NUTRIENT UPTAKE AND DISTRIBUTION IN SUBTERRANEAN CLOVER DURING RECOVERY FROM NUTRITIONAL STRESSES

II.* EXPERIMENTS WITH SULPHUR

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Summary

Young subterranean clover plants, raised at three sulphur levels, were transferred to complete solutions and to solutions without sulphur and then grown for a further 7 days.

The rates of sulphur uptake, expressed on a root weight basis, were considerably higher for previously deficient plants than for non-deficient plants. The roots retained approximately the same percentage of the sulphur taken up by the plant, irrespective of the sulphur status at transfer. Considerably more sulphur was distributed to old leaves than to young leaves of previously deficient plants, while in non-deficient plants sulphur was preferentially distributed to new shoots formed after transfer.

In a similar experiment with ^{35}S little or no retranslocation was found of radioactive sulphur accumulated in older leaves of previously deficient plants. For plants raised at an optimum sulphur level part of the sulphur in older leaves was redistributed to new leaves. No retranslocation of root sulphur was observed.

Plants grown without an external sulphur supply showed no net losses of sulphur from existing leaves. Sulphur recovered in new leaves formed after transfer of deficient plants to solutions without sulphur had been derived from old petioles. In plants that were not deficient at transfer to solutions without sulphur, most of the sulphur in new leaves had been derived from the roots.

I. Introduction

The first paper of this series (Bouma 1967*b*) examined the uptake and distribution patterns of phosphorus after transfer of subterranean clover plants, raised at different phosphorus levels, to complete solutions and to solutions without phosphorus.

The present paper reports the results of similar experiments with sulphur. The results are discussed and compared with those for phosphorus.

II. Methods

In the first experiment young subterranean clover plants were raised at three sulphur pretreatment levels (S_1, S_2, S_3 — 0·2, 0·5, and 4 p.p.m. sulphur respectively). When the plants were 23 days old they were transferred to complete solutions and to solutions without sulphur. They remained in these solutions for a period of 7 days.

In the second experiment seedlings were pretreated at three sulphur levels for a period of 14 days. The sulphur levels were 0·25, 1, and 4 p.p.m. sulphur. Plants were then transferred to complete solutions which contained ^{35}S (2 μc/l) for 1, 2, or 3

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days immediately after transfer, or for the last 3 days of the 7-day recovery period. Two plants were harvested at the end of each uptake period and on day 7 for counting and for autoradiography. Further experimental details were as described in the first paper (Bouma 1967*b*).

