SOME CHEMICAL REACTIONS OF SULPHUR DIOXIDE AFTER ABSORPTION BY ALFALFA AND SUGAR BEETS[1]

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(WITH TWO FIGURES)

Introduction

When sulphur dioxide enters the intercellular space of a leaf it encounters moist surfaces upon which, on account of its great solubility in water, it is readily absorbed, forming, presumably in the first instance, sulphurous acid. The latter appears to be neutralized as it enters the cells by the inorganic and organic bases in the cells or it may unite with aldehydes to form addition products. Subsequently there is oxidation to sulphate. The rate of this oxidation is indicated by the fact that sulphur dioxide has been recovered from the leaf tissue by acid distillation for several hours after a severe fumigation (**3**). In fumigations with low concentrations of sulphur dioxide the oxidation to sulphate evidently occurs about as rapidly as absorption because in this case sulphur dioxide cannot be recovered by acid distillation. A small part of the sulphite or sulphate may be reduced and incorporated into organic sulphur compounds such as cystine and methionine, and others. Formation of cystine and methionine would suggest the enrichment of the protein with sulphur.

In this paper evidence is submitted regarding first, the neutralization reactions and second, the production of organic sulphur compounds. The work is based on long-continued, low-concentration fumigations of alfalfa in which acute leaf injury was largely avoided. Some data are also included for sugar beets similarly treated. In some of the experiments, the fumigations were continued until the older leaves had accumulated sulphate to the limit of their capacity and had become chlorotic. Additional evidence regarding the production of organic sulphur compounds, obtained by the use of radioactive sulphur as a tracer element, is reported in the following paper (**5**).

Experimentation

EFFECT OF ABSORPTION OF SULPHUR DIOXIDE ON THE BUFFER CAPACITY OF THE LEAVES

In 1935-1936 an extensive series of experiments was carried out at Logan, Utah, on the fumigation of alfalfa with sulphur dioxide. These experiments have already been described in part (**8**). As one phase of this investigation a chemical study of the leaf tissue was undertaken, for the purpose of determining the disposition of the sulphur dioxide after absorption. Total sulphur analyses of these plots were published earlier (**8**) and a sulphur fractiona-

[1] This is the fifth of a series of papers on "The Effect of Prolonged Low Concentrations of Sulphur Dioxide Upon Plants."

tion is presented later in this paper (tables II and III). It is of interest to know how the plants reacted to the acidity of these relatively large quantities of acid gas.

