THE CONTRIBUTION BY LEAVES OF DIFFERENT AGE TO NEW GROWTH OF SUBTERRANEAN CLOVER PLANTS FOLLOWING THE REMOVAL OF A SULPHUR STRESS

II.* UPTAKE AND DISTRIBUTION OF SULPHUR

By D. Bouma,† Z. Titmanis,‡ and E. A. N. Greenwood‡

[Manuscript received 8 March 1972]

Abstract

Plants were grown in three sulphur pretreatment solutions (0·25, 1, and 4 p.p.m.: S_1, S_2, and S_3) and transferred to full solutions (= S_3) 32 days after sowing. On the same day the five oldest trifoliate leaves were shaded in one-third of the plants, in another third these leaves were removed and the remainder of the plants were left untreated (controls).

Between days 32 and 35 sulphur uptake by S_1 and S_2 plants was greater than by S_3 plants. Shading and particularly defoliation reduced sulphur uptake, but sulphur pretreatment effects remained unchanged.

The distribution of total sulphur during the same period was predominantly to the older treated group of leaves in the control and shaded S_1 plants, but shifted to new leaves which emerged between days 32–35 as the sulphur status at transfer increased. In the defoliated S_1 plants sulphur distribution shifted to the younger leaves existing at transfer but not to newly formed leaves. Differences in root sulphur were relatively small.

In most plant parts the marked increases in total sulphur between days 32 and 35 were attributable to an accumulation of sulphate sulphur. The changes in the organic sulphur content of the dry matter were small. The greatest increase in organic sulphur occurred in the younger, sulphur-deficient, emerged leaves of the S_1 plants, while there was no change, or a small loss, from the older unimpaired leaves of S_1 and S_2 plants respectively.

The results indicated a restricted mobility of organic as well as sulphate sulphur. There was no evidence for any significant redistribution of sulphur between plant parts. The results were consistent with the conclusion of Part I of this series that during the development of a sulphur deficiency the metabolic activity of the younger leaves declined while that of the older leaves remained relatively unaffected, at least initially.

I. Introduction

In Part I of this series (Bouma, Greenwood, and Dowling 1972) it was concluded that during the first 3 days of recovery from sulphur stress, assimilates for new growth arose from current photosynthesis in the relatively unimpaired mature leaves, supplemented by mobilization in younger emerged leaves. The latter showed a decline in growth and some chlorosis due to the previous sulphur shortage. This conclusion was based on the results of an experiment in which, after transfer of

* Part I, *Aust. J. biol. Sci.*, 1972, **25**, 1147–56.

† Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T. 2601.

‡ Division of Plant Industry, CSIRO, Rural Sciences Laboratory, Private Bag, P.O. Wembley, W.A. 6014.

plants raised at different sulphur levels to complete solutions, a comparison was made of the changes in net assimilation rates and in dry matter distribution between leaves of different age in intact plants and in plants in which the five oldest trifoliate leaves were individually shaded or removed. Although they were relatively small, the differences in response during the first 3 days after transfer to complete solutions determined the growth patterns in later stages of the experiment.

In view of the differences in the contribution by leaves of different ages to the assimilate pool for new growth, it was thought that there could also be significant differences in the pattern of sulphur distribution, but in a direction opposite to that for the products of carbon assimilation. Because of their greater sulphur shortage, the younger emerged leaves might show a net import of sulphur, while at least some export might occur from mature, unimpaired leaves. This hypothesis was tested in the work reported here.

II. Methods

Full experimental details were described before (Bouma, Greenwood, and Dowling 1972). Briefly, *Trifolium subterraneum* L. (cv. Mt. Barker) was grown at three sulphur pretreatment levels in nutrient solutions [0·25, 1 and 4 p.p.m. (= S_1, S_2, and S_3 respectively).] On the 32nd day after sowing, plants grown at S_1 and S_2 levels were transferred to the S_3 level, and plants in each sulphur pretreatment were divided into three groups. In one group shades of aluminium foil were placed over the first five trifoliate leaves (fully expanded). In the second group the corresponding leaves were cut off at the base of the petiole, while in the third group the leaves were left untreated. These leaf treatments are referred to as shaded, defoliated, and control respectively. At the same time, the leaves younger than the treated ones were tagged (numbers varied between 4 and 7).