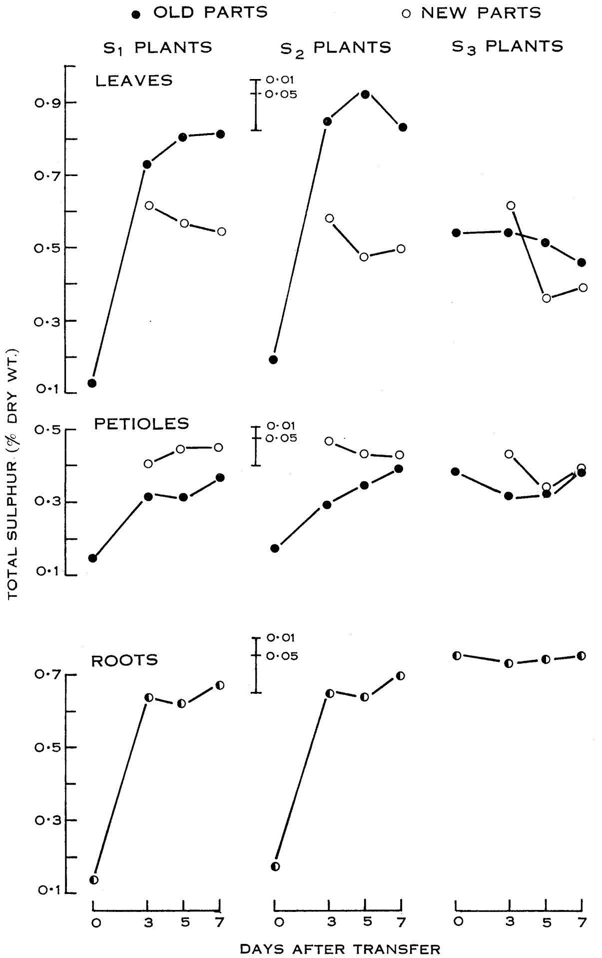

Fig. 1.—Changes in relative sulphur contents after transfer of plants raised at three sulphur levels (S_1, S_2, S_3) to complete nutrient solutions (expt. I). Minimum differences for significance at $P < 0 \cdot 05$ and $P < 0 \cdot 01$ are shown.

III. Results

(*a*) *Experiment I*

(i) *Uptake and Distribution of Sulphur after Transfer to Complete Solutions*

The changes in relative and absolute sulphur contents after transfer of plants raised at three sulphur levels to complete solutions are shown in Figures 1 and 2 respectively. The distribution percentages of sulphur to the different plant parts are shown in Table 1.

The transfer of sulphur-deficient plants (S_1 and S_2) to complete solutions resulted in considerable increases in relative sulphur of all plant parts. The increases were particularly pronounced in existing leaves and in roots and occurred mainly during

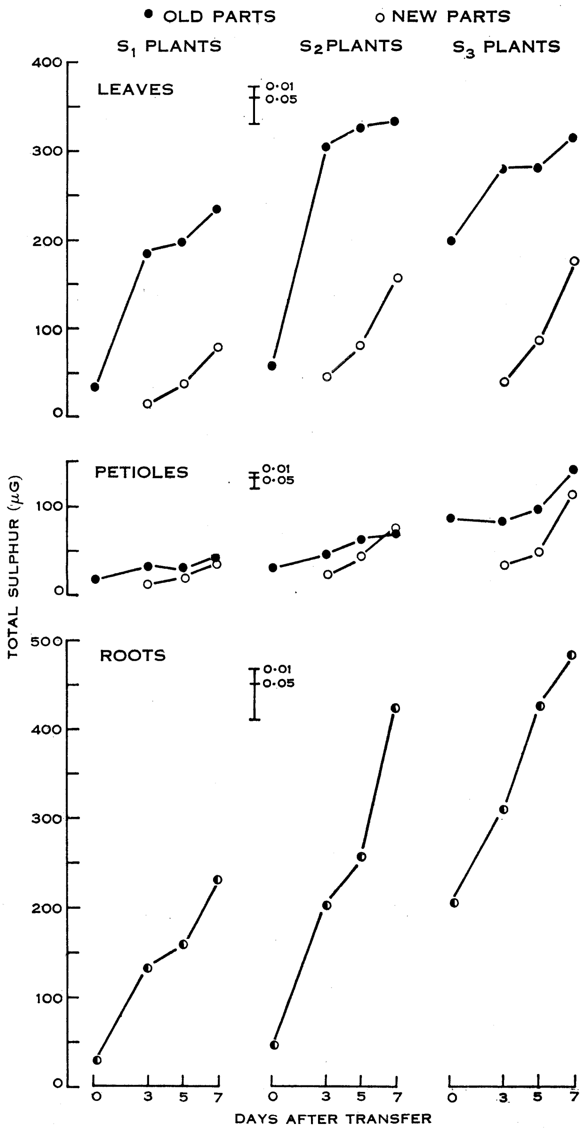

Fig. 2.—Changes in absolute sulphur contents after transfer of plants raised at three sulphur levels (S_1, S_2, S_3) to complete solutions (expt. I). Minimum differences for significance at $P<0\cdot05$ and $P<0\cdot01$ are shown.

the first 3 days of recovery. On day 3 and subsequent harvests relative sulphur in older leaves of S_1 and S_2 plants greatly exceeded that of the new leaves and also the relative sulphur contents of old and new leaves of plants that had not been deficient (S_3).

