EFFECTS OF WATERING AND FERTILIZATION ON CARBOHYDRATE RESERVES IN SUGAR MAPLE SEEDLINGS

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Abstract

Sugar maple seedlings, grown under three nutrient and three moisture levels, were analyzed after three growing seasons for starch and ethanol-soluble sugars. Analytical procedures are detailed in the appendix. Fertilization did not affect carbohydrate levels in stems or roots. Water stress caused a significant reduction in the amount of carbohydrates in stems and roots; it had no significant effect on carbohydrate concentration in roots, but it significantly increased carbohydrate concentration in stems.

INTRODUCTION

Because plants rely on stored carbohydrates for shoot elongation in the spring, eultural practices that promote carbohydrate accumulation would be expected to stimulate subsequent growth. In a previous paper (Carl et al. 1973), we described some of the effects soil mosture and nutrient levels had on seedling growth of sugar maple (*Acer saccharum* Marsh.). In this report, we provide information on the status of reserve carbohydrates (starch and ethanol-soluble sugars) at the end of the third growing season for these same seedlings.

METHODS

We grew half-sib sugar maple seedlings in 20-cm plastic pots filled with soil from the B horizon of a Paxton loam and imposed three nutrient and three soil moisture levels in all combinations (nine treatments) with nine seedlings per treatment. The three nutrient levels were: (1) low—no nutrient added; (2) medium—1-liter equivalent of a complete Hoagland solution added to each pot; and (3) high—2-liter equivalents of a complete Hoagland solution added to each pot. The three levels of soil moisture were: (1) low—soil moisture maintained at 8 to 18 percent; (2) medium—soil moisture maintained at 18 to 28 percent; and (3) high—soil moisture maintained at 28 to 38 percent.

We harvested the seedlings after leaf-fall of the third growing season, and analyzed stems and roots separately for starch and ethanol-soluble sugar content. Analytical procedures are detailed in the appendix.