At harvest (22, 32, 35, 39, and 43 days after sowing) plants were separated into shoots (aerial parts) and roots. The shoots were further separated into leaves and petioles. The leaves included the leaflets and petiolules. The petioles included the stipules and the short stem. Leaves and petioles were further separated into the following groups on the basis of leaf treatment on the day of transfer to full solution (day 32).

(1) Shaded leaves and petioles (five oldest trifoliates) including also unifoliates and cotyledons (fraction I).

(2) Leaves and petioles younger than those of fraction I, existing and tagged on day 32 (fraction II).

(3) Leaves and petioles emerging after day 32 (fraction III). The short stem was included in the petioles of this fraction.

(4) Roots.

There were 12 pots per treatment on each harvest occasion and two plants per pot. At harvests plants were kept in a refrigerator until separated and then rapidly dried in a vacuum oven (70°C). Plant parts were bulked in groups of six replicates. Total sulphur was determined by the method described by Steinbergs *et al.* (1962). Sulphate sulphur was extracted from 0·1 g material by shaking for 0·5 hr with 50 ml of 0·01M calcium chloride. 2 ml of 5% barium chloride was added to an aliquot containing 10–50 μg sulphur. Volume was reduced to 0·5 ml by evaporation. Supernatant was removed through a sintered-glass filter-stick of porosity 4. The barium sulphate precipitate was washed three times with 1-ml lots of barium chloride. The sulphate was reduced to sulphide and determined as methylene blue (Johnson and Nishita 1952). Organic sulphur was taken as total sulphur minus sulphate sulphur.

Results are presented on a relative basis (percentage of dry matter) and on an absolute basis (micrograms per plant).

III. Results

(a) *Changes in Relative Sulphur Content*

(i) *Effects of Sulphur Supply*

On day 32, the relative sulphur content of all plant fractions was greater at S_3 than at S_2 and S_1 and was greater for fraction I than for fraction II at all sulphur levels (Fig. 1). Petiole sulphur has not been presented as it agreed closely with leaf sulphur.

At S_1 relative sulphur in fraction II leaves was very low (0·14%) and these leaves were the first to become pale and chlorotic. Apparently, sulphur was not readily translocated from older to younger leaves.

The effect of the sulphur stress was further reflected in the low proportion of sulphate sulphur in all S_1 plant parts on day 32, where it could be determined (Fig. 1; Table 1). The proportion of sulphate sulphur increased with the sulphur status of the plant at transfer.

Table 1

RELATIVE SULPHUR CONTENTS OF THE DIFFERENT PLANT PARTS

The first value of each pair is the organic sulphur content and the value in parenthesis is the sulphate content. — denotes insufficient material for analysis

Plant part	Sulphur level	Relative sulphur content (% dry matter)			
		Day 32	Day 35: control	Day 35: shaded	Day 35: defoliated
Older leaves (fraction I)	S_1	0·19 (0·01)	0·19 (0·40)	0·22 (0·31)	—
	S_2	0·33 (0·15)	0·28 (0·40)	0·29 (0·42)	
	S_3	0·33 (0·42)	0·33 (0·49)	0·33 (0·54)	
Younger leaves (fraction II)	S_1	0·14 —	0·25 (0·40)	0·22 (0·53)	0·25 (0·57)
	S_2	0·28 —	0·34 (0·29)	0·35 (0·38)	0·34 (0·56)
	S_3	0·36 (0·12)	0·37 (0·19)	0·39 (0·25)	0·40 (0·30)
New leaves (fraction III)	S_1	—	0·24 (0·18)	0·27 (0·24)	0·28 (0·23)
	S_2	—	0·27 (0·16)	0·30 (0·19)	0·34 (0·28)
	S_3	—	0·30 (0·10)	0·30 (0·15)	0·33 (0·15)
Roots	S_1	0·11 (0·01)	0·12 (0·45)	0·16 (0·50)	0·17 (0·44)
	S_2	0·16 (0·10)	0·19 (0·36)	0·15 (0·53)	0·13 (0·60)
	S_3	0·15 (0·78)	0·15 (0·73)	0·19 (0·83)	0·11 (0·91)
S.E. ($P = 0·05$)		0·03 (0·02)	0·02 (0·04)	0·03 (0·05)	0·04 (0·03)