On day 3 relative sulphur in the roots of S_1 and S_2 plants almost reached the high values found for the roots of the S_3 plants and changed little during the remainder of the 7-day period. Relative sulphur in roots, petioles, and existing leaves of S_3 plants changed little during the period after transfer. The differences in relative sulphur between old and new shoots of S_3 plants were small compared with those for S_1 and S_2 plants.

Absolute sulphur in old leaves of S_1 and S_2 plants increased approximately six times during the first 3 days after transfer. Increases in absolute sulphur later during the recovery were significant, but considerably smaller than before. A large demand for sulphur in the roots is indicated by the very marked increases in absolute sulphur of these organs. This applies particularly to the S_2 plants, which showed a tenfold increase in root sulphur between day 0 and day 7.

TABLE 1

DISTRIBUTION OF SULPHUR TO DIFFERENT PLANT PARTS FOR THE PERIODS 0–3 AND 3–7 DAYS AFTER TRANSFER TO COMPLETE SOLUTIONS EXPRESSED AS A PERCENTAGE OF THE INCREASE FOR THE WHOLE PLANT

Experiment I

Plant Part	Period 0–3 Days			Period 3–7 Days		
	S_1 Plants	S_2 Plants	S_3 Plants	S_1 Plants	S_2 Plants	S_3 Plants
New leaves	6	9	15	25	26	28
New petioles	4	4	13	11	12	17
Old leaves	51	51	33	20	6	6
Old petioles	4	3	−2	4	5	12
Roots	36	33	41	40	51	36

The distribution indices shown in Table 1 emphasize these trends and show clearly that the distribution of sulphur to the different plant parts depended to a large extent on the sulphur status of the plant at transfer. During the first 3 days after transfer the roots retained almost the same proportion of the sulphur taken up by the plant. In plants that had not been under stress (S_3) a considerable part of the upward movement of sulphur was directed to new shoots, while in previously deficient plants far greater amounts were distributed to old shoots than to new shoots. The existing leaves of S_1 and S_2 plants received eight and six times as much sulphur respectively as the new leaves. For the period 3–7 days after transfer the S_2 plants were comparable to those raised at S_3, the distribution of sulphur to new shoots being greater than to older shoots. The new leaves of the S_1 plants received only a little more sulphur than the older leaves.

(ii) *Redistribution of Sulphur after Transfer to Solutions without Sulphur*

Table 2 shows that the redistribution of sulphur after transfer of S_1, S_2, and S_3 plants to solutions without sulphur depended to some extent on the severity of the existing stress at transfer. At all pretreatment levels sulphur in older leaves was the least mobile sulphur source in the plant. For plants which were already deficient at transfer (S_1 and S_2), root sulphur was also not available to meet the demands for sulphur in new growth. Nearly all the sulphur recovered in new shoots of S_1 and S_2 plants had been derived from sulphur mobilized in older petioles. For the S_3 plants, roots as well as old petioles lost nearly half of their sulphur. This was apparently sufficient to enable the plants to continue growth during the 7-day period at nearly the same rate as the corresponding plants in complete solutions (Bouma 1967*a*).

TABLE 2

ABSOLUTE SULPHUR CONTENTS OF PLANT PARTS 0, 3, AND 7 DAYS AFTER TRANSFER OF PLANTS RAISED AT THREE SULPHUR LEVELS TO SOLUTIONS WITHOUT SULPHUR

