

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

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Abstract

During the first 3 days after transfer of moderately sulphur-deficient plants (S_1) to full nutrient solutions, the relative growth rate (R_W) was considerably lower than that of plants raised at higher sulphur levels (S_2 and S_3). This was reflected in a lower leaf area ratio of the S_1 plants, and particularly in a reduction of nearly 50% in the net assimilation rate (E_A). Net losses in dry matter from younger emerged leaves and petioles accounted for 25% of the dry matter in new leaves and petioles of S_1 plants produced during this period.

Shading of the five oldest trifoliate leaves on the day of transfer reduced E_A at all sulphur levels by about the same absolute amount. However, E_A of the shaded S_1 plants was now only 21% of E_A for the shaded S_3 plants. In S_1 plants dry matter losses from younger emerged and shaded leaves and petioles accounted for 64% of the dry matter in new leaves and petioles produced during the first 3 days of recovery. No net losses occurred in S_2 and S_3 plants.

Removal of the five older trifoliate leaves at transfer caused a reduction in R_W at S_1 and S_2 levels, but only a reduction in E_A of the S_1 plants. E_A of the defoliated S_1 plants, attributable to the younger emerged leaves, was only 32% of that for the defoliated S_3 plants.

Assimilates for new growth during the first 3 days of recovery from a sulphur stress were considered to arise from current photosynthesis in mature, relatively unimpaired leaves and from mobilization in the younger emerged leaves. Although these early treatment effects were small, the consequent changes in growth patterns were large.

There were no net losses of dry matter from the roots.

I. INTRODUCTION

In previous growth studies with subterranean clover plants, it was shown that the decline in relative growth rate caused by a moderate sulphur stress was associated with a decline in net assimilation rate and leaf area ratio (Bouma 1967). The effect on net assimilation rate was confirmed by direct measurements of CO_2 exchange by whole plants (Bouma 1967, 1970).

During the initial stages of recovery from a sulphur stress, photosynthesis remained lower than in non-deficient plants, and existing leaves and petioles lost sufficient dry matter to account for a significant proportion of the dry matter in newly emerging leaves and petioles. The extent of the dry matter loss, as well as of

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the depression in photosynthesis, appeared to depend on the severity of the previous sulphur stress (Bouma 1967), suggesting that the importance of mobilization in existing plant parts as a source of assimilates for new growth may also increase with the severity of a previous stress.

Although net photosynthesis by whole plants declined at the onset of a sulphur stress, limited observations suggested that the younger leaves, in which the chlorosis typical for sulphur deficiency developed first, began to lose their photosynthetic activity before the older leaves. These results could point to differences in the contribution by older and younger emerged leaves to new growth during the recovery from stress conditions. The mature leaves, with a higher net assimilation rate and minimal demands on those assimilates for their own growth, might be able to contribute to the assimilate pool for new growth by continued or renewed photosynthesis, without any significant net loss of dry matter. The mobilization in emerged leaves and petioles, referred to above as another important source of assimilates for new growth, could then be attributable to younger leaves and petioles. If there are such differences between emerged leaves of different age in their contribution to new growth, then there could also be differences in the patterns of sulphur uptake and distribution between these groups of leaves. However, these patterns might be in a direction opposite to those for dry matter. The younger leaves could show a net import of sulphur because of their greater sulphur shortage, while at least some net export of sulphur might occur from the relatively unimpaired mature leaves.

These hypotheses were examined in the work reported in the present series of papers. This was done by comparing, during the recovery from different levels of sulphur stress, intact plants with plants in which mature emerged leaves were either individually shaded, to prevent them from contributing by photosynthesis to the assimilate pool, or removed from the plant to prevent their contribution by mobilization as well as by photosynthesis. The first paper of this series deals with the dry matter relationships between emerged leaves of different age and new growth, while the second paper (Bouma, Titmanis, and Greenwood 1972) presents the patterns of sulphur uptake and distribution.

