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Root Temperature and Carbohydrate Status of Young Cotton Plants[1]

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ABSTRACT

Tests were conducted to determine the effects of root temperature on carbohydrate status of leaves, epicotyls, hypocotyls, and roots of young cotton plants when the tops remained warm. Glucose, fructose, and sucrose were separated by paper chromatography and estimated colorimetrically. Reducing sugars, total sugars, and starch were also estimated by standard colorimetric tests.

Low root temperatures caused rapid increases in sugar contents of all plant parts. Hypocotyls contained the most sucrose, whereas epicotyls contained the most glucose, fructose, and total sugars. Roots contained the lowest concentrations of glucose and fructose.

Low root temperatures also increased the starch contents of leaves, epicotyls, and hypocotyls, except that a root temperature of 10 C caused wilting and a very low starch content of leaves. Roots contained very little starch.

Since starch as well as sugars accumulated at low root temperatures, the accumulations of sugars were not likely caused by starch breakdown, except in wilted leaves on plants whose roots were chilled at 10 C. On the basis of other reports, the effects of temperature on respiration rate and rate of conversion of sugars to other compounds appear to be more probable causes of the observed carbohydrate accumulations.

Additional index words: **Fructose, glucose, moisture stress, paper chromatography, starch, sucrose.**

SUGARS tend to accumulate during cool weather, partly because of conversion of starch to sugar (14). We reported earlier (8) that sugars accumulated in cotton when the entire plants were cooled. However, if the roots were kept warm sugars accumulated only slightly even though the tops were chilled. This response indicated that root temperature may exert a considerable effect on carbohydrate status of cotton plants. Root temperature is frequently lower than air temperature during the spring, after rains, and after irrigation. We were interested in sugar content of cotton seedlings because of a possible relationship between sugars and susceptibility to seedling diseases (8 and article in preparation).

In this paper we report the effects of four root temperatures on the concentration of glucose, fructose, sucrose, reducing sugars, total sugars, and starch in roots, hypocotyls, epicotyls, and leaves of young cotton plants when air temperature was not a variable.

MATERIALS AND METHODS

Plant Culture

Cotton seeds (*Gossypium hirsutum* L. cv. 'Parrott') were germinated in moist vermiculite. Uniform seedlings were transferred to an aerated complete liquid nutrient solution for greenhouse culture. Root and top temperatures were unregulated for 19 days. The nutrient solutions were then renewed and root temperature treatments of 10, 15, 20, and 30 C were initiated. Replicated plant samples were collected after 0, 1, 2, and 4 days of growth under controlled root temperature conditions. Air temperature was not a variable and averaged 26.5 C during the 4-day experimental period; the average maximum was 31.2 C and the average minimum was 21.8 C.

Upon harvest, the plants were rinsed successively with tap water and distilled water; divided into leaf blades plus cotyledons (hereafter called leaves), epicotyls plus petioles, hypocotyls, and roots; and freeze-dried. The dried tissues were stored over $CaCl_2$ at —26 C. Before analyses, the tissues were ground to pass a 60-mesh screen and stored at room temperature over silica gel during analyses.

Sugar Estimation

Sugars were removed from 100-mg samples by three successive extractions with 10-ml portions of 80% ethanol at 80 C for 10 min each. The extracts were centrifuged and pooled and the ethanol was removed *in vacuo* at 75 to 80 C with a rotary evaporator. The aqueous residue was made to 20 ml, transferred to a centrifuge tube, and shaken vigorously with 2 ml of chloroform to remove residual lipids and proteins (20). This suspension was centrifuged and portions of the upper phase used for sugar determinations. Reducing sugars were estimated by the ferricyanide method (18). Total sugars were estimated by the same method after hydrolysis in 0.5 N H_2SO_4 for 30 min. at 100 C and subsequent neutralization with NaOH.

Chromatography

Samples of 100 mg from each of the four replications were pooled and, because of the larger size of the pooled samples, extracted four times with 80% ethanol at 80 C. Ethanol was removed *in vacuo* as outlined above, but the samples were centrifuged without chloroform. The supernatant fractions were dried *in vacuo* and the sugars were transferred with 2 ml of water to glass centrifuge tubes. The samples were then shaken with 0.2 ml of chloroform and centrifuged to remove residual lipids and protein.

