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CARBOHYDRATE STATUS AS A MEASURE OF SEEDLING QUALITY

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ABSTRACT--Seedlings depend on reserve carbohydrates from the time they are lifted until photosynthesis is sufficient to meet the demands of growth and respiration. If carbohydrate reserves are inadequate to meet respiratory demands associated with cold storage and outplanting, the seedling will die. If carbohydrates are stored as starch, the relative amount can be estimated by the degree to which iodine stains a cross section of the stem or root. For precise assays of carbohydrates including both sugars and starch. more sophisticated methods are required.

5.1 *INTRODUCTION*

Plant physiologists sometimes discuss the carbon fixed by plants as if it were money. They can calculate exactly the "cost" of building a leaf and can estimate the cost of maintaining the leaf over a period of time (e.g.. Mooney and Gulmon 1979). Moreover, by projecting total photosynthesis throughout the leaf's lifespan, they may calculate the return on the carbon invested in constructing the leaf.

In this paper. I shall focus on the seedling's savings account--its carbohydrate reserves. The initial "deposit" and depletion rate of these reserves help explain why seedlings perform as they do after undergoing various cultural treatments, storage, and outplanting.

Duryea, M. L. (ed.). 1985. Proceedings: Evaluating seedling quality: principles, *procedures, and predictive* abilities *of* major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis. ISBN 0-87437-000-0

In discussing carbohydrate reserves, I shall first provide a general sketch of the kinds of carbohydrates and how they are accumulated and depleted. I shall then provide a detailed discussion of techniques for measuring carbohydrates. Last, I shall discuss how carbohydrate status is likely to relate to field performance.

5.2 KINDS OF CARBOHYDRATES

The carbohydrates are a diverse group of compounds sharing common building blocks--the sugars. Within this group are hundreds of compounds and, in the case of the sugars. those compounds are rapidly interconverted (Kramer and Kozlowski 1979). This complexity can be reduced, however, by grouping the carbohydrates according to their functions in the plant (Farrar 1980). I shall discuss them simply as the structural carbohydrates (e.g.. cellulose), the reserve carbohydrates (e.g.. starch), and the sugars (Fig. 1).

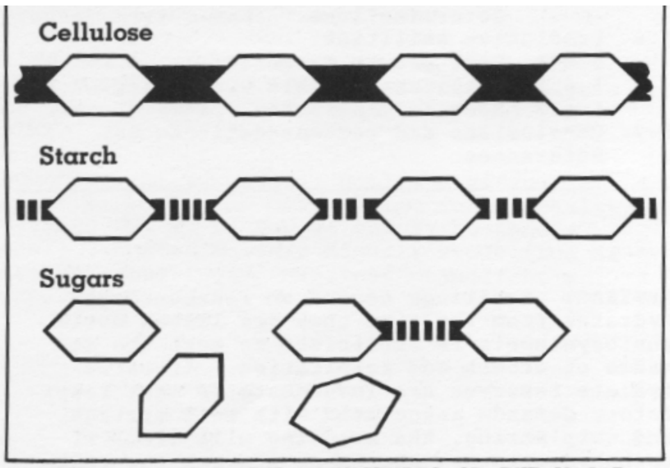

FIGURE 1. STYLIZED STRUCTURES OF THE MAJOR GROUPS OF CARBOHYDRATES. HEXAGONS REPRESENT INDIVIDUAL SUGAR MOLECULES, AND THICKNESS OF THE BANDS CONNECTING THEM INDICATES THE STRENGTH OF CHEMICAL BONDS HOLDING THE SUGARS TOGETHER.

5.2.1 Structural carbohydrates

In the seedling's carbon budget. the structural carbohydrates represent a non-refundable investment of carbon. Carbon spent by the plant to build structure is thereafter unavailable for other uses.

Cellulose is the predominant structural carbohydrate in trees (Kramer and Kozlowski 1979). It is a linear chain (or polymer) of glucose molecules that cross-link with adjacent cellulose molecules. This cross-linkage gives the plant much of its strength. Cellulose. like other structural carbohydrates, is found almost exclusively outside the cell membrane in the cell walls.

The hemicelluloses are a second important group of structural carbohydrates occurring in the cell walls. They include complicated polymers consisting of a wide variety of sugars (Kramer and Kozlowski 1979). There has been some speculation that hemicelluloses may at times serve as reserve materials in trees (Priestley 1962, Kimura 1969, Meyer and Splitstoesser 1971, Glerum and Balatinecz 1980. Kile 1981), but the evidence is inconclusive. Their exact function in the cell wall is not yet known (Glerum 1980).

Finally. the pectins. which are chains of modified and unmodified galacturonic acid molecules, appear in the middle lamellae between cells. They serve as an intercellular glue and are apparently not used as reserve substances.

5.2.2 Reserve carbohydrate

Starch is the most important reserve carbohydrate. It comprises the "savings account" from which a seedling can draw if respiratory or growth expenses exceed photosynthetic income.

Like cellulose. starch is composed of joined glucose molecules. Unlike cellulose, the starch molecule is easily disassembled by enzymes, a process that releases its constituent sugars. Starch occurs within special structures--amyloplasts and chloroplasts-inside the cells. In a rice grain or potato tuber. starch concentrations may reach 70% of the dry weight (Jenner 1982). In conifer needles (Ericsson 1979) and fine roots (Ericsson and Persson 1980). starch concentrations may reach 30% of the dry weight. On the other hand. starch concentrations may drop to near zero in a root or leaf about to be shed (Marshall 1984).

