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# $SO_4^{2-}$ Deprivation Has an Early Effect on the Content of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase and Photosynthesis in Young Leaves of Wheat[1]

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**Wheat (*Triticum aestivum* cv Chinese Spring) supplied with 0.45 mM $SO_4^{2-}$ for 14 d with relative growth rates (RGR) of 0.22 to 0.24 $d^{-1}$ was deprived of S for 7 to 8 d. There was no significant effect on RGR or leaf development (leaf 2 length was constant; leaf 3 expanded for 2–4 d; leaf 4 emerged and elongated throughout the experiment) during the S deprivation. In controls the net assimilation rate (*A*) closely reflected leaf ontogeny. S deprivation affected *A* in all leaves, particularly leaf 4, in which *A* remained at 8 to 10 μmol $CO_2$ $m^{-2}$ $s^{-1}$, whereas in controls *A* rose steadily to >20 μmol $CO_2$ $m^{-2}$ $s^{-1}$. In leaf 2, with a fully assembled photosynthetic system, *A* decreased in S-deprived plants relative to controls only at the end of the experiment. Effects on *A* were not due to altered stomatal conductance or leaf internal $[CO_2]$ ($[C]_i$); decreases in the initial slope of $A/[C]_i$ curves indicated an effect of S deprivation on the carboxylase efficiency. Measurement of Rubisco activity and large subunit protein abundance paralleled effects on *A* and $A/[C]_i$ in S-deprived leaves. Negative effects on photosynthesis in S-deprived plants are discussed in relation to mobilization of S reserves, including Rubisco, emphasizing the need for continuous S supply during vegetative growth.**

Young shoot tissues are usually recognized as the most susceptible to the disruption of normal growth and metabolism when the external supply of $SO_4^{2-}$ becomes limited. This is especially the case when the $N_2$ supply to the plant is adequate (Robson and Pitman, 1983; Marschner, 1995). The effects are usually explained in terms of the restrictions placed on the redistribution of endogenous sources of S from the older leaves. It has been demonstrated, for instance, that the efflux of $SO_4^{2-}$ located in leaf vacuoles is slow, so that older leaves may contain stores of $SO_4^{2-}$, whereas younger leaves are $SO_4^{2-}$ deficient (Clarkson et al., 1983; Bell et al., 1994, 1995). It is evident, however, that the major fraction of S in leaves is in protein and that Rubisco is the principal component of this fraction in many species.

Mobilization of the S-containing amino acids in Rubisco could be a major internal source of S in the absence of any other vegetative storage protein. In *Lemna minor* L., S starvation has been shown to lead to the degradation of Rubisco even in cultures supplied with adequate (5 mM) $NO_3^-$ (Ferreira and Teixeira, 1992). This behavior is somewhat contrary to the accepted view that S starvation does not initiate the senescence program in most higher plants and that S remains relatively immobile unless N is also deficient (Robson and Pitman, 1983; Sunarpi and Anderson, 1996a, 1996b, 1997). Lowering Rubisco in antisense experiments, particularly at high light intensity, reduces photosynthesis and growth (Stitt et al., 1991; Hudson et al., 1992; Krapp et al., 1994; Eckhardt et al., 1997). However, at lower light intensities, leaves can sometimes lose much of their Rubisco without affecting their photosynthetic capacity or the RGR of the plant (Quick et al., 1991, 1992).

Previous work with intact, young leaves of sugar beet (*Beta vulgaris*) revealed that photosynthesis (measured as *A*) was unaffected until $[SO_4^{2-}]$ fell to less than 250 μg $g^{-1}$ dry weight over a period of 2 to 3 d after $SO_4^{2-}$ deprivation (Terry, 1976). The effect on *A* was related to a decrease in chlorophyll content, but Rubisco activity also declined in chloroplasts isolated from leaves at a similar stage of $SO_4^{2-}$ starvation.

In the present paper we examined the relationship between Rubisco concentration and activity in leaves of different developmental stages during an episode of S deprivation. This behavior is correlated with the *A* of the leaves. The very early impact on photosynthesis and Rubisco in young, expanding leaves underlines the need for cereal plants to have a continuous external supply of S.