Sulphur Pretreatment Level	Days after Transfer	Sulphur Content of Old Leaves (μg)	Sulphur Content of Old Petioles (μg)	Sulphur Content of New Leaves (μg)	Sulphur Content of New Petioles (μg)	Sulphur Content of Roots (μg)
S_1	0	32	20	—	—	27
	3	36	10	3	4	25
	7	34	10	5	3	26
Net loss or gain		2	−10	5	3	−1
S_2	0	58	30	—	—	42
	3	53	15	12	5	43
	7	56	12	12	6	46
Net loss or gain		−2	−18	12	6	4
S_3	0	197	85	—	—	203
	3	208	51	29	15	142
	7	190	46	106	47	104
Net loss or gain		−7	−39	106	47	−99

(*b*) *Experiment II*

Figure 3 shows the uptake and distribution of ^{35}S after 1, 2, or 3 days in complete solutions containing radioactive sulphur and the subsequent redistribution of the tracer at the end of the 7-day period.* Figure 3 also shows the distribution of

* The plants in this experiment were smaller than those of the corresponding experiment with phosphorus (Part I), which made it difficult to separate old and new shoots on day 2. The new shoots on day 2 were therefore included with the old shoots. Although this introduced some bias, it does not affect the results because new shoots were separated from old shoots on day 3.

^{35}S on day 7 in the treatment where the tracer was present during the last 3 days of the period.

The transport of ^{35}S to the shoots was slow compared with that of ^{32}P found in the previous paper. The amounts of radioactive sulphur translocated to the shoots approximately doubled between day 1 and day 3 at all pretreatment levels. The quantities recovered in the shoots of the S_2 plants were always greater than those for S_1 and S_3 plants.

Of the ^{35}S present in the old shoots of S_1 plants on the first day after transfer nothing was retranslocated to new shoots between day 1 and day 7. The small amounts present in new shoots on day 7 were probably derived from the roots. S_1

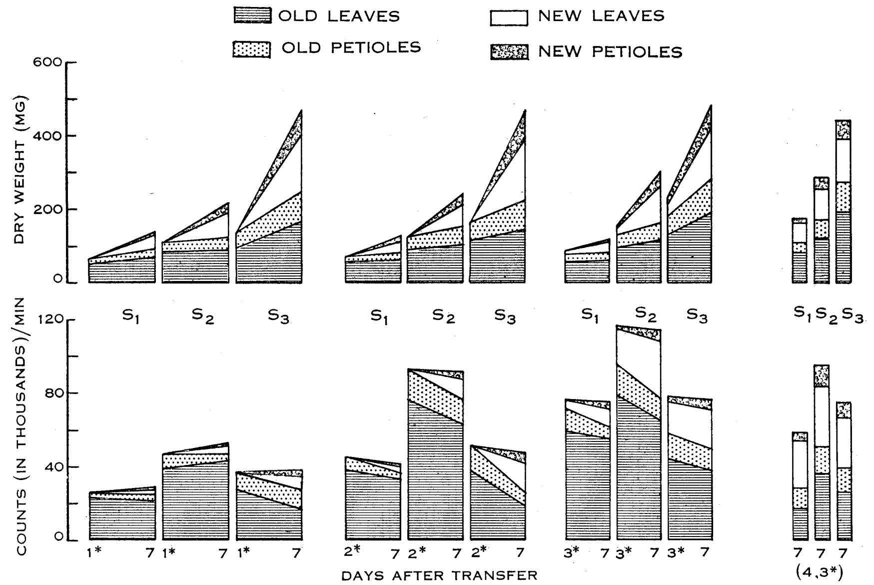

Fig. 3.—Changes in dry weight and distribution of ^{35}S during a period of 7 days after transfer of plants raised at three sulphur levels to complete solutions. ^{35}S was present for 1, 2, or 3 days immediately after transfer or during the last 3 days. Plants were grown in tracer-free solutions for the remainder of the 7 days. The treatments are shown as follows (from left to right, respectively): 1*, 7; 2*, 7; 3*, 7; 4, 3* (expt. II), the asterisks referring to the number of days in tracer solutions.