5.2.3 Sugars

The sugars are the metabolically active carbohydrates and are involved in all biosynthetic and maintenance activities. They are the only form of carbohydrate transported from place the place within the plant (Kramer and Kozlowski 1979). They can be regarded as the seedling's "pocket money."

The sugars also serve as the major carbohydrate for storage when temperatures are low. Any starch present is likely to be converted to sugar during cold-hardening (Kramer and Kozlowski 1979) because the sugar acts as an antifreeze within the cells.

5.3 CARBON INPUTS AND OUTPUTS

5.3.1 Photosynthesis

Photosynthesis is the process whereby CO2 is drawn from the atmosphere and converted to simple sugars. The energy source for this process is sunlight. Photosynthesis represents the plant's income, its paycheck. All other chemical reactions within the plant depend upon the "burning" of these sugars to release energy. This burning of sugars, releasing CO2, is what we mean by respiration. Respiration can be separated into that required for construction of tissue and that associated with maintenance of tissue. The relationships between each of these processes and the various kinds of carbohydrates are shown in Figure 2.

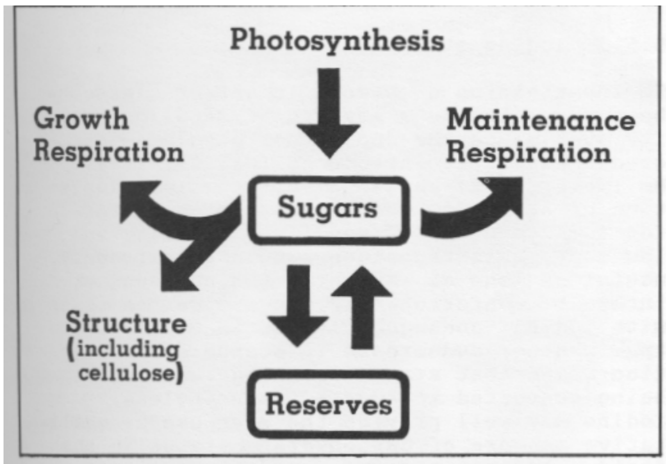

FIGURE 2. RELATIONSHIP BETWEEN THE VARIOUS TYPES OF CARBOHYDRATES AND THE PROCESSES OF GROWTH AND MAINTENANCE RESPIRATION.

5.3.2 Growth respiration

The seedling has two costs associated with growing new tissue. The greatest cost is that of the carbon actually being placed into the new tissue--for example, the sugars that are placed into cellulose polymers. The second cost is for the sugars burned to provide energy for synthesis of new molecules. In the case of cellulose, this is simply a matter of joining sugars. To make more complicated compounds, however, requires considerably more energy. The C02 released in these processes is attributed to "growth respiration." The rate of growth respiration depends upon the growth rate and the biochemical composition of the tissue. Note that by this definition, growth is an irreversible commitment of resources. Because it is not irreversible, an increase in weight as a result of starch accumulation is not growth: the starch can be burned and the weight lost.

5.3.3 Maintenance respiration

Respiration rates never fall to zero in living tissue; there is a cost associated with tissue maintenance. This cost is usually termed "maintenance respiration." It is associated with the burning of sugars to yield the energy needed for maintenance processes. These processes include replacement and repair of enzymes and cell membranes as well as transfer of materials from cell to cell. Because enzymes and membranes are replaced more frequently at higher temperatures, maintenance respiration increases exponentially with increasing temperature, approximately doubling with every 10°C increase (Penning de Vries 1975).

5.4 ACCUMULATION AND USE OF RESERVES

As discussed earlier, the seedling's only source of sugars, its only income, is through photosynthesis. The seedling does not necessarily use its sugars immediately, however. In fact, a large proportion of the sugar is often stored as reserves. The exact amount being stored depends in large measure upon the rates of photosynthesis and respiration (Kramer and Kozlowski 1979). Therefore, reserve accumulation is favored by anything that (1) increases photosynthesis, (2) decreases growth, or (3) decreases rates of maintenance respiration. Alternatively, any of the opposite effects will reduce reserve accumulation and eventually begin to deplete reserves. Changes in light intensity, temperature. nutrition, or growth rates will alter these three major processes, resulting in predictable changes in reserves, as discussed in the following section.

5.4.1 Light

Within a limited range, photosynthesis is sharply increased by increasing irradiance. If such an increase does not cause an equivalent increase in growth, starch will accumulate (Berry and Downton 1982). Alternatively. placing a seedling in darkness causes it to deplete its reserves, especially if growth continues (Fig. 3). Only by using reserves accumulated during the day can seedlings continue to grow at night (Kramer and Kozlowski 1979). High seedbed densities necessarily

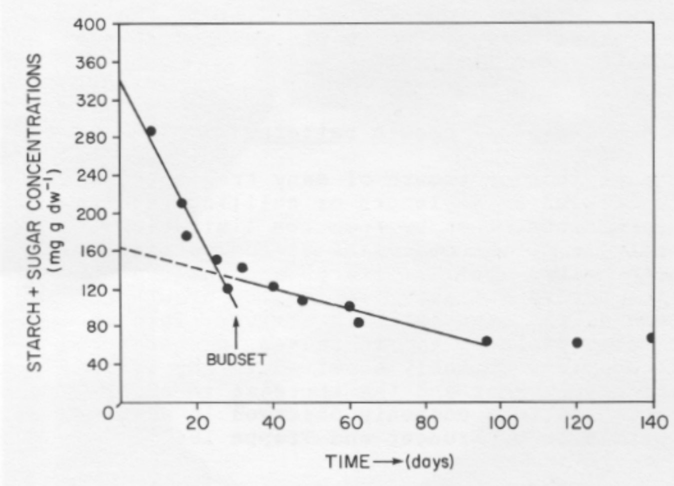

FIGURE 3. STARCH AND SUGAR DEPLETION IN DOUGLAS-FIR SHOOTS MAINTAINED IN DARKNESS. REGRESSION LINES BEFORE AND AFTER BUDSET ARE SIGNIFICANTLY DIFFERENT ($\alpha = 0.01$).

reduce the amount of light available to each seedling and may reduce starch and sugar reserves.

