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Accumulation of Simple Sugars in Developing Cotton Bolls as Influenced by Night Temperatures[1]

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ABSTRACT

Cotton plants (*Gossypium hirsutum* L. var. Dunn 56 C) growing in the field were subjected to four different night temperature regimes (10, 15, 20, and 25 C) during the boll development phase. Total and individual sugars were determined on developing bolls ranging in age from 0 to 45 days. Total soluble sugars were found to be directly associated with temperature, with the lowest concentration occurring in the 10-C treated bolls, and the highest concentration in the 25-C treated bolls for the first 15 days. After 15 days this relationship was reversed. Definite time-temperature relationships that were apparently associated with the cessation of fiber elongation and the initiation of secondary wall formation were noted. The principal sugars present were glucose, fructose, and sucrose. The concentration of each varied with boll age and temperature treatment.

Additional index words: **Glucose, Fructose, Sucrose.**

THE development of the cotton (*Gossypium hirsutum* L.) boll proceeds in two distinct phases: a period of enlargement and a period of maturation (9, 10). These two phases roughly correspond with the developmental sequence of the fiber, i.e., elongation and secondary wall formation (9, 11).

Temperature has been shown to play a primary role in determining the rate at which each of these developmental phases proceeds. As early as 1930, Hawkins and Serviss (6) reported that temperature below the optimum for plant growth retarded fiber elongation. More recently, the work of Gipson and co-workers (3, 4) has established that the rate of fiber elongation is extremely temperature sensitive. Working with night temperature as a variable, they found that the rate of elongation was retarded by temperatures below 20 C, and that the extent of the effect became more pronounced as temperature was decreased below this level.

The influence of temperature on secondary wall formation is no less critical. Gipson and co-workers (2, 5) showed that both rate and amount of cellulose synthesis were governed by the prevailing temperature during the synthesis process. Chemical properties of the cellulose produced were also influenced by temperature. Hessler, Simmons, and Lane (8) found that late-season cotton grown under cool night temperatures contained less crystalline and more amorphous cellulose. Hessler, Lane, and Young (7) in a later study found that cotton grown under sub-optimum temperatures showed a close relationship between the cellulose-reducing sugar ratio and temperature deficiency. Their data showed that low temperatures during the boll development period caused reduced cellulose synthesis and the accumulation of sugars in the boll. During boll development, while the temperature was high, cellulose continued to increase, while sugars decreased. To them, the fact that cellulose was low and sugars high when the temperature was adverse for growth indicated a temperature block to cotton fiber development.

Since temperature exerts such a pronounced effect on the rate of cellulose synthesis, its effect could be mediated through the accumulation or formation of simple sugars in the developing cotton boll. Thus, the relative amount and kinds of sugars in the developing boll may be the key to the cause of reduced cellulose synthesis and the resulting immature fiber. An insight into the effect of temperature on sugar metabolism in the developing cotton boll could lead to better understanding the mechanism whereby temperature affects boll development. Therefore, our objective was to study the relationship between boll sugars and various night temperatures.

MATERIALS AND METHODS

This research was conducted during the 1970 growing season on field-grown cotton (*Gossypium hirsutum* L. var. Dunn 56 C). Cultural practices currently considered best for production of irrigated cotton were used throughout the study.

Since only night temperature effects were studied, field chambers that would exert a minimum influence on the daytime environment were required. The chambers utilized were those described and used by Gipson (5). Night temperature control was initiated July 16, when the first white blooms were noted, and was continued until the first frost, which occurred October 9, 1970. Four temperature treatments (10, 15, 20, and 25 C) were employed during the season, with temperature control maintained nightly from approximately sunset to sunrise. Due to the size and number of chambers required, each temperature treatment was not replicated. There were, however, approximately 75 plants within each temperature treatment from which boll samples were collected.

Blooms were tagged daily and bolls of ten consecutive ages ranging from day of bloom to 45 days were collected from each temperature regime on September 9, which was 55 days after initiation of the treatments. Immediately after harvesting, the bolls were placed in a deep freeze (—20 C) and stored until analyzed. All bolls collected for each age and each treatment were divided into three replications for sugar analyses. The bracts were removed and approximately 25 g of tissue of each replicate was macerated and extracted using 70% acetone. The extract was filtered and cleared of all ionic fractions using ion exchange resins. Total soluble sugars were determined colorimetrically using the anthrone reagent (13). Gas chromatographic analysis of the trimethylsilyl ether derivatives was conducted to determine the individual sugar components of each sample (14). The gas chromatograph was equipped with dual columns and dual H_2 flame ionization detectors. Two 1.83 m × 0.32 cm (6′ × ⅛″) ID stainless steel columns were packed with 3% QF-1 coated on 100-200 mesh Chromosorb W (HP). Nitrogen was used as the carrier gas and the flow rate was set and maintained at 40 cc/min. Following injection, the column oven was maintained at 120 C for 4 min, then programmed for a linear increase of 8 C/min to a final temperature of 220 C. At least

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three 1.0-μliter aliquots from each sample were injected and peak areas, as a measure of component concentration, were determined using a digital integrator. The percentage of each component was calculated from the sum of components.

