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Effects of Defoliation and Drought on ROOT FOOD RESERVES in SUGAR MAPLE Seedlings

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THE PROBLEM

THE ARTIFICIAL defoliation of sugar maple *(Acer saccharum* Marsh.) can cause a marked decline in root food reserves, especially starch, and an increase in the levels of the reducing sugars, fructose and glucose. Defoliation can also bring on the dieback-decline syndrome in sugar maples *(Parker and Houston 1968)*.

Because drought, too, can bring on dieback symptoms, the question arose whether drought (or a combination of drought and defoliation) has the same effect as defoliation on root food reserves and reducing sugars.

Two experiments designed to examine this question were conducted in 1968-69. In one, the effects of both drought and defoliation were examined; in the second, defoliation was eliminated as a variable. This paper is a report on those experiments.

Root food reserves are defined as the material, consisting of proteins, lipids, and carbohydrates (including starch), which is extracted with methanolic trichloroacetic acid. Certain cell-wall constituents removed by other and older methods are not included in this definition *(Priestley 1965)*.

THE EXPERIMENTS

Methods

Experiment I.—Ninety forest-grown sugar maple seedlings, about 6 years old and 18 inches tall, were dug in May 1967 and planted in 8-inch plastic pots containing forest topsoil. The seedlings were grown under partial shade and were watered frequently during the summer of 1967. In autumn they were heeled in and mulched with pine needles.

In mid-June 1968 the soil in all pots was brought to field capacity by soaking from above. The pots were covered with two layers of black plastic, which were slit to fit around the stems and were bound to the pots with string. A masonite cover, slotted to fit the stem, was placed over the plastic on each pot, and the slot was covered with masking tape.

The 90 seedlings were divided into 3 groups of 30 each (table 1). Seedlings in the first group were not defoliated; those in the second group were defoliated once, on 1 July 1968; and those in the third group were defoliated twice, once on 1 July and again on 5 August.

Three watering regimes were used for all groups: (1) 10 seedlings in each group were watered once a week (watered), (2) 10 were watered once every 2 weeks (intermittently watered), and (3) 10 were not watered (unwatered). The seedlings that were watered and not defoliated served as the control group.

The pots, with contents and covers, were weighed after thorough watering to determine their initial total weight. They were then weighed every week, partly to determine the levels to which water should be added in the watered and intermittently watered pots, and partly to determine the point—presumed to be the wilting point—at which weight loss ceased in the unwatered plants.

The pots were arranged randomly on shelves in a screened enclosure that was covered with a layer of clear polyethylene.

All seedlings were harvested on 6 September, 1968; this was before leaf-fall. Roots were removed and kept frozen at —15°C. for 3 months before they were analyzed.

Food reserves (FR) were determined in the thicker part of the root, 7 to 10 cm. below the root collar. A modification of Priestley's *(1965)* analytical procedure was used.

Table 1.—*Food reserves in sugar maple roots, as percent of dry weight*

Treatment	Non-defoliated	Once defoliated	Twice defoliated	Average
Watered	30.1	29.6	23.5*	27.6
Intermittently watered	27.0	30.5	26.4	28.0
Unwatered	28.7	31.6	25.1	28.4
Average	28.6	30.6	25.0*	—

*Significantly different from controls (nondefoliated, watered) at probability of 0.90.

Enough 3-mm.-thick disks were cut from the root to fill a 12-ml. weighing bottle. The bottle containing root disks was capped and weighed to determine fresh weight, was oven-dried for 48 hours at 105°C., and was reweighed to 0.001 g. to determine pre-extraction weight.

To perform an extraction, the contents of a weighing bottle were placed in a 250-ml. flask that was attached through a ground glass joint to a water-cooled condenser. The contents were then refluxed for 2 hours in 150 ml. of 5 percent trichloroacetic acid (TCA) solution in 35 percent methanol. The liquid portion was suction-filtered off and discarded; the root pieces were transferred to the thimble of a Soxhlet apparatus and extracted with 170 ml. of once-distilled tap water for 8 hours.

The sample was dried in the thimble at 70°C. for 1 hour, placed in a weighing bottle, and oven-dried at 105°C. for 48 hours. All samples were cooled 20 minutes in a desiccator before capping and weighing.

Original (pre-examination) dry weight minus dry weight after extraction was used as a measure of food reserves. Weight loss was expressed as a percentage of original dry weight.

