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RELATIONSHIP OF SOLUBLE CARBOHYDRATES TO AGE OF CORN COB PARENCHYMA TISSUE*

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Abstract—The 80% ethanol-soluble carbohydrate content of corn cob parenchyma tissue was determined on a per cell basis before and during the period of cell death. In four of the five populations studied, a period of cell elongation was followed by a drop in per cell sugar concentration to a very low level, following which cell death began. After this period, the total sugar concentration increased to a maximum, then decreased, and in some populations, increased again.

INTRODUCTION

Following the finding that spread of stalk rot pathogens in corn (*Zea mays*) is limited to areas of dead parenchyma cells,[1] biochemical studies of parenchyma aging and death were undertaken. As part of these investigations, it was found that corn cob parenchyma tissue free of vascular tissue was a suitable model for studies of tissue senescence and death, but that, in this tissue, the periods of cell elongation and cell death overlapped.[2] In a subsequent investigation,[3] it was found that, because of this overlap, a per cell basis of data analysis was a valid basis for physiological and biochemical studies. In this work, the relationship of sugar concentrations to cell death in corn cob parenchyma tissue has been investigated on a per cell basis.

RESULTS

Cell death in the cob parenchyma tissue, as indicated by a decrease in density,[1] began between days 5 and 9 after silking in all five populations (Fig. 1). Densities were approximately 0·95 g/cm^3 at day 0 and dropped to as low as 0·37 g/cm^3 in population WF9 × 38-11 on day 20.

Changes in per cell concentrations of D-glucose, D-fructose, sucrose and total sugar were calculated on a per cell basis (Figs. 2–6). In each population there was a period, at some time during the first 5 days following silking, in which the total sugar per cell reached a very low concentration. This period was usually followed by a period of near maximum concentration of total sugar, in some cases 25 times higher than that of the period of minimum sugar concentration. A comparison of this period of low sugar concentration with the period of cell elongation and the onset of cell death shows that in Mo 940, 38-11, WF9 × 38-11 and Hy_2RF × 07RF, the elongation period preceded the drop in

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[1] A. J. Pappelis and F. G. Smith, *Phytopath.* **53**, 1100 (1963).
[2] J. N. BeMiller, D. C. Johnson and A. J. Pappelis, *Phytopath.* **59**, 989 (1969).
[3] J. N. BeMiller, D. C. Johnson and A. J. Pappelis, *Phytopath.* **60**, 513 (1970).

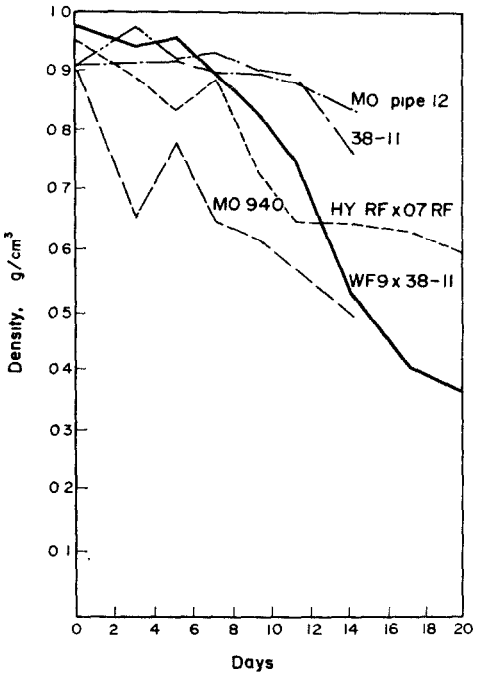

FIG. 1. DENSITY OF CORN COB PARENCHYMA TISSUE VS. DAYS FOLLOWING SILKING IN ALL VARIETIES.

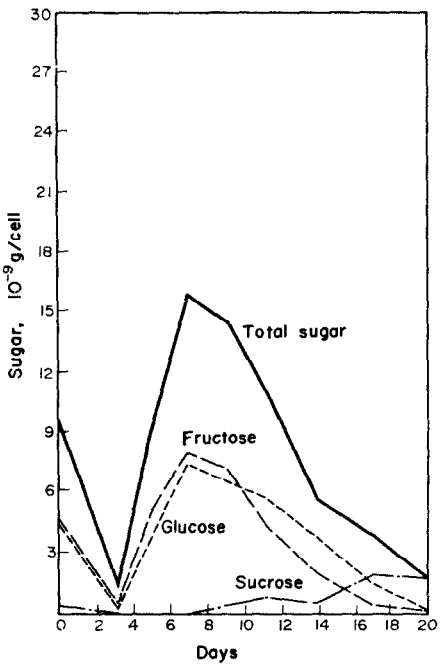

Fig. 2. CONCENTRATION CHANGES OF TOTAL SUGAR, GLUCOSE, FRUCTOSE AND SUCROSE VS. DAYS FOLLOWING SILKING, WF9 × 38–11.

