CLIMATIC EFFECTS IN THE METABOLISM OF THE SUGAR BEET[1]

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INTRODUCTION

In the summer of 1919 the senior writers undertook an investigation of nitrogenous metabolism in leaves of the sugar beet, which has been reported briefly (*17, p. 17*).[3] It was the purpose of that work to determine whether protein synthesis occurred simultaneously with carbon assimilation or ensued in the following period of darkness. The results of earlier investigation on this point indicate the first of these relations. Kosutany (*11*) found a little greater percentage of soluble protein in leaves of *Vitis riparia* at 3 a. m. than at 3 p. m. On the other hand, Suzuki (*22*), employing leaves of several plant species, found at 6 p. m. a decided excess of this constituent over the percentage at 6 a. m. Schulze and Schütz (*18*), basing results upon both the number of leaves sampled and the weight of fresh material, concluded that with younger leaves of boxelder (*Acer negundo*) proteins migrated at night from the seat of synthesis. Similarly, Pigorini (*16*) found a nocturnal decline of more than 11 per cent in the protein nitrogen of leaves of mulberry (*Morus alba*). These results were based on weights of fresh tissue. According to Chibnall (*7*), this is the most accurate method of expressing the results. He found (*8*) with the runner bean (*Phaseolus vulgaris*) a decline of nearly 2 per cent of foliar protein nitrogen at night.

ANALYTICAL PROCEDURE

Inasmuch as preliminary tests (*24*) had indicated the impossibility of preserving the tissue without alteration of the soluble protein, the freshly sampled material was extracted directly. The leaf surface was dried by absorbent paper and brushed free of foreign material. After dissecting out the midribs the blade tissue was rapidly chopped fine, thoroughly mixed and replicate samples of 20 or 25 gm. were taken for moisture determination. Replicate samples of 10 gm. were used for determining total nitrogen. A 100-gm. portion of the tissue was triturated rapidly in a large porcelain mortar with the addition of an equal weight of washed, fine, spherical sand. About 5 c. c. of ether was added to promote plasmolysis, and water was added as required to give proper consistency for trituration. This took about 15 minutes. The pulverized tissue was then transferred

[1] Received for publication Oct. 14, 1925; issued July, 1926. Published with permission of the director of the Wisconsin Agricultural Experiment Station.

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[3] Reference is made by number (italic) to "Literature cited," p. 75.

to a double layer of cheesecloth and extracted with successive portions of water to a volume of about 1,800 c. c., wringing out each portion of wash water. This extract was passed through a layer of paper pulp nearly 0.5 cm. thick on a Buchner funnel, with the aid of gentle suction. Petioles and roots were similarly treated. When washed to a volume of 2 liters, the filtrate was usually quite transparent and green to brown in color. Aliquots of 500 c. c. were brought quickly to boiling and coagulated with a few drops of 10 per cent acetic acid. This precipitate of the soluble protein was filtered out and determined by the Kjeldahl-Gunning method, the time required from sampling of the tissue to boiling of the extract being about one hour. This period is of importance in relation to a subsequent discussion of enzymatic action. The extent of extraction specified here had been found to remove all but traces of soluble aplha-amino nitrogen compounds.

The residual solution from the separation of soluble protein was employed for determination of sugars and of nitrogen present as amino acids and other forms. Separate aliquots from the 2 liters of filtered extracts served for the determination of total soluble nitrogen. Reducing sugars were determined by the Shaffer-Hartmann method (*19*), and sucrose by the Herzfeld process for inversion (*5, p. 266*).

It appears that the amount of nitrogen, apparently in the form of soluble protein, may depend upon the degree of grinding of the tissue, as well as upon the methods of filtering. The writers have found that this constituent is relatively independent of the thickness of paper pulp employed as a filter. Comparison of the composition of extract with that obtained by use of a mill for grinding, as employed by Osborne (*14*) with frozen tissue, is given in Table 1. The writers applied the mill to the fresh, unfrozen tissue.

Microscopic examination indicated that the walls of practically all cells were ruptured by the use of a mortar for grinding. The results show that the mill treatment recovered 10 to 15 per cent more of the total nitrogen in soluble form than did use of the mortar. This is accounted for largely by the increased dispersion of protein. That a similar effect can be obtained by regrinding the extracted tissue in the mortar method is indicated by preliminary tests. The other differences in composition of extract by the two methods of grinding are too small to appear significant.

TABLE 1.—*Composition of extract obtained from sugar-mangold leaves by mortar and pestle, compared with use of the Nixtamal mill*

[Values are percentages of dry matter]

	Sample A		Sample B	
	Mortar	Mill	Mortar	Mill
Total nitrogen	5.40	5.40	5.42	5.42
Soluble nitrogen	3.62	4.20	3.42	4.28
Coagulable nitrogen	2.70	3.28	2.40	3.16
Other soluble nitrogen	.92	.92	1.02	1.12
Alpha amino nitrogen	.35	.22	.31	.22
Reducing sugars	3.00	3.05	2.45	2.60
Sucrose	1.40	1.55	1.60	1.35

A phase of this process of recovering the soluble constituents which should receive special attention is the possibility of inversion of sucrose during the period between sampling and heating the extract for recovery of soluble proteins. To test this matter, extracts obtained by maceration in a mortar, in which the time elapsed until inactivation of enzymes by boiling did not exceed 30 minutes, were compared with those obtained from corresponding leaves killed immediately after sampling by immersion in boiling alcohol (*13*). $CaCO_3$ was added promptly to the extracts. The data appear in Table 2.

TABLE 2.—*Comparison of recovery of sugars from sugar-beet leaves by direct extraction with alcohol and delayed extraction with water*

[Values are percentages of dry matter]

	Time of sampling					
	Morning		Noon		Night	
	Direct	Macerated	Direct	Macerated	Direct	Macerated
Reducing sugars	2.85	2.90	4.74	4.83	1.40	1.49
Sucrose	1.70	1.71	1.35	1.43	2.00	2.10

These results show that no inversion occurred during the time required by the extraction method employed in the present investigation. Similar conditions were found to hold for petiole and root tissues. Preliminary trials over longer periods indicated that inversion of sucrose in leaf tissue does not become appreciable at room temperatures until after one hour. This gives good grounds for assuming that marked variations in the distribution of sugars in the present case existed in the tissues as sampled.

RESULTS OF 1919

Samplings from many plants were taken at about sunrise and sunset. Only two leaves were taken from a single plant, and these were intermediate in age to the foliage as a whole. The climatic data are those recorded at the weather station, located about 1.2 kilometers east of the beet field and at some 30 meters greater elevation than the beet field.

