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SULPHUR NUTRITION OF COTTON[1]

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(WITH ONE FIGURE)

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The role of sulphur in the metabolism of the tomato, and some other plants, was the object of a critical study by NIGHTINGALE *et al.* (**14**) in 1932. This work was followed by a succession of three papers by S. V. EATON (**8, 9, 10**) dealing respectively with soybeans, sunflowers, and black mustard. In each of the foregoing studies, attention was given to the distribution of sulphur between selected plant parts and to the effects of sulphur deficiency on carbohydrate and nitrogen metabolism. A large measure of emphasis was placed by the authors on proteolysis. BEESON (**2**) has shown by his analyses that the total sulphur in plants tends to approach or may exceed in concentration that of total phosphorus. COOPER and MITCHELL (**5**) found that cotton plants cultured under various conditions contained an average of 0.28% of sulphur and 0.24% of phosphorus on the dry weight basis. In a few instances (**4, 12, 18**), cotton has been shown to respond under field conditions to sulphur fertilization. The fact that attention to the sulphur relations in the nutrition of cotton has been somewhat limited finds one explanation in that sulphur is an ingredient in superphosphate as well as in some of the nitrogen and potash fertilizers.

The present study of the sulphur nutrition of the cotton plant has tended to follow lines of inquiry similar to those followed by Nightingale *et al.* and Eaton. In the discussion of the cotton data, differences and similarities are pointed to among the foregoing five plants. The data on cotton include: (1) distribution and forms of sulphur in cotton plants grown on a fertile soil; (2) the effects of sulphur deficiency on growth and mineral accumulation, and (3) the forms and concentrations of sulphur, carbohydrates and nitrogen in plants grown in sand cultures with two and with five levels of sulphate supply. In each experiment, analyses were made before and after the plants had flowered. Consideration is given to the effects of sulphur supply on fruitfulness.

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Analytical methods

Carbohydrates: The methods used in determining the various fractions have been outlined elsewhere (**7**).

Nitrogen: Total nitrogen (a) was determined by the Kjeldahl method after preliminary reduction with reduced iron powder. Soluble nitrogen (b) was similarly determined and includes all nitrogenous compounds extractable from the dried tissue with water at 80° C. Soluble organic nitrogen (c) was determined upon an aliquot of the extract obtained in (b) omitting the nitrate-reduction step. Nitrate-N was calculated as the difference between (b) and (c). Alpha-amino nitrogen was determined upon an aliquot of (b) using the Van Slyke method. Protein nitrogen was calculated as the difference between (a) and (b).

Sulphur fractions: Duplicate one and two gram tissue samples were Soxhlet-extracted with 80% ethanol. Sulphur determinations were made on both the extracts and alcohol-insoluble residues. Sulphate sulphur in one of the duplicate extracts was determined in the usual manner after precipitation as $BaSO_4$ and the evaporation of the alcohol. The total sulphur content of the second extract was determined by the official (**1**) magnesium nitrate method. The difference between total and sulphate sulphur was designated soluble organic sulphur. One of the alcohol-insoluble residues was refluxed with 2.5% HCl for two hours, and sulphate sulphur in the resulting extract again determined as $BaSO_4$. Total sulphur was determined on the other alcohol-insoluble residues, as before, and the difference between total and sulphate sulphur was taken as a measure of the protein sulphur. Sulphate was calculated as the sum of the alcohol-soluble and -insoluble sulphates. Total sulphur represents the sum of the three sulphur fractions. When fractionation of sulphur was limited to total organic and sulphate sulphur, the former was calculated as the difference between total and sulphate sulphur.

Other constituents: Calcium, magnesium, and phosphorus were determined (the latter colorimetrically) by official (**1**) methods. The potassium determination followed the WILCOX (**15**) gravimetric procedure. The iron determination was by the Walker thiocyanate procedure as given by YOE (**17**).

Experimental procedure

DISTRIBUTION AND FORMS OF SULPHUR ACCUMULATED IN PLANTS ON SOIL

The Stoneville 2B plants, providing the data for table I, were planted April 19, 1949, and grown outdoors in three-gallon jars of manured Houston Black Clay. In the preflower stage (three weeks in advance of the first flower), the leaves contained 87% of the total sulphur in the plants. Fifteen days after flowering, the dry weight per plant had increased 4.7 fold but the total sulphur per plant had increased only 2.3 fold. At this time the leaves, buds, and bolls contained 88% of the total sulphur in the plants. The leafy

TABLE I

CONCENTRATION OF SULPHUR FRACTIONS IN VARIOUS PARTS OF COTTON PLANTS GROWN ON FERTILE SOIL. DRY WEIGHT PER PLANT AND PERCENTAGES ON DRY WEIGHT

Plant parts	Dry weight	Sulphate S	Soluble org. S	Protein S	Total S
	gms.	%	%	%	%
Preflower, 43 days:					
Leaves	6.5	0.80	0.06	0.17	1.03
Stems and petioles	3.3	.15	.02	.06	.23
Root	2.0	.04	.03	.03	.10
Entire plant	11.8	.49	.05	.12	.66
Early fruiting, 79 days:					
Leaves	17.4	.55	.06	.14	.75
Stems and petioles	16.3	.05	.02	.03	.10
Root	11.9	.03	.01	.04	.08
Reproductive	9.8	.14	.03	.08	.25
Entire plant	55.4	.22	.02	.08	.32
Mature seed:					
Kernels	....	.02	.04	.31	.37

bracts of the buds and bolls constitute a substantial part of the weight of these organs but only in the instance of protein S was the concentration of any sulphur fraction as high as in the leaves. In the mature seed kernels, protein sulphur constituted 84% of the total sulphur. In the vegetative tissues this percentage was characteristically much lower. At both the first and second samplings, table I, sulphate S was the dominant form. Between the two samplings, there were no striking changes in the proportions of sulphate, soluble organic, or protein sulphur.