The total soluble sugar concentration data were analyzed for statistical significance using analysis of variance with the means at each 5-day age interval compared using Duncan's New Multiple Range Test (12).

RESULTS AND DISCUSSION

Total boll sugars as a function of night temperature and boll age are shown in Fig. 1. Statistical analysis of the data indicated that for the first 15 days after anthesis differences in sugar concentrations were found to be significantly influenced by temperature. The 25-C treated bolls contained the greatest amount of soluble sugars, and the 10-C treated bolls, the least amount. After 15 days this trend was reversed and the bolls of lower-temperature treatments contained significantly higher quantities of free sugars as compared to the 25-C treatment.

At anthesis (day 0) a pronounced temperature effect was already apparent, with the 25-C treated bolls containing twice the amount of sugars as found in the 10- and 15-C treated bolls. Although no data were obtained for the squares, a simple interpolation of the four temperature curves of Fig. 1 would suggest an appreciable sugar differential due to temperature in the late square stage. These lines also suggest that sugars must have been increasing rapidly in the older squares under all temperature treatments.

After anthesis, during the early stages of boll development, the sugar concentration increased at all temperatures, but more rapidly under higher temperatures. The difference in the rate of sugar accumulation resulted in a distinct time-temperature relationship. Bolls produced under 10-C night did not attain the maximum sugar concentration until 25 days age. Under 15-, 20-, and 25-C nights, maximum sugar levels were attained at 20, 15, and 10 days of age, respectively. Thus, for each 5 degrees C that night temperature was increased between 10 and 25 C, the time required to reach a peak soluble carbohydrate level was decreased by 5 days. In each case the maximum sugar level was attained about the midway point of the fiber elongation period (Table 1). Once the maximum concentration was reached, however, it did not remain static, but began to decline immediately. At the highest temperature this decline occurred quite rapidly. This probably indicates that simple sugars continue to accumulate in the boll until the rate of utilization exceeds the rate of accumulation.

The rapid decline in soluble sugars probably suggests that growth-induced metabolic changes are occurring. The rate of boll growth has been found to be extremely fast during this same time period. Boll development, as measured by increasing diameter, has been found to be only 25% complete 10 days postanthesis, but to be essentially complete 15 to 18 days after anthesis (15). This rapid increase in carpel wall growth could be causing the observed rapid decline in soluble sugars under the 25-C night temperature conditions. The rate of boll growth could be limited at lower temperatures by the availability of soluble sugars.

Another possible explanation for the decline in sugar concentration would involve the formation of

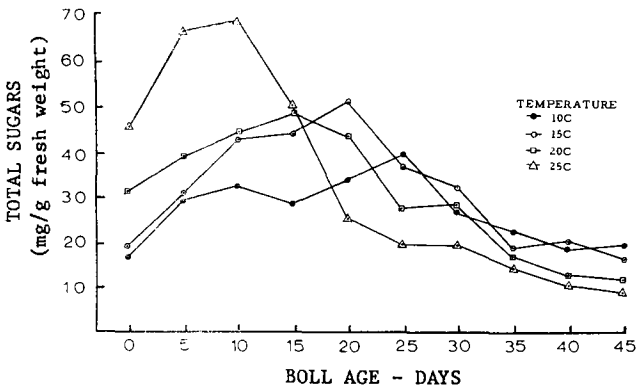

Fig. 1. Total sugar concentrations in various age cotton bolls developed under different night temperatures.

Table 1. Night temperature, boll age interactions on attainment of maximum sugar levels and fiber length.

Night temp, C	Boll age, days, at: Maximum sugar level	Maximum fiber length	Which sugars level off
10	25	40	40-45
15	20	33	35-40
20	15	28	25-30
25	10	26	20-25

precursor materials for cellulose synthesis. Since cation and anion exchange resins were used to clear the filtrate prior to colorimetric analysis, phosphorylated sugars would have been removed and would not have been detected in the total sugar analysis. Consequently, one would expect a decline in total simple sugars at this stage if they were being incorporated into precursor material for cellulose synthesis. Since the decline of simple sugars was greatest in the high temperature regimes and less in the low temperature regimes, it is probable that less precursor material was being formed under low temperatures. This, in turn, should result in a reduced rate of cellulose synthesis, which has been observed by Gipson and Ray (5).