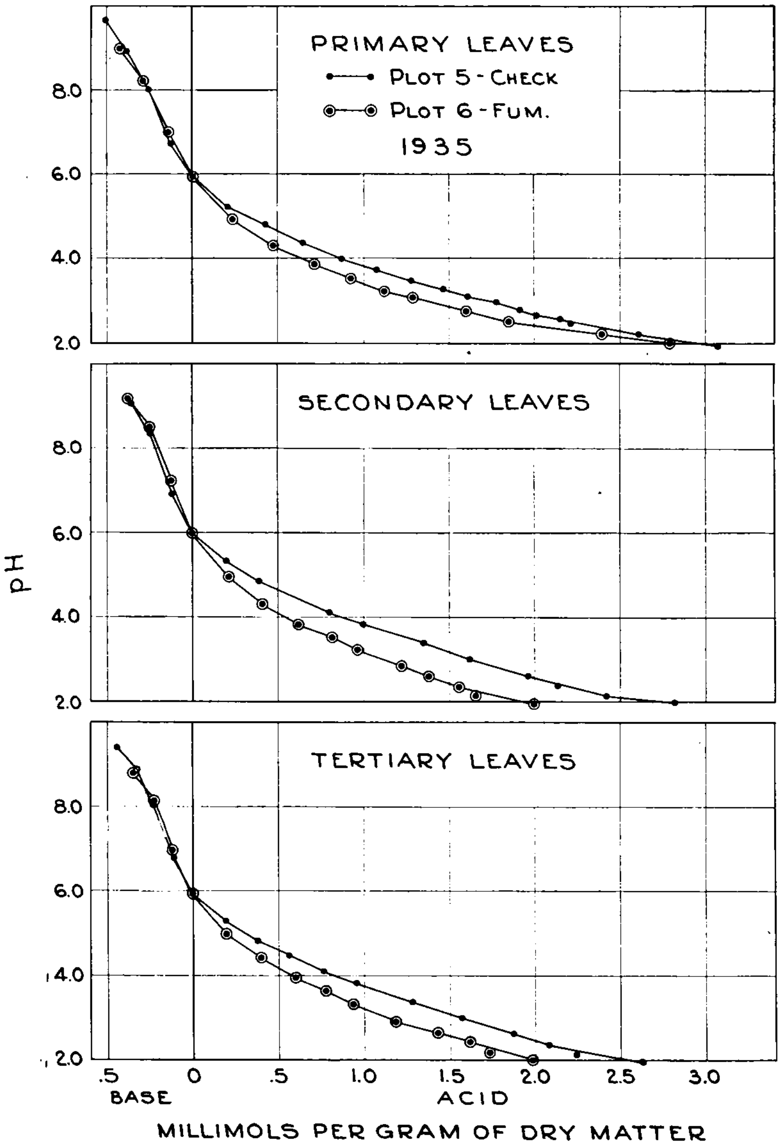

FIG. 1. Titration with sodium hydroxide and hydrochloric acid, using the glass electrode, of well-macerated leaf tissue from an unfumigated plot and a plot fumigated with sulphur dioxide.

In many earlier experiments the pH of juice expressed from fresh leaves—usually after freezing—has been determined on samples taken immediately before and immediately after a fumigation with sulphur dioxide.

With alfalfa the maximum decrease observed was 0.3 pH unit, though with dahlias, a decrease of 1.0 pH unit has been observed. When injury did not occur there was little or no change of pH. This was definitely true in the long-continued, low-concentration fumigation experiments.

It was realized of course, that local concentrations of acid may have existed in the leaves even though the pH of the expressed juice was unchanged, but it was not feasible to study this matter. Instead, the buffer capacity of leaf tissue was investigated by making a number of electrometric titrations of well-macerated material, using an assembly of a glass electrode and Lindemann electrometer.[2] Typical results are presented in figure 1, which gives the titrations of the primary, secondary, and tertiary leaves for the unfumigated plot 5, and the corresponding fumigated plot 6 in 1935. Similar results were obtained for other pairs of plots. The values in figure 1 have been corrected for the diluting effect of the titrating liquid. The initial pH of all the samples was 5.8 to 5.9. On the alkaline side, the buffer capacity was small and not appreciably affected by the fumigation treatment; but on the acid side, the absorbed sulphur dioxide definitely reduced the buffer capacity.

The titrations for plots 5 and 6 and also for plots 7 and 8, 1935, are analyzed in table I which gives the amounts of acid and their ratios required to cause certain changes of pH in the check and fumigated samples. Table I also gives the sulphur increment of the fumigated material as found by analysis (see table II) and the resulting calculated acidity ascribable to the absorbed sulphur dioxide. Finally these values are divided into the differences between the titration values of fumigated and check samples to give the percentage of acidity accounted for by the titrations.

The buffer capacity of the fumigated leaves ranged from 54 to 83 per cent. of the corresponding check leaves, according to the age of the leaves and the magnitude of the pH change considered. In the titration to pH 3, the ratios of buffer capacity ranged from 67 to 83 per cent. In every case the secondary leaves had smaller ratios than the others, that is, the fumigation effect was greatest in the secondary leaves. Only 10 to 15 per cent. of the acid added by the fumigation treatments affected the titration to pH 5, but the titration to pH 3 accounted for 30 to 39 per cent. of the sulphur dioxide acidity in the case of the primary leaves, and 44 to 56 per cent. in the secondary and tertiary leaves.

It is interesting to note that the older fumigated primary leaves, with larger increments of sulphur, showed less reduction of buffer capacity than the secondary leaves, which suggests that there was a slow reaction in the leaf tending to restore the buffer capacity. No adequate explanation of this reaction is available at this time. Some translocation of the sulphur occurred and change to organic sulphur took place to a very limited extent as is shown later. When the leaves were ashed, considerably more sulphate and less

[2] The authors are indebted to Messrs. D. PETERSON and G. F. SOMERS for making these titrations.

