured in this study. Winjum (1963) showed that starch in Douglas-fir seedling tops increased from a winter-base level of about 150 mg per gram dry weight to about 240 mg per gram at the spring peak. Since he used a hydrochloric acid extraction method (personal communication), hemicellulose was probably included in the "starch" fraction. Increment between base level and spring peak in Winjum's study is about the same as found in this study where the more starch-specific perchloric acid extraction method was used. Similarity of results reached by different methods provides indirect evidence that increases were mainly in starch, not in hemicellulose. However, in sampling older Douglas-fir needles, Clements (1938) showed spring increase and decrease in a nonstarch fraction of acid-hydrolyzable carbohydrate; and Worley (1937) found a midwinter rise and fall in reducing action for a starch-free fraction extracted with hydrochloric acid. Possibly timing and quantity of hemicellulose utilization change with plant age.

One important role of seedling food reserves may be inferred by the nearly

coincident timing during winter and early spring of carbohydrate peaks and maximum "root regeneration potential" as reported by Stone *et al.* (1962), Winjum (1963), and Lavender (1964). Level of carbohydrate reserves may be one internal factor influencing "root regeneration potential" as well as the relation between lifting date and seedling field survival observed by Lavender (1964) and Sissingh (1964).

Douglas-fir seedlings differ from deciduous trees in yearly patterns of carbohydrate reserves. Starch accumulates in deciduous stems in summer and early fall and is converted to sugar with the advent of cool weather (Kraybill *et al.* 1930, Siminovitch *et al.* 1953). In Douglas-fir seedlings, there is little increase in starch concentration during summer, and the late fall and winter increase in sugars appears due to continued photosynthesis without concomitant use of the photosynthate. Appreciable wintertime photosynthesis has been observed in large Douglas-fir and ponderosa pine trees (Helms 1965, Fritts 1966). Both Douglas-fir and deciduous trees convert sugars to starch before spring growth.

Alternation of root growth and shoot growth is a striking phenological phenomenon. Root activity preceded bud break, practically stopped while shoots elongated rapidly, and then resumed as rate of shoot growth diminished. Diameter growth, particularly for Wenatchee seedlings, also appeared to alternate with root and shoot growth. However, diameter measurements did not distinguish between hydration changes and true growth, which might account for the observed increase in diameter preceding bud break. Top growth of Willamette seedlings substantially exceeded top growth of Wenatchee seedlings, but their root activity was lower, especially during July and August when lammas growth occurred. These several observations make it appear that rapid growth by both roots and shoots simultaneously was beyond the substrate-supplying capabilities of these seedlings. Some observers have described similar growth alternation (Kaufman 1965, Romberger 1963). In *Pinus strobus* seedlings, translocation of photosynthate from tops to roots occurred in an alternating pattern which appears to coincide with peak growth periods (Shiroya *et al.* 1966). Recent evidence indicates that growth alternation may occur in conditions of high, but not of low, soil moisture (Lister *et al.* 1965).

Application

The information from this study supports some current cultural practices and suggests how others might be improved to increase survival of planting stock. For example, nursery stock is customarily not lifted and outplanted after buds begin to swell in the spring because it often survives poorly. Its low survival may be less directly related to bud break *per se* than to ebbing root activity that may remain low until summer. Inability to regenerate a root system rapidly at the planting site probably increases mortality of seedlings from drought or other factors.

Late fall lifting of stock to be placed in cold storage also appears to be a reason-

able practice. Late lifting not only minimizes storage duration, but also allows prelifting buildup of food reserves. Such buildup should provide more respiratory substrate for use during storage and more residual food reserves to support vigorous growth after outplanting.

It would also seem reasonable that spring-lifted stock should be dug prior to the surge of root activity and held in cold storage until outplanted. Food reserves would go into root growth at the planting site rather than in the nursery bed, thus conserving the energy represented by the many new roots lost during lifting and handling. Aldhous (1964), in England, obtained higher survival rates for February- and early March-lifted 1-0 Douglas-firs than for those lifted later, though the difference was not significant. Data of Lavender (1964) and Sissingh (1964) show the same trends.

With insight gained from this study, timing of these and other nursery operations can now be tested with a more fundamental understanding of the seedling processes involved.

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