5.4.2 Moisture

As plant moisture stress develops, growth is reduced long before there is any reduction in photosynthesis (Hsaio 1973). Consequently, starch concentrations increase sharply under mild moisture stress (Vartanian 1981). Of course, once a drought becomes severe, photosynthesis is also halted. Accumulation of starch under mild water stress provides a means by which a plant may better survive a period in which photosynthesis has been halted (Levitt 1972. Vartanian 1981).

5.4.3 Nutrition

In some species, mild nutrient deficiencies also reduce growth more than they reduce photosynthesis (Ariovich and Cresswell 1983). New growth requires major nutrient inputs, whereas photosynthesis can continue with nutrients already in place. For these reasons. a decline in nutrient availability will result in starch accumulation (Ariovich and Cresswell 1983). Improved nutrient availability, on the other hand. may increase growth rate and reduce accumulation of reserves (Waring et al. 1984, unpubl.).

5.4.4 Temperature

Temperature affects photosynthesis as well as growth and maintenance respiration. Photosynthesis and growth peak at temperatures around 15°-25°C (Lavender and Overton 1972, Kramer and Kozlowski 1979); however, rates of maintenance respiration continue to rise exponentially to about 45°C (Penning de Vries 1975). Low temperatures can kill rapidly growing tissues and may also disrupt the photosynthetic apparatus in the foliage (Linder and Lohammar 1981).

5.4.5 Seasonal growth patterns

The periods of growth of many tree species are controlled by daylength or chilling requirements rather than by resource limitations. Analysis of the processes discussed earlier nonetheless applies; starch and sugar accumulate before and after periods of growth for as long as photosynthesis is active. This accumulation explains the increases in starch concentrations commonly observed in the spring and late summer and the increase in sugar concentrations commonly observed in autumn (Little 1970, Krueger and Trappe 1967).

5.5 PROCEDURES FOR DETERMINING CARBOHYDRATE CONTENT

As stated earlier, the carbohydrates comprise a complex group of compounds, which I have simplified for this discussion by grouping them according to function. To a certain extent, they can be analyzed according to these functional groups as well. I will present below a simple test for detecting the presence of starch with iodine, a more complex analytical procedure for quantifying hexose sugar and starch contents, and will briefly mention methods for analyzing individual sugars.

5.5.1 Iodine staining

Iodine staining of starch in intact tissue has been suggested as a measure of seedling quality ever since the subject of physiological grades was first introduced (Wakeley 1948). Hellmers (1962) showed almost complete depletion of starch in shoots kept in cold storage for 4-1/2 months. Given its simplicity and low cost, this technique should be extremely useful as long as starch is the compound of interest. Unfortunately, sugars do not stain with iodine: consequently, it is difficult to know whether a decrease in starch concentration means that starch is being respired or being converted to sugars. Nonetheless, iodine may well provide the most useful qualitative measure of carbohydrate status in the field. Iodine staining would be of little use, however, during the winter, when starch is converted to sugars. Because conversion to sugars occurs to a lesser extent in roots than in shoots (Kramer and Kozlowski 1979), iodine staining might be useful even during the winter if applied to root tissues.

5.5.2 Extractions

Determination of the amounts of starch and sugar in a tissue requires that the starch and sugars be completely extracted. Because such extraction has proven difficult in the past, estimates of starch and sugar concentrations have varied widely. Sugars can be extracted with ethanol (Association of Official Analy tical Chemists 1980), methanol (Priestley 1962), or methanol:chloroform:water (MCW) (Dickson 1979). Hot 80% ethanol has been most usual (Fig. 4). The alcohol extract contains phenols, organic acids, and pigments as well as the sugars. It is necessary to remove these contaminants to determine the sugar concentrations colorimetrically. The non polar pigments (including chlorophyll) and the phenols can be partially removed by a 100% acetone extraction prior to the ethanol extraction. The remaining non polar contaminants can then be removed by adsorbing them out of the ethanol extract with insoluble polyvinylpyrollidone (Sanderson and Perera 1966). Organic acids can then be removed by precipitation with neutral lead acetate (Sanderson and Perera 1966); a clear, nearly colorless extract is left (Fig. 5). Solvent fractionation (addition of chloroform to MCW extract) may also be used to rid the extract of non-polar contaminants (Dickson 1979).

After the soluble sugars have been removed.