TABLE I

EFFECT OF ABSORBED SULPHUR DIOXIDE ON THE BUFFER CAPACITY OF THE LEAVES OF ALFALFA

Plots	Leaf	S absorbed by fumigated leaves		HCl required to change dry leaf tissue from pH 5.9					Absorbed acidity accounted for
				to pH	in Check plot	in Fumigated plot	in Difference	Ratio	
		%	millimols/gm.		millimols/gm.	millimols/gm.	millimols/gm.	%	%
5 and 6	Primary	1.75	1.09	5.0	0.33	0.22	0.11	67	10
"	Secondary	1.55	0.97	5.0	0.31	0.19	0.12	61	12
"	Tertiary	1.26	0.79	5.0	0.28	0.19	0.09	68	11
7 and 8	Primary	1.50	0.94	5.0	0.31	0.20	0.11	65	12
"	Secondary	1.42	0.89	5.0	0.28	0.15	0.13	54	15
"	Tertiary	0.88	0.55	5.0	0.29	0.21	0.08	72	15
5 and 6	Primary	1.75	1.09	4.0	0.90	0.65	0.25	72	23
"	Secondary	1.55	0.97	4.0	0.87	0.54	0.33	62	34
"	Tertiary	1.26	0.79	4.0	0.85	0.57	0.28	67	36
7 and 8	Primary	1.50	0.94	4.0	0.85	0.59	0.26	69	28
"	Secondary	1.42	0.89	4.0	0.81	0.49	0.32	61	36
"	Tertiary	0.88	0.55	4.0	0.80	0.62	0.18	78	33
5 and 6	Primary	1.75	1.09	3.0	1.72	1.39	0.33	81	30
"	Secondary	1.55	0.97	3.0	1.58	1.10	0.48	70	50
"	Tertiary	1.26	0.79	3.0	1.56	1.13	0.43	72	55
7 and 8	Primary	1.50	0.94	3.0	1.56	1.19	0.37	76	39
"	Secondary	1.42	0.89	3.0	1.49	0.99	0.50	67	56
"	Tertiary	0.88	0.55	3.0	1.42	1.18	0.24	83	44

carbonate remained in the residue from the fumigated than from the unfumigated samples but the inorganic bases were present to about the same extent in the two. This suggests that neutralization was largely accomplished by the organic bases such as amino groups or other nitrogen compounds.

THE SULPHUR FRACTIONATION

In addition to investigating the neutralization reactions, the fractionation of the leaf sulphur into a number of different types of compounds was undertaken. A critical study of the method of fractionating the sulphur is presented in detail elsewhere (**4**). A summary of the procedure follows:

About 15 grams of freshly frozen material were ground in a mortar, then suspended in water and passed repeatedly through a laboratory homogenizer until a uniform suspension was obtained. Later the suspension was made in a Waring blendor without preliminary grinding. Aliquot portions were dried. Other aliquots were placed in a special digestion flask with excess of a suspension of magnesium oxide and cadmium chloride or hydroxide and boiled gently under reflux for 2 to 4 days in an atmosphere of hydrogen. This treatment hydrolyzed sulphydryl or disulphide groups, associated with cystine and closely related compounds in the proteins, to hydrogen sulphide which was precipitated as cadmium sulphide. After slow acidification of the reaction mixture with hot dilute hydrochloric acid, the hydrogen sulphide was swept out with hydrogen, collected in a zinc acetate absorber, and determined colorimetrically as methylene blue. This fraction is often called "labile" or "cystine" sulphur. Methylene blue was formed by adding 15 ml. of 0.25 per cent. p-aminodimethyl aniline in 20 per cent. (volume) sulphuric acid, followed immediately by 2 ml. of 12 per cent. ferric alum—without exposing the mixture to the air. The bottle was swirled during addition of the alum. The blue solution was compared in a colorimeter with a stable cuprammonium solution containing 0.60 per cent. hydrated copper sulphate and 0.0024 per cent. potassium dichromate in 0.4 N ammonium hydroxide. Careful adjustment of the dichromate was necessary in order to secure the correct shade of color. This solution matched the methylene blue over a limited range and was equivalent to 0.34 mg. sulphide sulphur per 100 ml. Four digestions could be run simultaneously. Since the methylene blue solutions were not stable, duplicate analyses of the corresponding check and fumigated samples were made at the same time.

The undissolved leaf material in the reaction flask was filtered off and analyzed for sulphur in the Parr bomb to give "acid insoluble organic sulphur." The filtrate was treated with barium chloride to remove the inorganic sulphate. The filtrate from the latter was made alkaline, evaporated to dryness in a large nickel crucible, and the residue was fused with sodium peroxide to destroy organic matter. The sulphate thus produced was determined gravimetrically to give "soluble organic sulphur."