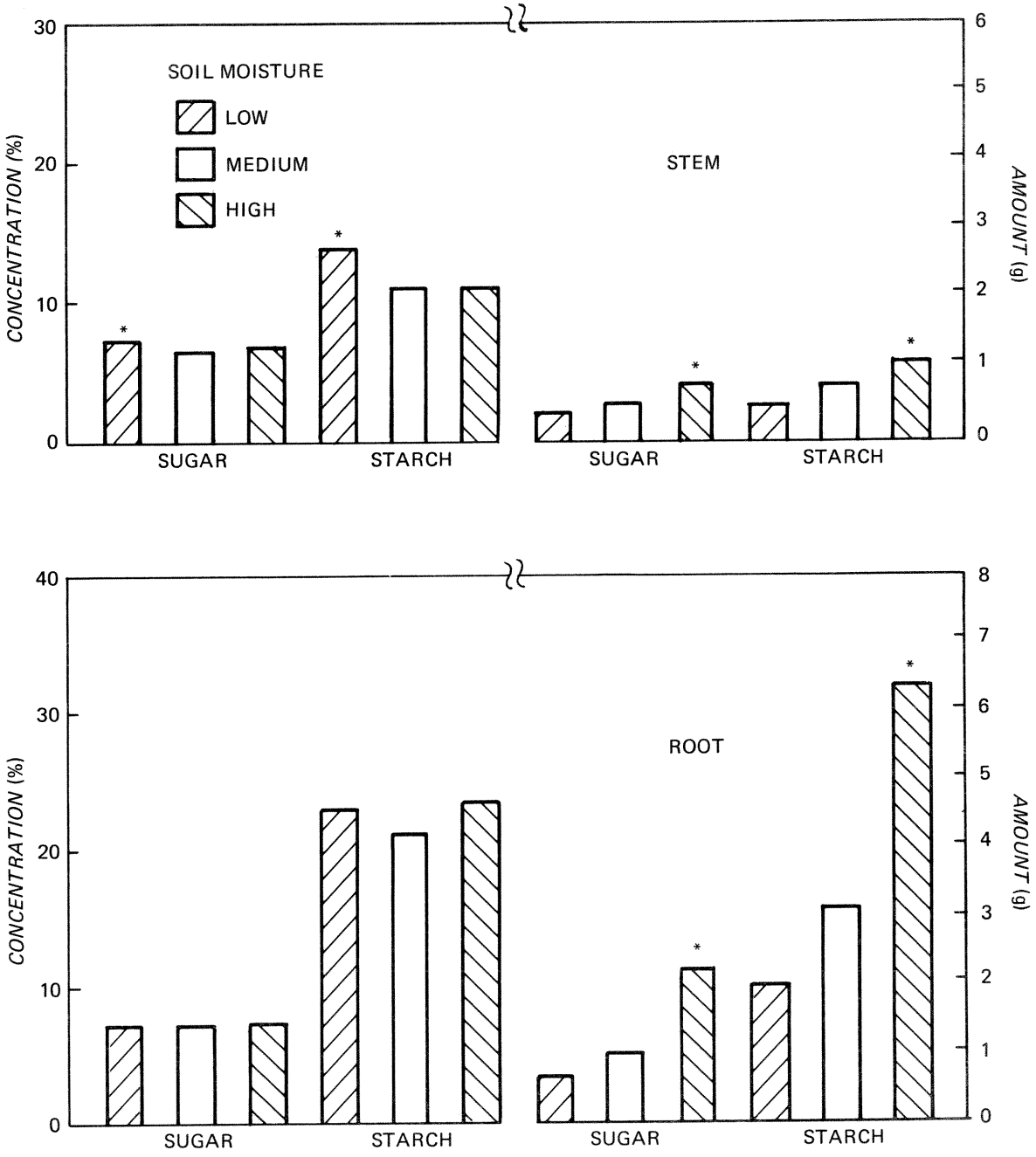
RESULTS AND DISCUSSION

Carbohydrate levels are commonly reported in terms of concentration (percent dry weight), but this may be misleading because changes in carbohydrate levels may be masked by differences in plant biomass. Consequently, we express sugar and starch content both in concentration and in absolute amounts (Table 1).

Table 1.—Weight (in grams) and concentration (in percent) of starch and ethanol-soluble sugar levels in 3-year-old sugar maple seedlings, by treatment.

Treatment	Stem sugar		Stem starch		Root sugar		Root starch	
	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt
Low nutrients								
Low H_2O	7.3	13.3	15.9	29.8	6.6	46.8	23.4	165.5
Med H_2O	6.1	51.3	9.5	76.6	6.7	144.8	22.5	461.8
High H_2O	5.8	50.4	11.1	90.2	7.6	178.2	24.2	558.4
Medium nutrients								
Low H_2O	7.0	29.2	11.6	50.6	7.6	91.6	21.8	245.7
Med H_2O	6.4	38.7	9.0	53.9	7.6	104.7	20.7	269.8
High H_2O	6.8	83.9	10.4	118.1	7.1	212.7	21.9	577.7
High nutrients								
Low H_2O	7.6	16.0	13.0	31.4	6.9	58.4	22.2	178.6
Med H_2O	6.0	28.4	12.7	58.7	6.8	63.8	19.5	205.2
High H_2O	6.3	78.0	9.5	103.2	7.1	237.3	23.4	763.0

Figure 1.—Effect of soil moisture on reserve carbohydrates in 3-year-old sugar maple stems and roots. An asterisk indicates that the value for that watering level is significantly different from the other two levels according to the Student-Newman-Kuels test.



Fertilizers had no significant effect on the concentration or amount of carbohydrates in stems or roots, and there were no significant interactions between fertilizers and water. There was, however, a good deal of variation within treatments, and this may have masked significance. In 1973 we reported (Carl et al.) that fertilizers did not significantly improve growth of these seedlings. As hypothesized in that paper, the potting soil and/or the added water may have contained sufficient nutrients to mask the effects of the additional fertilizer.

Watering had a significant effect on carbohydrate reserves. Stems and roots grown under low water levels had less than half as much sugar and starch as did those grown in moist soil (Fig. 1). This is undoubtedly the result of reduced photosynthesis in seedlings grown under severe water stress (Kramer 1969). And, because subsequent growth depends upon carbohydrate reserves, well-watered seedlings were substantially larger (Carl et al. 1973).