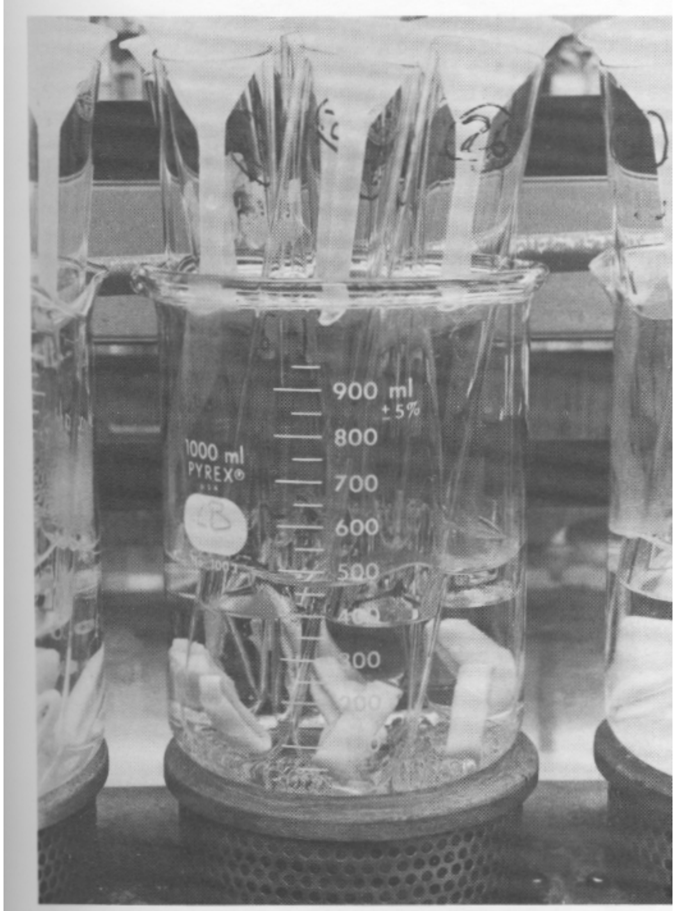

FIGURE 4. APPARATUS FOR EXTRACTING SUGARS FROM TISSUE WITH 80% ETHANOL.

starch can be extracted from the residue (Fig. 6). Enzymes are often used in this extraction because of their specificity (Smith 1969. Haissig and Dickson 1979). Unfortunately, the starch-extraction enzymes are easily deactivated by any phenols remaining in the tissue or in solution and therefore may not yield complete extractions (Ebell 1969). This problem is apparently much more severe in conifer tissues than in the crop plants for which most of these methods were developed. Haissig and Dickson (1979) may have circumvented this problem by extracting with MCW prior to starch extraction. but their method has not been rigorously checked for completeness of extraction.

Starch can also be extracted by acids, usually perchloric or sulfuric. Acid concentrations should be chosen carefully, however, as perchloric acid concentrations used in the past were recently demonstrated to hydrolyze cellulose (Hansen and Moller 1975). Prolonged extractions with 35% perchloric acid will provide complete extraction of all starch. This extraction also removes a small amount of structural carbohydrate, which should be corrected for. The correction can be made by precipitating the starch out of solution with iodine (Hassid and Neufeld 1964). by measuring the carbohydrate concentrations of the extract at the point at which iodine-staining disappears, or by allowing the tissue to exhaust its reserves by storing it in darkness at high temperature.

5.5.3 Non-specific carbohydrate determinations

Total hexose sugar concentrations of sugar extracts and starch extracts can be determined with a spectrophotometer by measuring color developed in the reaction with anthrone (Yemm and Willis 1954) (Fig. 7). The major hexose sugars appear to yield very similar color development per glucose equivalent.

5.5.4 Specific carbohydrate determinations

Finally, if one wants to identify specific sugars in the extracts, it is necessary to use chromatographic techniques. Alternatively. glucose concentrations can be determined from color development after adding glucose oxidase to the solutions (Ebell 1969). Given the ready interconversions among sugars and the utility of the anthrone reaction, however. there seems little point in trying to identify specific sugars in assessing seedling quality.

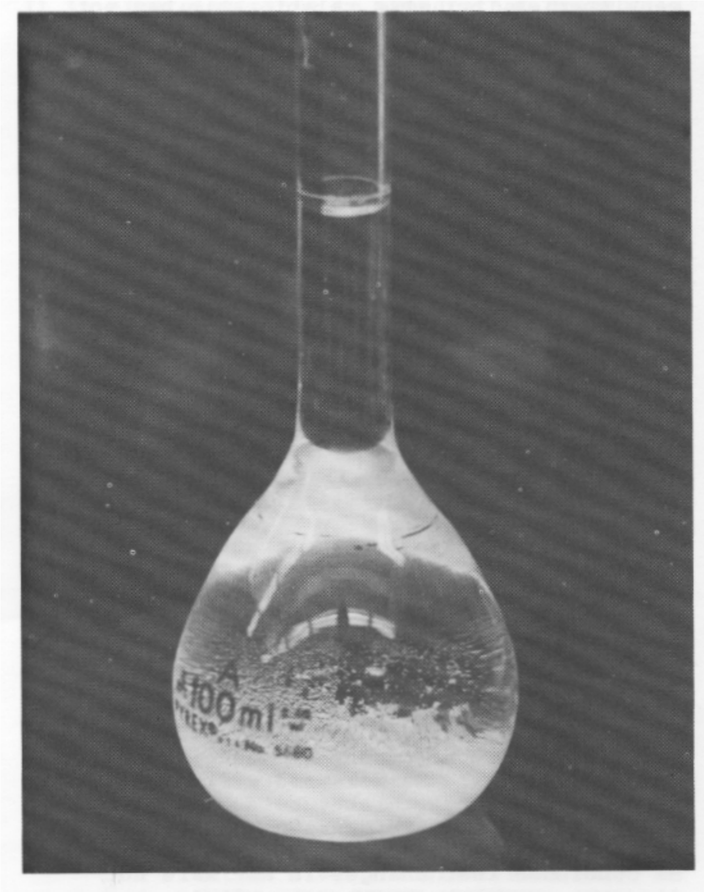

FIGURE 5. ETHANOL EXTRACT AFTER PRECIPITATION OF ORGANIC ACIDS WITH LEAD ACETATE.