## MATERIALS AND METHODS

### Culture of Plants at RUU

Seeds of wheat (*Triticum aestivum* cv Chinese Spring) were surface sterilized and washed in aerated, distilled

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Abbreviations: *A*, net assimilation rate; $[C]_i$, leaf internal $[CO_2]$; $g_s$, stomatal conductance; LARS, Long Ashton Research Station; LSU, large subunit; RGR, relative growth rate; RUU, University of Utrecht.

water for 4 h before being placed on layers of paper toweling saturated with nutrient solution (see below). Trays containing the seeds were kept in the dark at 20°C for 48 h; the trays were then uncovered and the seedlings were left to grow until the mesocotyl was at least 10 mm in length. Seedlings were transferred to 30-L polyethylene tanks and supported in an opaque cover by discs of polyurethane foam.

The culture solution contained the following salts: $KNO_3$ (2.0 mM), $Ca(NO_3)_2$ (0.5 mM), $MgSO_4$ (0.45 mM), $KH_2PO_4$ (0.3 mM), and micronutrients comprising the following salts: FeNaEDTA (4.9 μM), $Cu(NO_3)_2$ (0.16 μM), $MnCl_2$ (3.6 μM), $ZnCl_2$ (0.77 μM), KCl (5.0 μM), $H_3BO_3$ (9.2 μM), and $(NH_4)_6Mo_7O_{24}$ (0.016 μM). The conditions in the growth room were: PPFD, 500 μmol $m^{-2}$ $s^{-1}$; RH, 70%; light period, 16 h (7:00 AM–11:00 PM); and temperature, constant at 20°C.

$SO_4^{2-}$-deprivation treatment was started on one-half of the plants (four tanks) when the plants were 14 d old; $MgCl_2$ was substituted for $MgSO_4$.

### Culture of Plants at LARS

Wheat seeds were germinated on sheets of wet paper toweling in the dark at 22°C. The seedlings were transferred to PVC supports mounted in the lid of a 50-L tank and grown hydroponically at 20°C/18°C (day/night) with a 16-h photoperiod in a controlled-environment room. The nutrient solution contained the following salts: $MgSO_4$ (0.45 mM), $Ca(NO_3)_2$ (0.45 mM), $KH_2PO_4$ (0.3 mM), and $KNO_3$ (1.5 mM), and micronutrients were supplied as described above. For the −S nutrient solution, $MgSO_4$ was replaced with $MgCl_2$ at the same concentration. The PPFD at the leaf surface was 550 to 600 μmol $m^{-2}$ $s^{-1}$ and the RH was maintained at 70%/80% (light/dark periods). The nutrient solution was replaced as necessary until the plants were 11 d old, when one-half of the tanks received fresh nutrient solution as before and the other half received $SO_4^{2-}$-free medium. Plants were at the three-leaf stage at this time (day 0) and the third leaf was tagged for subsequent identification. The respective nutrient solutions were renewed once over the time course of the experiment.

### Measurements of Photosynthesis

Measurements of photosynthesis were made between 9:00 AM and 12:00 PM (2–5 h into the photoperiod). Three randomly selected control or −S plants were placed with their roots in pots with approximately 250 mL of culture solution (+S or −S) and quickly transferred to the IR gas-analysis setup. Gas-exchange measurements were carried out in an open system as described in Poot et al. (1997). Differences in partial pressures of $CO_2$ and $H_2O$ between air entering and leaving three parallel cuvettes were measured with a $CO_2/H_2O$ analyzer (model LI-6262, Li-Cor, Lincoln, NE). Gas-exchange parameters were calculated according to the method of von Caemmerer and Farquhar (1981). The conditions within the cuvettes were as follows: leaf temperature, 19.5 to 20°C; vapor pressure difference, 0.5 kPa; boundary layer conductance, 10 mol $m^{-2}$ $s^{-1}$; PPFD, 1170 to 1200 μmol $m^{-2}$ $s^{-1}$. For each set of determinations, three leaves of the same position and pretreatment were used, providing three independent replicates.

The area of leaf selected for measurement spanned the midpoint of the lamina length. The cuvette was 7 cm wide and the measuring area was 3.5 to 7 $cm^2$, depending on the stage of leaf development and leaf position. $A$ and $g_s$ were measured at saturating light intensity (1200 μmol $m^{-2}$ $s^{-1}$ PPFD). On five sampling occasions, the value of $A$ was determined at a range of external $[CO_2]$; the variation of $A$ with $[C]_i$ was used to estimate the efficiency of Rubisco. After measurements had been made by the IR gas analyzer, the limits of the segment in the cuvette were carefully marked, the segment cut out, its area was measured with a leaf area meter (Li-Cor), its dry weight was determined, and the specific leaf area was calculated.