The sugars were separated by descending paper chromatography on large sheets of Whatman No. 1 paper, the ends of which were serrated to insure uniform flow of solvent off the ends. Five 50-μl portions of extract were applied as 5-cm (2-inch) streaks, 2.5-cm (1 inch) apart, along the origin. The chromatograms were developed with n-butanol, 95% ethanol, and water (52:32:16, v:v:v) for 48 hours at room temperature. After drying, each chromatogram was cut into five strips. The center and outside strips were sprayed with a p-anisidine-diphenylamine reagent (2) and heated at 80 C for 5 min. These marker strips were realigned with the unsprayed strips to locate the sugars. The areas which contained sucrose, glucose, and fructose were cut out and the sugars eluted in test tubes with water.

Glucose and fructose were estimated by the ferricyanide method (7, 18). Sucrose was filtered and estimated by the phenol-H_2SO_4 method (5) for most samples. However, tests conducted during the analyses showed that sucrose could be hydrolyzed and determined by the ferricyanide method without interference by cellulose fibers from unfiltered samples (7).

Starch Determination

Starch was extracted with $HClO_4$ (after sugars were extracted with 80% ethanol), precipitated with I_2KI, and purified with ethanol-NaOH and ethanol-NaCl (9). The starch was then dissolved in water and portions used in the phenol-H_2SO_4 test (5) which gave more reproducible results than the anthrone test (9).

RESULTS AND DISCUSSION

Data in Fig. 1-4 and 10-12 are expressed as averages of four replications. Vertical lines at each point represent twice the standard error of the mean for that point.

Low root temperatures increased both reducing and total sugar contents of all plant parts (Fig. 1-4). In leaves, the greatest accumulations occurred at a root

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temperature of 10 C, but this was not true for epicotyls, hypocotyls, and roots where, in general, sugar contents were highest at a root temperature of 15 C. Sugars accumulated rapidly and tended to reach a maximum within 2 days. Epicotyls contained the highest concentration of sugars, over 20% on a dry weight basis after 2 days at the 15 C root temperature (Fig. 2). Hypocotyls were next with 15% sugars (Fig. 3).

Sucrose content appeared somewhat higher, especially in epicotyls, after 2 days when the root temperature was 15 C than when it was 10 C (Figs. 5-8). Sucrose content of leaves was also higher at a root temperature of 20 C than 15 C (Fig. 5). This decline in sucrose at the lower root temperatures was accompanied by increases in glucose and fructose contents of leaves, suggesting either hydrolysis of sucrose or a decreased rate of sucrose synthesis. Leaves contained relatively low levels of sucrose (Fig. 5 and 9), whereas roots contained very low levels of glucose and fructose (Fig. 8 and 9).

After two days at a root temperature of 15 C, leaves contained the least and hypocotyls contains the most sucrose (Fig. 9). Epicotyls and roots contained high and approximately equal amounts of sucrose. Epicotyls contained by far the highest concentrations of glucose and fructose and also contained much more sucrose than leaves. The higher content of all three sugars in epicotyls than in leaves may have resulted from active accumulation of sugars by phloem tissue. Bieleski (3) reported that phloem actively accumulated