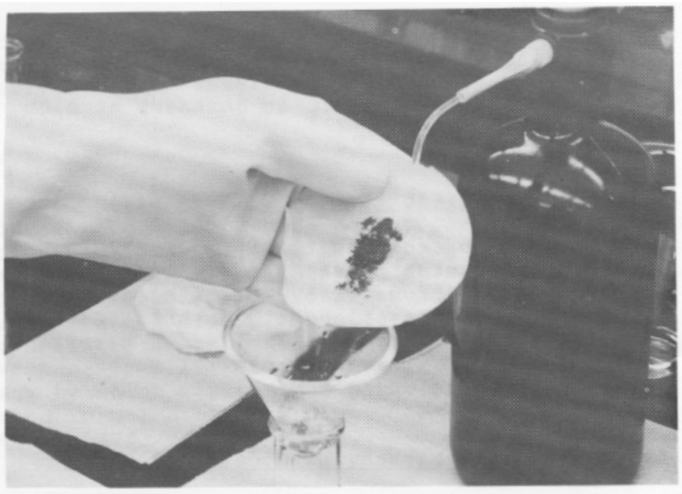

FIGURE 6. WASHING THE ETHANOL-EXTRACTED RESIDUE INTO 35% PERCHLORIC ACID TO EXTRACT STARCH.

5.6 PREDICTIVE ABILITIES

The literature relating seedling growth and survival to carbohydrate status is contradictory. Correlations between survival and carbohydrate status have been demonstrated for many species, but this relationship has not been found consistently [see Ritchie and Dunlap (1980) and Duryea and McClain (1984) for reviews]. There are a number of problems in interpreting the literature. First, the diverse carbohydrate extractions and the analyses used in these studies vary in completeness of extraction and in what carbohydrates they extract. Second. differences in growth conditions, handling, storage, and planting conditions could easily induce or alleviate carbohydrate stress in the seedlings. Just as one would not expect a fertilizer response if the added nutrients were not limiting growth, so one should not expect a carbohydrate response if carbohydrate status were adequate. In the following section I will discuss how carbohydrate status affects seedling growth and survival.

5.6.1 Root growth potential

It has long been recognized that root growth is essential to the survival of the planted seedling, and a specialized literature has developed around the measurement of "root growth potential" (RGP) of seedlings (Ritchie and Dunlap 1980). It was long thought that the major effect of improved carbohydrate status was an increase in RGP (Wakeley 1948. Ritchie 1982): however. Ritchie (1982) has recently presented strong evidence against this hypothesis. He measured a steady decline in "non-structural carbohydrates" (presumably starch and sugars) in seedlings in cold storage. although RGP rose sharply and then fell. He concluded that changes in RGP were not directly correlated with concomitant changes in concentrations of reserve carbohydrates. Many experiments involving girdling, shading, and defoliation suggest that root growth, at least in conifers, is dependent upon current photosynthate rather than reserves (Richardson 1958, van den Driessche 1978, Zaerr et al. 1973. Marshall 1984).

5.6.2 Respiratory costs

If carbohydrate reserves are not related to RGP, then how else could they improve seedling growth and survival? Carbohydrate status presumably measures different aspects of seedling quality than does RGP. RGP, being closely related to bud dormancy (Ritchie and Dunlap 1980), seems to be a measure of readiness to grow. Carbohydrate status, on the other hand, may be more a measure of capacity to maintain tissue function under stress, i.e., to withstand periods when respiratory losses exceed photosynthetic income. If so. then the effects of carbohydrate status on growth and survival might appear only when seedlings are forced to draw heavily upon their reserves (Little 1970).

It should be possible to calculate the point at which the carbohydrate balance "goes into

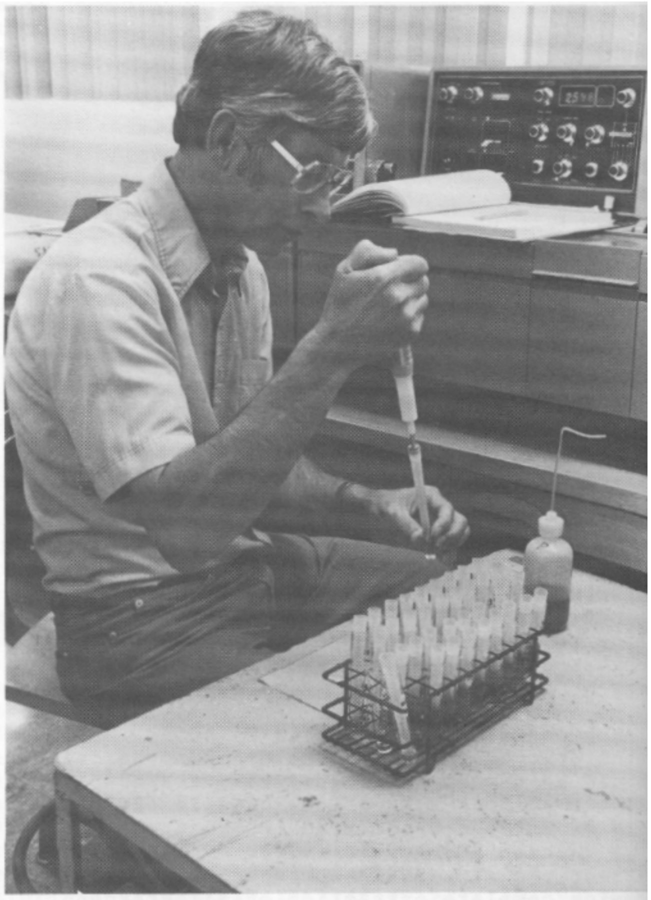

FIGURE 7. DETERMINING SUGAR OR STARCH CONCENTRATIONS BY MEASURING COLOR DEVELOPMENT WITH A SPECTROPHOTOMETER.

the red" by using a process much like balancing a checkbook. Starting with a measured starch and sugar concentration, respiratory expenditures could be subtracted and photosynthetic deposits could be added as the seedling goes through its lifting, storage, and planting cycle. Moreover, it should be possible to work in the opposite direction, to calculate the total respiratory costs a seedling is likely to incur, to define that total as a threshold concentration (Ronco 1973) and then attempt to grow seedlings with carbohydrate reserves in excess of the threshold.