In Table 3 are assembled the climatic and analytical data of this period. A value of 20 per cent has been assumed to approximate the average nitrogen content of soluble compounds other than proteins, amino acids, and ammonia, reported here as "rest-soluble N." All of the values for nitrogenous compounds were based on the dry matter. In this particular case the values for sugar were obtained by the Defren-O'Sullivan method. Therefore they probably exceed the true reducing sugars, and are of questionable significance. The climatic and analytical data are assembled in Figure 1.

It appears from the graphs that the time of day was less significant than temperature in determining differences of composition. The limited and questionable data for reducing sugars seem to be directly related to temperature variations. The soluble protein varies in an inverse manner to changes of temperature, and with particularly

marked effect on the cold morning of August 27. This is true also of the other higher forms of soluble, assimilated nitrogen designated as "rest-soluble." The insoluble protein varies in the same directions as temperature, while α-amino nitrogen varies with temperature in the afternoon samplings, but inversely to it in the morning. Ammonia varies in the manner of temperature throughout, but amide follows the course of amino acid. For some reason not apparent, the data for the evening of September 6 are erratic throughout as compared with other dates. It may be noted that a medium degree of radiation coincides with a high temperature efficiency on this date.

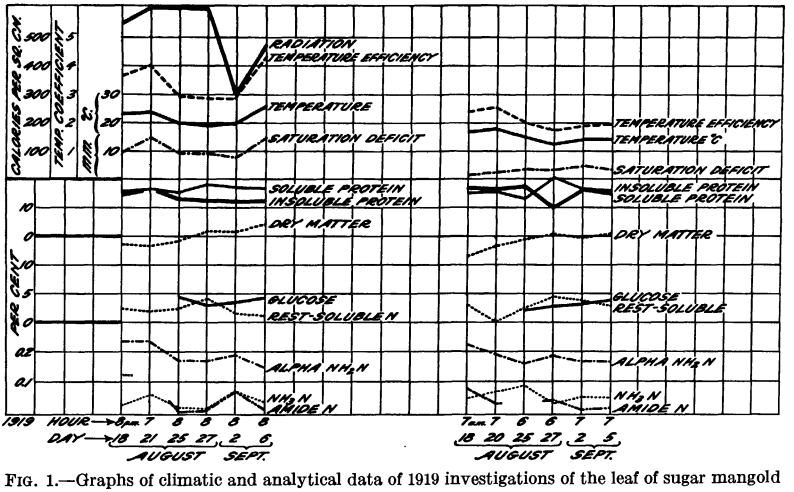

FIG. 1.—Graphs of climatic and analytical data of 1919 investigations of the leaf of sugar mangold

TABLE 3.—*Climatic data and analytical results for investigation of sugar-beet leaves, 1919*

Date	Climatic data				Dry matter	Analytical data of dry matter						
	Temperature	Temperature coefficient	Radiation calories per square centimeter	Vapor pressure deficit		Reducing sugars	Insoluble protein	Soluble protein	Rest-soluble N×5	α-Amino N	Amide N	Ammonia N
	°C.			Mm.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Aug. 18, 7 a. m	17.5	2.5	------	1.9	11.9	------	16.9	14.6	2.9	0.22	0.08	0.05
8 p. m	23.5	3.7	556	9.5	13.7	------	16.6	16.9	2.3	.24	.12	.03
Aug. 20, 7 a. m	17.8	2.5	------	2.3	13.4	------	16.7	15.2	.2	.19	.03	.07
Aug. 21, 7 p. m	24.4	4.0	597	14.3	13.6	------	17.3	17.4	2.1	.25	------	.06
Aug. 25, 6 a. m	14.4	2.0	------	4.0	14.6	2.5	17.0	12.5	2.6	.16	------	.08
8 p. m	20.0	2.9	592	9.4	14.6	4.1	13.5	15.6	2.5	.17	.01	.02
Aug. 27, 6 a. m	12.8	1.8	------	3.1	15.2	3.3	9.8	20.5	4.8	.19	.04	.03
8 p. m	19.4	2.8	585	9.2	16.1	3.2	12.0	18.5	4.1	.17	.02	.02
Sept. 2, 7 a. m	13.9	1.9	------	5.2	14.9	3.2	15.5	16.3	3.3	.17	.02	.05
8 p. m	20.0	2.9	284	7.8	16.1	3.4	11.8	17.9	1.5	.19	.06	.06
Sept. 5, 7 a. m	14.4	2.0	------	2.8	15.3	3.6	15.2	15.0	3.2	.17	.02	.05
Sept. 6, 8 p. m	26.1	4.5	480	14.1	17.3	3.9	12.4	16.3	1.0	.15	.02	.03

RESULTS OF 1920

The dates of sampling in this season were necessarily widely separated. This should be kept in mind in considering irregularities of the analytical data, particularly as regards the widely separated final date of September 29. The data are given in Table 4, and are shown graphically in Figure 2.

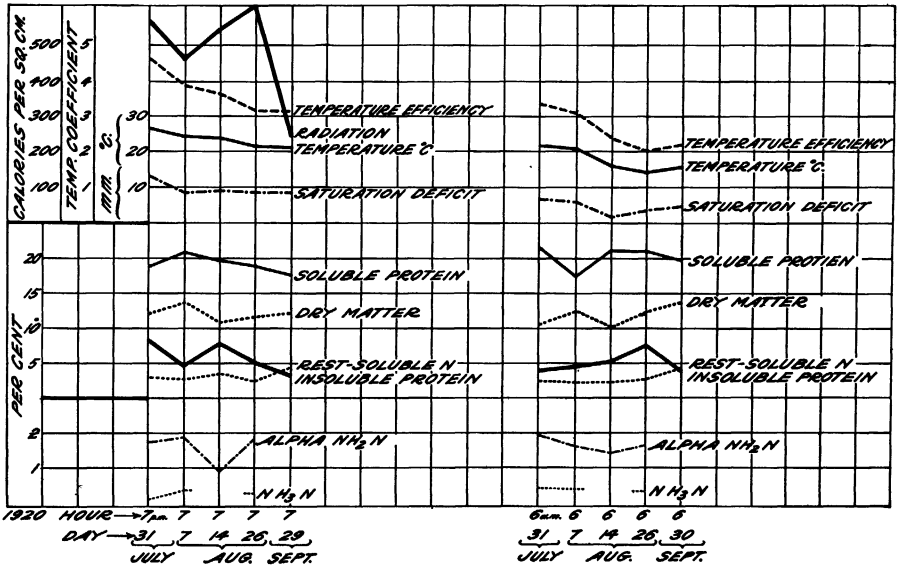

FIG. 2.—Graphs of climatic and analytical data of 1920 investigations of the leaf of sugar mangold

TABLE 4.—*Climatic data and analytical results for investigation of sugar-beet leaves, 1920*