II. METHODS

(a) *Plant Culture and Treatments*

Subterranean clover seeds (*Trifolium subterraneum* L. cv. Mt. Barker) were sown in washed river sand (15 May 1967). After 7 days uniform seedlings were placed in pretreatment nutrient solutions at three sulphur levels, 0.25, 1, and 4 p.p.m. (S_1 , S_2 , and S_3 respectively). Pretreatment was continued until 32 days after sowing when all plants were given the S_3 sulphur level. At that stage, the plants in each sulphur pretreatment were divided into three groups. In one, shades of aluminium foil were placed over the five oldest trifoliate leaves; in another the five oldest leaves were cut off at the base of the petiole; in the third group the leaves were untreated. The groups are referred to as shaded, defoliated, and control respectively. At the same time (day 32) the emerged leaves younger than those shaded or defoliated were identified by a small ring around the petioles, where this was physically possible. Their numbers varied between four and seven per plant.

The experiment was carried out in a glasshouse with a day temperature (9 a.m.—6 p.m.) of 24°C and a night temperature of 19°C. The average solar radiation for the period 0–43 days from sowing was 215 cal cm⁻² day⁻¹, while the average for the experimental period (days 32–43) was 216 cal cm⁻² day⁻¹.

Further cultural and experimental details were as described by Bouma (1967).

(b) Harvest Procedure

At harvest plants were separated into shoots (aerial parts) and roots. The shoots were further separated into leaves and petioles. The leaf fraction included the petiolules and the leaflets. The petiole fraction included the ligules and the short stem. Leaves and petioles were separated into the following groups on the basis of leaf treatments:

- (1) Shaded leaves and petioles (five oldest trifoliates), or the corresponding parts in the controls. For ease of presentation the cotyledons and unifoliate leaf and petiole were included with the corresponding shaded fraction. This did not significantly affect the results. This combined fraction is referred to as older leaves, petioles, or shoots, or fraction I. Of course, the corresponding leaves and petioles (five oldest trifoliates) were removed in the defoliated treatment and only the cotyledons and unifoliate leaf were left after harvest 2 on day 32. Figures 1 and 2 illustrate the proportions of dry weight removed in the defoliation treatment.
- (2) Leaves and petioles younger than those of fraction I, existing at the time of transfer to complete solutions, identified by a small ring around the petioles (see above). These are referred to as fraction II.
- (3) Leaves and petioles emerging after transfer to full solutions on day 32 (fraction III).
- (4) Roots.

Plant samples were dried at 70°C in a vacuum oven.

There were 12 pot replicates, and two plants per pot. Harvests were taken on days 22, 32, 35, 39, and 43 after sowing. Leaf areas were determined by comparing individual leaves with a set of photographic standards (Williams, Evans, and Ludwig 1964).

(c) Growth Analysis

The functions and concepts of growth analysis (Williams 1946; Watson 1952) were used to examine the growth responses in the present experiment. The following symbols are used:

- A = leaf area (cm²);
 W = dry weight (mg) per plant (shoot and root);
 W_L = dry weight (mg) of leaves per plant;
 W_L/W = leaf weight ratio;
 R_A = relative rate of leaf area expansion (day⁻¹);
 R_W = relative growth rate (day⁻¹);
 E_A = net assimilation rate (mg cm⁻² day⁻¹);
 A/W = leaf area ratio (cm² mg⁻¹).

By definition:

$$R_W = E_A \times A/W;$$

$$A/W = W_L/W \times A/W_L.$$

III. RESULTS

(a) Establishment of Sulphur Stress

There was no significant effect of sulphur on the dry weight of leaves, petioles, and roots at day 22 or again at day 32 (Fig. 1). Effects of sulphur on changes in leaf area, although more pronounced, followed the same trends as those for leaf dry weight and are therefore not shown. When leaves were partitioned into the different age classes on day 32, the dry weight of young leaves (fraction II) of S₁ plants was less than that of S₂ and S₃ plants (Fig. 2). Thus it was evident that on day 32 there was no sulphur stress at S₂ and only a mild and recently developed stress in the younger emerged leaves of the S₁ plants.