PLANTS IN SAND CULTURES SUPPLIED WITH MINUS S AND PLUS S SOLUTIONS

This planting of Stoneville 2B cotton was made in washed quartz sand in 30 tall four gallon stone jars in the greenhouse on March 14, 1949. After thinning to two seedlings per jar, half of the jars were supplied with millimolar concentrations of salts as follows: 5 $Ca(NO_3)_2$, 5 KNO_3, 2 $MgSO_4$, 1 KH_2PO_4 (the foregoing is Hoagland's solution), and 1 NaCl. The other jars received a like solution except that $MgCl_2$ was substituted for the $MgSO_4$. Both lots received 5 p.p.m. of boron, 0.5 p.p.m. of manganese, 0.05 p.p.m. of zinc and 0.01 p.p.m. of copper. Iron was supplied as magnetite mixed with the sand. De-ionized water and C.P. salts were used. The manufacturer's analyses indicated that the non-sulphate salts added 0.1 p.p.m. of SO_4 to the nutrient solutions.

New solutions were added always in amounts sufficient to produce drainage and in equal quantities to both the plus and minus S plants. Toward the end of the experiments, the nutrient-solution additions amounted to two liters per day. Only occasionally, on days of high transpiration, were the nutrient solutions supplemented by additions of de-ionized water.

One plant was removed from each jar for analyses on April 20, 36 days after planting, and the second one was taken on May 18, 64 days after planting. The dry weight per plant of the minus S plants increased from one gram at the first sampling to nine grams at the second sampling. The plus S plants increased from six grams to 46 grams. There was little proportionate difference in the gain with age between the plus and minus plants. Plant heights in the minus and plus groups were respectively 19 and 34 cm. at the first sampling and 31 and 59 at the second; *i.e.*, the SO_4 deficiency in this experiment reduced plant height by about half.

At both the first and second samplings, all minus S leaves were greenish yellow whereas all plus S leaves were green. In the minus S plants, there was no evident gradation of color between young and old leaves. The cotyledons of the minus S plants dropped between the first and second sampling and the oldest true leaves began to show deterioration. Leaves of corresponding age were much smaller on the minus S than on the plus S plants. The chemical data from this experiment are presented in table II.

Carbohydrates: At 36 days, remarkably little reducing and sucrose sugars were found in the tops of the minus S plants. At 64 days, no titratable sugars were found in the minus S leaves. The stems and petioles contained only one fifth as much sugar as was found in the plus S stems and petioles. On the other hand, the concentrations of starch and hemicellulose were reduced only moderately by sulphur deficiency at the first sampling and little or none at the second sampling.

Sulphur: In their various parts, the minus S plants contained only a quarter to an eighth as high percentage of total sulphur as the plus S plants.

Nitrogen: The minus S plant parts accumulated in the order of twice as high concentrations of nitrate and soluble organic nitrogen as did the plus S plants; the only exception being nitrate in the roots. On the other hand the plus S leaves contained almost twice as high concentrations of protein nitrogen as did the minus S leaves. This increase in protein N was not shown by the stems and roots.

Mineral elements: The minus S leaves accumulated nearly equal extra percentages of phosphorus and magnesium, but these accumulation effects were slight in the other tissues. There was some extra concentration of calcium in all minus S tissues. Potassium and iron concentrations were unaffected.

Effects through five levels of SO_4 supply

This experiment was conducted in the same manner as the foregoing one except that $MgCl_2$ was replaced with $MgSO_4$ in successive steps to produce five culture solutions containing 0.1, 1.0, 10, 50, and 200 p.p.m. of SO_4 ion. The planting was made in the greenhouse on March 17, 1950, and consisted of six jars for each SO_4 level.