sugar concentration by about 2 days, which in turn preceded the onset of cell death (drop in density) by approximately 2 days (Table 1) (Figs. 2–5). In Mo Pipe 12, the period of low sugar concentration occurs at or before cell elongation (Fig. 6).

Glucose and fructose concentrations almost paralleled each other throughout the sampling period in all varieties. Sucrose, however, was at very low concentration until after day 5 and until as late as day 11 in some varieties, at which time it began to increase, in some cases exceeding glucose or fructose concentration at some time prior to elongation and then again following elongation, after which time it dropped to various levels depending upon the variety.

TABLE 1. TIME OF MINIMUM SUGAR, ELONGATION, AND ONSET OF DENSITY DROP IN DAYS FOLLOWING SILKING

Variety	Elongation period (day)	Onset of density drop (day)	Minimum sugar concentration per cell (day)
Mo 940	0–5	7	5
$Hy_2RF \times 07RF$	0–5	5–9	5
38–11	0–5	9	5
WF9 × 38–11	0–3	7	3
Mo Pipe 12	0–3	5–9	0

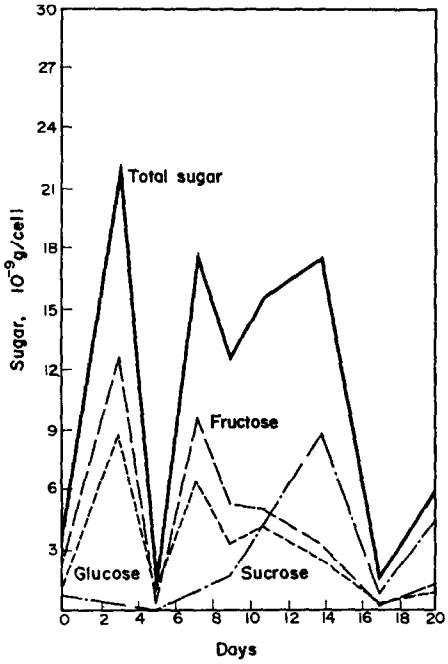

Fig. 3. CONCENTRATION CHANGES OF TOTAL SUGAR, GLUCOSE, FRUCTOSE AND SUCROSE VS. DAYS FOLLOWING SILKING, $Hy_2RF \times 07RF$.

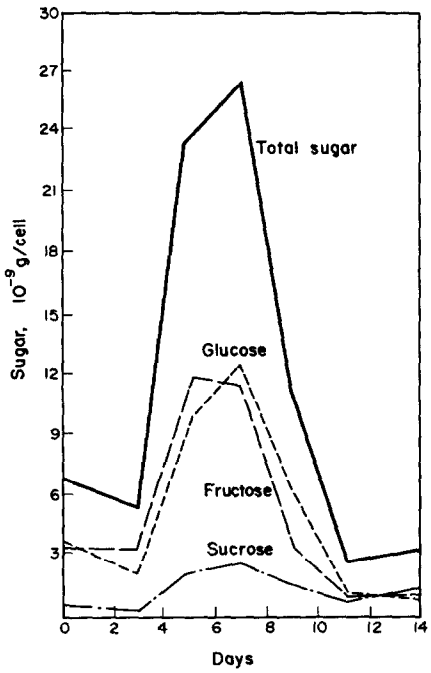

Fig. 4. CONCENTRATION CHANGES OF TOTAL SUGAR, GLUCOSE, FRUCTOSE AND SUCROSE VS. DAYS FOLLOWING SILKING, 38–11.

DISCUSSION

The data from these experiments do not allow any conclusions as to the cause or triggering of senescence in corn cob parenchyma tissue. They do show trends which are consistent with one of the several theories of senescence.