Unfortunately, few measurements of the magnitude of these respiratory costs are currently available. Nonetheless. I shall work through two hypothetical cases, using published data where possible and pointing out where additional measurements are needed.

A seedling enters the winter months having stored a certain amount of carbohydrate. As discussed earlier, this amount varies with growing conditions. The seedling represented by the upper line in Figure 8 is lifted in November with a reserve carbohydrate concentration of 20% *by dry* weight. This seedling is placed in cold storage for 4 months. After it has been in cold storage for several days, its rate of maintenance respiration falls to approximately 0.4 mg CH_2O per gram per day (van den Driessche 1979, Ritchie 1982) and remains at this level for the remainder of the 4 months. At the end of the storage period, its reserve carbohydrate concentration will be 15%.

One can assume that the planted seedling must wait about 3 weeks to achieve a positive carbon balance. This delay occurs because the soil is cold, a state that limits water uptake. and because the photosynthetic apparatus has been disorganized by storage and by any frosts that may occur (McCracken 1978). During this time, the seedling must continue to meet its maintenance respiration costs, an activity that brings the reserve carbohydrate concentration down to 13%. In addition, the seedling must invest a large amount of photosynthate in preparing leaves for photosynthesis and buds for shoot elongation (Gordon and Larson 1968). This investment may require as much as 5 mg of carbohydrate per day for perhaps a week. Such an estimate is little more than a guess--measurements are needed. If it is valid. the carbohydrate concentration would decline to 9%.

Once photosynthesis begins, a great quantity of starch can be deposited in the shoots within a few days. With such deposits, the starch concentration can exceed 25% before budbreak occurs (Ericsson et al. 1983). Nonetheless, the amounts of reserves in seedlings apparently do not greatly exceed the amounts needed to begin photosynthesis in the spring.

This point can be illustrated by another example. The concentration of reserve carbohydrates in a second seedling is represented by the lower line in Figure 8. This seedling is lifted when its reserve carbohydrate concentration is only 14%--6% lower than the first seedling. Perhaps it was grown in an overcrowded seedbed or was allowed to set bud too late in the year. The seedling is stored at a higher temperature than the first seedling and consequently depletes its reserves much more rapidly. The seedling is planted at 5% reserve carbohydrate, and its remaining reserves are exhausted while the seedling is preparing to begin photosynthesis. The seedling dies because it is unable to meet its maintenance requirements.

5.6.4 Long-term effects

Carbon debts seem to carry over from one developmental phase to the next and from one growing season to the next. Thus, Engelmann spruce seedlings planted with poor carbohydrate status suffered high mortality the following winter because they were unable to develop sufficient cold-hardiness (Ronco 1973). In a Swedish study. Scots pine seedlings outplanted late in summer with reduced carbohydrate status still had significantly reduced growth the third growing season after planting (Ericsson et al. 1983).

It is also reasonable to expect that drought resistance will be reduced as a result of low carbohydrate status. Ritchie (1982) suggested that such reductions might occur because of an inability to adjust osmotically and retain positive cell turgor at low xylem water potentials. I have also found that fine root mortality under drought conditions can be attributed to a simple inability to meet maintenance requirements during periods of drought (Marshall 1984). Theoretically, the same could be true of whole seedlings.

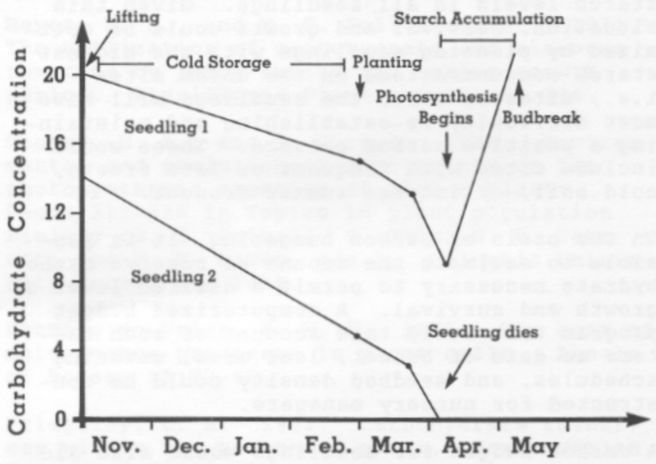

FIGURE 8. HYPOTHETICAL STARCH AND SUGAR PATTERNS OF TWO SEEDLINGS DURING THE LIFTING, STORAGE, AND PLANTING CYCLE. ONE SEEDLING (TOP) SURVIVES: THE OTHER (BOTTOM), WITH INADEQUATE CARBOHYDRATE RESERVES, DOES NOT.