### Growth Analysis

Plants that had been used for measurements of photosynthesis plus three others from each treatment (giving six replicates) were separated into the following components: root, lamina of leaf 2, lamina of leaf 3, lamina of leaf 4, and the remaining shoot. Tissues were dried at 70°C for 48 h before determining dry weights. Note that leaf 4 emerged during the course of the experiment.

### Preparation of Leaf Extracts

Leaves were excised, wrapped in aluminum foil, and immersed in liquid $N_2$. Samples were stored at −80°C until required. Individual samples were then ground to a fine powder with a mortar and pestle in liquid $N_2$. Approximately 100 mg of accurately weighed tissue was transferred to a microfuge tube and 5 μL/mg of ice-cold extraction buffer (100 mM Hepes, pH 7.7, containing 20 mM KCl) was added prior to mixing. The homogenate was centrifuged at 5°C for 90 s at 13,800$g$ (maximum), and the supernatant was transferred to a fresh ice-cold microfuge tube. This procedure was repeated, and the clarified extract was assayed immediately and then stored at −20°C. The pellets were retained for chlorophyll determination (see below).

### Biochemical Analyses

Rubisco carboxylase activity was assayed at 25°C according to the method of Keys and Parry (1990) using an $O_2$ electrode chamber as the reaction vessel. In a total volume of 1.0 mL, the reaction mixture contained 100 mM Bicine, pH 8.2, 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$, 185 kBq, 330 μM D-ribulose-1,5-bisphosphate, and leaf extract equivalent to 8 mg of fresh-frozen weight. Enzyme activity was determined by initiating the reaction with RuBP after a 5-min preincubation of the extract in the reaction mixture.

### Chlorophyll Determination

The pellets remaining after Rubisco extraction were extracted with 80% acetone and the chlorophyll content was

determined according to the method of Mackinney (1941) and Arnon (1949).

### SDS-PAGE and Polypeptide Quantification

Frozen extracts were thawed and mixed with an equal volume of sample buffer (120 mM Tris, pH 6.8, 20% [w/v] glycerol, 4% [w/v] SDS, 100 mM DTT, and 0.02% [w/v] bromophenol blue) and agitated at 37°C for 2 min prior to separation of the proteins in a 15% (w/v) acrylamide/0.1% (w/v) bisacrylamide minigel (Hawkesford and Belcher, 1991). Protein was detected by staining with Coomassie blue R-250. Gels were imaged using a gel-documentation system (GelDoc, Bio-Rad). Reflected light images were captured with a video camera, and volumes (densities) of individual bands were determined using the Molecular Analyst software (Bio-Rad). Local background subtraction was applied to minimize any gel-to-gel or within-gel variation.

### Inorganic Ion Analysis

The anions $SO_4^{2-}$ and $NO_3^-$ were measured in hot-water (>90°C) extracts of oven-dried plant material. Approximately 30 mg of material was extracted in 2 mL of water. A dilution of the extract was injected into the sampling loop of an anion HPLC system (Dionex, Sunnyvale, CA).