Betterton[4] reported a high positive correlation between mg of reducing sugar per cm^3 and density ($r = 0{\cdot}92$) and mg of total sugar per cm^3 and density ($r = 0{\cdot}85$) in parenchyma tissue of corn stalks. He indicated that, although this correlation existed, the drop in density in most cases slightly preceded the sugar decrease and, therefore, concluded that an increased permeability of the cytoplasmic membrane allowed water loss before the onset of sugar loss. He also observed that the sucrose concentration was not well correlated with the density drop, but rather increased during the period of cell death, then decreased. The data from corn cob parenchyma tissue after seven days following silking show that, in most populations, reducing sugar per cell is correlated with density, whereas sucrose or total sugar per cell is not (Table 2).

Betterton[4] did not observe the early abrupt decrease of total and reducing sugar to a very low concentration followed by an increase to almost the original level preceding the density drop. The decrease may be related to rapid cell elongation, for the stalk tissue used in the earlier study had completed elongation. Alternatively, it is possible that Betterton failed to observe this fall and rise in total and reducing sugar because his sampling dates were spaced at seven day intervals, rather than the 2–3-day intervals used in this work, or

[4] H. O. BETTERTON, M. S. Thesis, Southern Illinois University, Carbondale, 99 pp. (1963).

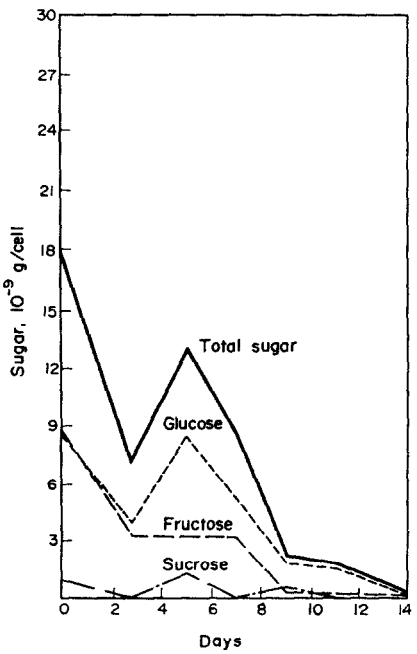

Fig. 5. Concentration changes of total sugar, glucose, fructose and sucrose vs. days following silking, Mo 940.

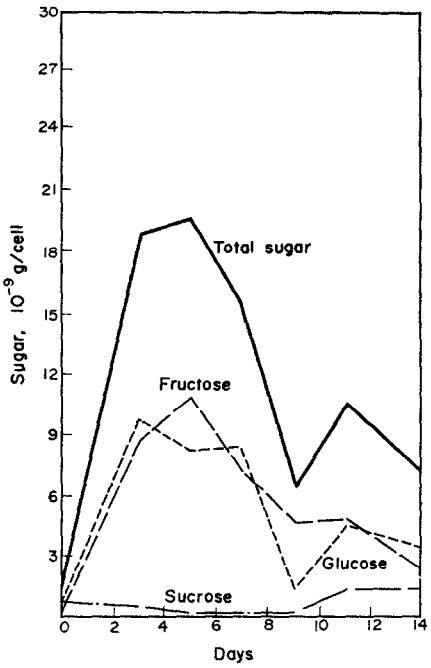

Fig. 6. Concentration changes of total sugar, glucose, fructose and sucrose vs. days following silking in Mo pipe 12.

that the concentration of sugars in the vascular bundles of corn stalks masked changes in parenchyma cells and other cell types outside the bundles.

Since per cell concentrations of sugars in corn cob and stalk parenchyma tissue decrease gradually after the onset of cell death, the changes are likely to be the result of rather than the cause of senescence. The most significant aspect of sugar concentration as related to cell death is the abrupt drop preceding cell death. It could be concluded that, since the period of low sugar coincides in most cases with the end of the elongation period (Table 1), the pool of free sugars is being exhausted for synthesis of cellulose, other cell wall poly-

Table 2. Correlation coefficients for the relation of per cell sugar concentrations to tissue density

Variety	Time period (days after silking)	Correlation coefficients			
		Glucose	Fructose	Sucrose	Total sugar
WF9 × 38–11	7–20	0·99	0·98	−0·86	1·0
Hy_2RF × 07RF	7–17	0·79	0·81	−0·51	0·40
38–11	7–11	1·0	1·0	0·96	1·0
Mo Pipe 12	3–14	0·77	0·93	−0·82	0·78
Mo 940	0–14	0·56	0·87	0·77	0·73

saccharides, lignin and other cell components[3] and that cell to cell translocation is disrupted during this period of rapid elongation and reestablished later. The possibility that these changes are only a reflection of what is occurring in other parts of the plant, such as the filling of newly formed kernels, is not eliminated. Whatever the reason for this period of very low sugar concentration, because of the subsequent return to near normal concentrations, we do not believe that it supports the theory of Leopold[5] which attributes the triggering of senescence to a nutrient drain resulting in decreased synthesis of necessary plant substituents.