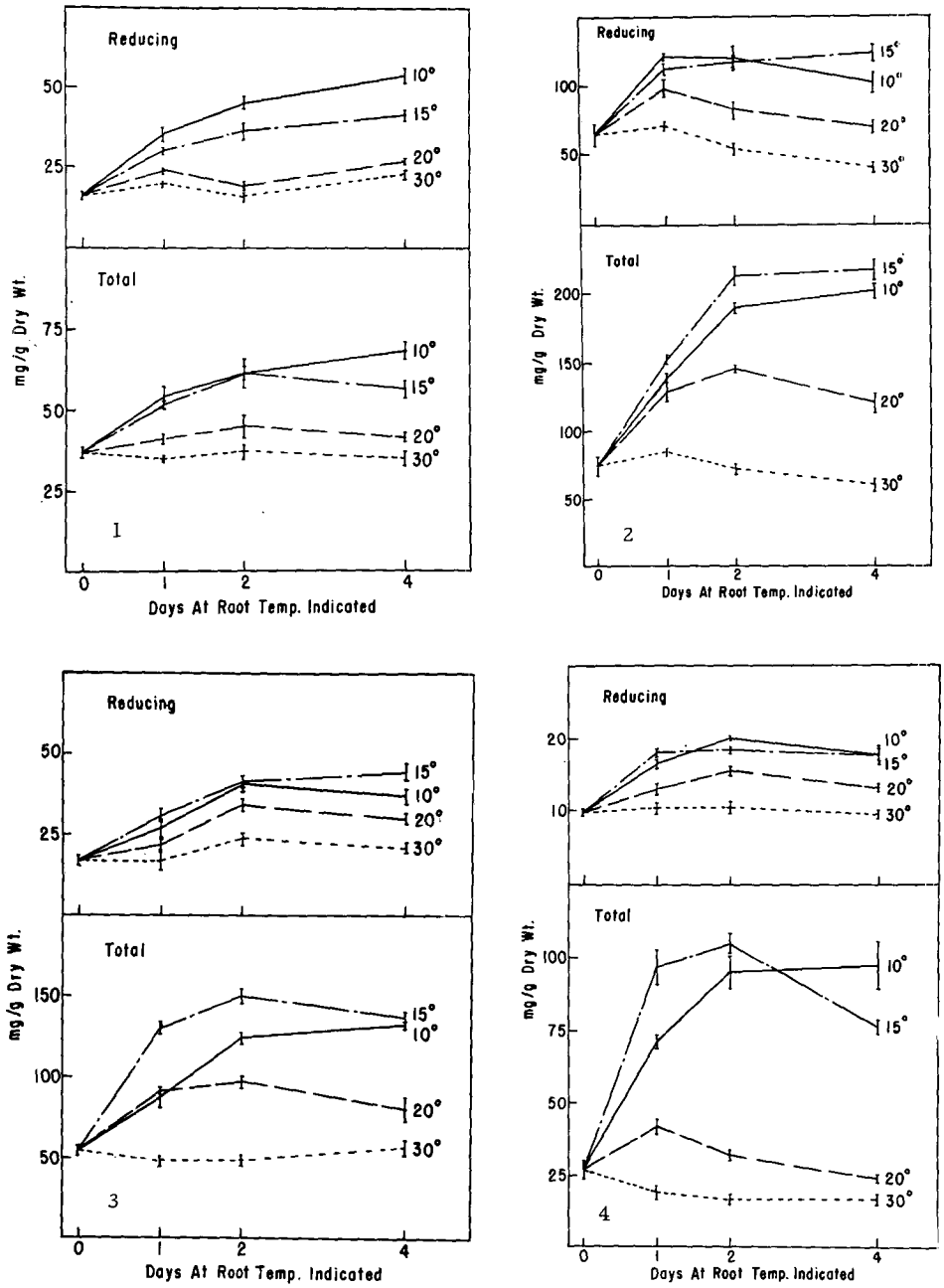

Fig. 1-4. Reducing and total sugars in (1) leaves, (2) epicotyls, (3) hypocotyls, and (4) roots as influenced by number of days at root temperatures of 10, 15, 20, and 30 C. The data are averages of four replications.