5.7 CONCLUSIONS AND RECOMMENDATIONS

Seedlings are largely unable to photosynthesize during the period from lifting until several weeks after planting, when they have adjusted to their new surroundings and conditions again become suitable for photosynthesis. During this time, they must depend upon their carbohydrate reserves to meet maintenance respiration requirements, to repair tissue damage, and to repair the photosynthetic machinery. In budgeting terms, they have no income but have continuing costs and must pay those costs out of previous savings. Any conditions that substantially reduce those savings prior to lifting, during storage, or after outplanting--either by reducing the initial amount of savings or by increasing the rate at which the savings are depleted--will reduce growth and, ultimately, survival. A seedling without reserves and without photosynthetic income is a dead seedling.

Accumulation of reserves prior to lifting is favored by any treatment that inhibits growth more than it inhibits photosynthesis. Such treatments include mild water stress, mild nutrient stress, or low temperatures. Starch and sugar also accumulate under conditions favoring high photosynthetic rates such as high light levels or low seedbed densities.

Approximate starch concentrations can be determined by iodine staining. However, for quantitative determination of reserves, there is no substitute for laboratory analysis. Both of these techniques could be used immediately after seedlings are lifted in the fall, again after cold storage, and again after outplanting. The state of the seedling could therefore be monitored closely and handling could be adjusted accordingly.

Of course, it may not be possible to maximize starch levels in all seedlings. Given this situation, survival and growth could be optimized by planting seedlings with the highest starch concentrations on the worst sites-i.e.. sites on which the seedlings will have most difficulty re-establishing and maintaining a positive carbon balance. These would include sites with frequent or late frosts, cold soil, or intense summer drought.

On the basis of carbon budgeting, it is possible to estimate the amount of reserve carbohydrate necessary to permit a desired level of growth and survival. A computerized budget program that would take account of such factors as date of budset. leaf area, watering schedules, and seedbed density could be constructed for nursery managers.

A carbon budget for seedlings would also aid regeneration silviculturists in determining the most probable cause of death on a given site. Although seedling mortality is often blamed directly on water stress, carbon budgeting indicates that the primary effect of water stress is to shut down photosynthesis. This stoppage throws the seedling back on its reserves--for as ling with enough starch reserves should be able to withstand a long drought period. Cause of death could easily be determined by doing "autopsies" on dead seedlings--staining for or extracting starch and sugar. If no starch or sugar is present, the seedlings probably starved to death, rather than dying as a direct result of the drought. Such a distinction is important because a seedling with greater reserves might have survived. From these autopsies, nursery managers could determine whether improving the carbohydrate status of seedlings would increase their survival.

ACKNOWLEDGMENTS

I would like to express my gratitude to Drs. David DeYoe. Mary Duryea. Gary Ritchie. and Richard Waring for their excellent reviews.

REFERENCES

Association of Official Analytical Chemists. 1980. Official methods of analysis of the Association of Official Analytical Chemists. 13th ed. Washington. D.C. 1018 p.

Ariovich. D., and C. F. Cresswell. 1983. The effect of nitrogen and phosphorus on starch accumulation and net photosynthesis in two variants of Panicum maximum Jacq. Plant. Cell, and Environment 6:657-664.

Berry, J. A., and W. J. S. Downton. 1982. Environmental regulation of photosynthesis. Pages 263-343 in Photosynthesis: development, carbon metabolism, and plant productivity. Vol. II. Academic Press, N.Y.

Dickson, R. E. 1979. Analytical procedures for the sequential extraction of 14-C-labeled constituents from leaves, bark and wood of cottonwood plants. Physiologia Plantarum 45:480-488.

Duryea, M. L., and K. M. McClain. 1984. Altering seedling physiology to improve reforestation success. Pages 77-114 in Seedling physiology and reforestation success (M. L. Duryea and G. N. Brown, eds.). Proceedings physiology working group. Technical session. Society of American Foresters national convention, Portland, Oregon. Oct. 16-20, 1983. Martinus Nijhoff/Dr. W. Junk Publishers. The Hague/Boston/Lancaster.

Ebell. L. F. 1969. Specific total starch determinations in conifer tissues with glucose oxidase. *Phytochemistry* 8:25-36.

Ericsson. A. 1979. Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needle on 20-year-old Scots pine trees (Pinus sylvestris). Physiologia Plantarum 45:270-280.

Ericsson, A., and H. Persson. 1980. Seasonal changes in starch reserves and growth of fine roots of 20-year-old Scots pines. Pages 239-

250 in structure and function of northern coniferous forests--an ecosystem study (T. Persson, ed.). Ecological Bulletins (Stockholm) 32.

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. Swedish University of Agricultural Sciences. Faculty of Forestry, Uppsala. Studia Forestalia Suecica No. 165. 17 p.

Farrar. J. F. 1980. Allocation of carbon to growth, storage, and respiration in the vegetative barley plant. Plant, Cell, and Environment 3:97-105.

Glerum. C. 1980. Food sinks and food reserves in temperate climates. New Zealand Journal of Forestry Science 10:176-185.

Glerum, C., and J. J. Balatinecz. 1980. Formation and distribution of food reserves during autumn and their subsequent utilization in jack pine. Canadian Journal of Botany 58:40-54.

Gordon. J. C., and P. R. Larsen. 1968. The seasonal course of photosynthesis, respiration, and distribution of 14-C in young *Pinus resinosa* trees as related to wood formation. Plant Physiology 43:1617-1624.

Haissig, B. E., and R. E. Dickson. 1979. Starch measurement in plant tissue using enzymatic hydrolysis. Physiologia Plantarum 47:151-157.

Hansen. J., and I. Moller. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochemistry 68:87-94.