Starch was estimated from microscopically examined hand-cut cross-sections of roots stained with I_2-KI. The amount of starch present was expressed as the percentage of the total cross-sectioned area occupied by starch-filled cells.

Experiment II.—In the second experiment, three groups of 17 seedlings each were dug in the autumn of 1968. None of these seedlings was defoliated. The three watering regimes used in the summer of 1969 were the same used in the first experiment.

The seedlings, in uncovered pots, were placed on boards resting on the ground and were protected from rainfall by a polyethylene canopy about 10 feet above them and open on the sides.

On 16 July, 1969 the roots of eight seedlings, selected randomly from each of the three groups, were dug up, cut off, frozen, and analyzed for sugars by means of paper chromatography *(Parker 1962)*. Starch was determined as above.

The remaining seedlings (nine per treatment) were harvested on 19 August and were analyzed for sugars and starch.

Results

Experiment I.—Food reserves in the roots of the seedlings that were watered and defoliated twice averaged 23.5 percent of the total root dry weight (table 1). This was significantly lower (at a probability of 0.90) than the food reserves of the control group (seedlings watered once a week and not defoliated), which averaged 30.1 percent of the total root dry weight. However, the food reserves of the seedlings in the group that was watered and defoliated once averaged 29.6 percent—not significantly different from the food reserves of the control group.

When all the watering regimes were pooled, the food reserves of all defoliated seedlings were significantly lower than those of the controls.

Seven of the 10 nondefoliated and unwatered seedlings dried to the wilting point. The three that did not were omitted from analysis. The food reserves of the seven unwatered and wilted seedlings were not significantly different from those of the controls, although the average food reserves were lower on the average than those of the controls (table 1). Two control seedlings were omitted because the soil dried out during the experiment. Another control seedling died and was omitted.

The soil of most of the defoliated and unwatered seedlings did not dry out enough to reach the wilting point, apparently because of the reduced transpiration in the defoliated seedlings.

Table 2.—*Estimates of starch in root sections as a percentage of the cross-section area occupied by cells containing starch (experiment I)*[1]

Treatment	Non-defoliated	Once defoliated	Twice defoliated	Average
Watered	90	30	5	42
Intermittently watered	75	55	15	48
Unwatered	65	40	20	42
Average	77	42	13	—

[1]Significances not computed because estimates were based on visual microscopic observations.

No significant differences in food reserves were found among defoliated seedlings in the different watering regimes (table 1).

Starch averaged 13 percent in seedlings defoliated twice, 42 percent in seedlings defoliated once, and 77 percent in seedlings that were not defoliated (table 2). Starch levels also were different between nondefoliated seedlings that were watered (90 percent), unwatered (65 percent), and intermittently watered (75 percent).

Experiment II.—There were significantly (0.95 probability) greater amounts of fructose and glucose in the roots of unwatered seedlings than in the roots of watered seedlings in both the July and August harvests (fig. 1). Although sucrose was greater in

Figure 1.—Percent fructose, glucose, and sucrose, based on root dry weight, for the three watering regimes: water (WAT.), intermittently watered (INT.), and unwatered (DRY) from mid-June to time of harvest (16 July or 19 August). Significant differences in reducing sugars were found between watered and both intermittently watered and unwatered seedlings in July, and between unwatered and both intermittently watered and watered seedlings in August.