In contrast to its inhibitory effects on the weight of carbohydrate reserves, water stress caused a small but statistically significant increase in the concentration of sugar and starch in the stem (Fig. 1). Normally, a substantial portion of the carbohydrates produced is assimilated into secondary plant constituents such as cellulose. In stems, severe water stress appears to inhibit the conversion of carbohydrates into these secondary products more than it inhibits the production of carbohydrates; consequently, the concentration of stem carbohydrates increases. Brown and Blaser (1970) reached a similar conclusion is a study of orchard grass (*Dactylis glomerata*). In maple roots, the accumulation of carbohydrates and the assimilation of secondary products were equally inhibited by water stress, for treatment had no significant effect on root sugar or starch concentration.

Restricting soil moisture may be of benefit for nursery-grown seedlings in that it may cause a slight increase in stem carbohydrate concentration which may make the seedlings more resistant to frost injury (Levitt 1956). If sugar maple seedlings grown under very moist conditions are indeed more susceptible to frost injury, this can probably be corrected by reducing the watering schedule in late summer.

Appendix
Procedures for Quantitative Analysis of Ethanol-Soluble Sugars and Starch

TREATMENT OF PLANT MATERIAL

Plant material is frozen immediately after collection (to prevent interconversion of carbohydrates) and stored at −15°C. Another method of preparing samples is to cut the material into small pieces, place approximately 2-g samples in glass jars, and fix the samples with hot 80% ethanol. The jars are then capped and stored at room temperature until samples are analyzed; all material not included in the sample is dried and weighed for subsequent calculation of total amounts of carbohydrates.

EXTRACTION AND ANALYSIS

Extraction and colorimetric procedures have been adapted from Morris (1948), Hodge and Hofreiter (1960), Horwitz (1960), Ward and Johnston (1962), and Witherell (1963).

Ethanol-soluble sugars

1a. If the material has been frozen, grind it in a Wiley mill, using a 10-mesh screen. Place approximately 2 g of the ground material in a 500-ml round-bottomed flask and add approximately 100 ml of 80% ethanol.

1b. If the material has been stored in ethanol, pour the entire contents (plant material plus ethanol) into a 500-ml round-bottomed flask and add enough ethanol to make approximately 100 ml.

2. Place the flask in a heating mantle, connect it to a condenser, and reflux the ethanol for 1 h, with the transformer adjusted so the ethanol will boil. A series of these units can be set up so that several samples can be analyzed simultaneously.

3. Weigh a 60-ml fritted glass funnel that has been oven dried and cooled in a desiccator.

4. Decant the ethanol through the funnel and collect it in a filtering flask. Use a vacuum to facilitate filtration; a water aspirator is satisfactory for all vacuum needs.
5. Add 50 ml of 80% ethanol to the residue in the round-bottomed flask, and reflux for 1/2 h.
6. Repeat Steps 4 and 5. Collect all the filtrate in the same flask.
7. Pour the entire contents of the round-bottomed flask into the funnel and rinse the flask, residue, and funnel with 80% ethanol. Save the filtrate and residue.
8. Oven dry the fritted funnel containing the residue, cool it in a desiccator, and weigh. Save the residue for starch analysis.
9. Transfer the filtrate (Step 7) to an evaporating flask, and reduce the filtrate to about 15 to 20 ml on a flash evaporator. The flash evaporator should be operated with the water-bath temperature at 40°C, and at maximum vacuum.
10. Transfer the concentrated filtrate to a 50-ml volumetric flask.
11. Rinse the evaporating flask several times with small quantities of distilled water. Add each rinse to the volumetric flask.
12. Make the filtrate up to 50 ml with distilled water.
13. Weigh 2 g of Rexyn 101[1] and 3 g of Rexyn 203[1] into a 250-ml Erlenmeyer flask, and add the 50 ml of filtrate.
14. Clarify the filtrate by shaking gently on a mechanical shaker for 3 h.
15. Pass the filtrate through a 60-ml fritted funnel into a 125-ml filtering flask, using vacuum.
16. Transfer a 5-ml aliquot of the clarified filtrate into a 200-ml volumetric flask, and make up to volume with distilled water.
17. Place a 5-ml eliquot of the 200-ml dilution into each of two 1-in colorimeter tubes, and prepare a reference blank tube containing 5 ml of distilled water.
18. Place the tubes in a cold water bath, and add 10 ml of anthrone reagent[2] gently down the side of each tube, so that two separate layers are formed. A repeating pipet can be used to save time.
19. After all the tubes have been prepared, shake them thoroughly, and place them in a boiling water bath for 15 min. Evaporation can be reduced by covering the mouth of each tube with a large glass marble.
20. Cool the samples quickly to room temperature in a cold water bath, wipe the tubes clean, and read percent transmittance on a colorimeter set at 540 nm. Use the reference blank to adjust the colorimeter for 100% transmittance. If solutions are too dark for accurate colorimetric readings, adjust the aliquot size (Step 16); aliquot size must be known for subsequent calculations.