Transfer of the S_1 and S_2 plants to complete solutions ($= S_3$) on day 32 increased relative sulphur of all plant parts. The increases were greatest at S_1 and least at S_3 (Fig. 1). Most of the increase in relative sulphur between day 32 and 35 was due to an accumulation of sulphate sulphur in all plant parts existing at transfer and this was more apparent the lower the sulphur status on day 32 (Table 1). There was little change in organic sulphur of fraction I leaves and roots in any of the sulphur pretreatments between day 32 and 35 (Table 1). However, the fraction II leaves of

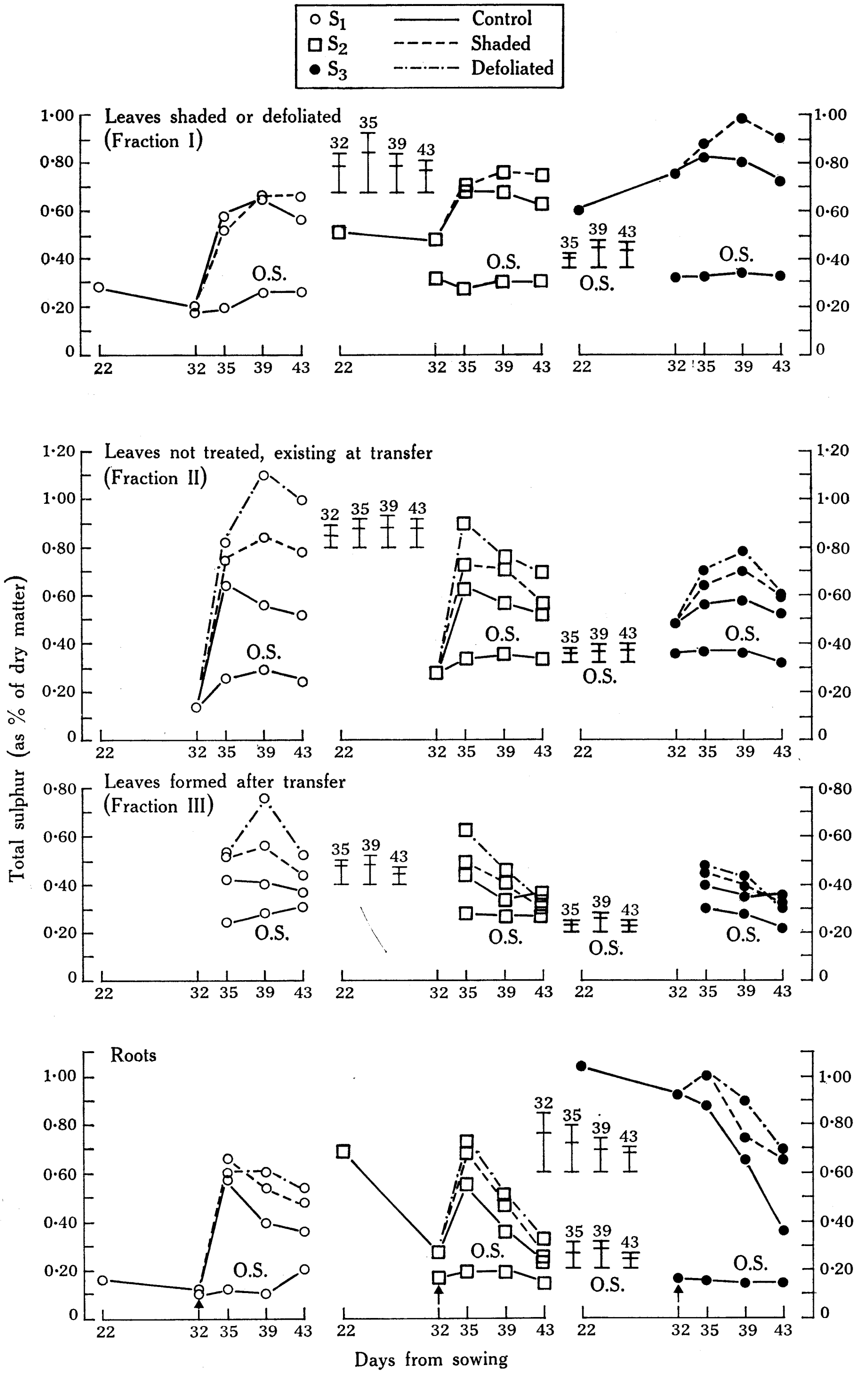

Fig. 1—Changes in the relative contents of total sulphur of different plant parts (% dry matter). Organic sulphur (O.S.) is shown for the control plants only. Day 32 (day of transfer to full solutions) is shown by arrow.