Further evidence for a sulphur stress in S_1 control plants is seen in the delayed recovery of fraction I and II leaves and in the dry weight of fraction III leaves during

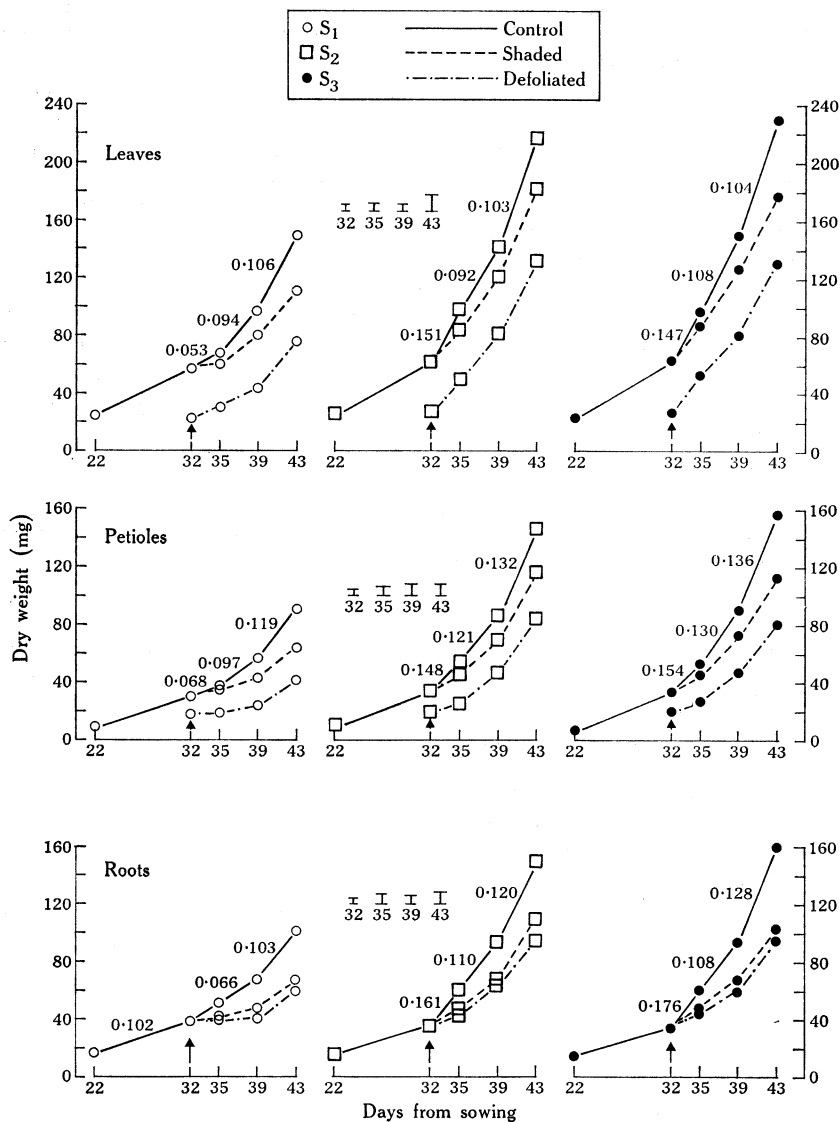


Fig. 1.—Effects of changes in sulphur supply and of partial shading or defoliation on the dry weights of principal plant parts. Full supply of sulphur and leaf treatments were given on day 32 (arrow). Least significant differences for days shown are indicated by vertical bars ($P = 0.01$) in all graphs. These do not apply to comparisons involving plant parts reduced in size by the defoliation treatment. In this figure, for example, the L.S.D. values apply to all root comparisons, but only to control and shaded leaves and petioles. Relative growth rates per day for different plant parts (controls only) are shown on the slopes for the harvest intervals concerned.

the first 3 days after removal of the sulphur stress. The magnitude of the lag in growth may be inferred from the values of R_w given in Figures 1 and 2 for the control

plants. The delay has been shown by Bouma (1967) to be due to a delay in the recovery of E_A .