Date	Climatic data				Dry matter	Analytical data of dry matter				
	Temperature	Temperature coefficient	Radiation calories per square centimeter	Vapor pressure deficit		Insoluble protein	Soluble protein	Rest-soluble N×5	α-Amino N	Ammonia N
	°C			Mm.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
July 31, 6 a. m.	21.7	3.30	-------	6.9	11.0	4.3	21.9	2.6	0.20	0.05
7 p. m.	26.7	4.67	570	12.2	12.5	8.1	18.8	3.1	.17	.02
Aug. 7, 6 a. m.	21.1	3.17	-------	6.3	12.8	4.8	17.5	2.2	.16	.04
7 p. m.	23.9	3.85	459	8.4	12.8	4.6	21.3	2.7	.18	.04
Aug. 14, 6 a. m.	16.7	2.33	-------	1.7	10.0	5.2	21.3	2.2	.14	.06
7 p. m.	23.3	3.70	535	9.6	11.3	7.5	19.4	3.6	.09	-------
Aug. 26, 6 a. m.	14.4	2.00	-------	3.4	12.5	7.5	21.3	2.5	.17	.03
7 p. m.	21.1	3.17	590	8.9	13.2	5.0	18.1	2.5	.18	.03
Sept. 30, 6 a. m.	16.1	2.25	-------	5.2	14.8	3.8	19.4	4.5	-------	-------
Sept. 29, 7 p. m.	21.1	3.17	254	8.5	14.5	3.1	17.5	4.5	-------	-------

In general, soluble protein varied in a manner inverse to temperature changes. This agrees with the results of 1919. Insoluble protein varied somewhat in the same direction as temperature in the evening samplings, but inversely so in the morning. The rest-soluble nitrogen varied irregularly, and apparently insignificantly. α-Amino nitrogen varied in a manner diametrically opposite to its behavior in 1919—that is, in the opposite direction to temperature in the afternoon but directly so in the morning. The data for NH_3 are too few to be significant. It is apparent that the scattered dates of sampling failed to produce consistent analytical results. The marked increase of rest-soluble nitrogen with advance of the season (September 29 to 30) is striking, and simulative of the low-temperature effect on proteins in 1919.

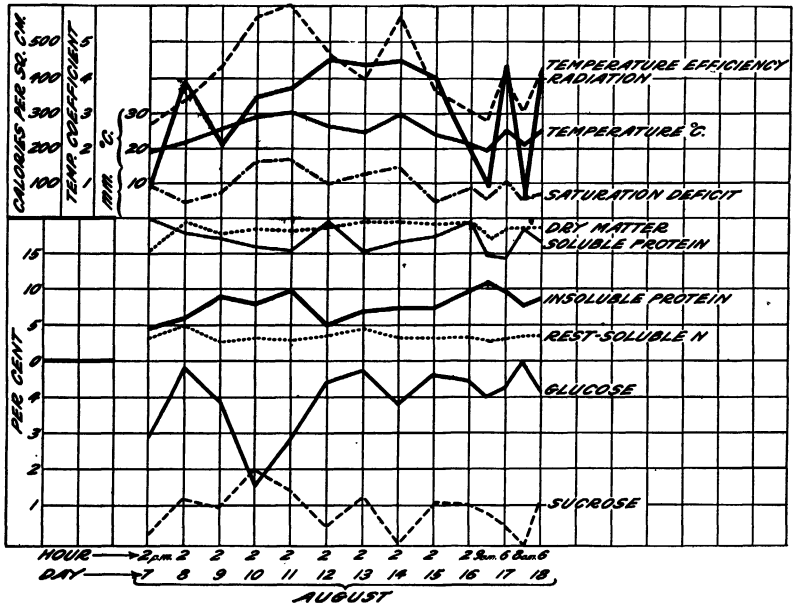

FIG. 3.—Graphs of climatic and analytical data of 1923 investigations of the leaf of sugar beet

RESULTS OF 1923

In 1923 the services of several workers were available. Even with this unusual amount of assistance it was necessary to abandon detailed separation of the nitrogenous constituents in order to maintain a schedule of two or more samplings per day. More importance was attached, however, to the uninterrupted daily samplings and analyses of petioles and roots, in addition to leaf blades. It appears, also, that it was possible to select more favorable hours of the day than in previous seasons for obtaining marked and significant differences of composition. The data are presented in Tables 5 to 8, and Figures 3 to 8, inclusive.

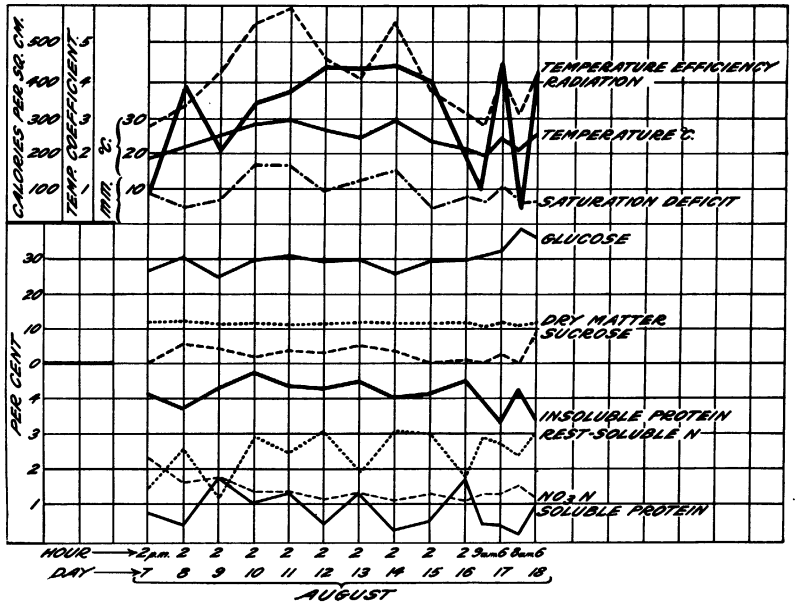

FIG. 4.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

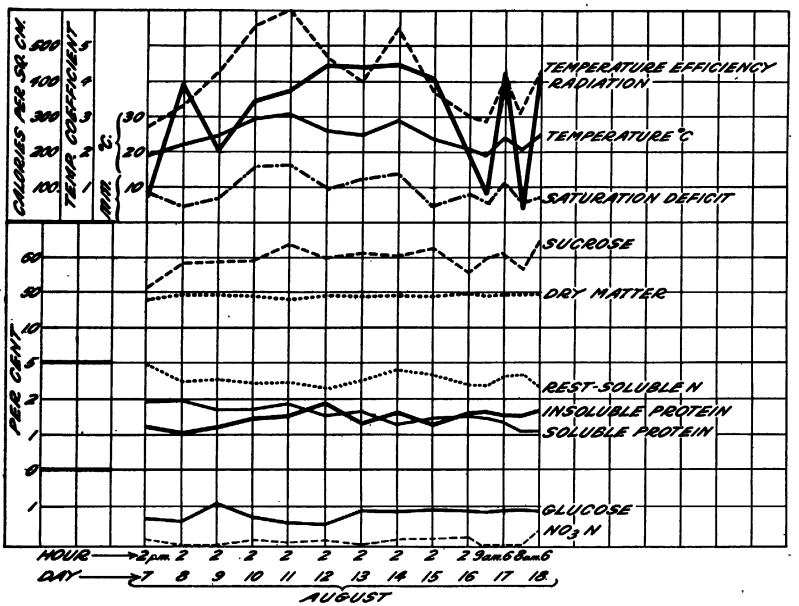

FIG. 5.—Graphs of climatic and analytical data of 1923 investigations of the root of sugar beet