Subsequent study of this procedure (**4**) has shown that small amounts of sulphite and sulphate are produced from cystine by the mild alkaline

TABLE II

TOTAL SULPHATE, AND ORGANIC SULPHUR IN THE DRY LEAVES OF ALFALFA AT THE CONCLUSION OF EACH FUMIGATION EXPERIMENT AT LOGAN, UTAH

PLOTS	YEAR	SO_2 FUMIGATION		LEAF SAMPLE	SULPHATE SULPHUR		TOTAL SULPHUR		ORGANIC SULPHUR*		SULPHUR INCREMENT DUE TO FUMIGATION	
		DURATION	AVE. CON.		CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	SULPHATE	TOTAL
		hours	*p.p.m.*		%	%	%	%	%	%	%	%
3 and 4	1935	1078	0.141	Composite	0.23	1.23	0.48	1.52	0.25	0.29	1.00	1.04
5 and 6	1935	628	0.188	Tertiary	0.12	1.40	0.37	1.63	0.25	0.23	1.28	1.26
		338	0.291	Secondary	0.16	1.71	0.42	1.97	0.26	0.26	1.55	1.55
				Primary	0.23	1.92	0.51	2.26	0.27	0.34	1.69	1.75
7 and 8	1935	528	0.236	Tertiary	0.16	1.03	0.43	1.32	0.27	0.29	0.87	0.89
				Secondary	0.25	1.61	0.52	1.94	0.27	0.33	1.36	1.42
				Primary	0.35	1.81	0.63	2.13	0.28	0.32	1.46	1.50
3 and 4	1936	12	0.822	Composite	0.24	0.54	0.50	0.81	0.26	0.27	0.30	0.31
5 and 6	July 1936	21	0.309	Composite	0.26	0.54	0.51	0.81	0.25	0.27	0.28	0.30
5 and 6	Sept. 1936	132	0.348	Upper Sec.	0.21	1.41	0.45	1.67	0.24	0.26	1.20	1.22
				Upper Prim.	0.33	1.48	0.58	1.75	0.25	0.27	1.15	1.17
				Lower	0.43	1.13	0.63	1.39	0.20	0.26	0.70	0.76
9 and 10	1936	42	0.429	Upper	0.23	0.72	0.52	1.01	0.29	0.29	0.49	0.49
				Lower	0.35	0.81	0.58	1.04	0.23	0.23	0.46	0.46
11 and 12	1936	25	0.600	Upper	0.31	0.75	0.60	1.05	0.29	0.30	0.44	0.45
				Lower	0.44	0.95	0.68	1.27	0.24	0.32	0.51	0.59
				Average	0.27	1.19	0.53	1.47	0.26	0.29	0.92	0.95

* Total minus sulphate sulphur.

digestion, in addition to the sulphide; also the products of the reaction can be more conveniently and more accurately determined by iodometric titration than by methylene blue. While the colorimetric labile sulphur values are doubtless somewhat low (about 10 per cent.), every effort was exerted to make them comparable, and reliable conclusions can probably be drawn from them.

Table II gives a description of the samples and their respective histories; also the total sulphur and sulphate sulphur of check and fumigated plots. The difference between sulphate and total sulphur in each case is assumed to be organic sulphur. The differences between the total sulphur values of check and fumigated plots represent sulphur dioxide absorption. Since these differences are only slightly greater than the differences between the corresponding sulphate values, it is apparent that the absorbed sulphur dioxide was largely converted into sulphate. In spite of the fact that the fumigated samples contained two to twelve times as much sulphate as their checks, they contained only slightly larger amounts of organic sulphur. These latter differences, though small, are highly significant statistically, by the test of FISHER's "t." The organic sulphur values also covered a comparatively narrow range (0.20 per cent. to 0.34 per cent.) in the different plots. Some of the older primary leaves had less organic sulphur than the younger leaves.

In table III the organic sulphur fraction is divided into three parts: labile, acid soluble, and acid insoluble sulphur. In most cases the sum of these fractions agreed well with the organic sulphur in table I. The soluble fraction was usually larger than the other two, but the differences were not great. In general the fumigated leaves had more of each fraction than the check leaves. This was invariably true of the labile fraction. On the average, the insoluble and soluble fractions showed increases in the fumigated plots of 5 and 9 per cent., respectively, and the labile sulphur an increase of 16 per cent.