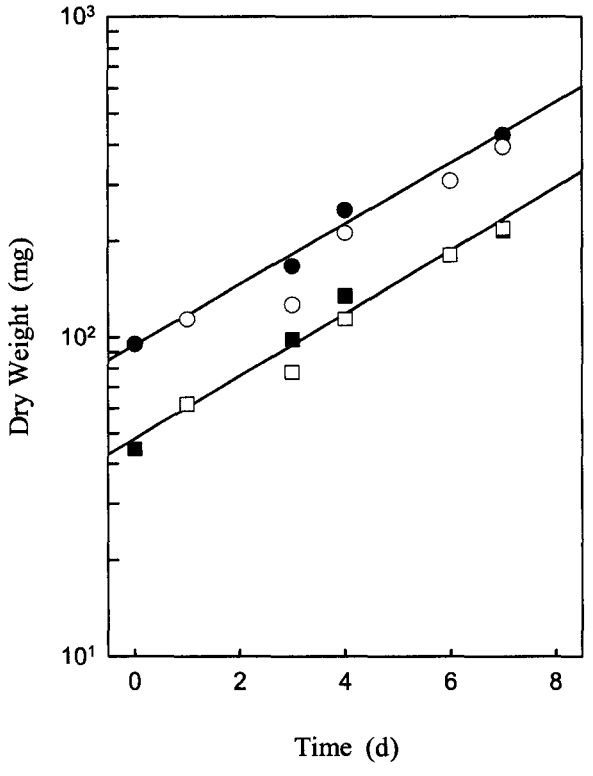


**Figure 1.** RGR of roots and shoots of wheat. Data are dry weights of whole shoots (●, ○) and roots (■, □) throughout the duration of the experiment at RUU in the control (+S) plants (filled symbols) and in the S-deprived plants (open symbols).

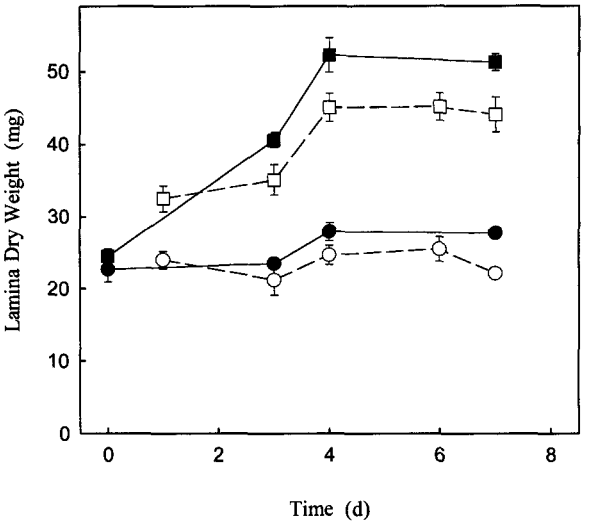


**Figure 2.** Effect of removal of S supply on lamina dry weights of leaves 2 and 3 of wheat. ● and ○, Leaf 2; ■ and □, leaf 3 with control (+S) plants (filled symbols) and S-deprived plants (open symbols). Values do not include leaf sheaths. Error bars are ± SE ($n$ = 6).

## RESULTS

### Growth and Form of the Plants Grown at the Two Sites

Culture conditions were similar at the two sites and the plants were at similar stages of development during the experimental period of 7 to 8 d. The RGR of shoots was 0.22 and 0.24 $d^{-1}$ at LARS and RUU, respectively. The root-to-shoot dry weight ratios were 0.58 and 0.55 at LARS and RUU, respectively.

### Growth during $SO_4^{2-}$ Deprivation

When log dry weights of shoots from controls and −S plants used in the photosynthesis measurements made at RUU were plotted against time, the data fitted to a single straight line (Fig. 1). Similarly, there were no differences between the growth of roots in the two treatments. There was no significant change in the root:shoot dry weight ratio in this experiment.

### Pattern of Leaf Growth

There was a well-marked pattern in the development of leaves of the control plants (Fig. 2), which was correlated with the $A$ (see below). At the beginning of the experiment, leaf 2 had almost completed its expansion and its weight increased by 22%. Leaf 3 doubled its dry weight during the first 4 d of the experiment and then stopped increasing in weight. Leaf 4 emerged and expanded throughout the latter part of the experiment. A similar pattern was observed in measurements of leaf length of plants grown at LARS (Fig. 3) and was used to measure Rubisco activity. The length of leaf 2 did not change, that of leaf 3 increased for 2 d, whereas leaf 4 emerged between d 0 and d 2 and continued elongating until d 6.