the S_1 plants showed a considerable increase in organic sulphur over this period. There was also a small increase in organic sulphur in fraction II leaves at S_2, but no change at S_3. This finding supports the hypothesis that sulphur stress developed first in the younger leaves.

In comparable treatments total sulphur of fraction III leaves was lower than that of leaves of fractions I or II (Fig. 1). However, the difference was largely due to the accumulation of sulphate sulphur in the leaves of fractions I or II, since all fractions showed similar concentrations of organic sulphur in the dry matter. The difference in total sulphur between leaves of different ages persisted throughout the experiment in all treatments (Fig. 1; Table 2).

TABLE 2

ORGANIC AND SULPHATE SULPHUR CONTENTS OF THE THREE LEAF FRACTIONS (I, II, III) AND THE ROOTS ON DAY 43

Treatment	Sulphur level	Organic sulphur (% dry matter)				Inorganic sulphur (% dry matter)			
		I	II	III	Roots	I	II	III	Roots
Control	S_1	0·27	0·22	0·31	0·20	0·30	0·26	0·06	0·15
	S_2	0·31	0·33	0·33	0·14	0·32	0·19	0·03	0·08
	S_3	0·33	0·31	0·21	0·14	0·40	0·20	0·04	0·22
		S.E. = 0·02*				S.E. = 0·01*			
Shaded	S_1	0·23	0·26	0·26	0·22	0·44	0·52	0·17	0·25
	S_2	0·33	0·27	0·23	0·12	0·42	0·30	0·06	0·12
	S_3	0·29	0·28	0·23	0·27	0·61	0·31	0·09	0·39
		S.E. = 0·02*				S.E. = 0·02*			
Defoliated	S_1	—	0·27	0·27	0·22	—	0·72	0·25	0·31
	S_2	—	0·23	0·24	0·13	—	0·47	0·09	0·20
	S_3	—	0·28	0·21	0·28	—	0·31	0·10	0·41
		S.E. = 0·02*				S.E. = 0·04*			

* $P = 0 \cdot 05$.

(ii) *Effects of Shading and Defoliation*

In general, these treatments caused greater increases in relative sulphur content of plant parts compared with the controls (Fig. 1). This applied particularly to the leaves of fractions I and II, and more so the greater the initial sulphur stress. Shading and defoliation had least effect on root sulphur. Table 1 shows that there was no effect of shading or defoliation on organic sulphur in any of the plant parts and that the increase in total sulphur was entirely due to an accumulation of sulphate sulphur.

(b) *Sulphur Uptake and Distribution*

Pretreatment effects were clearly reflected in the differences in the absolute sulphur content of the principal plant parts between days 22 and 32 (Fig. 2) even though dry weights of plants were similar. Transfer to full solutions caused immediate and marked increases in absolute sulphur of all plants parts, but particularly of leaves and roots. Shading had little effect on absolute sulphur of leaves, petioles,