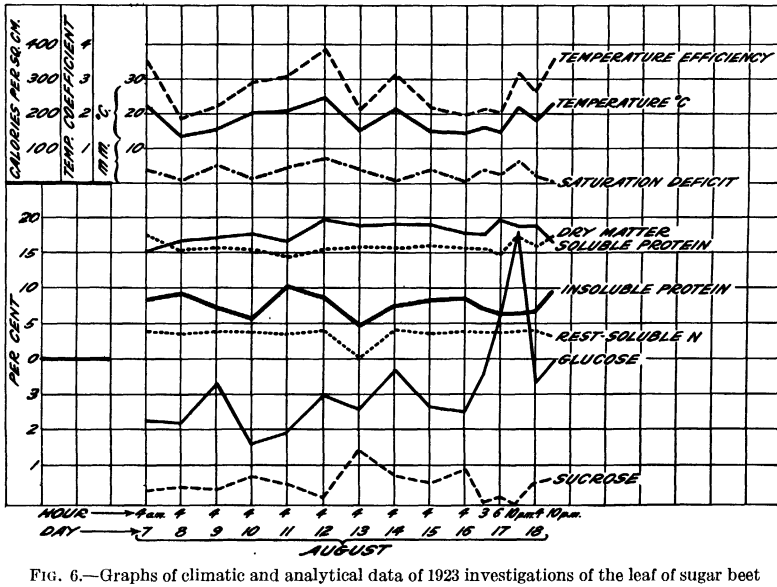

FIG. 6.—Graphs of climatic and analytical data of 1923 investigations of the leaf of sugar beet

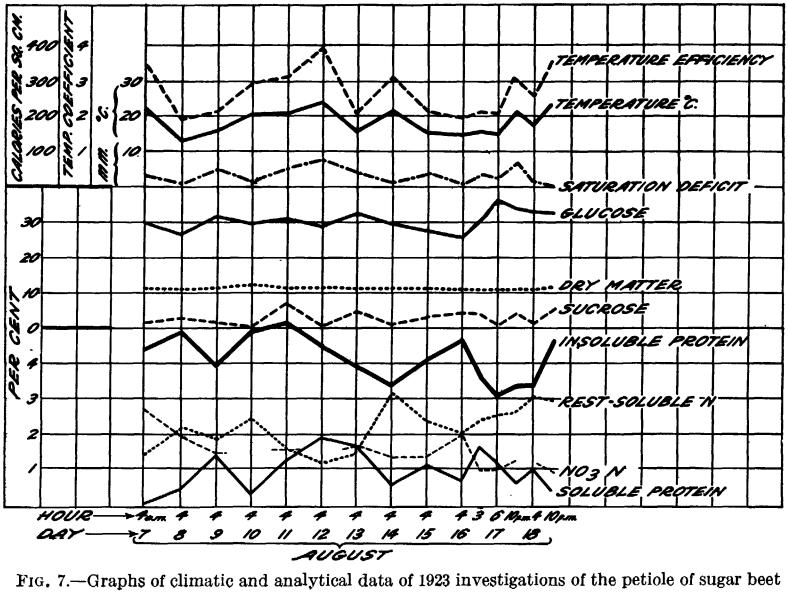

FIG. 7.—Graphs of climatic and analytical data of 1923 investigations of the petiole of sugar beet

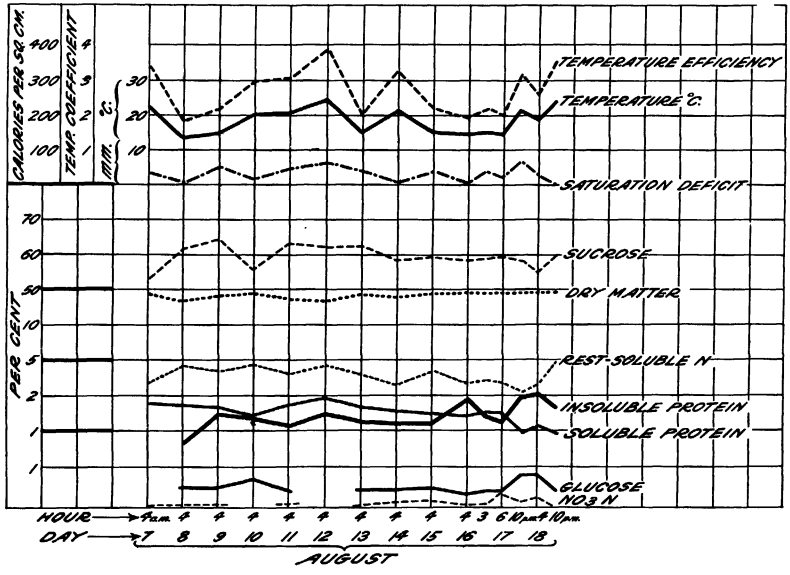

FIG. 8.—Graphs of climatic and analytical data of 1923 investigations of the roots of sugar beet

