CARBOHYDRATE PRODUCTION IN HEALTHY AND IN BLIGHTED SPINACH

By RODNEY H. TRUE, *Physiologist in Charge*, and LON A. HAWKINS, *Physiologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

Spinach plants affected with the blight show many symptoms pointing to a derangement of functions concerned with the carbohydrates, among which are the yellowish-green color and the sweetish taste. It was therefore deemed important to investigate these constituents in normal and in diseased plants. The material was taken from commercial fields near the Virginia Truck Experiment Station. The plants were carefully dug with a spade, and the soil adhering to the roots was quickly washed off. To reduce the translocation of products, the plants were divided into roots and tops, and after being loosely packed in market baskets were covered with paper and stored in a building in diffused light. The collection took place late in the forenoon on February 5, 1915, a clear day.

The samples were kept in a cool place while in transit and were taken to the laboratory at 7. 30 the next morning, where they were given immediate attention.

Starch, sucrose, and reducing sugars were determined by the usual methods. The results of these determinations are presented in Table I as percentages calculated on both fresh and dry weights of tops and on the fresh weights of the roots.

It will be noted that the samples of diseased tops have a somewhat greater percentage of dry matter than the healthy tops.

The reducing sugars under the conditions here given are clearly less abundant in the tops of blighted plants than in the normal samples, while in the roots but a trace is present in either type of material. The situation with reference to the sucrose in the tops, however, is quite the reverse, the diseased plants containing a considerably greater quantity than those in health. This difference is so great as to give a much higher total sugar content for the pathological material, a fact which in part accounts for the strikingly sweet taste found in the latter plants. It is of interest, however, to note that no noticeable sweetness is found in the normal leaves, although in the material collected in 1915 they contain nearly 80 per cent as much total sugars and nearly 65 per cent as much sucrose. Since the taste of sweetness is interfered with by a variety of other taste sensations, it is possible that certain substances having a marked taste may be present in the normal material and absent in that affected by the disease. On tasting the fresh material in the field it seemed that the characteristic "spinach" taste so strongly marked in the healthy leaves was almost lacking in the sweet diseased leaves.

TABLE I.—*Carbohydrates in healthy and in blighted spinach*

Carbohydrate.	Spinach tops.								Spinach roots, 1915.	
	Wet weight.				Dry weight.					
	Normal		Diseased.		Normal.		Diseased.		Wet weight.	
	1915	1916	1915	1916	1915	1916	1915	1916	Normal.	Diseased.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Dry weights of material.	.....	12. 53	.....	17. 43	.....	.....	.....	.....	.....	.....
	.....	12. 87	.....	16. 96	.....	.....	.....	.....	.....	.....
	11. 90	13. 42	11. 90	16. 10	.....	.....	.....	.....	.....	.....
	11. 50	13. 95	12. 50	18. 56	.....	.....	.....	.....	.....	.....
Average.....	11. 70	13. 19	12. 20	17. 26	.....	.....	.....	.....	.....	.....
Reducing sugars.....	. 49	. 28	. 52	. 26	4. 10	2. 23	4. 26	1. 49	.....	.....
	. 70	. 26	. 47	. 29	5. 98	2. 01	3. 85	1. 71	Trace.	Trace.
	. 69	. 25	. 45	. 26	5. 89	1. 86	3. 68	1. 61	.....	.....
	. 49	. 22	. 44	. 24	4. 10	1. 58	3. 60	1. 29	.....	.....
Average.....	. 59	. 25	. 47	. 26	5. 02	1. 89	3. 85	1. 50	.....	.....
Total sugars..... ...	1. 46	2. 48	2. 08	4. 77	12. 47	19. 79	17. 04	27. 36	.....	.....
	1. 36	1. 88	2. 18	5. 22	11. 62	14. 61	17. 86	30. 37	4. 07	6. 42
	1. 16	1. 60	1. 89	5. 49	9. 91	11. 92	15. 49	34. 10	5. 94	4. 17
	2. 03	2. 33	1. 83	4. 74	17. 36	16. 70	15. 00	25. 53	.....	.....
Average.....	1. 50	2. 07	1. 99	5. 05	12. 84	15. 69	16. 35	29. 26	5. 00	5. 30
Sucrose calculated by difference.....	. 93	2. 20	1. 50	4. 51	7. 94	17. 56	12. 29	25. 87	.....	.....
	. 63	1. 62	1. 64	4. 93	5. 44	12. 60	13. 44	29. 07	.....	.....
	. 45	1. 35	1. 38	5. 23	3. 84	10. 06	11. 31	32. 49	.....	.....
	1. 47	2. 11	1. 33	4. 50	12. 56	15. 12	10. 90	24. 24	.....	.....
Average.....	. 88	1. 82	1. 46	4. 79	7. 44	13. 80	11. 98	27. 76	.....	.....
Starch.....	. 95	1. 06	1. 53	1. 72	8. 11	8. 45	12. 54	9. 87	.....	.....
	. 79	1. 13	1. 51	1. 93	6. 75	8. 78	12. 37	11. 38	2. 03	2. 26
	. 73	. 99	1. 48	1. 57	6. 24	7. 38	12. 13	9. 75	2. 51	2. 58
	. 88	. 99	1. 51	1. 98	7. 52	7. 10	12. 37	10. 67	.....	.....
Average.....	. 84	1. 04	1. 51	1. 80	7. 15	7. 88	12. 35	10. 43	2. 27	2. 42