A statistical analysis of the differences between check and fumigated plots by the method of FISHER's "t," has been made and the corresponding values of P are inserted in table III. The effect of the fumigation was highly significant statistically in the case of the labile sulphur and the total organic sulphur, but the significance was not so definite in the other two comparisons, especially in the insoluble organic fraction. It is evident that the fumigations probably contributed some sulphur to all organic fractions, and certainly contributed to the labile fraction, though the absolute amounts were small.

LABILE SULPHUR IN THE 1939-41 ALFALFA EXPERIMENTS

Labile sulphur analyses have been made on the leaves of all the crops of alfalfa grown in the sand culture experiments in 1939-40-41. These experiments have already been described in part (6, 7). The leaf samples were harvested and frozen as before. The 1939 and 1941 samples were analyzed soon after harvest, but the 1940 samples were not analyzed until 1942. The

TABLE III

FRACTIONATION OF THE ORGANIC SULPHUR IN THE DRY LEAVES OF ALFALFA AT THE CONCLUSION OF EACH FUMIGATION EXPERIMENT AT LOGAN, UTAH

PLOTS	YEAR	LEAF SAMPLE	LABILE OR CYSTINE SULPHUR		WATER SOLUBLE SULPHUR		WATER IN-SOLUBLE SULPHUR		TOTAL		ORGANIC SULPHUR FROM TABLE I	
			CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED	CHECK	FUMIGATED
			%	%	%	%	%	%	%	%	%	%
3 and 4	1935	Composite	0.078	0.089	0.112	0.135	0.082	0.092	0.272	0.316	0.25	0.29
5 and 6	1935	Tertiary	0.082	0.103	0.110	0.118	0.063	0.073	0.255	0.294	0.25	0.23
		Secondary	0.084	0.102	0.105	0.124	0.074	0.072	0.263	0.298	0.26	0.26
		Primary	0.078	0.100	0.117	0.127	0.067	0.068	0.262	0.295	0.27	0.34
7 and 8	1935	Tertiary	0.085	0.100	0.107	0.119	0.082	0.086	0.274	0.304	0.27	0.29
		Secondary	0.087	0.104	0.129	0.113	0.084	0.091	0.300	0.308	0.27	0.33
		Primary	0.082	0.102	0.117	0.128	0.084	0.084	0.283	0.314	0.28	0.32
3 and 4	1936	Composite	0.070	0.100	0.079	0.078	0.113	0.096	0.262	0.274	0.26	0.27
5 and 6	July 1936	Composite	0.066	0.073	0.092	0.095	0.119	0.116	0.277	0.284	0.25	0.27
5 and 6	Sept. 1936	Upper sec.	0.079	0.080	0.094	0.092	0.096	0.101	0.269	0.273	0.24	0.26
		Upper prim.	0.069	0.071	0.112	0.101	0.092	0.103	0.273	0.275	0.25	0.27
		Lower	0.054	0.057	0.080	0.133	0.074	0.077	0.208	0.267	0.20	0.26
9 and 10	1936	Upper	0.078	0.084	0.103	0.096	0.063	0.101	0.244	0.281	0.29	0.29
		Lower	0.063	0.065	0.090	0.084	0.075	0.072	0.228	0.221	0.23	0.23
11 and 12	1936	Upper	0.082	0.092	0.114	0.118	0.110	0.112	0.306	0.322	0.29	0.30
		Lower	0.062	0.074	0.073	0.119	0.102	0.110	0.237	0.303	0.24	0.32
		Average	0.075	0.087	0.102	0.111	0.086	0.091	0.263	0.289	0.256	0.283
		Fumigated/check	....	1.16	....	1.09	....	1.05	....	....	....	....
		Probability value, P, from Fisher's "table of t"	....	<0.01	....	0.07	....	0.10	....	<0.01	....	<0.01