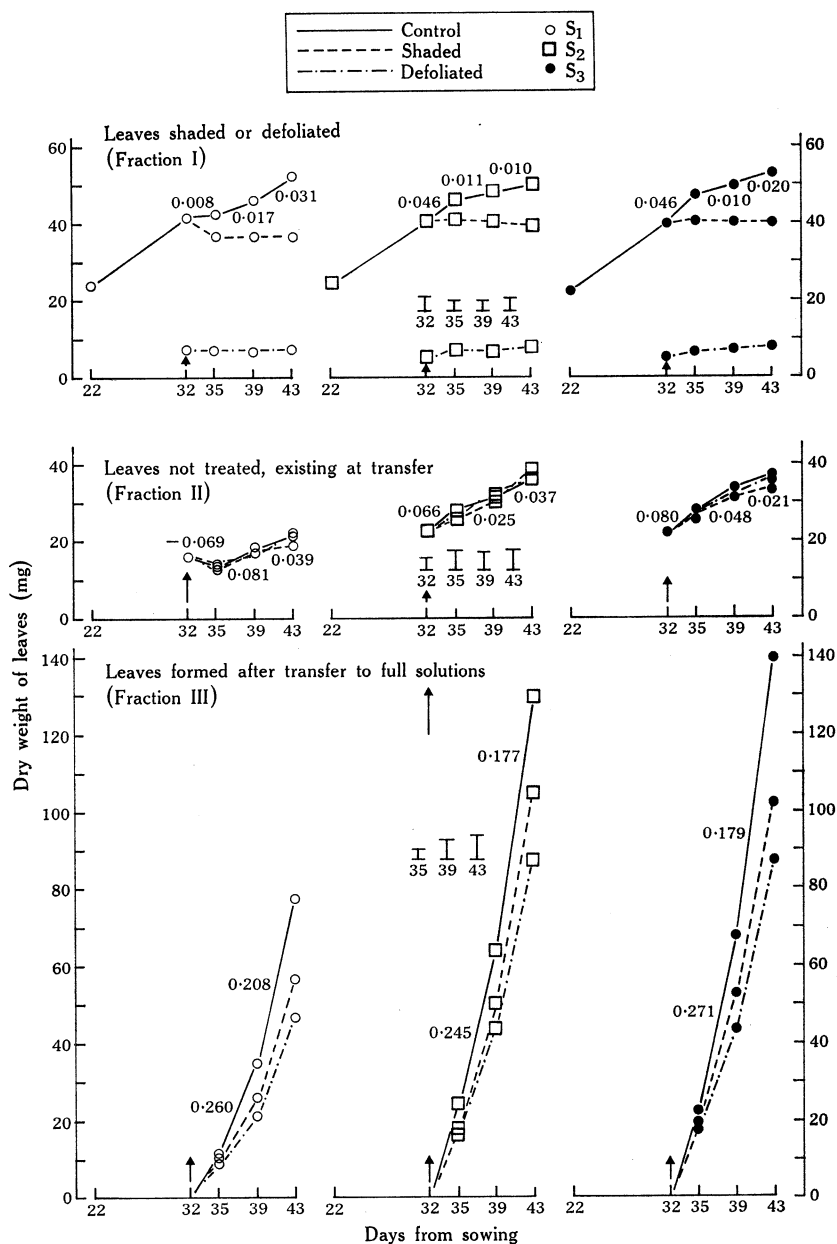


Fig. 2.—Effects of changes in sulphur supply and of partial shading or defoliation on the dry weights of the three groups of leaves (see Section II for explanation). Relative growth rates per day for the three groups of leaves (controls only) are shown on the slopes for the harvest intervals concerned.