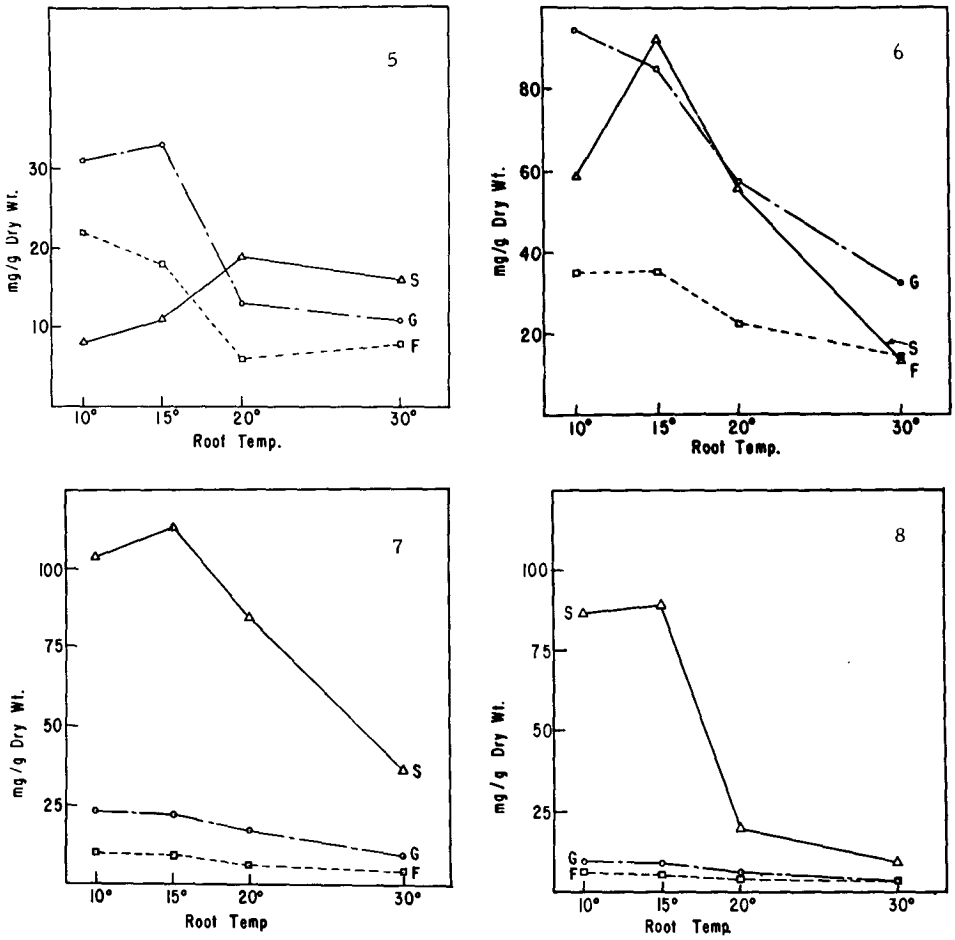

Fig. 5-8. Sucrose, glucose, and fructose contents of (5) leaves, (6) epicotyls, (7) hypocotyls, and (8) roots after 2 days at root temperatures of 10, 15, 20, and 30 C.

sucrose against a high concentration gradient. Perhaps glucose and fructose are also accumulated.

Accumulation of sugars in many plants when the weather turns cool is due, at least in part, to conversion of starch to sugars (14). But, the accumulation of sugars in cotton which resulted from low root temperature probably was not due to starch conversion except at the 10 C root temperature where the leaves contained even less starch than those from plants whose roots were warmed to 30 C (Fig. 10). In contrast, a 15 C root temperature resulted in higher concentrations of starch than any other root temperature (Fig. 10-12).

The striking contrast in starch contents of leaves at root temperatures of 10 C and 15 C may have been due to severe physiological drought at the 10 C root temperature. Leaves of plants whose roots were chilled at 10 C remained wilted during the experimental period; those whose roots were chilled at 15 C wilted slightly but recovered after the first day. Cotton plants become progressively less able to absorb adequate amounts of water as the root temperature is lowered (1, 12). Eaton and Ergle (6) found that drought caused large reductions in starch content of cotton leaves. Starch contents of epicotyls and hypocotyls responded strangely to a root temperature of 10 C; they remained low for the first 2 days and then increased rapidly to levels almost as high as those at a root temperature of 15 C (Fig. 11 and 12). This rapid increase in starch

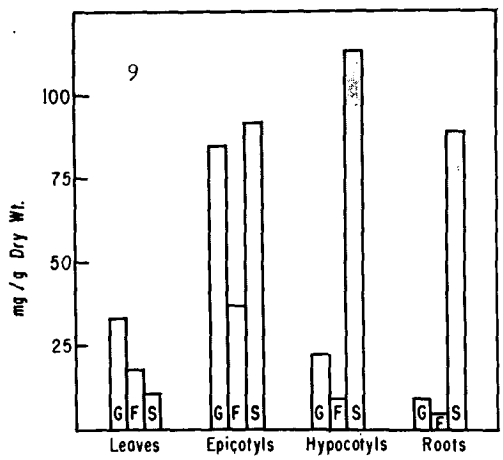

Fig. 9. Glucose, fructose, and sucrose contents of leaves, epicotyls, hypocotyls, and roots after 2 days at a root temperature of 15 C.

content of epicotyls and hypocotyls was accompanied by a slight decline in reducing sugars (Fig. 2 and 3). Perhaps these plants became somewhat adapted to cold roots and the moisture stress became less, possibly due to accumulation of osmotically active substances such as sugars and amino acids. Starch contents of roots were less than 2 mg/g dry weight and were too low to measure accurately.