TABLE IV

LABILE SULPHUR IN THE LEAVES OF THE 1939 ALFALFA CROPS

Crop	High pH						Low pH					
	High S		Medium S		Low S		High S		Medium S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
	%	%	%	%	%	%	%	%	%	%	%	%
1st	0.135	0.128	0.122	0.136	0.107	0.128	0.132	0.132	0.115	0.131	0.115	0.132
2nd	0.123	0.123	0.122	0.130	0.084	0.115	0.124	0.123	0.128	0.127	0.083	0.124
3rd	0.124	0.125	0.129	0.133	0.086	0.109	0.124	0.134	0.132	0.129	0.090	0.123
4th	0.134	0.131	0.136	0.146	0.076	0.132	0.145	0.153	0.131	0.142	0.075	0.141
Average	0.129	0.127	0.127	0.136	0.088	0.121	0.131	0.135	0.126	0.132	0.091	0.130
P, from Fisher's "t"	....	0.35	....	0.02	....	0.03	....	0.25	....	0.28	....	0.03

iodometric method was used. The data for 1939 are given in table IV and for 1940-41 in table V and figure 2. The tables also summarize the experimental treatments. When adequate amounts of sulphur were available for

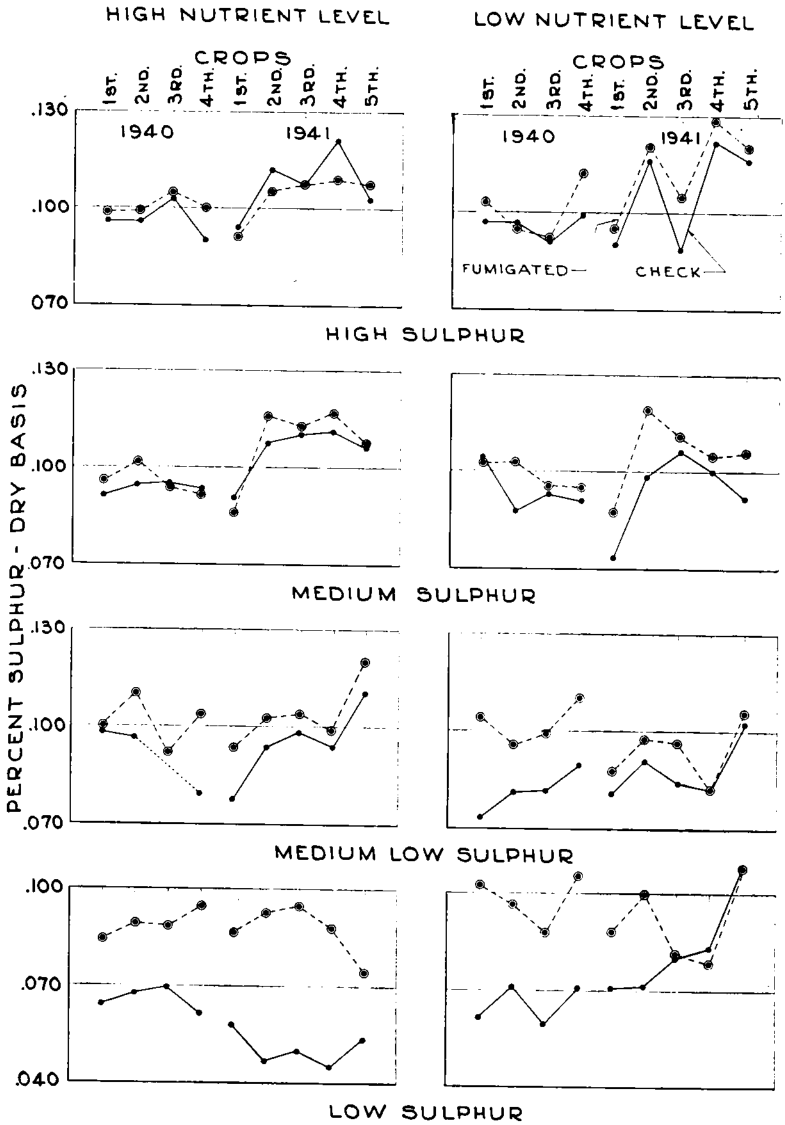

FIG. 2. Labile sulphur in alfalfa leaves grown in the sand culture experiments of 1940-41, with two levels of concentration of the principal nutrients; four levels of sulphate concentration in the nutrient solution; and with and without fumigation with sulphur dioxide.

the nutrition of the alfalfa, the amount of labile sulphur generally exceeded 0.09 per cent. and the average amount was 0.130 per cent. in 1939, 0.098 per cent. in 1940, and 0.105 per cent. in 1941. These values are somewhat higher