TABLE 5.—*Climatic data of sugar-beet sampling, 1923 (figs. 3 to 8)*

Date	Temperature	Temperature efficiency index	Vapor pressure deficit	Radiation, calories per square centimeter [2]	Date	Temperature	Temperature efficiency index	Vapor pressure deficit	Radiation, calories per square centimeter
	°C.		Mm.			°C.		Mm.	
Aug. 7, 4 a. m.	22.8	3.6	2.9	-------	Aug. 15, 4 a. m.	15.6	2.2	3.6	-------
2 p. m.	18.9	2.7	8.6	73	2 p. m.	23.3	3.7	4.5	403
Aug. 8, 4 a. m.	13.3	1.9	.9	-------	Aug. 16, 4 a. m.	13.9	1.9	.5	-------
2 p. m.	22.2	3.4	4.4	397	2 p. m.	20.6	3.1	8.5	207
Aug. 9, 4 a. m.	15.6	2.2	5.3	-------	Aug. 17, 3 a. m.	15.6	2.2	3.2	-------
2 p. m.	25.6	4.3	7.4	209	6 a. m.	14.4	2.0	2.3	-------
Aug. 10, 4 a. m.	20.0	2.9	1.7	-------	9 a. m.	19.4	2.8	5.7	90
2 p. m.	29.4	5.7	16.8	339	6 p. m.	25.0	4.2	10.3	435
Aug. 11, 4 a. m.	20.6	3.1	5.2	-------	10 p. m.	21.1	3.2	6.9	-------
2 p. m.	30.6	6.1	17.3	373	Aug. 18, 4 a. m.	18.3	2.6	2.0	-------
Aug. 12, 4 a. m.	23.9	3.9	7.1	-------	8 a. m.	20.6	3.1	5.4	40
2 p. m.	26.7	4.7	9.4	450	6 p. m.	25.6	4.3	6.8	411
Aug. 13, 4 a. m.	15.0	2.1	3.8	-------	10 p. m.	22.8	3.6	.0	-------
2 p. m.	24.4	4.0	12.3	434					
Aug. 14, 4 a. m.	21.1	3.2	.9	-------					
2 p. m.	29.4	5.7	14.3	447					

TABLE 6.—*Analytical data of sugar-beet leaf, 1923 (figs. 3 and 6)*

Date	Dry matter	Composition of dry matter				
		Insoluble protein	Soluble protein	Rest-soluble N×5	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m	17.5	8.1	15.0	3.3	2.3	0.3
2 p. m	15.7	4.9	20.0	3.0	2.9	.2
Aug. 8, 4 a. m	15.4	9.0	16.8	2.8	2.1	.4
2 p. m	19.0	6.2	18.1	3.1	4.8	1.2
Aug. 9, 4 a. m	16.0	7.5	17.3	2.9	3.3	.3
2 p. m	17.9	8.6	17.4	2.6	3.8	1.0
Aug. 10, 4 a. m	15.5	5.6	17.8	3.4	1.6	.7
2 p. m	18.0	7.8	16.5	3.3	1.4	1.9
Aug. 11, 4 a. m	14.0	10.0	16.1	3.0	1.9	.5
2 p. m	17.8	9.7	15.4	3.0	2.8	1.4
Aug. 12, 4 a. m	15.4	7.9	19.2	3.7	3.0	.2
2 p. m	18.4	4.8	19.1	3.6	4.3	.5
Aug. 13, 4 a. m	15.9	4.3	18.3	.2	2.6	1.4
2 p. m	19.2	6.9	15.3	4.5	4.7	1.3
Aug. 14, 4 a. m	15.6	7.1	18.9	3.6	3.7	.7
2 p. m	19.4	7.4	17.0	3.2	3.8	.0
Aug. 15, 4 a. m	16.1	8.0	18.3	3.4	2.6	.5
2 p. m	18.5	7.5	17.5	3.1	4.6	1.1
Aug 16, 4 a. m	15.5	8.2	17.6	3.5	2.4	.5
2 p. m	18.4	9.9	15.9	2.8	4.4	1.1
Aug. 17, 3 a. m	15.5	7.0	17.6	3.5	3.6	.0
6 a. m	14.6	6.2	19.7	3.4	5.1	.2
9 a. m	17.1	11.8	14.4	2.8	4.0	.9
6 p. m	18.2	9.8	14.1	3.0	4.3	.5
10 p. m	17.1	6.5	18.3	3.7	7.6	.0
Aug. 18, 4 a. m	16.3	6.8	18.8	3.8	3.2	.5
8 a. m	17.5	7.6	18.4	3.5	5.0	.0
6 p. m	17.8	8.6	16.6	3.5	4.2	1.1
10 p. m	17.3	9.9	16.6	3.1	4.0	.6

TABLE 7.—*Analytical data of sugar-beet petiole, 1923 (figs. 4 and 7)*

Date	Dry matter	Composition of dry matter					
		Insoluble protein	Soluble protein	Rest-soluble N×5	NO_3	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m	11.7	4.4	0.1	1.5	2.6	30.0	1.7
2 p. m	12.2	4.1	.7	1.5	2.4	27.0	1.0
Aug. 8, 4 a. m	11.5	4.9	.5	2.2	2.0	26.3	2.9
2 p. m	12.7	3.8	.4	2.6	1.6	30.4	6.4
Aug. 9, 4 a. m	11.9	3.9	1.5	1.8	1.6	31.3	1.7
2 p. m	12.1	4.3	1.8	1.2	1.8	25.8	5.4
Aug. 10, 4 a. m	12.9	4.9	.3	2.5	----------	29.2	.0
2 p. m	12.2	4.8	1.0	2.9	1.3	29.6	2.4
Aug. 11- 4 a. m	11.2	5.2	1.3	1.6	1.6	30.8	7.2
2 p. m	11.9	4.4	1.3	2.5	1.4	31.1	4.4
Aug. 12, 4 a. m	12.0	4.4	1.9	1.2	----------	28.5	.6
2 p. m	12.2	4.3	.4	3.1	1.2	29.8	3.2
Aug. 13, 4 a. m	11.4	3.9	1.4	1.6	1.7	32.8	5.1
2 p. m	12.5	4.6	1.3	1.8	1.4	30.0	6.0
Aug. 14, 4 a. m	11.2	3.4	.5	3.2	1.3	29.8	1.4
2 p. m	12.4	4.1	.3	3.2	1.2	26.3	3.7
Aug. 15, 4 a. m	11.4	4.1	1.1	2.3	1.4	28.5	3.0
2 p. m	11.8	4.1	.6	3.0	1.3	29.6	.4
Aug. 16, 4 a. m	11.2	4.7	.6	1.9	2.0	26.8	4.7
2 p. m	12.0	4.6	1.8	1.8	1.1	29.8	1.6
Aug. 17, 3 a. m	11.2	3.5	1.6	2.4	1.0	31.3	4.3
6 a. m	10.9	3.0	1.1	2.6	1.0	36.6	.1
9 a. m	11.1	3.9	.5	2.8	1.3	31.1	.0
6 p. m	12.2	3.3	.4	2.7	1.3	32.8	2.7
10 p. m	11.2	3.3	.7	2.7	1.3	34.1	3.7
Aug. 18, 4 a. m	11.0	3.3	.9	3.1	----------	32.3	1.5
8 a. m	11.3	4.3	.2	2.4	1.6	38.7	.0
6 p. m	11.9	3.4	.9	3.0	1.2	26.1	9.6
10 p. m	12.0	4.6	.3	2.9	0.9	32.2	5.3