Symptoms: At the time of the first sampling (the 35th day after planting) there was a step by step increase in the intensity of the green color of

TABLE II

DRY WEIGHT AND SULPHUR, CARBOHYDRATE, NITROGEN AND MINERAL ELEMENTS IN COTTON PLANTS AT 36 AND 64 DAYS WITH AND WITHOUT ADDED SULPHATE. DATA EXPRESSED AS PERCENTAGE OF DRY WEIGHT

Age and plant part	Dry weight (gm.)		Carbohydrates							
			Reducing sugars		Sucrose		Starch		Hemicellulose	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	1.0	5.8	0.07	1.13	0.08	0.84	3.77	5.47	6.36	8.36
Early fruiting, 64 days:										
Leaf blades*	4.3	21.3	0.00	1.16	.00	1.44	8.70	8.86	7.45	7.28
Stems, petioles	3.1	18.6	.45	2.63	.29	2.43	9.90	11.68	14.18	14.58
Roots	1.6	5.8	2.36	2.13	3.04	3.84	17.26	14.59	12.74	13.55

	Sulphur (total)		Nitrogen							
			Nitrate		Solu. org.		Protein		Total	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	0.11	0.86	1.44	0.63	1.51	0.67	1.23	2.38	4.18	3.68
Early fruiting, 64 days:										
Leaf blades*	.12	.91	0.17	0.07	1.83	0.59	1.55	2.65	3.55	3.31
Stems, petioles	.04	.17	.20	.14	1.45	0.39	1.58	1.28	3.23	1.81
Roots	.02	.12	.09	.16	.67	.29	0.41	0.42	1.17	.87

	Mineral Elements									
	Phosphorus		Potassium		Calcium		Magnesium		Iron	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	0.37	0.35	4.06	3.83	4.47	3.61	0.88	0.79	....	....
Early fruiting, 64 days:										
Leaf blades*	0.56	.26	3.63	3.34	4.00	2.77	.58	.28	0.026	0.023
Stems, petioles	.24	.17	3.30	3.37	1.39	.91	.23	.18	....	....

*Samples included all leaves.

the leaves with increasing SO_4 supply. In the 0.1 and 1.0 p.p.m. levels, yellowing appeared within the first two weeks after planting. At 35 days, the plants supplied with 200 p.p.m. of SO_4 were greener than those supplied with 50 p.p.m. By 65 days, the color of the 50 and 200 p.p.m. SO_4 leaves was equal. At 65 days, the cotyledons of the 0.1 and 1.0 p.p.m. plants had abscised, and the oldest leaves were showing some deterioration.

Growth: Corresponding with the increasing intensity of chlorophyll color, step by step increases are shown at 35 days (table III) in both the fresh weight of tops and plant height (fig. 1) through the full series of sulphate supply levels. At 65 days, the plants in 50 and 200 p.p.m. of SO_4 had approximately equal weights. The height of plants was reduced more by sulphur deficiency in this experiment than in the previous one; at 65 days, the height of plants with 0.1 p.p.m. of sulphate was only one quarter

of the height of the 200 p.p.m. plants. The differentiation of main-stalk nodes was reduced to one half by sulphate deficiency. At the 0.1 SO_4 level, there were no fruiting or vegetative branches over 10 cm. long. Fifty parts per million of SO_4 gave as good growth and fruiting at 65 days (fig. 1) as did 200 p.p.m. Through the five levels of SO_4 supply there were respectively 0.5, 3.2, 15.8, 23.4, and 22.5 developing bolls per plant. As shown in table III sulphate supply did not influence relative fruitfulness, *i.e.*, there was no effect on the partition of growth between vegetative and fruiting activities. Similar relations have been shown elsewhere in the instance of nitrate supply.

Carbohydrates: Neither the entire tops at 35 days nor the leaf blades and main stalks at 65 days of the plants in either the 0.1 or 1.0 p.p.m. sul-

TABLE III

GROWTH AND FRUITING OF COTTON PLANTS AS INFLUENCED BY SULPHATE SUPPLY. AVERAGE VALUES PER PLANT

Treatment SO_4	Fresh tops	Height	Main-stalk nodes	Branches*		Relative fruitfulness**
				Fruiting	Vegetative	
p.p.m.	*gm.*	*cm.*	*number*	*number*	*number*	
			35 days—preflowering stage			
0.1	8.3	16	6.5	....	....	....
1	13.2	22	6.7	....	....	....
10	28.1	35	8.2	....	....	....
50	34.5	38	8.8	....	....	....
200	45.5	43	9.3	....	....	....
			65 days—early fruiting stage			
0.1	13	24	9.7	0.0	0.0	
1	50	41	12.0	0.2	0.0	4.0
10	237	86	15.6	5.2	0.0	3.8
50	350	100	17.8	8.2	1.2	4.0
200	345	103	18.0	8.3	0.8	3.8

* Length of 10 cm. or more.

** Number of developing bolls per 100 grams of fresh stems and leaves.

phate levels (table IV) contained titratable amounts of either reducing or sucrose sugars. Also, in general accord with the previous experiment, the percentage of starch was reduced moderately in entire plants by 0.1 p.p.m. of sulphate at 35 days and in the leaf blades at 65 days but not in the main stalks. The percentage of hemicellulose was essentially unaffected by the level of SO_4 supply.

Nitrogen: The percentage of total nitrogen was always greater when the SO_4 supply limited growth. The high accumulations of NO_3 and soluble organic nitrogen found at low SO_4 levels gave way to an accumulation of protein nitrogen at the higher SO_4 levels. Only in the instance of the woody stems did the protein N fail to increase with the increase in sulphur supply.