(b) Effects of Shading and Defoliation

The dry weight of the shoots shaded or removed on day 32 amounted to 53% of the total shoot dry weight. Increases in plant dry weight after day 32 were smaller in the shaded than in the control treatment, and smaller still in the defoliated treat-

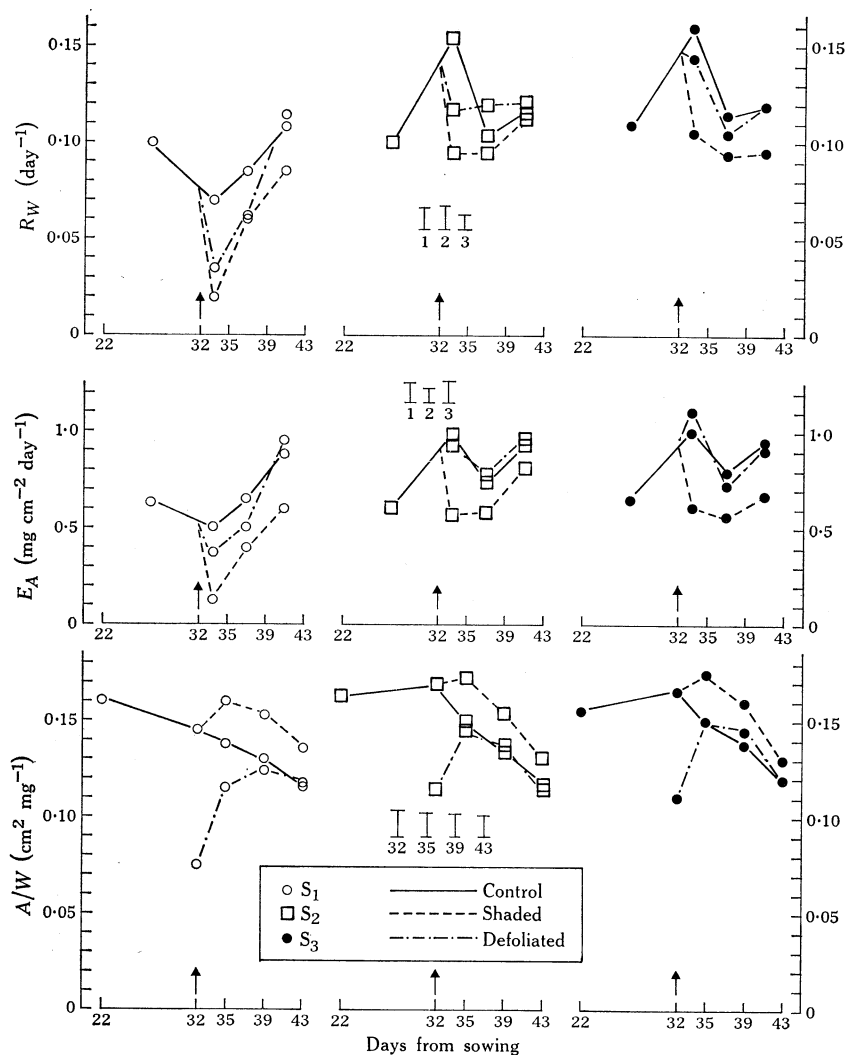


Fig. 3.—Changes in the derived growth functions R_W , E_A , and A/W during the course of the experiment. L.S.D. applies to the three harvest intervals in the case of R_W and E_A .

ment, irrespective of sulphur status on day 32 (Fig. 1). Leaf treatment had no significant effect on dry weights of fraction II leaves, but dry matter accumulation by fraction III leaves declined in the order control, shaded, and defoliated treatment (Fig. 2). Fraction I leaves continued to accumulate dry weight after day 32 in the

controls of all sulphur pretreatments. In the shaded treatment, however, these leaves of the S_1 plants showed a net dry matter loss between day 32 and 35, while at S_2 and S_3 dry weights of fraction I leaves remained unchanged.