TABLE V

LABILE SULPHUR IN THE LEAVES OF THE 1940-41 ALFALFA

Crop	High nutrient level								Low nutrient level							
	High S		Medium S		Medium low S		Low S		High S		Medium S		Medium low S		Low S	
	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated
1940	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1st	0.097	0.103	0.104	0.103	0.073	0.104	0.061	0.102	0.096	0.099	0.092	0.096	0.099	0.101	0.064	0.084
2nd	0.097	0.099	0.095	0.102	0.097	0.111	0.068	0.089	0.097	0.095	0.088	0.102	0.081	0.096	0.071	0.097
3rd	0.103	0.105	0.095	0.094	....	0.092	0.069	0.089	0.091	0.092	0.093	0.095	0.082	0.099	0.059	0.088
4th	0.090	0.100	0.093	0.092	0.080	0.105	0.061	0.095	0.099	0.112	0.090	0.095	0.090	0.111	0.070	0.106
Ave.	0.097	0.102	0.097	0.098	0.083	0.103	0.065	0.094	0.096	0.100	0.091	0.097	0.088	0.102	0.066	0.094
1941																
1st	0.094	0.092	0.091	0.086	0.078	0.094	0.058	0.087	0.090	0.095	0.083	0.093	0.080	0.087	0.071	0.088
2nd	0.112	0.106	0.108	0.116	0.094	0.097	0.047	0.093	0.116	0.120	0.098	0.119	0.104	0.107	0.071	0.100
3rd	0.107	0.107	0.110	0.113	0.099	0.105	0.050	0.095	0.089	0.105	0.107	0.111	0.084	0.097	0.080	0.083
4th	0.105	0.109	0.112	0.117	0.094	0.099	0.045	0.088	0.122	0.129	0.100	0.105	0.082	0.082	0.083	0.079
5th	0.103	0.108	0.107	0.108	0.111	0.121	0.053	0.074	0.116	0.120	0.095	0.106	0.102	0.106	0.107	0.108
Ave.	0.104	0.104	0.106	0.108	0.095	0.103	0.051	0.087	0.107	0.114	0.097	0.107	0.090	0.096	0.082	0.092
Ave. 1940-41	0.101	0.103	0.102	0.103	0.091	0.103	0.057	0.090	0.102	0.108	0.094	0.103	0.089	0.098	0.075	0.093
P, from Fisher's "t"	....	0.25	....	0.20	....	0.01	....	0.01	....	0.01	....	0.01	....	0.01	....	0.01

than the 1935-36 values which were probably about 10 per cent. low, due to the method of analysis as already suggested. The 1940 figures are probably low also due to two years' storage before analysis.

When sulphate was deficient in the nutrient solution, the labile sulphur went down to a minimum value of 0.045 per cent. With few exceptions the fumigated crops had more labile sulphur than the corresponding check crops. These differences were small with adequate sulphur nutrition of the plants through the roots; but the differences were larger under conditions of sulphur deficiency. Statistical significance is indicated for these differences in the case of the "medium sulphur, high pH" and both "low sulphur" pairs

TABLE VI

LABILE SULPHUR IN THE LEAVES AND ROOTS OF 1943 SUGAR BEETS

NUTRIENT CONCENTRATION LEVEL	S IN NUTRIENT SOLUTION	LABILE SULPHUR IN					
		LEAVES			ROOTS		
		CHECK	FUMIGATED	P*	CHECK	FUMIGATED	P*
	p.p.m.	%	%		%	%	
High	18	0.094	0.100		0.0111	0.0108	
	11	0.098	0.101		0.0110	0.0113	
Low	13	0.106	0.118		0.0109	0.0117	
	11	0.102	0.101		0.0095	0.0099	
Ave.		0.100	0.105	0.17	0.0106	0.0109	0.29
High	0.6	0.041† 0.065‡	0.101		0.0094† 0.0093‡	0.0128	
	0.6	0.037† 0.059‡	0.085		0.0110† 0.0102‡	0.0126	
Low	0.6	0.080	0.105		0.0089	0.0102	
	0.5	0.079	0.095		0.0122	0.0131	
Ave.		0.065	0.097	0.02	0.0103	0.0122	0.04

* Probability value from Fisher's "table of t."
† Poor plants.
‡ Fairly vigorous plants.

of plots in 1939, and for all the 1940-41 pairs except "high" and "medium" sulphur in the high nutrient. Nearly all the fumigated crops had normal amounts of labile sulphur.