Sulphur: Between the 0.1 and 200 levels of SO_4 supply there occurred in

the leaves a 100 fold gain in sulphate, a threefold gain in soluble organic, and a twofold gain in protein sulphur. The tendency for the cotton plant to accumulate sulphate in its leaf cells (but not in stem cells) seems quite remarkable. Elsewhere (**6**) it has been shown that the leaf sap of cotton

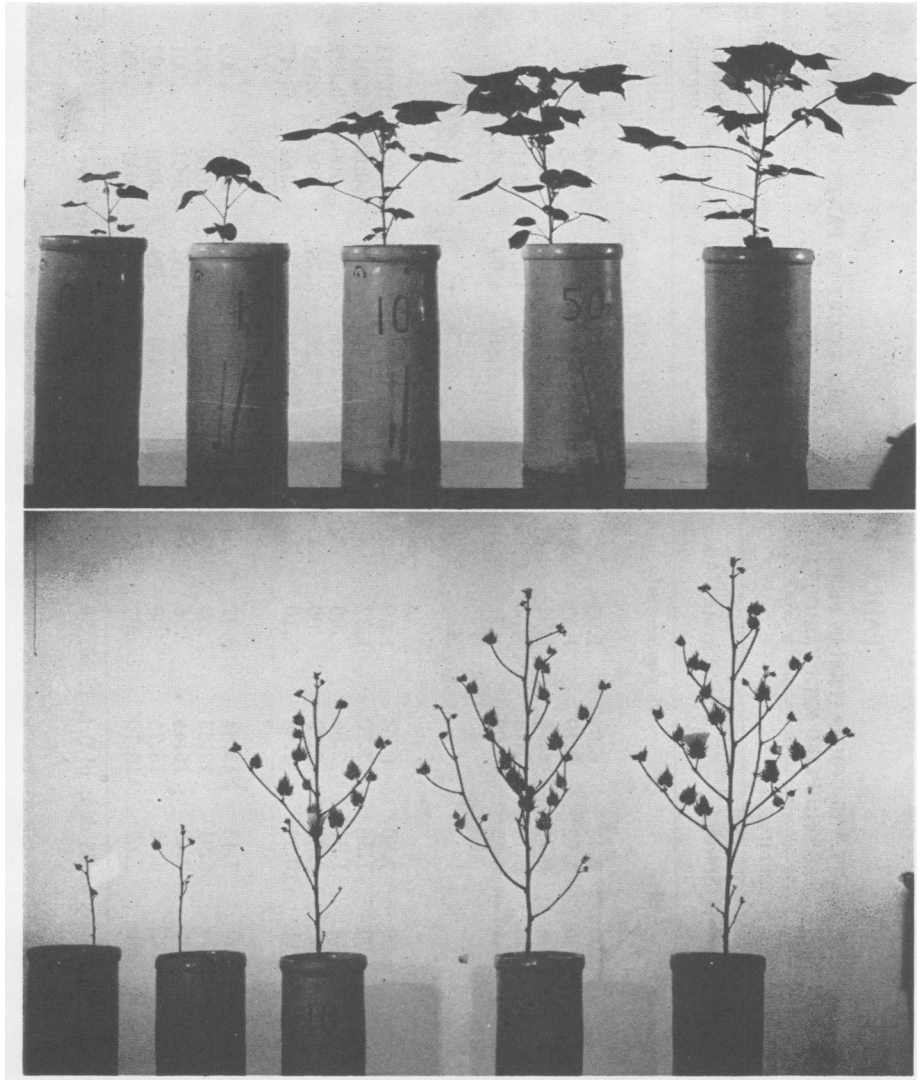

FIG. 1. Cotton plants at 35 days (above) with 0.01, 1, 10, 50 and 200 p.p.m. (C) SO_4 and (below) at 65 days defoliated.

grown on a solution containing 250 milliequivalents per liter SO_4 (3,933 p.p.m. of S) has a 0.44 normal concentration of total S expressed as sulphate.

The data of table IV are rearranged in table V to bring out certain sulphur and protein relations. The chloroplasts of the leaves of sudan grass

TABLE IV

DRY WEIGHT IN GRAMS PER PLANT AND COMPOSITION IN PERCENTAGES ON DRY WEIGHT OF PARTS OF COTTON PLANT SUPPLIED WITH VARIOUS LEVELS OF SULPHATE