(c) *Changes in the Derived Growth Functions*

Over the first harvest interval R_W differed little between sulphur levels (Fig. 3). Over the second harvest interval, however, R_W of the control plants increased by about 0.05 per day at S_2 and S_3 but declined by 0.03 per day at S_1 in spite of the transfer to complete solutions at the beginning of the interval. Defoliation, and particularly shading, caused a marked reduction in R_W over the subsequent harvest interval. This happened in all sulphur pretreatments, but R_W declined to very low values at S_1 . There is a clear indication that the effect of defoliation on R_W depended on the sulphur status of the plants on day 32. At S_3 defoliation caused a 10% reduction in R_W over the subsequent harvest interval only. The corresponding reduction at S_2 was 25%, but at S_1 as much as 72% and 30% over the two harvest intervals after transfer respectively.

The effects of treatments on E_A were similar to those for R_W (Fig. 3). Shading caused a marked depression in E_A in all sulphur pretreatments, as would be expected. It is noteworthy that shading reduced E_A by similar absolute amounts in all sulphur pretreatments, suggesting that the contribution by the five mature leaves to the assimilate pool was about the same at S_1 as at S_3 . The low value of E_A for the shaded S_1 plants clearly reflected the marked effect of the sulphur stress on the assimilatory capacity of the younger emerged leaves (fraction II). This is further supported by the effects of defoliation. There was little or no effect on E_A of the S_2 or S_3 plants, but in the S_1 plants the removal of the five oldest leaves caused a 26% and 21% reduction in E_A over the first and second harvest interval after transfer respectively. Since sulphur deficiency lowers the rate of dark respiration (Bouma 1971), it appears that effects on E_A were largely a reflection of the differences in the photosynthetic capacity of the older and younger emerged leaves.

The ratio A/W for the control plants differed little between sulphur pretreatments, apart from somewhat lower values at S_1 on days 32 and 35. Shading caused an immediate increase in A/W in all sulphur pretreatments and the values remained well above those for the control plants throughout the experiment. This increase in A/W was largely the result of an increase in the ratios of A/W_L , there being little change in the ratio W_L/W .

The fall in A/W in the defoliation treatment was a reflection of the removal of leaf tissue in this treatment. The values had returned to those of the control plants in all sulphur pretreatments by the end of the experiment.

IV. DISCUSSION

The present results confirm that recovery, even from a mild sulphur stress, can be delayed by a severe restriction in the supply of assimilates due to reduced photosynthesis (Bouma 1967).

The importance of mobilization in existing plant parts as an alternative source of assimilates for new growth during recovery is illustrated in Table 1. In the control S_1 plants 25% of the increases in dry weight of roots and new shoots over the first

3 days of recovery could be accounted for by a net loss from the younger shoots only (fraction II). The reason why there was no net loss of dry matter from the older leaves is probably related to the differences in photosynthetic activity between older and younger leaves. In preliminary experiments (unpublished) younger, chlorotic leaves showed a decline in net CO_2 uptake but not the older, still green leaves. This is supported by treatment effects on E_A in the present experiment. Shading reduced E_A by approximately similar amounts in all sulphur pretreatments (Fig. 3). However, the absolute value for E_A was only $0.13 \text{ mg cm}^{-2} \text{ day}^{-1}$ at S_1 , compared with $0.62 \text{ mg cm}^{-2} \text{ day}^{-1}$ for the shaded S_3 plants. Shading of the older leaves caused a net loss of dry matter from these leaves, which, together with the loss from the younger leaves, could have accounted for as much as 64% of the dry matter in new growth of the S_1 plants (Table 1). The reduction in E_A due to shading of the S_2 and S_3 plants (Fig. 3), was apparently not sufficient to induce a net loss of dry matter from existing plant parts. The effects of shading on E_A and A/W (Fig. 3) agree with those found by Blackman and his co-workers (e.g. Blackman and Wilson 1951; Blackman and Black 1959).