Table VI summarizes labile sulphur values for the leaves and roots of sugar beets in 1943. These experiments were carried out like the alfalfa experiments in 1939-40-41. Small seedlings were transplanted on Aug. 7 and Aug. 14. The "high nutrient" plots had solutions in which the proportions and concentrations of the principal constituents were those suggested by ARNON and HOAGLAND (1). The "low nutrient" plots had similar solutions in which the concentration was 30 per cent. during the very early stages of growth, and later 10 per cent. of the "high nutrient" plots. "High sulphur" indicates about 11 to 18 p.p.m. sulphate sulphur in the nutrient solution; "low sulphur," about 0.6 p.p.m. The fumigation treatment averaged 0.186 p.p.m. sulphur dioxide applied 5.2 hours daily on 60 days between August 19 and November 2. Eight plots comprised the experimental unit, and the experiment was duplicated on the other eight plots. Growth on most of the plots was quite uniform. On November 1st two representative leaves were taken from each plant, giving a composite plot sample of 50 leaves. These were cut into small pieces after removing the midrib, well mixed, and quickly frozen in tight containers. The "high nutrient, low sulphur" check plots exhibited very poor growth, indicating marked sulphur deficiency and lack of balance in the nutrient solution, but they each had a few plants that were much better than the rest. These were sampled separately.

The root samples were prepared by carefully trimming off the leaves around the crown and running the beets through a pulping machine which very effectively broke up the tissue to a fine suspension. The pulp was well mixed and a sample was frozen in a tight bottle until used. One-hundred-gram portions of this material were analyzed for labile sulphur.

The labile sulphur values for sugar beet leaves in table VI are about the same as for alfalfa leaves in table V, and the relationships between the treatments are quite similar for the two plants: that is, the sulphur-deficient leaves had appreciably smaller amounts of labile sulphur than those adequately supplied either from the nutrient solution or from sulphur dioxide, and the fumigation treatments raised the labile sulphur level of the deficient leaves nearly to normal.

The roots had only about one-tenth as much labile sulphur as the leaves, but the general relationships to the treatments were similar in roots and leaves. The differences due to fumigation for both roots and leaves were statistically significant only in the low-sulphur pairs of plots.

In the "high nutrient-low sulphur" unfumigated plots, the plants that made the best growth had more labile sulphur in the leaves (but not in the roots) than the poorer plants. It is of interest to note that these two check plots had poorer plants and lower labile sulphur values in the leaves than did the "low nutrient-low sulphur" plots, suggesting that sulphur deficiency is more serious with a high nutrient level than with a low level and indicating that the balance of the nutrients in a solution is important as well as the concentration.

Finally it may be stated that the labile sulphur analyses in the sand cultures confirm the earlier experiments in soil in showing that some of the absorbed sulphur dioxide is reduced and incorporated into cystine or closely related organic sulphur compounds.

Summary

A chemical study has been made of the alfalfa leaf samples obtained in the alfalfa fumigation experiments of 1935-36, and 1939-41, and also the 1943 work with sugar beets.

1. The prolonged low concentration fumigations with sulphur dioxide did not change appreciably the pH of the expressed leaf juice.

2. These treatments reduced appreciably the buffer capacity of the leaves as shown by electrometric titration.

3. Organic bases in the leaf were probably principally responsible for the neutralization of the absorbed acid.

4. The data suggest a slow restoration of the buffer capacity.

5. The absorbed sulphur dioxide was changed principally to sulphate.

6. A small amount of organic sulphur was produced including cystine or related "labile" sulphur compounds, and probably also other soluble and insoluble organic sulphur compounds.

7. Labile sulphur was consistently higher in the fumigated plots of 1935-36 than in the unfumigated plots. It was generally higher in the later work also. When the nutrient solution was deficient in sulphate, the fumigated leaves had considerably more labile sulphur than the unfumigated leaves. The differences between the labile sulphur values of the fumigated and unfumigated plots were small when the sulphur available to the roots was adequate.

8. The roots of sugar beets had only about one-tenth the labile sulphur in the leaves, but showed differences due to the different sulphur treatments, similar to those shown by the leaves.

9. The high nutrient-low sulphur unfumigated plots had very low labile sulphur values in the leaves both of alfalfa and of sugar beets—considerably lower in fact than the low nutrient-low sulphur plots, suggesting a lack of balance as well as deficiency in the former.

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