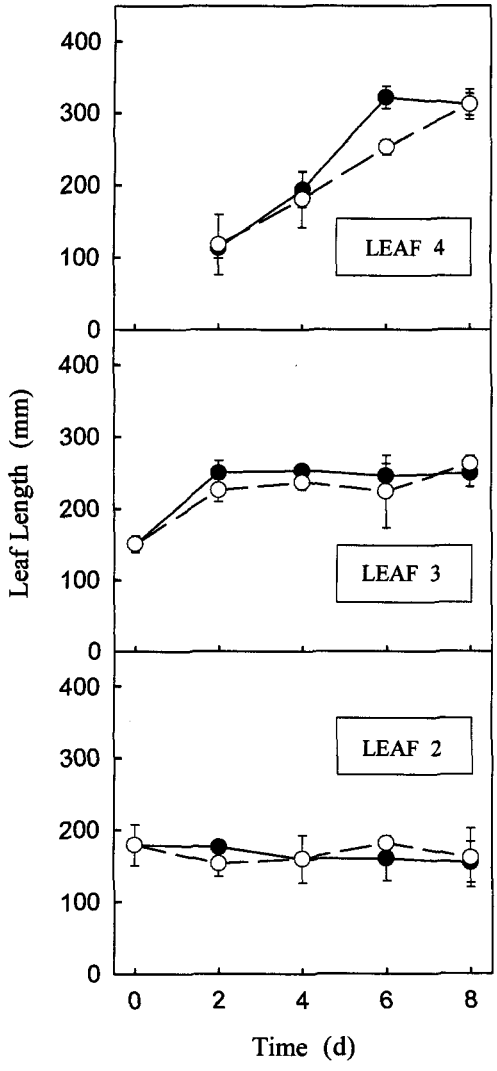


**Figure 3.** Effect of $SO_4^{2-}$ deprivation on lengths of leaves 2, 3, and 4 of wheat. Leaves from +S plants (●) or S-deprived plants (○) were excised at the junction of the lamina and the leaf sheath. Data are means of leaf lengths from three plants ± SE.

The above pattern of events was not strongly perturbed by $SO_4^{2-}$ deprivation. There was no significant effect on leaf length (Fig. 3), but the dry weights of leaf 2 and leaf 3 were significantly lower in comparison with controls after d 3 (Fig. 2).

### *A*

In control (+S) leaves there were distinct patterns of change in *A* associated with leaf expansion (Fig. 4). In leaf 2, which was essentially fully expanded at the outset, *A* remained constant, whereas in leaf 3 it was at a markedly lower value than in leaf 2 on d 0, but increased to a maximum value after 5 d. In leaf 4 when first measured on d 3, *A* was less than one-half of that in leaf 2 but increased throughout the experimental period as the leaf increased in length, weight, and mass per unit area. The −S treatment had a marked effect on the values of *A* in all leaves at the end of the experiment. The effect was most pronounced in leaf 4, in which *A* did not increase significantly above the initial value of about 10 $\mu$mol $m^{-2}$ $s^{-1}$.

In leaf 3 of −S plants *A* increased over the first 4 d but then declined; a similar decline was seen between d 2 and 7 in leaf 2. The pattern of response of *A* also bears a close resemblance to the data on Rubisco activity (see below).

The $g_s$ measured on the leaves used for the photosynthesis measurements at 1200 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD and ambient $CO_2$ (data not shown) reflected the same developmentally related pattern seen for leaf growth (Fig. 3). The values of $g_s$ were lower while leaves 3 and 4 were expanding (300–350 mmol $m^{-2}$ $s^{-1}$) compared with when they reached full expansion, when they had $g_s$ values of 550 to 600 mmol