SO_4 supply p.p.m.	Dry weight*	Carbohydrates				Nitrogen					Sulphur			
		Reduc. sugar	Sucrose	Starch	Hemicellulose	Nitrate	Amino	Soluble organic	Protein	Total	Sulphate	Soluble organic	Protein	Total
35 days—early bud stage														
Tops														
0.1	1.0	0.00	0.00	2.43	6.02	1.95	....	1.55	1.39	4.89	0.004	....	....	0.132
1	1.6	0.00	0.00	5.92	7.19	1.55	0.41	1.19	2.10	4.84	.005	....	....	.146
10	4.0	1.83	1.06	6.74	6.86	.92	0.17	.73	2.43	4.08	.080	....	....	.286
50	5.0	1.96	.56	6.14	7.13	.78	0.19	.73	2.56	4.07	.341	....	....	.542
200	6.8	2.11	.51	6.45	7.09	.77	0.18	.64	2.68	4.09	.837	....	....	1.057
65 days—early boll stage														
Leaves**														
0.1	1.1	0.00	0.00	7.60	2.64	1.39	0.57	2.23	0.96	4.58	0.003	0.014	0.094	.111
1	2.4	0.00	0.00	7.36	2.70	1.37	....	2.21	1.28	4.86	.003	0.014	0.101	.118
10	3.4	1.10	0.44	11.31	2.86	.06	.37	1.19	2.56	3.81	.009	0.022	0.147	.178
50	4.7	1.25	1.81	9.81	2.72	.00	.17	.51	3.25	3.76	.096	0.043	0.213	.352
200	4.7	1.32	2.11	10.34	2.38	.10	.16	.45	3.20	3.75	.362	0.047	0.198	.607
Main stalks														
0.1	0.5	0.00	0.00	7.43	13.84	0.97	1.01	3.11	.79	4.87	.000	.002	.036	.038
1	1.9	0.00	0.00	6.91	13.83	.60	1.01	2.98	.38	3.96	.000	.001	.034	.035
10	10.4	4.84	1.05	9.42	15.44	.26	.06	.39	.38	1.03	.001	.001	.040	.042
50	14.5	5.73	2.02	6.42	15.75	.08	.04	.30	.41	.79	.008	.007	.049	.064
200	13.9	5.96	1.90	7.62	15.70	.07	.02	.28	.41	.76	.011	.008	.077	.096

*Fresh weight (grams) of entire tops at 65 days of 0.1, 1, 10, 50, and 200 p.p.m. SO_4 treatments respectively: 13, 50, 237, 350, 345.

**Upper 5 per plant.

beyond the early stage of development have been found by HANSON *et al.* (**11**), in keeping with work on other plants, to contain upwards to 40% of the total leaf protein. It was also found that the chloroplasts contained in the order of 70% of the total protein sulphur of the leaves. The data of table V indicate that under conditions of sulphur deficiency the leaf proteins are much richer in sulphur than they are when the sulphur supply is ample; thus implying that under sulphur starvation and partial chlorosis the chloroplasts have, so to speak, a first call on the available sulphur. This idea is apparently supported by the finding that the stem proteins (storage with few chloroplasts) increased in their sulphur content from 0.73 to 3.00% as the SO_4 supply increased, whereas the leaf proteins decreased from 1.57 to 0.94% sulphur.

TABLE V

PROTEINS AND SULPHUR CONTENT OF PROTEINS IN LEAVES AND STEMS OF COTTON PLANTS AT 65 DAYS

Sulphate supply, p.p.m.	Leaves		Stems	
	Proteins (N × 6.25)	Sulphur in proteins	Proteins (N × 6.25)	Sulphur in proteins
	%	%	%	%
0.1	6.00	1.57	4.94	0.73
1	8.00	1.26	2.38	1.42
10	16.00	0.92	2.38	1.68
50	20.31	1.05	2.56	1.91
200	20.00	0.94	2.56	3.00

Discussion

As indicated in the introduction, the procedure followed in the present study of the sulphur nutrition of cotton has features in common with those used by EATON in his studies with the soybean (**8**), the sunflower (**9**), and black mustard (**10**). The latter studies, in turn, followed along lines similar to the earlier work of NIGHTINGALE *et al.* (**14**) on the sulphur metabolism of the tomato. In all cases analyses were made of stem tissues and usually the concentrations of various carbohydrates and nitrogen, as well as sulphur fractions were determined. An opportunity is thus provided for evaluating the sulphur reactions of the cotton plant in terms of the comparative physiology of the five species. Points common to the five plants provide for generalizations, and the differences provide illustrations of variations which may occur in reaction paths. The tabulations of the foregoing in table VI furnish the principal basis for the review.

Before reviewing the data of table VI, it should be noted that each of the investigators selected or developed a different base nutrient. The contrasts in the concentrations and proportions of ions, as well as differences in the frequency of solution renewal, may account for some of the variations in plant responses which might not have been experienced had all the spe-

TABLE VI

CHARACTERISTICS OF TOMATO (13), SOYBEAN (8), SUNFLOWER (9), BLACK MUSTARD (10), AND COTTON PLANTS GROWN ON SULPHATE DEFICIENT SOLUTIONS RELATIVE TO HIGH SULPHATE SOLUTIONS. THE CHEMICAL DATA ARE FOR THE PLANT STEMS EXPRESSED AS PERCENTAGES OF DRY WEIGHT