plants grown with ^{35}S for 2 or 3 days after transfer retranslocated small amounts of radioactive sulphur, which were sufficient to account for the small increases of the tracer in new shoots formed between day 2 and day 7, or between day 3 and day 7. Even for the S_1 plants that had been in tracer solutions for 3 days after transfer, the amounts of ^{35}S found on day 7 in new leaves were small compared with those in old leaves. The translocation pattern for S_2 plants was similar to that for S_1 plants. The tracer taken up in old shoots during the first 24 hr was not retranslocated to other plant parts during the following 6 days in tracer-free complete solutions. The additional tracer taken up during the second and third day permitted some retranslocation, which was sufficient to account for the increases in new shoots during the

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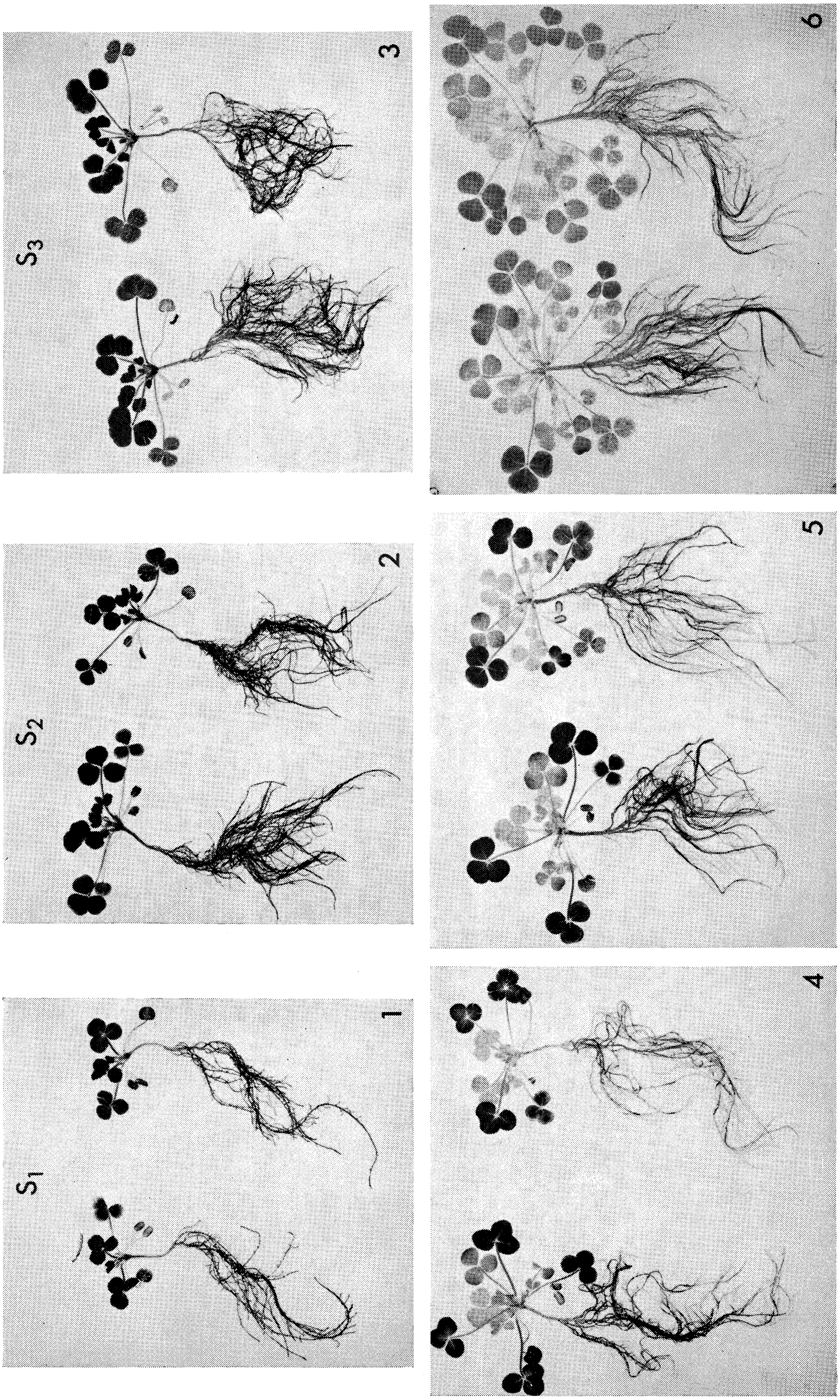