Starch

1. Grind the coarse residue obtained from the sugar extraction (Step 8 of sugar analysis).
2. Weigh out a known amount (approximately 0.15 g) of the ground residue in a 50-ml round-bottomed polyethylene centrifuge tube.
3. Place a magnetic stir rod in the centrifuge tube.
4. Add 10 ml of 4.7N perchloric acid, drop by drop, from a buret, stirring vigorously on a magnetic stirrer for 10 min.
5. Centrifuge at approximately 3,700 rpm for 5 min.
6. Decant the liquid, filtering through a 15-ml coarse fritted funnel, into a chilled 125-ml filtering flask immersed in ice water. Use vacuum to facilitate filtration.
7. Repeat Steps 4, 5, and 6 twice more. Collect all the filtrate in the same filtering flask.
8. Transfer the total filtrate to a 50-ml volumetric flask, and make up to volume with distilled water.
9. Measure a 5-ml aliquot into another 50-ml volumetric flask. Prepare a separate reference flask by adding 5 ml of distilled water to a 50-ml volumetric flask. Treat the sample and reference flasks as follows:

[1] Fisher chemicals. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U. S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

[2] Prepare anthrone reagent by adding 1 gm anthrone powder to a 500-ml volumetric flask, and make up to volume with 95% sulfuric acid.

10. Add a drop of phenolphthalein indicator (1% solution in 95% ethanol).
11. Add 2N sodium hydroxide until a pink color appears.
12. Add 2N acetic acid, a drop at a time, until pink color disappears, and then add an additional 2.5 ml.
13. Add 0.5 ml of 10% potassium iodide solution. Use a repeating pipet.
14. Add 5.0 ml of 0.0125N potassium iodate solution. Use a repeating pipet.
15. Shake the flasks to mix the contents thoroughly. Wait 5 min, and make up to volume with distilled water.
16. Pour about 5 ml of the liquid from each flask into each of two 1/2-in colorimeter tubes, and read percent transmittance with the colorimeter set at 590 nm. Use the reference tube to adjust the colorimeter for 100% transmittance. If solution is too dark for accurate colorimetric readings, adjust the aliquot size (Step 9); aliquot size must be known for subsequent calculations.

STANDARDS AND CALCULATIONS

Sugar

Prepare a 0.1 mg/ml sugar solution by adding 100 mg of sucrose to a 1,000-ml volumetric flask, and make up to volume with distilled water. Prepare a series of known sugar concentrations by adding aliquots (0 to 50 ml) of this solution to 50-ml volumetric flasks, and make up to volume with distilled water.

Pour two 5-ml samples of each standard concentration into 1-in colorimeter tubes. Then treat according to Steps 18, 19, and 20 of the sugar analysis.

To calculate the sugar content in a sample, relate the percent transmittance readings obtained from the sample material to those obtained from the standards. This can be done graphically or mathematically. Because of the dilution in Steps 16 and 17 of the sugar analysis, this value must be multiplied by a conversion factor to determine the total amounts of sugar in the sample.

Starch

Since starches from different plant species vary in their amylase content, it would be desirable to use as a standard a sample of pure starch from the species being analyzed (Ward and Johnston 1962). Nielsen (1943) gives a method for extracting starch for standardization if it is desired. If relative values for a particular species are satisfactory, standards can be prepared from potato starch. This is the procedure we used.

Prepare a 1.0 mg/ml starch solution by adding 20 ml of 4.7N perchloric acid to 100 mg of starch in a beaker. Stir for 10 min (make sure starch is completely dissolved), and make up to volume in a 100-ml volumetric flask. Place a series of aliquots from 0.2 to 3.0 ml of this solution in 50-ml volumetric flasks, and proceed as outlined in the starch analysis, beginning with Step 10.

Methods for calculating starch concentration and amount are similar to those outlined for sugar. The only difference is in the method of obtaining total sample weight. Sugars have already been removed before the starch samples are weighed; therefore, the sample weight obtained in Step 2 of the starch analysis sould be corrected for this.

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