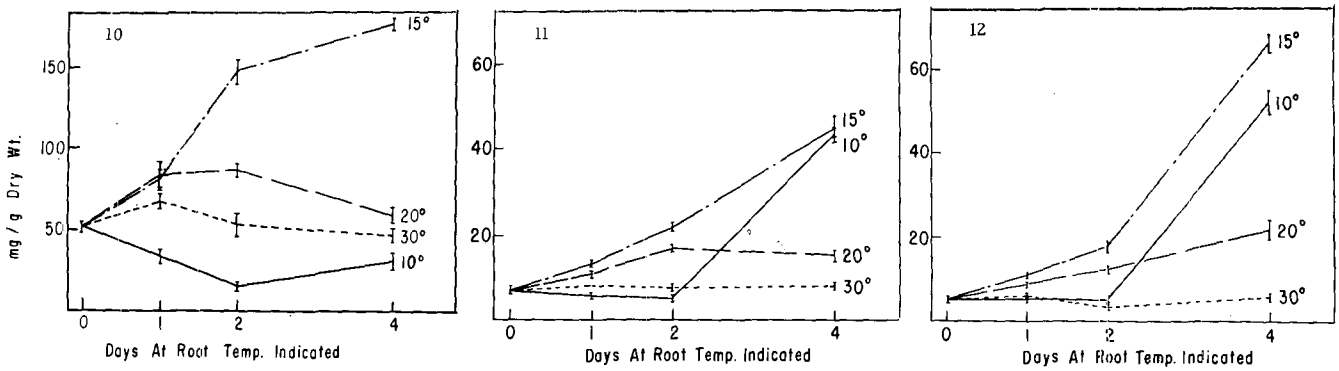

Fig. 10-12. Starch contents of (10) leaves, (11) epicotyls, and (12) hypocotyls as influenced by number of days at root temperatures of 10, 15, 20, or 30 C. Data for roots are not included because roots contained too little starch to measure accurately. The data are averages of four replications.

Considering the fact that the roots comprised only 12.5% of the total dry weight of the plants, root temperature had a strong influence on carbohydrate content of the entire plants. There are probably at least two reasons for this, respiration rate and rate of conversion of sugars to other compounds. Respiration rate of cotton roots is relatively high and strongly influenced by temperature (21). However, conversion of sugars to other compounds for use in the roots and for export to the tops may be even more important than respiration in altering carbohydrate content of plants.

Roots are highly active in nitrogen metabolism (13, 15, 16, 22). For example, Vickery et al. (22) concluded that intensity of ammonium assimilation was greatest in roots and lowest in leaves. Mothes (15) regarded roots as the primary site of nitrogen assimilation. Kursanov (13) reported that up to 50% of photosynthate labeled with C^{14} moved from leaves to roots of *Cucurbita pepo* where it was rapidly converted to organic acids, amino acids, and amides. Almost half of the labeled material was then returned to the tops. Guinn has found at least 12 amino acids in xylem exudate of decapitated cotton plants (unpublished).

Roots may also produce hormones. Itai and Vaadia (10) and Kende (11) were able to isolate substances with cytokinin activity from root exudate of sunflower. Phillips and Jones (19) and Carr et al. (4) reported gibberellin-like activity in bleeding sap of certain plants. Although the amount of carbohydrate used in producing such growth substances is small, the regulatory effects in the utilization of carbohydrates for growth may be important. For example, cytokinins influence nucleic acid and protein synthesis (17). It seems reasonable to suppose that low root temperature would slow all metabolic activities in roots. Therefore, if roots do produce cytokinins, low root temperature could decrease the rate of utilization of carbohydrates even in the leaves by slowing nucleic acid and protein synthesis.

Whether or not cotton roots produce cytokinins or gibberellins is open to speculation. Our data indicate only that the temperature of cotton roots influences carbohydrate status of the entire plant.

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