The starch content of the diseased tops is somewhat more than double that of the normal material. In the roots, both total sugars and starch were practically alike in both types of material.

From these results it appears justifiable to conclude that the cause of injury does not affect the machinery of photosynthesis or the materials used in carbohydrate manufacture to such an extent as to stop production. That this is carried on with equal efficiency in all parts, or with normal efficiency even in any part of the leaf, however, can not be stated. Indeed, the yellowish-green color representing an apparent reduction of chlorophyll would seem likely to go with a decreased activity in the

photosynthetic function. This condition recalls that of tobacco leaves when "mature" for cutting. The color changes to a more yellowish green, the leaves take on the brittle character seen in the diseased spinach and like it become gorged with starch.

It would hardly be safe to assume that photosynthetic activity is not impaired in the blighted plants in spite of the accumulation of carbohydrates. It is quite possible that impairment may be the case and that accumulation results from some interference with carbohydrate utilization.

In view of the destructive action of oxidases on diastatic enzyms, reported by Woods (*51*) in the case of tobacco mosaic, it was thought possible that here a somewhat similar situation was present. Since Bunzell in his investigation on this subject found the oxidase reaction with most reagents to be somewhat more intense in the diseased material, both leaves and roots, than in normal samples, it was thought necessary to determine the comparative diastatic activity of juices from these two types of material. The fresh leaves, after being ground in a mortar, were placed in a flask having a volume of 250 cc. and digested for 24 hours with 100 cc. of glycerin in an ice box at a temperature of about 10° C. This was then made up to volume, strained, and 50 cc. of the solution was added to 25 cc. of 1 per cent soluble starch. Controls were made in the same way from each sample to which no starch paste was added. One cc. of toluol was added to each flask to prevent the action of microorganisms. All preparations were allowed to stand at 30° C. for 48 hours, after which they were removed, cleared with lead acetate, made up to 100 cc. and filtered. The data given in Table II show the quantity of reducing sugar present in the preparations containing starch paste in excess of the controls from the same samples.

TABLE II.—*Diastatic activity in normal and in blighted spinach*

Date of collection.	Quantity used.	Glucose.	
		Normal leaves.	Blighted leaves.
	Gm.	*Gm.*	*Gm.*
February, 1915	100	0.316	0.308
Do	100	.304	.312
Do	100	.340	[a] .528
Average		.320	.310
March, 1916	50	.0130	.0142
Do	50	.0167	.0155
Do	50	.0172	.0121
Average		.0117	.0106

[a] Extracted with larger volume and calculated to the same basis as the others.

These results seem to point to the absence of any marked difference in the starch-digesting capabilities of normal and blighted spinach. This being the case it would seem to be indicated that the cause of carbohydrate accumulation should be sought in the deeper-lying metabolic processes in connection with which carbohydrates are utilized.

To recapitulate, it appears that in spinach-blight the process of carbohydrate manufacture is not inhibited, although it may be retarded. The reducing sugars are practically absent from the roots of all plants, while in the tops the normal plants contain somewhat more than the diseased. Both sucrose and starch are present in the leaves of the blighted plants in markedly greater quantity than in those of the normal plants. They are found in the roots of both healthy and diseased plants in approximately like quantities.

Determinations of diastatic activity failed to bring out any marked difference between healthy and diseased plants.

It is indicated that carbohydrate accumulation is due not to a breakdown of digestion but to some partial failure in the subsequent metabolic processes in connection with which carbohydrates are used.