TABLE 8.—*Analytical data of sugar-beet root, 1923 (fig. 5 and 8)*

Date	Dry matter	Composition of dry matter					
		Insoluble protein	Soluble protein	Rest-soluble N×5	NO_3	Glucose	Sucrose
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Aug. 7, 4 a. m.	18.4	----------	1.8	2.4	0.0	----------	53.3
2 p. m.	18.0	1.1	1.9	2.9	.2	0.7	51.9
Aug. 8, 4 a. m.	17.4	0.6	1.8	2.9	.0	.5	63.0
2 p. m.	18.6	1.0	1.9	2.5	.0	.6	58.1
Aug. 9, 4 a. m.	17.9	1.6	1.7	2.7	.1	.5	64.7
2 p. m.	18.5	1.3	1.7	2.6	.0	1.1	59.2
Aug. 10, 4 a. m.	18.6	1.3	1.3	2.9	----------	.6	56.9
2 p. m.	18.2	1.5	1.7	2.4	.2	.7	59.0
Aug. 11, 4 a. m.	17.3	1.1	1.8	2.6	.1	.4	63.0
2 p. m.	17.6	1.6	1.8	2.4	.1	.6	63.3
Aug. 12, 4 a. m.	16.9	1.5	1.9	2.9	----------	----------	61.5
2 p. m.	18.3	1.9	1.6	2.3	.2	.5	60.0
Aug. 13, 4 a. m.	17.8	1.2	1.7	2.6	.0	.4	61.8
2 p. m.	18.3	1.3	1.7	2.5	.04	.9	61.4
Aug. 14, 4 a. m.	17.6	1.2	1.6	2.3	.2	.4	58.5
2 p. m.	18.7	1.6	1.3	2.8	.2	.9	60.0
Aug. 15, 4 a. m.	18.2	1.2	1.4	2.8	.2	.4	59.3
2 p. m.	18.7	1.3	1.4	2.7	.2	.9	62.5
Aug. 16, 4 a. m.	18.8	1.8	1.4	2.3	.04	.3	58.0
2 p. m.	19.5	1.6	1.6	2.4	.2	.9	55.1
Aug. 17, 3 a. m.	17.9	1.4	1.6	2.5	.2	.4	58.6
6 a. m.	18.1	1.2	1.5	2.4	.4	.4	59.6
9 a. m.	18.9	1.7	1.4	2.4	.0	.8	59.6
6 p. m.	19.1	1.5	1.3	2.6	.0	.9	60.9
9 p. m.	18.4	2.0	0.9	2.1	.2	.8	58.1
Aug. 18, 4 a. m.	18.6	2.1	1.1	2.3	.3	.8	55.0
8 a. m.	19.2	1.5	1.1	2.7	.04	.9	56.0
6 p. m.	19.2	1.6	1.1	2.2	.4	.8	64.0
10 p. m.	19.3	1.7	0.9	3.1	.0	.4	60.0

Computations were made of the coefficients of correlation between individual climatic factors and various plant constituents. In only one case, however, was a significant relation found—namely, soluble protein of the leaf showed a negative correlation with temperature of $r = -0.390 \pm 0.106$. It is not surprising that in the case of the sugars at least, the averaging of values for periods of light with those for darkness failed to show correlations. One might well anticipate that synthetic effects of the former period would be offset by assimilative processes of the latter. In view of these conditions the graphs have been divided, as for the data of preceding seasons, into periods corresponding to light and darkness.

EFFECTS IN DAYLIGHT

EFFECTS IN THE LEAF

It seems that one of the most important features of the present investigation of the leaf concerns reducing sugars. The values for afternoon samplings show (fig. 3) that the percentage of reducing sugars expressed as glucose follow, in general, the variations of solar radiation. The depressions of reducing sugars on the 10th and 14th days, when radiation was at a high level, are associated with marked increased of the temperature efficiency. These relations seem to indicate that temperatures approaching 30° C. serve to reduce the apparent synthetic effects of a high plane of illumination. Comparison with the data for 1919 (fig. 1) indicates that radiation is here producing its maximum effect, and that somewhat under 5 per cent of reducing sugars may represent the maximum accumulation by this species of plant under these particular climatic conditions. This agrees with Campbell's values from the

sugar mangold (*6*), and those of Davis, Daish, and Sawyer obtained in the warm weather of August (*9*). The latter, however, found up to 12 per cent of hexoses in the cool weather of October.

Sucrose varied irregularly. Over a considerable portion of the period investigated it followed the course of reducing sugars, but at other times, especially from August 9 to 12, it varied inversely as the latter sugar. In view of these results it appears that glucose, as the preponderant form of reducing sugars, fluctuates more like a primary product of carbon assimilation than does sucrose.

The soluble protein in the leaf blades varied distinctly in a direction inverse to temperature. This is in agreement with the results of the previous seasons. Insoluble protein varied irregularly, but, with the exception of the period August 14 to 17, it fluctuated parallel to temperature, and hence inversely to soluble protein. This suggests a relation logically to be expected—namely, interconversion between soluble and insoluble protein. It appears significant, also, that insoluble protein varies inversely as reducing sugars, suggesting the possibility of the sequence: Hexose—soluble protein—insoluble protein. The relatively slight variations of rest-soluble nitrogen are also in the direction of those for reducing sugars, suggesting a direct relation of such carbohydrates to synthesis of these forms of nitrogenous compounds.

As the dry matter is relatively constant and shows no pronounced effects of saturation deficit of the air, it may be concluded that the latter factor had little influence in producing the effects analyzed here.

EFFECTS IN THE PETIOLE

In the petiole the variations of dry matter are insignificant, the effects of the saturation deficit of the air appearing to be negligible. The percentage of reducing sugars shows relatively little variation, but there is a distant correspondence to variations of reducing sugars in the leaf. It is partially related in the inverse direction to temperature changes. The small amounts of sucrose vary roughly in the same direction as reducing sugars. They therefore correlate with the monosaccharides of the leaf and not with its sucrose content. It appears from the data that invert sugar is the migratory form of carbohydrates, as generally observed by others.

The soluble protein in the petiole varies irregularly, but seems to be inversely related to radiation. Insoluble protein is generally variable with the soluble form. The rest-soluble nitrogen is distinctly related inversely to soluble protein. It appears to be positively related to temperature, hence varying inversely to the fluctuations of reducing sugars. Nitrate shows an inverse relation to reducing sugars and to rest-soluble nitrogen, while largely parallel to soluble protein. It thus appears that NO_3 is transformed to rest-soluble forms of nitrogen as hexoses accumulate, but that the soluble and insoluble proteins diminish simultaneously.