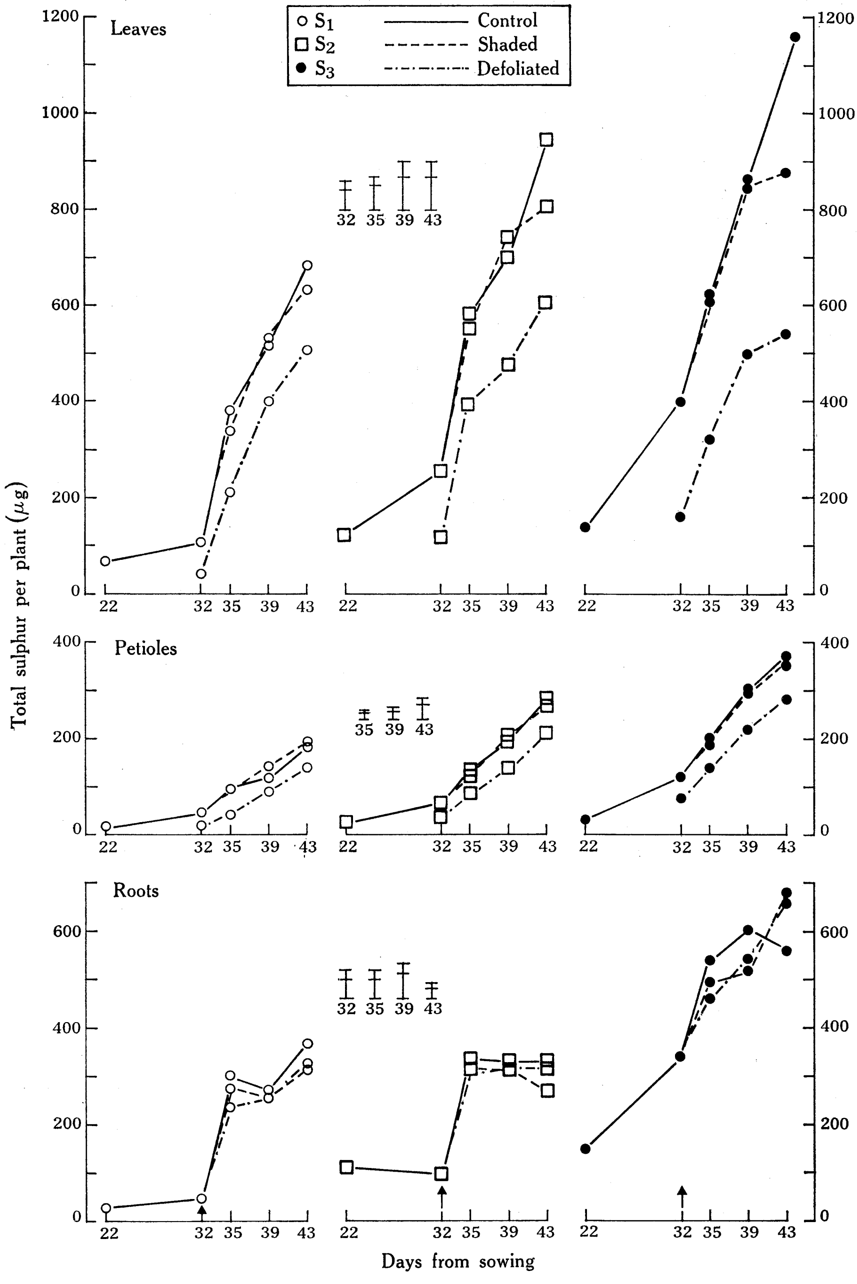

Fig. 2.—Absolute total sulphur contents of leaves, petioles, and roots.

or roots during the first stages of recovery. However, at the last harvest there was less leaf sulphur in the shaded treatment than in the control, particularly at S_3.

Defoliation also had little effect on root sulphur but the differences in leaf sulphur caused by defoliation on day 32 increased with time.