	Cotton	Tomato	Soybean	Sunflower	Black mustard
Weight reduction	95%	substantial	35%	75%	60%
Yellowing of leaves	all	lower leaves first	upper leaves first	all	upper leaves first
Leaf deterioration	lower leaves	....	lower leaves	....	....
Stem elongation	reduced 60%	normal	reduced slightly	reduced 33%	reduced 60%
Stem thickening	reduced	reduced	reduced	reduced	reduced
Sulphur in stems:					
Soluble in alc.	reduced 75%	increased	....	increased	reduced 75%
Protein	reduced 50%	reduced	....	large reduction	none present
Total organic	reduced 55%	slight reduction	unchanged	large reduction	reduced 75%
Nitrogen accumulation:					
NO_3	greatly increased	high	usually increased	slight increase	reduced
Sol. organic	greatly increased	increased 40%	great increase	large increase	increased
Protein	increased 90%	slightly higher	30% increase	20% reduction	reduced
Carbohydrate accumulation:					
Reducing sugars	reduced to 0	"high"	large reduction	reduced 75%	great reduction
Sucrose	reduced to 0	"high"	decrease	reduced 75%	great increase
Starch, dextrin.	no change	"high"	moderate increase	increased 50%	great increase
Hemicellulose	reduced slightly	....	little change	increased 50%	some increase
Minerals:					
Potassium	no change	low	....	....	....
Calcium	increased 50%	increased	....	....	....
Magnesium	increased 30%	....	....	....	....
Phosphorus	increased 40%	....	....	....	....

cies been given like substrates and like environments. By reason of these variations in growth conditions, similarities in the plant responses gain weight as being primary, whereas the departures may lose some of their significance. In all the investigations C.P. salts were used. The minus S culture solutions could not have been alike as regards the level of SO_4 supply. Nor could they have remained alike very long when subjected to SO_4 uptake by the plants, particularly in view of differences in frequency of solution renewal.

Of the five species, the greatest growth depression in minus S solutions was shown by cotton which was followed by sunflower. It seems probable that the sulphur requirement of cotton is especially high. This conclusion is indicated by the fact that even the 1.0 p.p.m. SO_4 solution (table V) gave an 85% reduction in growth which is greater than that found for sunflowers in EATON'S (**9**) minus S solution.

The leaves of the cotton and sunflower were yellow from the base to the apex of the plants, without significant variation in intensity. There was yellowing in some of the leaves of tomato, soybean, and mustard but this symptom developed in the younger leaves late in the growth of these plants. CHAPMAN and BROWN (**3**) have noted the same characteristic in citrus. Yellowing of leaves, accompanied by growth depression, is in all probability the most general symptom of sulphur deficiency. In cotton leaves, the yellowish green color extends over the entire blade without gradation between the veins and outer mesophyll. It seems probable that this may also be a general characteristic in the other plants, otherwise the pattern of the yellowing would have been called to attention. Nightingale *et al.*, and Eaton directed attention to the similarity between the leaf color of minus S plants and that of plants low in nitrogen; this observation applies also to cotton. The fact that yellowing developed in the upper and younger leaves of soybean and mustard after considerable growth may indicate only that the SO_4 of the nutrients did not reach a sufficiently low level to cause yellowing until the plants were advanced in size and the SO_4 demand had become heavy. The yellowing of the lower leaves of the minus S tomato, in advance of the upper leaves, provides evidence in favor of the contention of Nightingale *et al.* that sulphur can be withdrawn from old tissues for re-use in meristems. On the other hand, if proteolysis is involved in sulphur deficiency, then in soybean and mustard it must be very slow. In the instance of these latter plants, no mention is made of a loss of chlorophyll in lower leaves at the time the yellow leaves were developing near tops of the plants. Nightingale *et al.* make a point of the observation that the yellowing of the older tomato leaves was not associated with a change in the appearance of the protoplasm.

Abscission of the old leaves of minus S cotton plants, including the cotyledons, was usually preceded by the development of necrotic margins and irregular dark colored dead areas in the mesophyll. Eaton notes for the soybean that there was a tendency for the lower leaves, especially in the

minus sulphur plants, to die and fall off. The area of four minus S soybean leaves was found to be only half that of four plus S leaves. The differences in leaf areas, were similarly marked in cotton and are indicated also in the photographs of tomato, sunflower, and mustard included in the publications cited.

Inasmuch as the longevity of a leaf must be conditioned by its physiological status, it seems doubtful that yellowing, or a shorter life, of leaves even though they are low in some mineral element, is conclusive evidence of the withdrawal of a deficient element. Expressed as percentages of green matter Nightingale *et al.* show little difference in the total sulphate free S between either the upper or lower stems or between the lower blades of plus S and minus S tomato plants, but there was a contrast between the upper blades in the two solutions.

Nightingale *et al.*, as does KRAYBILL *et al.* (**13**), note little if any effect of sulphur deficiency on the elongation of tomato stems. A slight shortening is noted on soybean, a substantial shortening for sunflower. There were marked effects of sulphur deficiency on the elongation of mustard and cotton. In all plants the stems were thin and inclined to be woody.

Of the five species, only the soybean failed to show some reduction in the percentage of total organic sulphur. Tomato and sunflower had increased, rather than reduced, the percentages of alcohol-soluble sulphur. There were reductions in protein S in cotton, tomato, and sunflower, but mustard showed no change. Mustard seems to be unique in that Eaton found it to contain no alcohol-insoluble sulphur (protein sulphur).