Hassid, W. Z., and E. F. Neufeld. 1964. Quantitative determination of starch in plant tissues. Pages 33-36 in Methods in carbohydrate chemistry, Vol. IV (R. L. Whistler. B. J. Smith. J. N. Bemiller, and M. L. Wolfrom, eds.). Academic Press. New York.

Hellmers. H. 1962. Physiological changes in stored pine seedlings. Tree Planters' Notes 53:9-10.

Hsiao, T. C. 1973. Plant responses to water stress. Annual Review of Plant Physiology 24:519-570.

Jenner. C. F. 1982. Storage of starch. Pages 700-747 in Encyclopedia of plant physiology. new series. Vol. 13A (A. Pirson and X. H. Zimmerman. eds.). Springer-Verlag. Berlin.

Kile, G. A. 1981. Annual variations in soluble sugars, starch, and total food resources in *Eucalyptus obliaua* roots. Forest Science 27:449-454.

Kimura, M. 1969. Ecological and physiological studies on the vegetation of Mt. Shimigare. VII. Analysis of production processes of young *Abies* stand based on the carbohydrate economy. Botanical Magazine (Tokyo) 82:6-19.

Kramer. P. J., and T. T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York.

Krueger, K. W.. and J. M. Trappe. 1967. Food reserves and seasonal growth of Douglas-fir seedlings. Forest Science 13:192-202.

Lavender. D. P., and W. S. Overton. 1972. Thermoperiods and soil temperatures as they affect growth and dormancy of Douglas-fir seedlings of different geographic origin. Oregon State University. Forest Research Laboratory. Corvallis. Research Paper 13. 26 p.

Levitt. J. 1972. Water stress. Pages 322-446 in Responses of plants to environmental stresses. Academic Press, New York.

Linder. S.. and T. Lohammar. 1981. Amount and quality of information on CO_2-exchange required for estimating annual carbon balance of coniferous trees. Pages 73-87 in Understanding and predicting tree growth (S. Linder, ed.). Swedish University of Agricultural Sciences. College of Forestry. Uppsala. Sweden. Studia Forestalia Suecica No. 160.

Little. C. H. A. 1970. Seasonal changes in carbohydrate and moisture content in needles of balsam fir *(Abies balsamea).* Canadian Journal of Botany 48:2021-2028.

Marshall. J. D. 1984. Physiological control of fine root turnover. Ph.D. thesis. Oregon State University, Corvallis. 98 p.

McCracken, I. J. 1978. Carbon dioxide uptake of pine seedlings after cool storage. Forest Science 24:17-25.

Meyer, M. M., and W. E. Splitstoesser. 1971. The utilization of carbohydrate and nitrogen reserves by *Taxus* during its spring growth period. Physiologia Plantarum 24:306-314.

Mooney. H. A., and S. Gulmon. 1979. Environmental and evolutionary constraints on the photosynthetic capacity of higher plants. Pages 316-338 in Topics in plant population biology (0. T. Solbrig. S. Jain. G. B. Johnson, and P. H. Raven. eds.). Columbia University Press. New York.

Penning de Vries, F. W. T. 1975. The cost of maintenance processes in plant cells. Annals of Botany 39:77-92.

Priestley, C. A. 1962. Carbohydrate resources within the perennial plant. Commonwealth Bureau of Horticulture and Plantation Crops. East Malling. Maidstone, Kent. Technical Communication No. 27. 116 p.

Richardson. S. D. 1958. Bud dormancy and root development in *Acer saccharinum.* Pages 409-425 in The physiology of forest trees (K. V. Thimann, ed.). Ronald Press, New York.

Ritchie, G. A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Canadian Journal of Forest Research 12:905-912.

Ritchie. G. A., and J. R. Dunlap. 1980. Root growth potential: its development and expression in forest tree seedlings. New Zealand Journal of Forestry Science 10:218-248.

Ronco. F. 1973. Food reserves of Engelmann spruce planting stock. Forest Science 19:213-219.

Sanderson, G. W.. and P. M. Perera. 1966. Removal of polyphenolic compounds interfering with carbohydrate determinations in plant extracts with an insoluble polyphenol adsorbent. The Analyst 91:335-336.

Smith. D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Research Division. College of Agricultural and Life Sciences, University of Wisconsin, Madison. Research Report 41. 11 p.

van den Driessche. R. 1978. Seasonal changes in root growth capacity and carbohydrates in red pine and white spruce nursery seedlings. Pages 6-19 in Proceedings of the IUFRO symposium on root physiology and symbiosis (A. Riedacker and J. Gagnaire-Michard. eds.).

van den Driessche. R. 1979. Respiration rate of cold-stored nursery stock. Canadian Journal of Forest Research 9:15-18.

Vartanian. N. 1981. Some aspects of structural and functional modifications induced by drought in root systems. Plant and Soil 63:83-92.

Wakeley, P. C. 1948. Physiological grades of southern pine nursery stock. Proceedings of the Society of American Foresters Annual Convention 1948:311-320.

Waring. R. H., A. J. S. McDonald. S. Larsson. T. Ericsson. A. Wiren. E. Arvidsson, A. Ericsson, and T. Lohammar. 1984. Controlling internal chemistry of plants for studies of herbivory and microbial inoculation. Submitted to Oecologia.

Yemm. E. W., and A. J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57:508-514.

Zaerr. J. B., D. P. Lavender. R. K. Hermann. and G. B. Sweet. 1973. Factors affecting root growth in Douglas-fir seedlings. Abstract from: Plant Physiology. Program and Abstracts. Annual meeting. University of Calgary. Alberta. June 17-21. 1973. Vol. 51. Supplement, p. 20.