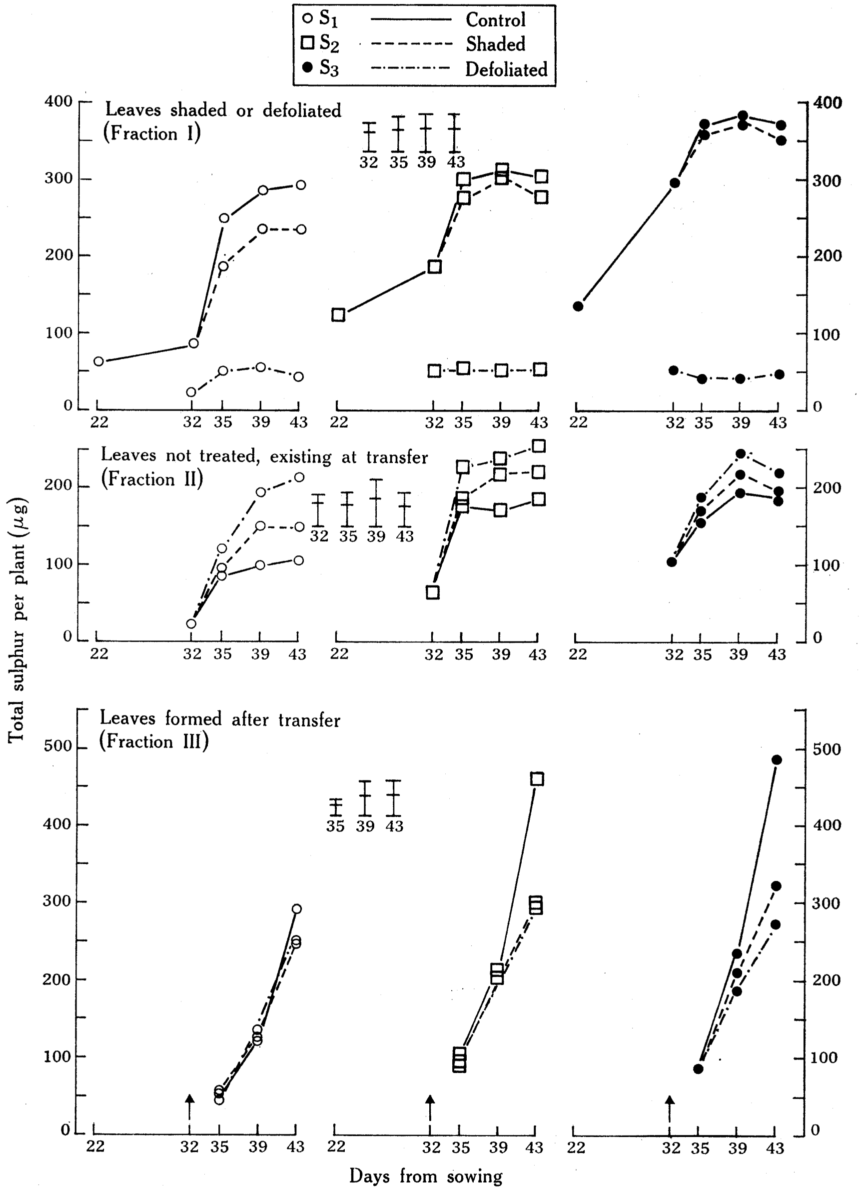

Fig. 3.—Absolute total sulphur content of the three different groups of leaves.

Although the overall effect of shading on the leaves as a whole was small, at least initially (Fig. 2), shading greatly influenced the initial distribution of sulphur between the different groups of leaves (Fig. 3). Shading reduced the amount of sulphur moving to the leaves of fraction I, it increased sulphur transported to fraction

II leaves, particularly in the S_1 plants, but had little initial effect on transport to fraction III leaves. Removal of fraction I leaves caused an even greater increase in sulphur translocated to fraction II leaves than did shading.

In Part I of this series (Bouma, Greenwood, and Dowling 1972) it was concluded that the effects of shading and defoliation on growth found at the end of the experiment were largely determined in the period immediately after the initiation of those treatments on day 32. We examined the corresponding early effects on sulphur uptake and distribution to the various plant parts (Table 3). On day 32, the S_1 plants contained relatively large amounts of sulphur in the shoots of fraction I. The importance of younger shoots and roots as sinks for sulphur increased with the sulphur supply before day 32. Transfer to full solutions on day 32 resulted in a greater sulphur uptake by the S_1 and S_2 plants than by the S_3 plants, irrespective of leaf treatment.

TABLE 3

TOTAL SULPHUR CONTENT ON DAY 32 AND THE INCREASES BETWEEN DAY 32 AND DAY 35

Shoot refers to leaf+petiole. See Section II for explanation of subdivision of shoots

Plant part	Sulphur level	Total sulphur on day 32 (μg)	Increases between day 32 and 35 (μg)		
			Control	Shaded	Defoliated
Old shoots (I)	S_1	99	190	138	42
Younger shoots (II)		31	70	79	98
New shoots (III)		—	91	92	69
Roots		47	253	232	192
Whole plant		177	604	540	401
Old shoots (I)	S_2	231	117	94	0
Younger shoots (II)		88	115	120	175
New shoots (III)		—	166	145	145
Roots		95	237	222	219
Whole plant		414	635	581	537
Old shoots (I)	S_3	368	96	77	3
Younger shoots (II)		158	40	51	69
New shoots (III)		—	167	154	155
Roots		335	206	159	126
Whole plant		861	509	441	353