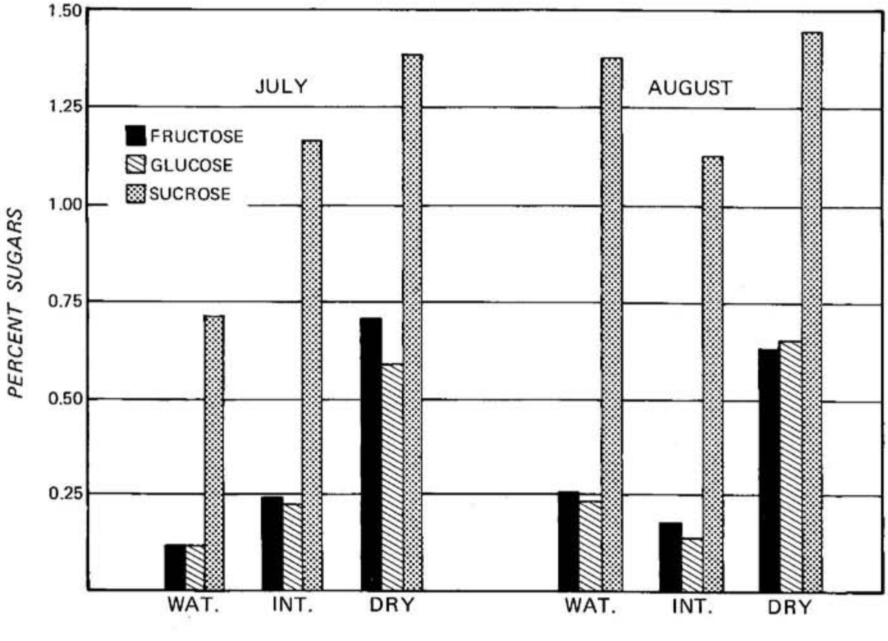

Table 3.—*Estimates of starch in root sections as a percentage of cross-section area occupied by cells containing starch (experiment II)*[1]

Treatment	July 16	August 19
Watered	90	95
Intermittently watered	95	95
Unwatered	60	25

[1]Significance was not computed because estimates were based on visual microscopic observations.

July in unwatered than in watered seedlings, this difference was not significant at 0.90 probability. Traces of raffinose and stachyose occurred occasionally in the extracts, but were not estimated quantitatively.

Starch was lower in August in the unwatered seedlings than in the intermittently watered or watered seedlings. Such differences were not so clearcut in July (table 3), indicating a definite decline in starch in the unwatered seedlings between the July and August harvests.

DISCUSSION

The results of the two experiments show that drought and defoliation have similar effects on carbohydrates in sugar maple roots: both treatments lower starch levels and raise reducing sugar levels. Sucrose may also increase, but the increase was not statistically significant in our results.

Although defoliation reduces total food reserves, drought does not seem to affect them very much. Perhaps the food reserves in the unwatered plants were maintained longer because drought does not abruptly curtail the photosynthetic capacity of the plant, but defoliation does. There also might have been no net change in the food reserves in the unwatered seedlings because carbohydrates not present as starch were present as sugar. Thus the starch decline in unwatered seedlings is not simply a case of root starvation, but most likely is the result of a carbohydrate shift from starch to soluble sugars.

The results of a number of earlier studies are similar to those presented here. Levitt *(1956)* concluded from his review, which dealt mostly with herbaceous plants, that starch was usually converted to sugar during drought unless injury occurred. This conversion is often associated with increased activity of amylases *(Vaadia et al. 1961)*. According to Eaton and Ergle *(1948)*, starch was converted to hexose sugars in the tops of cotton plants during a drought, and sucrose and starch as well as hexose sugars increased in the roots. In prolonged drought, carbohydrate percentage in the whole plant doubled.

Henrici *(cited by Stocker 1960)* found that levels of reducing sugars in lucern (alfalfa) leaves rose until the leaves had completely wilted; then the level declined. Starch underwent a general decline that began early in the experiment. The decline of reducing sugars might be explained by respiratory loss *(Vaadia et al. 1961)*. In loblolly pine (*Pinus taeda* L.) subjected to drought, there was a marked increase in reducing sugars, nonreducing sugars, and total carbohydrates, and a decrease in starch *(Hodges and Lorio 1969)*. But is was felt that the sugar increases were primarily the result of decreased growth, not of starch hydrolysis.

The starch and sugar changes reported in our study probably occur in many other eastern deciduous tree species, besides sugar maple, when they are subjected to drought. A number of chestnut oak *(Quercus prinus* L.) seedlings were included in experiment II, although the data are not presented. In July, no clear differences were found between the levels of reducing sugars in unwatered and control chestnut oak seedlings, but in August the levels of reducing sugars in unwatered seedlings showed a marked increase. Sucrose concentration was not affected by the various watering regimes. By August, starch was clearly lower in the roots of unwatered seedlings than in those of controls.

This increase in reducing sugars after stress brought on by defoliation or drought may help to explain why certain microorganisims capable of metabolizing specific sugars may attack such stressed trees. There is evidence that such microorganisms attack trees affected by defoliators more often than they do healthy trees

(Houston and Kuntz 1964), and it seems clear that drought also can be a contributing factor to the dieback-decline syndrome.

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