Possibly because of differences in the amount of woody elements, as well as inherent differences between plants, the sulphur concentration in the stems of the five plants provide a poor index to sulphur requirements. The stems of minus S cotton, dry weight basis, contained 0.04% of total organic sulphur; tomato stems (mean of upper and lower segments) 0.12%; soybean stems 0.05%; sunflower (mean of upper, middle, and lower) 0.32%; and mustard stems 0.18%. Cotton and sunflower thus had respectively the lowest and highest concentrations of organic sulphur in their stems, and yet these two plants showed the greatest growth reductions. Organic sulphur percentages of leaves of minus S plants are reported in three instances: upper main stalk cotton leaves contained 0.11%, upper tomato leaves, 0.15%, and all leaves of soybean, 0.24%. The tomato, which of these three plants had the most organic sulphur in its stems, had next to the least in its leaves. The orgánic sulphur of cotton leaves was three times that of the stems, the tomato approximately equal, and the soybean five times. Furthermore, it seems especially noteworthy that so little difference should have been found in the total organic S content of the stems between the plus S and minus S treatments in both tomato and soybean plants. The conclusion is indicated that between species the relative sulphur content of neither stems nor leaves provides an index to growth requirements.

Although it is reasonable to assume and believe that proteolysis and the

re-use of sulphur occurs in S deficient plants, such a relation has been and may continue to be difficult to demonstrate. Not only is this the case because of translocation and the change in the weight of tissues during periods of darkness, but also because of the recent evidence by WOOD and CRUICKSHANK (**16**) that the sulphur containing amino acids, cystine in particular, are broken down in the cytoplasm during protein hydrolysis; amounts of sulphate equivalent to the sulphur containing amino acids appeared.

With only occasional exceptions, sulphur deficiency resulted in higher concentrations of the soluble forms of nitrogen in all tissues and plants. But no general rule can apply to protein formation. In the stems of cotton and soybean, protein concentrations were increased, in tomato there was little change, whereas in sunflower and mustard small reductions were indicated.

Nightingale *et al.* report both reducing and sucrose sugars to have been high in minus S tomato stems. Eaton found the sucrose of mustard to show a great increase. Sugars were reduced in soybean and sunflower and in cotton little or none could be found. Starch and hemicellulose, on the other hand were usually either increased or unchanged. Three tendencies of the minus S plants stand out in the foregoing: Sugars were depressed in three of the plants, starch and hemicellulose remained high, and soluble organic nitrogen increased. The low sugar effect was especially pronounced in cotton, and the increased concentration of soluble organic nitrogen was nearly as striking (table VII). If each nitrogen atom given in this table is assumed to have been associated with four C atoms and with a hydroxyl or another group, then it is apparent that the concentration of soluble organic constituents of the minus S tissues were several times greater than in the plus S tissues.

With the question in mind of whether sugars might be present in the leaves and stems of S deficient plants at night, samples were taken on August 28 from a few plants grown outdoors on the 0.1 SO_4 solution. The following results were obtained:

	1 P.M.	8 P.M.	2 A.M.
Leaves			
Total sugars	**0.47**	**0.36**	**0.05**
Starch	**2.69**	**2.84**	**1.33**
Stems plus petioles			
Total sugars	**0.01**	**0.01**	**0.00**
Starch	**1.19**	**1.85**	**.75**

It may be noted that appreciable sugars were not found at 2 A.M. in either the leaves or stems. But sugars were found in relatively low concentrations in the leaves at both 1 P.M. and 8 P.M. It remains unknown whether the presence of leaf sugars in this test, as distinct from those preceding it in the greenhouse, is attributable to the outdoor light intensity or to some less tangible factor.

In the low sulphate cotton plants: (1) Sugars were absent, with the exception just noted, in leaves. (2) Sugars in the stems were about one fifth normal in one experiment (table II) and absent in a second (table IV). (3) Sugars in the roots were normal. (4) Starch levels were nearly normal in leaves, stems and roots. (5) Threefold, or higher, concentrations of soluble organic nitrogen were found in leaves, stems, and roots. In view of these relations it seems unlikely that much sugar could have been translocated from the leaves for the formation of the starch and hemicellulose found in the stems; the possibility is thus indicated that the primary carbon transfer was in the form of carbon-nitrogen compounds. The amino nitrogen, as well as the total soluble organic nitrogen was especially high in the low sulphate plants. The chemical data indicate that in sulphur deficient cotton plants the sugar equilibriums are shifted strongly toward the amino acids and other organic N compounds on the one hand and toward starch

TABLE VII

TOTAL SUGARS AND SOLUBLE ORGANIC NITROGEN OF COTTON EXPRESSED AS PERCENTAGES OF DRY WEIGHT—TAKEN FROM TABLE IV

SO_4 supply p.p.m.	35 days		65 days			
	Tops		Leaf blades		Stalks	
	Total sugars	Soluble organic N	Total sugars	Soluble organic N	Total sugars	Soluble organic N
0.1	.00	1.55	.00	2.23	.00	3.11
1	.00	1.19	.00	2.21	.00	2.98
10	2.89	.73	1.54	1.19	5.89	.39
50	2.52	.73	3.06	.51	7.75	.30
200	2.62	.64	3.43	.45	7.86	.28

and hemicellulose on the other, with the sugars existing only as transitory compounds in concentrations too low to give measurable reducing activity.