Figure 4 shows the increments in sulphur of the different plant parts of Table 3 expressed as percentages of the total uptake by the plant over the same period (distribution percentage). The distribution percentage to the roots was not greatly affected by leaf treatment or sulphur pretreatment. The important sulphur pretreatment effect was to decrease sulphur translocation to the shoots of fraction I and to increase the proportion of sulphur distributed to fraction III shoots with increasing sulphur status at transfer. When shaded the distribution was essentially the same as for the control plants, there being relatively more sulphur translocated to fraction I shoots of the S_1 plants than to those of the S_2 and S_3 plants. It is noteworthy that removal of the five oldest trifoliate leaves caused an increase in sulphur distribution to younger shoots (fraction II) but not to the newly emerged shoots (fraction III).

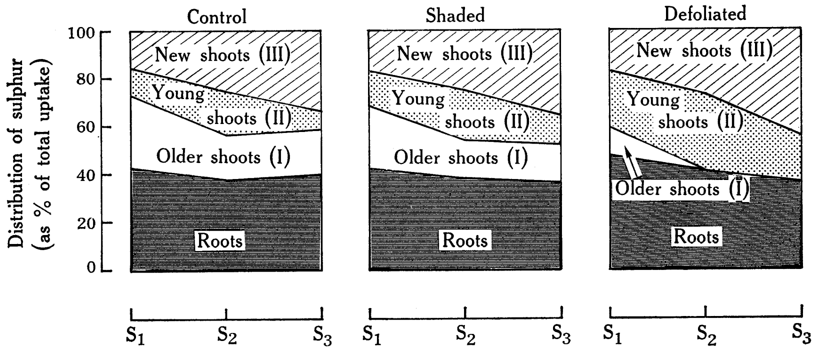

Fig. 4.—Distribution of sulphur to the different plant parts, expressed as a percentage of the total uptake by the plant, between days 32 and 35. Shoot refers to leaves plus petioles. See Section II for explanation of I, II, and III.

IV. Discussion

Earlier results (Bouma 1967*a*) and those of Part I in this series (Bouma, Greenwood, and Dowling 1972) have shown that new growth during the initial stages of recovery from a sulphur stress is restricted by a reduction in the net assimilation rate (E_A), to an extent determined by the severity of the previous sulphur shortage. Strong evidence was presented in Part I that assimilates for new growth, derived from current photosynthesis, arose mainly in mature, green, and relatively unimpaired leaves. Indirect evidence, derived from the comparison of intact control plants and plants in which the five oldest trifoliate leaves were shaded or removed, strongly suggested that most of the reduction in E_A was attributable to the younger emerged leaves. These leaves were somewhat chlorotic and showed net losses in dry matter sufficient to account for a significant proportion of the dry matter in new leaves formed during the first three days of recovery. The mobilization in the young emerged leaves appeared to increase with the severity of the previous sulphur stress.

The present results agree with the view that the older leaves (fraction I) remained unimpaired under the mild sulphur stress of the S_1 level and that most of its effect was reflected in the younger emerged leaves (fraction II). Table 1 showed that there was no increase in the organic sulphur content of the dry matter of fraction I leaves at S_1, between days 32 and 35, but a nearly twofold increase in the fraction II leaves. The decline in sulphur content of the dry matter under sulphur stress, first in sulphate sulphur and later also in organic sulphur, particularly in the younger emerged leaves (Table 1), is in agreement with other results, e.g. those of Ergle (1954) for cotton. While these effects of a sulphur deficiency are reasonably well documented, there is little information on the relation between sulphur uptake and distribution and the production and redistribution of dry matter during the initial stages of recovery from stress conditions. On the basis of the differences in the flow of carbon assimilates to and from mature and younger emerged leaves, established in Part I of this series, it was thought that some export of sulphur from older leaves was likely, while most of the flow of sulphur after transfer to complete solutions would be directed to the younger, most deficient emerged leaves.