Figs. 1–3.—Autoradiographs of plants raised at three sulphur levels (S_1, S_2, S_3) after 1 day in complete solutions containing 2 μc of ^{35}S per litre.
Figs. 4–6.—Autoradiographs of similarly treated plants after further growth for 6 days in tracer-free complete solutions. Exposure period 6 days for all plants.

following 5 or 4 days in solutions without ^{35}S. The quantities were, relatively speaking, similar to those found for S_1 plants. A greater mobility of sulphur in the old shoots of S_3 plants is suggested by the retranslocation of ^{35}S from these parts after day 1 and by the even greater amounts lost from the old shoots between day 2 and day 7.

The autoradiographs of Plate 1 clearly illustrate the differences in redistribution of ^{35}S after day 1, depending on the sulphur status of the plant on day 0. The four oldest trifoliate leaves of S_1 plants present on day 1 (Plate 1, Fig. 1) are clearly distinguishable on day 7 (Plate 1, Fig. 4) and appear nearly as black as on day 1. This is also the case for the S_2 plants (Plate 1, Figs. 2 and 5), though possibly less pronounced. At S_3, however, all older leaves are considerably lighter on day 7 (Plate 1, Fig. 6), than on day 1, although some of the older ones are still distinguishable. It appears, therefore, that the translocation of sulphur from older to younger leaves depended to a large extent on the sulphur status of the plant.

It is clear from Figure 3 that the translocation of ^{35}S from roots to shoots virtually ceased after transfer to tracer-free solutions. This was in marked contrast to the results for phosphorus where the upward movement of ^{32}P after transfer to solutions without radioactive phosphorus was quite considerable, and greater the severer the previous phosphorus stress.

The distribution of ^{35}S between older and new shoots was entirely changed when ^{35}S was applied during the last 3 days of the recovery period. The amounts of the tracer recovered in old leaves were considerably smaller than when it was applied during the first 3 days of recovery. Approximately equal amounts were distributed to old and new shoots of S_1 and S_2 plants as well as to those of S_3 plants.

IV. Discussion

The rates of sulphur uptake in experiment I, for the first 3 days after transfer to complete solutions, were calculated by using Williams' formula shown for phosphorus in Part I (Bouma 1967*b*). The values, expressed as μg sulphur/mg root weight/day, were 5·09, 5·97, and 2·48 for pretreatments S_1, S_2, and S_3 respectively. In experiment II (Fig. 3) the amounts of ^{35}S found in the shoots of S_1 plants were nearly the same as for S_3 plants, in spite of nearly twofold differences in plant size. The S_2 plants contained considerably more ^{35}S than those raised at S_3, on day 2 more than twice as much. These trends are essentially similar to those found for the uptake of phosphorus by plants raised at different phosphorus levels, which were shown to be largely governed by the phosphorus status of the plants at transfer (Part I, Bouma 1967*b*).

During the initial stages of recovery, the upward movement of sulphur was largely directed to leaves and petioles existing at transfer, while in non-deficient plants significant quantities were also distributed to new leaves and petioles. The percentage sulphur distributed to new shoots during the first 3 days after transfer in experiment I increased from 10 and 13% of the total taken up by the plant at S_1 and S_2, respectively, to 28% at S_3.

A preferential distribution to older leaves and petioles was also found for phosphorus in the experiments with plants recovering from phosphorus stresses

(Part I, Bouma 1967*b*). It appears reasonable to assume that the accumulation of nutrients in these organs was an essential feature of the recovery and resulted from the high internal demands caused by the previous starvation.