On the basis of the data of this study, which along with earlier studies[2,3] has shown that cell death begins about 7 days after silking (after the period of rapid cell elongation) in many varieties, including WF9 × 38-11 which seems to be good for these studies, it is recommended that future investigations concentrate on the period prior to this time.

EXPERIMENTAL

Tissue. Five populations of corn (*Zea mays* L.) (Mo 940, Hy_2RF × 07RF, Mo Pipe 12, 38–11 and WF9 × 38–11) were grown in Murphysboro, Illinois in 1967. Ears were tagged on the first day of silking (designated as day 0). Ears were then sampled on days 0, 3, 5, 7, 9, 11, 14, and with some populations also on days 17 and 20. Parenchyma tissue was removed from the entire length of the cob, in the younger ears by cutting away the kernels and the outer portion of the cob, and in the older tissue by using a cork borer small enough to fit inside the ring of vascular bundles. The tissue was weighed, and the volume was determined by water displacement in order to calculate the density. The tissue was then boiled in 80% EtOH for 10 min and macerated in an Omni-Mixer for 3–5 min. After filtration through a Buchner funnel, the filtrate was brought to a suitable volume with 80% EtOH and stored at room temp.

Sugar analysis. The method of Betterton[4] was used for the isolation of sugars as follows. An aliquot of the 80% EtOH extract representing 2–3 cm^3 of the original tissue was evaporated to 2–3 ml; 85 ml H_2O was added, and the mixture was heated to 80° to soften gummy precipitates. After cooling, 15 ml of 10% (0·26 M) neutral lead acetate was added to remove waxes and lipids.[6] After 10 min, the precipitate was removed by filtration, and 8–10 ml of 12·5% (0·92 M) KH_2PO_4 was added to the remaining solution to precipitate excess lead. The precipitate was removed by centrifugation at 500 *g* for 5 min. The centrifugate was stirred with a mixed anion-cation ion-exchange resin [Amberlite IR-45 (OH^-) and Amberlite IR-120 (H^+)] to remove remaining salts. The deionized solution was then evaporated to dryness to yield a sugar syrup.

This syrup was dissolved in pyridine by heating for 1 hr at just under the boiling point of 115°. The pyridine sugar solution was then quickly cooled and brought to volume in a 25-ml volumetric flask. One ml was removed for derivatization. To it was added 0·1 ml of hexamethyldisilazane and 0·1 ml of trifluoroacetic acid, and the solution was shaken to make the per(trimethylsylyl) derivative as described by Brobst and Lott.[7]

The detector and injection port temperatures of a GLC were set at 300°, and the column temp. was linear programmed at 4°/min from 150–240°. The column was packed with 5% SE-30 silicone gum rubber (methyl) on 60–80 mesh, acid-washed, dimethyldichlorosilane-treated Chromosorb W.

Since there was overlapping of the α-D-glucopyranose and glucofuranose peaks with a portion of the fructopyranose peak, the following method was used to calculate the area of each peak. Several standard D-glucose samples were derivatized as described above and chromatograms were made. The average percent of β-D-glucopyranose in the equilibrated mixture was calculated. Since the β-D-glucopyranose peak of the unknown samples was not involved in the overlap with the fructose peak, the β-D-glucopyranose area could be easily attained and, knowing its percentage of total glucose, the α-D-glucopyranose and glucofuranose areas could be calculated. This value, in turn, was subtracted from the total area of the glucofuranose, α-D-glucopyranose and fructose peaks leaving the area due to fructose alone. There was no overlap of any of the hexose peaks with the sucrose peak which had a much greater retention time. Standard deviation of the method was always less than 4% and usually less than 2·5%.

[5] A. C. Leopold, *Plant Growth and Development*, pp. 193–204, McGraw-Hill, New York (1964).
[6] W. E. Loomis, *Plant Physiol.* **1**, 179 (1926).
[7] K. M. Brobst and C. E. Lott, *Cereal Chem.* **43**, 35 (1966).

Key Word Index—Zea mays; Gramineae; corn cob; soluble carbohydrates; variation in senescing tissue