Considering the changes in total sulphur first (Table 3), it is evident that the total sulphur content of all plant parts increased considerably between days 32 and 35. This applied to all leaf treatments. Figure 4 showed that at S_1 a greater percentage of the sulphur taken up by the plant was distributed to older than to younger leaves. However, most of the increase in sulphur was sulphate sulphur (Table 1). Sulphate sulphur is usually regarded as storage sulphur (Eaton 1966) and it would, therefore, be more realistic to consider the adjustments during the first 3 days of recovery in terms of organic sulphur. The following comparison shows indeed that there was a marked increase in the organic sulphur content (μg) of the fraction II leaves, whereas there was little change at S_1 and S_2, for mature leaves (fraction I):

Level	Fraction I		Fraction II	
	Day 32	Day 35	Day 32	Day 35
S_1	80	79	22	33
S_2	132	125	64	96
S_3	131	151	89	103

It seems reasonable to conclude that these changes reflected the differences in metabolic demands during the recovery from sulphur stress, in line with the evidence based on the differences in the flow of carbon assimilates shown before.

The very high intake of sulphate sulphur over the period day 32–35 deserves some comment (Table 1; Fig. 1). The sulphur level in the full solutions (4 p.p.m. sulphur) was chosen to represent an optimum sulphur level under the prevailing experimental conditions. This level is $\frac{1}{16}$th of that commonly used in Hoagland type nutrient solutions. It does not appear likely therefore that the accumulation of sulphur (Table 3), of which most was sulphate sulphur, and which was greater at S_1 and S_2 than at S_3, was simply a reflection of a high sulphate level in the solutions. If this was the case there would have been some relationship with plant size. This was not so. Dry weights on day 32 were 138, 133, and 133 mg per plant, and leaf areas 22, 32, and 32 cm^2 at S_1, S_2, and S_3 respectively.

Although the present results probably reflect, at least in part, differences in internal demands or "sink strength" for sulphur, it is at this stage difficult to understand why this should express itself by the considerable accumulation of sulphate sulphur without any marked changes in the organic sulphur content of the dry matter of most plant parts. Earlier work (Bouma 1967*b*) has shown that most of the radioactive sulphur accumulated in the leaves during the first 24 hr of recovery was still present in those leaves 6 days later. In the same work it was shown that, over a period of 7 days after transfer of non-deficient plants (= S_3 of present work) to solutions without sulphur, there were no net losses of sulphur from existing leaves and all sulphur in new leaves had been derived from existing petioles and particularly from the roots. If most of the sulphur in the older leaves was sulphate sulphur, as the present results indicate, the question also arises why this does not circulate more freely in the plant. An answer to these questions must await further information on sulphur uptake and metabolism under conditions of changing sulphur supply.

From the point of view of assessing the sulphur status of the plant, it would appear that the younger leaves give a better reflection of the plant's response to changes in sulphur supply than the older leaves.

V. References

Bouma, D. (1967*a*).—Growth changes of subterranean clover during recovery from phosphorus and sulphur stresses. *Aust. J. biol. Sci.* **20**, 51–66.

Bouma, D. (1967*b*).—Nutrient uptake and distribution in subterranean clover during recovery from nutritional stresses. II. Experiments with sulphur. *Aust. J. biol. Sci.* **20**, 613–21.

Bouma, D., Greenwood, E. A. N., and Dowling, E. J. (1972).—The contribution by leaves of different age to new growth of subterranean clover plants following the removal of a sulphur stress. I. Origin and distribution of dry matter. *Aust. J. biol. Sci.* **25**, 1147–56.

Johnson, C. M., and Nishita, H. (1952).—Microestimation of sulphur in plant materials, soils and irrigation waters. *Analyt. Chem.* **24**, 736–42.

Steinbergs, A., Iismaa, O., Freney, J. R., and Barrow, N. J. (1962).—Determination of total sulphur in soil and plant material. *Analytica chim. Acta* **27**, 158–64.

Eaton, F. M. (1966),—Sulphur. In "Diagnostic Criteria for Plants and Soils". (Ed. Homer D. Chapman) (Univ. Calif., Div. Agric. Sci.)

Ergle, D. R. (1954).—Utilization of storage sulphur by cotton and the effect on growth and chloroplast pigments. *Bot. Gaz.* **115**, 225–34.