CHAPMAN and BROWN'S (3) results on the effect of sulphur deficiency on mineral accumulation provide background material for the present results with cotton. In orange leaves they found S deficiency to increase the percentage content of K, P, and Mg, as well as N, whereas Ca was reduced. In cotton, K showed little or no gain and Ca and Mg as well as P were increased. It would thus seem that in mineral relations, as well as in some other respects, a uniformity of reaction to S deficiency does not exist between species. The yellowing of cotton leaves evidently does not involve an iron relation; the leaves of cotton on plus S contained 0.023% iron and those on minus S 0.026%.

Summary

Cotton plants were grown on nutrient solutions low in sulphur. The leaves were chlorotic with symptoms resembling nitrogen deficiency. The stem elongation was retarded and leaf size reduced. The old leaves deteriorated.

Vegetative growth and number of bolls were increased proportionately with increasing sulphur supply, *i.e.*, there was no differential effect of sulphur on the two growth activities.

With sulphur deficiency, the concentrations of reducing sugars and sucrose in the leaves, and sometimes in the stems, were characteristically too low for measurement by the usual analytical procedures. Starch in all tissues was reduced only moderately if at all, and hemicellulose not at all.

Nitrate and soluble organic nitrogen accumulated in high concentrations; the increase in the latter was in the same order as the reductions in sugars. Protein nitrogen was greatly reduced by S deficiency in the tops of young plants and in the leaves of plants 65 days old. In the stems more protein was found in minus S than in high S plants.

Although S deficiency greatly decreased the amount of protein in the leaves, the percentage of S in this protein was higher, indicating that an unusually large part of the protein sulphur was localized in the chloroplasts.

The absence of sugars in the leaves of the low sulphate plants suggests that carbon was translocated from leaves to stems primarily in the form of organic nitrogen compounds and also that the sugars were transitory with the equilibriums shifted strongly towards amino acids on the one hand and towards starch on the other.

The discussion of the data is in a large part on the basis of the reactions of cotton to sulphur deficiency in comparison with the results of others who worked with tomato, soybean, sunflower, and mustard. A table summarizing the salient results with the five plants is presented. There were noteworthy differences in the reactions of the five plants.

In cotton, S deficiency resulted in extra accumulations of phosphorus, calcium, and magnesium. The accumulations of iron and potassium were changed little.

Little tangible symptomatic or biochemical evidence could be found of proteolysis and the re-use of sulphur. The biochemical difficulty of demonstrating sulphur re-use is indicated.

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LITERATURE CITED

1. ASSOCIATION OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. Fourth Edition. 1935.
2. BEESON, K. C. The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U. S. Dept. Agr. Misc. Pub. **369**: 1–164. 1941.
3. CHAPMAN, H. D. and BROWN, S. M. Effects of sulphur deficiency of citrus. Hilgardia **14**: 185–201. 1941.

4. Cooper, H. P. Nutritional deficiency symptoms in cotton. Soil Sci. Soc. Amer. Proc. **4**: 322–324. 1939.
5. Cooper, H. P. and Mitchell, J. H. Chemical composition of the cotton plant grown on different soil types. Soil Sci. Soc. Amer. Proc. **2**: 374–377. 1946.
6. Eaton, F. M. Toxicity and accumulation of chloride and sulphate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.
7. Eaton, F. M. and Egrle, D. R. Carbohydrate accumulation in the cotton plant at low moisture levels. Plant Physiol. **23**: 169–187. 1948.
8. Eaton, S. V. Influence of sulphur deficiency on the metabolism of the soybean. Bot. Gaz. **97**: 66–100. 1935.
9. Eaton, S. V. Influence of sulphur deficiency on metabolism of the sunflower. Bot. Gaz. **102**: 536–556. 1941.
10. Eaton, S. V. Influence of sulphur deficiency on metabolism of black mustard. Bot. Gaz. **104**: 306–315. 1942.
11. Hanson, E. A., Barrien, H. S., and Wood, J. G. Relations between protein nitrogen, protein sulphur and chlorophyll in leaves of Sudan grass. Australian Jour. Exptl. Biol. and Med. Sci. **19**: 231–234. 1941.
12. Harris, H. C., Bledsoe, R. W., and Calhoun, P. W. Responses of cotton to S fertilization. Jour. Amer. Soc. Agron. **37**: 323–329. 1945.
13. Kraybill, H. R. and Smith, T. O. Plant metabolism studies. New Hampshire Station, Bul. 212. 1924.
14. Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R. Effects of sulphur deficiency on metabolism in tomato. Plant Physiol. **7**: 565–595. 1932.
15. Wilcox, L. V. Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. Ind. and Eng. Chem., Anal. Ed. **9**: 136–138. 1937.
16. Wood, J. G. and Cruickshank, D. H. The metabolism of starving leaves. V. Changes in amounts of some amino acids during starvation of grass leaves; and their bearing on the nature of the relationship between proteins and amino acids. Australian Jour. Exptl. Biol. Med. Sci. **22**: 111–123. 1944.
17. Yoe, John H. Photometric Chemical Analysis. Vol. 1. John Wiley & Sons, Inc., New York. 1928.
18. Younge, O. R. Sulphur deficiency and its effect on cotton production on coastal plain soils. Soil Sci. Soc. Amer. Proc. **6**: 215–218. 1941.