EFFECTS IN THE ROOT

Variations of the dry matter in the root are negligible and incapable of modifying metabolism. The percentage of reducing sugars varies appreciably in the forepart of the period of observation, as if depressed by rising temperature, but it is almost constant later, despite sharp variations of both temperature and illumination. Sucrose shows a distinct correlation with radiation. As

might be expected in a storage organ highly charged with this reserve, the variations were proportionately much less than those of the leaf. This constituent seems to follow primarily the variations of reducing sugars of both leaf and petiole, rather than those of sucrose therein.

Soluble protein shows here a response generally inverse to temperature changes, while insoluble protein varied relatively directly with this factor. Where there are disagreements, as on August 11 to 13, the response seems to be delayed. The rest-soluble nitrogen varies irregularly. Aside from August 14 to 17, it appears to be inversely related to temperature, and hence correlated with the formation of soluble protein. The variations of nitrate are suggestive of an inverse relation to reducing sugars in the fore period and to rest-soluble nitrogen later. However, the amount is too small to be interpreted as being of significance, especially in view of the possible complication from equilibrium in the root between nitrate diffusing backward from the petiole and that undergoing translocation from the soil.

EFFECTS IN DARKNESS

EFFECTS IN THE LEAF

With the exception of August 9 and the afternoon hours of August 17, the percentage of reducing sugars in the leaf varies with temperature. The marked aberrations of August 9 and excessive effect of the evening of August 17 may well be due to the high illumination of the preceding daylight period. Sucrose varies consistently in a manner inverse to temperature, and hence inversely to reducing sugars. Variations of soluble protein show a tendency to follow temperature at the fore period of growth, but are not distinctly correlated therewith. With the exception of August 13 and 14, insoluble protein varies in a manner inverse to temperature changes. Thus, the proteins of the leaf tend to show by night a temperature response inverse to that of the daytime. The rest-soluble nitrogen shows only one distinct variation (on August 13), and that is parallel to the changes of temperature.

EFFECTS IN THE PETIOLE

The reducing sugars in the petiole vary irregularly with reference to temperature. Sucrose behaves similarly. Excepting the dates from August 11 to 13, soluble protein varied in an inverse relation to temperature. Insoluble protein also varied chiefly in the above manner. Thus these compounds show the same trend in the petiole as by day. The rest-soluble nitrogen varies quite irregularly. Nitrate trends toward an inverse relation to temperature. It therefore shows a rather distinct inverse relation to rest-soluble nitrogen and to reducing sugars.

EFFECTS IN THE ROOT

The percentage of reducing sugars in the root shows no correlation with climatic factors. Sucrose varies in a manner inverse to temperature changes. The latter sugar, therefore, follows its own variations in the leaf. Soluble protein shows a somewhat inverse relation to temperature, and the same is true of insoluble protein. Rest-soluble nitrogen is also irregular, but with a tendency to follow the course of temperature. The slight amounts and variations of nitrate can not be considered significant.

Of the preceding particulars, the following relations appear to be most definite: During exposure to daylight the reducing sugars increase in the leaf with illumination, when the temperature remains well below 30° C. Rest-soluble nitrogen varies parallel to reducing sugars. The relative amounts of these various compounds suggest the sequence: Reducing sugars—amino acids—soluble proteins—insoluble proteins. Simultaneously, the sugars of the petiole are determined somewhat by the variations of reducing sugar in the leaf, but the proteins show little correlation with climatic factors. The variations of nitrates and rest-soluble nitrogen are such as to indicate the sequence: Reducing sugars—rest-soluble nitrogen. Sucrose in the root responds to the variations of reducing sugars in leaf and petiole. Protein is stored in insoluble form at higher temperatures. In general, the results show the delayed response to be expected between leaf and root.

In darkness, the reducing sugars of the leaf increase at the expense of sucrose as the temperature rises. Simultaneously, the proteins also undergo hydrolysis and migrate to the petiole. In the latter organ the same status of proteins is maintained, indicating further migration of these compounds to the root at higher temperatures. The variations of nitrate in the petiole suggest that these, in conjunction with reducing sugars, are converted to rest-soluble forms, although this synthetic process may occur in the leaf blade. Sucrose in the root shows an inverse response to temperature during darkness, but proteins are less definitely influenced.

DISCUSSION

Since the early investigations of Brown and Morris, the nature of the first sugar produced in carbon assimilation of chlorophyllous plants has been a mooted question. These workers worked with leaves of nasturtium dried in a steam-heated oven. Fortunately, this tissue dries rapidly, so that the chance of caramelizing is relatively low. The analytical methods employed were the most reliable then extant. As a result of marked accumulation of sucrose during illumination by sunlight, while the reducing sugars varied irregularly, Brown and Morris (*2*) inclined to the belief that the former was the first sugar of photosynthesis. Parkin (*15*) followed the distribution of sugars in the leaf of the snowdrop (*Galanthus nivalis*). On exposure of etiolated plants and of detached leaves to daylight, sucrose increased very decidedly while the percentage of hexoses remained rather constant. The ratio of sucrose to hexoses decreased from the tip of the leaves toward the base, and increased as the season advanced. Parkin suggests it is quite possible that sucrose is the first tangible product of carbon assimilation.

Strakosch (*21*) observed, by means of the osazone reaction, that glucose seemed to be the first sugar formed in the mesophyll of the sugar-beet leaf. The reduction test with Fehling's solution upon tissue extracts showed a great preponderance of hexoses over sucrose in the leaf. Upon exposure to sunlight after prolonged etiolation the hexoses of the leaf were rapidly converted to sucrose. Similar observations led Dixon and Mason (*10*) to suggest that hexoses are synthesized in the protoplasm, sucrose being condensed therefrom and excreted into the vacuoles.

Campbell (*6*), on the basis of reliable methods of extraction and analysis, but with admittedly too few data to be conclusive, obtained

evidence that an increase of hexoses precedes that of sucrose in leaves of the sugar mangold with the advent of daylight periods.