Further examination of Fig. 1 reveals that in the later stages of boll development, the highest sugar content was associated with the lower temperatures. This is further evidence that the rate of mobilization of simple sugars into precursor material is possibly reduced under low temperatures. The results obtained in this study agree with the findings of Hessler, Lane, and Young (7) on sugar accumulation in cotton bolls under low temperatures.

Another interesting aspect of the temperature-time relationship is the boll age at which the total sugar content began stabilizing. The boll age when sugar content stabilization occurred corresponded to the time when the fiber attained maximum length (Table 1) and secondary wall thickening has been reported to begin (1, 10). The results obtained from this study suggest that fiber development, as governed by cellulose synthesis, is a function of both temperature and substrate availability. In the early stages of fiber development substrate availability may limit the rate of fiber elongation. During the latter stages of development, however, substrate is available, but temperature limits its conversion to cellulose probably by reducing the rate of precursor synthesis.

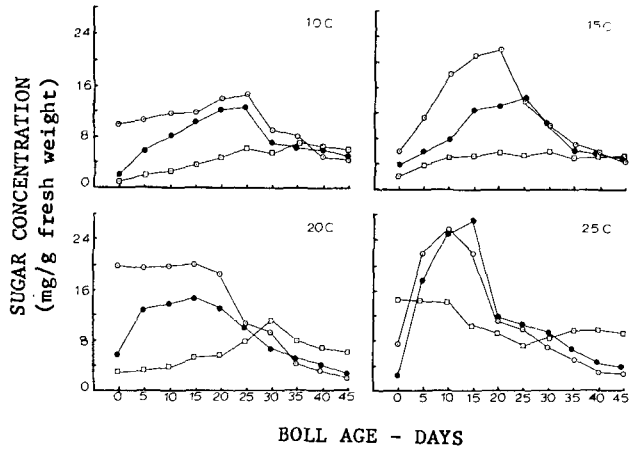

Fig. 2. The concentration (mg/g) of the three principal sugars (-○- glucose, -●- fructose, -□- sucrose) of cotton bolls developed under different night temperatures.

Table 2. The time-temperature relationship with respect to β-:α-D-glucose ratios in developing cotton bolls.

	Boll age, days			
	0	5	10	15
Temp.	β/α	β/α	β/α	β/α
10 C	.79	.74	.90	.90
15 C	1.00	.89	1.02	.91
20 C	1.07	1.35	1.21	1.14
25 C	1.74	1.54	1.56	1.49

Since total sugar content differed as a function of night temperature, a qualitative study to determine differences within the sugar fraction was undertaken. Four, five, and six carbon monosaccharides were identified, as well as sucrose, a dissaccharide. The greatest variation in concentration as a function of temperature occurred between glucose, fructose, and sucrose. The relationship between boll age, temperature, and concentration of each sugar are plotted in Fig. 2. It is apparent that glucose was the major sugar in the cotton bolls at all temperatures. Of the three principal sugars present, sucrose was found in the least quantity in the bolls grown under 10-, 15-, and 20-C night temperature treatments. In each of these treatments, the amount of sucrose found at anthesis was extremely low, with a gradual increase occurring in older bolls. In the bolls of the 25-C treatment, however, sucrose was the predominant sugar at anthesis, with the concentration three to five times greater than in bolls from the lower temperature regimes. If sucrose is the principal transport sugar in the cotton plant, one would expect lower concentrations under reduced temperatures where translocation is probably retarded.

Although no attempt was made to prevent mutarotation of the simple sugars prior to gas chromatographic analysis, some apparent temperature effects on sugar ratios still existed. The relationship between α- and β-D-glucose for the first 15 days of boll development appears very temperature dependent (Table 2). In the 10-C treated bolls there was more α-D-glucose as compared to β-D-glucose; as the night temperature was increased to 25 C, however, the relationship reversed and the ratio of β-:α-D-glucose increased. This ratio may be important in fiber elongation if the rate of cellulose synthesis in the primary cell wall of the fiber is being reduced by the availability of glucose. As temperature is increased the availability of substrate for cellulose synthesis also increases.

Although fiber development is probably the most important economic synthetic reaction occurring in the developing cotton boll, it is by no means the only reaction occurring. Seed development and carpel wall development are also occurring. These two processes also require large quantities of different types of soluble sugars. Therefore, the results obtained in this study are a reflection of total boll metabolism and not just of fiber development, although much of the data can be correlated to the known developmental phases and responses of the fiber.

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