TABLE 1
BALANCE SHEET OF THE DRY MATTER CHANGES IN THE DIFFERENT PLANT PARTS OVER
THE FIRST 3 DAYS AFTER TRANSFER (DAYS 32-35)

Sulphur pretreatment	Plant part	Dry matter change (mg)		
		Control treatment	Shaded treatment	Defoliation treatment
S_1	Old shoots (I)	1	-6	-1
	Younger shoots (II)	-9	-8	-4
	New shoots (III)	23	19	14
	Roots	13	3	0
	Net increase	28	8	9
S_2	Old shoots (I)	8	2	2
	Younger shoots (II)	5	0	0
	New shoots (III)	41	30	25
	Roots	23	10	6
	Net increase	77	42	33
S_3	Old shoots (I)	9	3	3
	Younger shoots (II)	6	5	4
	New shoots (III)	39	31	28
	Roots	25	12	9
	Net increase	79	51	44

By removing the older leaves (fraction I), the S_1 plants lost a photosynthetic source of assimilates (available in the control treatments) as well as a mobilizable source (available in the shaded treatment). Over the first harvest interval after transfer E_A of the defoliated S_1 plants, attributable to the younger emerged leaves, was only $0.38 \text{ mg cm}^{-2} \text{ day}^{-1}$ compared with a value of $1.11 \text{ mg cm}^{-2} \text{ day}^{-1}$ for the defoliated S_3 plants. In the latter plants defoliation had no effect on E_A compared

with the control S_3 plants. This is further evidence that most of the decline in E_A under condition of a sulphur stress was attributable to the younger emerged leaves. Most probably as a result the net increase in dry weight of the defoliated S_1 plants was only 32% of that for the control S_1 plants, while at S_3 the corresponding increase was as much as 56% (Table 1).

It is concluded that during the recovery from a sulphur stress assimilates for new growth were provided by current photosynthesis, originating mainly in mature relatively unimpaired leaves, supplemented by mobilization in younger emerged leaves and petioles (Table 1). It seems likely that the relative contribution by current photosynthesis in mature leaves would decrease, and that by mobilization in younger emerged shoots increase, with the severity of the preceding sulphur stress.

Working with cocksfoot Davidson and Milthorpe (1966) found that, at an adequate supply of nutrients, removal of all fully expanded laminae had no effect on the subsequent rate of leaf expansion compared with intact plants. However, at a low nutrient supply there was an appreciable effect. They suggested that the older expanded leaves affected regrowth after defoliation less by influencing the supply of photosynthate than by influencing the supply of mineral nutrients. When they removed the expanded portions of expanding leaves, however, subsequent leaf expansion was markedly reduced. They concluded from the differences in the effects caused by removing older and younger leaves that only the younger leaves are of importance as sources of assimilates to new leaf growth. The present results leave little doubt that in the dicotyledonous subterranean clover plant removal of the older expanded leaves will cause a reduction in subsequent leaf growth (Figs. 1, 2, and 3). This was evident irrespective of sulphur supply, but was particularly pronounced in those plants recovering from sulphur stress. In the latter plants, the older leaves were an important source of current photosynthates, while the younger leaves, of which some would still be expanding, provided at least some mobilizable assimilates.

Davidson and Milthorpe (1966) concentrated their study of the response of cocksfoot on leaf growth during the first few days after defoliation because they considered that subsequent growth rates depended on the initial development of the leaf surface. We agree with this point of view. The importance of an adequate sulphur status at the time of shading or defoliation was evident on the third day after treatment (day 35) and the subsequent differences in plant growth were, in the main, a reflection of those early and relatively small treatment effects.

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