The redistribution patterns of sulphur in plants recovering from sulphur stresses were characterized by a far more restricted mobility of sulphur than was the case for phosphorus. In experiment I (Fig. 2) no net losses were found from old shoots of S_1 or S_2 plants at any time during the recovery period. In experiment II no retranslocation occurred of ^{35}S taken up during the first day after transfer to complete solutions. Although some of the additional ^{35}S taken up in old shoots during the second or third day after transfer was retranslocated to new shoots, the amounts were small compared with the translocation of ^{32}P from older to new shoots in the previous experiments. The redistribution of sulphur after transfer of plants to solutions without sulphur (expt. I, Table 2) clearly indicated that sulphur, once captured in leaves, was virtually inaccessible for retranslocation to other plant parts. In progressive stages of sulphur stress the demand for sulphur by the new leaves was met by sulphur mobilized in the petioles and in the roots. These findings are in line with the observation that the chlorosis typical for sulphur deficiency occurred first in the younger trifoliate leaves. This has also been recorded by Eaton for a number of crops, for example, soybeans (1935), sunflowers (1941), and tomatoes (1951).

Reports on the mobility of sulphur and on the distribution patterns in plants are somewhat conflicting. Thomas *et al.* (1944) showed with radioactive sulphur that, under certain conditions, sulphur can be translocated as sulphate to younger plant parts when needed for growth. Wood (1942) considered that the translocation of sulphur depended on the sulphur status of the tissue and on the presence of meristematic or other tissues rapidly utilizing sulphates. Sulphur in the leaves of plants raised at adequate nutrient levels was thought to be relatively immobile and noticeable changes in the sulphur content of leaves would only occur under conditions of rapid synthesis or starvation. Thomas, Hendricks, and Hill (1950) applied radioactive sulphate to some of the leaves of lucerne and found a movement via the stems to the crown and then upward again to untreated parts of the shoots. Biddulph, Cory, and Biddulph (1956) claimed that the translocation of sulphur compares favourably with that of phosphorus and that it is freely mobile under a wide variety of nutrient conditions. Kylin (1953) found that deseeded wheat plants, grown for 3 days with radioactive sulphur when the first leaf was half-formed, had little radioactivity in the third leaf 24 days after treatment.

These conflicting results may be partly reconciled by suggesting, in line with the present evidence, a marked effect of the sulphur status of the plant on the availability of sulphur for retranslocation from one organ to another. There is clear evidence in Figure 3 that the mobility of ^{35}S increased as the recovery progressed; there is also evidence of an increase in mobility from S_1 to S_3. The sulphur levels in the present experiments were chosen after extensive preliminary work had shown that the S_3 level of 4 p.p.m. sulphur was approximately optimal for growth under the existing experimental conditions. In most of the experiments quoted above

Hoagland-type nutrient solutions were used, and these contain up to 16 times more sulphur than those of the present experiments.

It appears reasonable to assume that part of the sulphur translocated to leaves becomes fixed in structural compounds and that these are relatively inaccessible sources of sulphur for translocation to other plant parts. The demand for sulphur in these compounds would be particularly high in plants recovering from stress conditions, such as the S_1 plants of the present experiments. Sulphur taken up in excess of the demand in these "sinks" could conceivably be more readily available for redistribution to other plant parts. However, it appears from the present results that for plants raised at optimum sulphur levels, the sulphur fractions available for redistribution to other plant parts were small compared with those of phosphorus in the plants raised at optimum phosphorus levels in the previous experiments (Part I, Bouma 1967*b*). Both groups of experiments are comparable with respect to the range of nutritional pretreatments, age of plants, and all environmental conditions. With an external sulphur supply greatly in excess of the optimum, mobile sulphur, possibly as simple inorganic sulphates, may reasonably be expected to increase.

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