Davis, Daish, and Sawyer (*9*) present data for the distribution of carbohydrates in leaves of the mangold at intervals of two hours. These cover three days from August 26 to October 11. The variations of sucrose exceed those of the hexoses. On account of the low temperatures prevailing on their later dates of examination, their data (Table 9) are not comparable with those of the present writers. The results of August 26–27 are shown graphically in

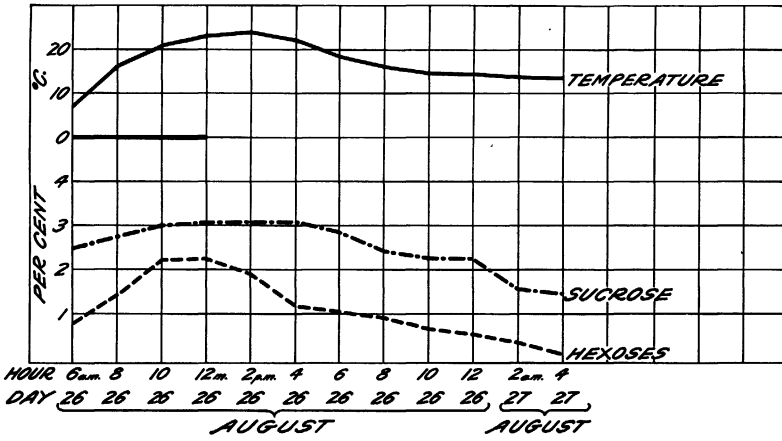

FIG. 9.—Graphs illustrating data of Davis, Daish, and Sawyer on distribution of carbohydrates in leaf of the mangold at intervals of two hours, Aug. 26–27

Figure 9. The percentage of hexoses declined after 10 a. m., when the temperature had reached 21°C. The total sugars also attained a maximum value by 10 a. m., and this plane was maintained until toward 2 p. m. Because of the generally greater variation of hexoses than of sucrose, these investigators consider that their results support the conception of the latter sugar as the primary one of carbon assimilation. As to the relative response of the sugars to the change from darkness to daylight, their results are inconclusive.

TABLE 9.—*Data from Davis, Daish, and Sawyer for Figure 9*

Date	Temperature	Hexose	Sucrose	Date	Temperature	Hexose	Sucrose
	°C.	*Per cent*	*Per cent*		°C.	*Per cent*	*Per cent*
Aug. 26, 6 a. m.	7.2	0.8	2.5	Aug. 26, 6 p. m.	18.9	1.0	2.8
8 a. m.	16.8	1.4	2.8	8 p. m.	16.1	.9	2.4
10 a. m.	21.1	2.2	3.0	10 p. m.	14.7	.9	2.2
12 a. m.	22.9	2.2	3.1	12 p. m.	14.4	.6	2.2
2 p. m.	23.9	1.9	3.1	Aug. 27, 2 a. m.	13.9	.4	1.6
4 p. m.	21.7	1.2	3.1	4 a. m.	13.3	.2	1.5

The accumulation of sucrose while hexoses are decreasing possibly may be explained by the respiratory effect of relatively high temperatures. Brown and Morris (*2*) estimated that glucose was utilized to a greater extent than fructose in respiration. Lindet (*12*) grew the embryo of barley on invert sugar, and found a utilization of 17 to 70 per cent more glucose than fructose. If this is true generally,

one can understand what prompted Boysen-Jensen (*1*) to make his observation on the accumulation of sucrose in germinating peas. If fructose accumulates, even though the plane of glucose is reduced, it would seem quite possible for the condition of equilibrium between these sugars to induce the formation of sucrose.

Thoday (*23*) quotes Broocks' results from weighings of leaf sections which indicate the cessation of photosynthesis at midday. Thoday ascribed this to engorgement of the leaf tissue with photosynthetic products. It would seem, recognizing limitations of Broocks' method of measurement and his unknown temperature relations, that the effect here discussed might have been due to an excess effect of respiration over synthesis. Spoehr (*20, pt. 2*) has shown the interrelation between respiration and photosynthesis of carbohydrates in leaves of the bean and sunflower. He found that an increase of photosynthesis definitely stimulated respiration. It appears that this effect must be taken into account in interpreting the results of others cited here and those of the present writers which indicate that high temperatures cause a depression in the percentage of reducing sugars in leaf-blade tissue. Moreover, if one considers the criticism of Brown and Heise (*3, 4*), the rate of carbon assimilation is not augmented much by an increase of temperature and it decreases with increasing illumination. Thus, in the writers' results for 1923 the overstepping of the most efficient light intensity may have operated in conjunction with higher temperatures to produce the limited content of reducing sugar attained. The writers interpret their results as indicating that these sugars are among the primary products appearing in carbon assimilation.

The writers recognize the claim of Chibnall (*7*) that computations based upon fresh weight of tissue overcome errors incident to determinations based upon dry weight. Nevertheless, they are unable to comprehend how the former method of calculation could alter the proportions of constituents when the moisture content fluctuates little. It is likewise difficult to conceive how allowance for large variations in moisture content could compensate differences in the rate of either synthesis or translocation of constituents in the leaf dry matter. Even the method of determining diurnal changes in tissue components by computation of absolute amounts per individual leaf can not be considered reliable, because of the possible removal or alteration of compounds contemporaneously with synthesis of the same. The latter factors might be compensated in part by includiug analyses of the other organs of the plant with those of the leaf. When computed to the basis of fresh weight of tissue, the writers' data of Table 6 show a corresponding increase of sugars in the afternoon. None of the relative diurnal values for other constituents are altered, except in the case of soluble protein, in which case the percentage is higher in the afternoon than in the morning on 9 of the 12 dates. As shown by their preceding statements, however, the writers do not consider this relation final proof of maximum synthesis of protein in daylight. In view of the limitations imposed upon separation of products of catabolism from those of anabolism, it seems necessary to consider simultaneous changes of composition in different organs of the plant. This situation greatly complicates the problem considered here. The writers have attempted to deal with it in the preceding survey of their results.

The results reported here give promise that it will be possible to determine the effects of specific climatic factors upon metabolism by means of chemical analysis of the plant tissue at frequent intervals, accumulating sufficient data to justify the computation of correlation coefficients. Perhaps conclusive results can be obtained in this way as surely as by the use of special equipment for the control of environmental factors, more particularly of temperature, atmospheric humidity, and illumination. The capacity of this type of equipment for plant yields must remain quite limited, except at exorbitant cost. Even when results have been tested under such artificial conditions, there remains the problem of determining to what extent the effects discovered obtain under the climatic complex to which field practice is subject.

SUMMARY

Results are presented for diurnal changes of chemical composition in leaf blades of the sugar mangold and sugar beet, and in the petiole and root of the latter plant.

The percentage of reducing sugars in the leaf blade increases with solar radiation, within limits. Temperature seems to be a limiting factor in the increase of these sugars when its value approaches 30° C.

The percentage of soluble protein in the leaf varies in a manner inversely related to temperature and therefore correlated with the fluctuation of reducing sugars. This constituent gave a correlation with temperature of -0.390 ± 0.106 for periods of light and darkness combined.

The foliar fluctuations of reducing sugars are distantly paralleled by the deposition of sucrose in the root, while relatively high temperatures increase the percentage of protein stored in this organ.

The relations in the petiole are such as to suggest that a high plane of reducing sugars in the plant, together with the presence of nitrates, leads to the formation of amino acids and rest-soluble nitrogen.

These results explain practical observations that cool, fair weather, such as that common to the autumn season, is favorable to the storing of high percentages of sugar in the root of the sugar beet.

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