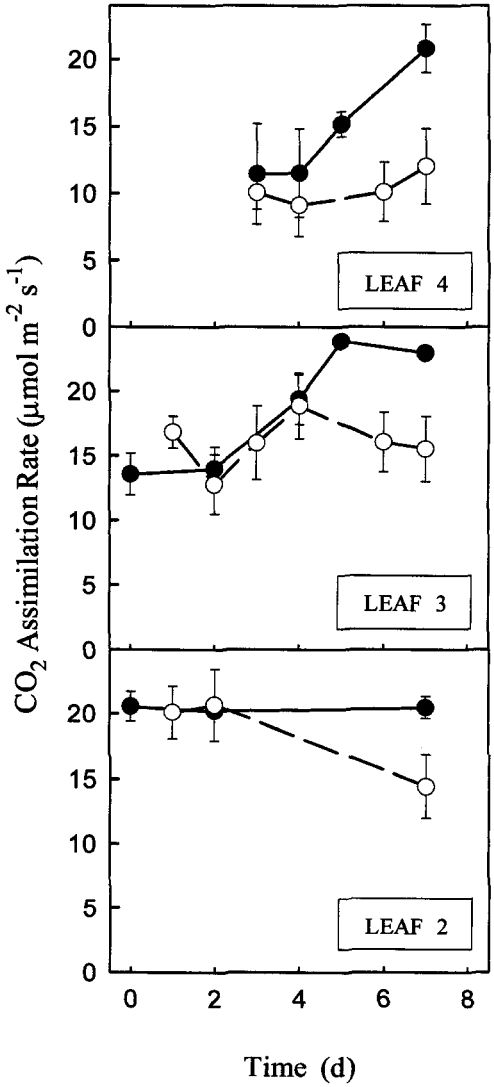


**Figure 4.** Effect of $SO_4^{2-}$ deprivation on $CO_2$ *A* of leaves 2, 3, and 4 of wheat. $CO_2$ assimilation rates (●, ○) in control (+S) leaves (filled symbols) and in S-deprived leaves (open symbols). Measurements at ambient [$CO_2$] and at 1200 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD. Values are the means of three replicates with ± SE for the $CO_2$ assimilation rates where greater than the symbol size.

**Table I.** *Initial slope of $A/[C]_i$ curves for leaves of wheat after growth for various periods with (+S) or without (−S) in the external solution*

SE of difference (20 df) = 0.1074; LSD (P = 0.05) = 0.224.

| Treatment | Leaf No. | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| | *initial slope μmol $m^{-2}$ $s^{-1}$ $Pa^{-1}$* | | |
| Control (+S) d 0 | 1.385 | 1.016 | — |
| Control (+S) d 5 | — | 1.706 | 1.039 |
| −S 1 d | 1.446 | 1.288 | — |
| −S 4 d | — | 1.289 | 0.599 |
| −S 6 d | 1.288 | 1.081 | 0.698 |

$m^{-2}$ $s^{-1}$. After full expansion, a decline in $g_s$ was recorded in leaves 2 and 3. There was no effect of the −S treatment on this pattern nor on the absolute values of $g_s$; evidently, the −S-dependent decreases in $A$ were unrelated to $g_s$.

### Carboxylase Efficiency

On a number of occasions the variation in $A$ with the $[C]_i$ was examined in three independent replicates on each occasion. If these two parameters are plotted, a curve is produced in which $A$ tends to saturate at high $[C]_i$. The initial slope of this curve can be used to estimate the efficiency of the carboxylase. It was found that the data could be fitted by an exponential curve of the form:

$$A = a - b^* \text{EXP}(-k^*[C]_i) \quad (1)$$

and this curve was fitted to each of the three independent replicate data sets. The percentage of variance accounted for by this fitted curve varied between 98.1 and 99.9%. The curve was fitted for estimation of the initial slope, not as an attempt to model the underlying processes. Using the fitted curves, the parameters and other derived values for each of the replicates were subjected to analysis of variance to test the significant differences between the different treatment combinations. There was a significant decrease in the initial slope of $A/[C]_i$ of −S leaf 3, relative to the +S treatment, on d 4 and 6. The difference was accounted for largely by an increase in slope of the controls over the 7-d period. On d 6, however, it was clear that the initial slope in −S leaf 3 was lowered in relation to both +S and −S on d 4. A more pronounced decrease in slope was observed in −S leaf 4 at this time (Table I). The marked increase in the initial slope between d 0 and 5 for the +S leaf 3 reflects the considerable increase in photosynthetic capacity occurring during this time in this leaf (Fig. 4).

The response of $A$ to light in leaf 4 was examined on d 6 (−S) and d 7 (+S). In the +S leaves, the value of $A$ increased by 25% over a range of PPFD, from 525 to 1240 μmol $m^{-2}$ $s^{-1}$, whereas in the −S leaf an insignificant increase in $A$ of only 7% was measured over the same PPFD range (data not shown).

### Rubisco Activity and Protein Content

Following a sampling protocol similar to that used in the gas-exchange experiments, the leaves of wheat plants grown at LARS were analyzed for their Rubisco activity. Figure 5 shows Rubisco activity in soluble extracts of individual leaves during the time course of the experiment. The Rubisco activity shown is that determined after a 5-min preincubation in the presence of $Mg^{2+}$ and $CO_2$ substrates (Keys and Parry, 1990). Initial Rubisco activites (see also Keys and Parry, 1990) were also determined and paralleled the total activities (data not shown), indicating no major change in the activation state of Rubisco. In all leaves Rubisco activity in the −S treatment declined significantly after d 2. The greatest effect was in leaf 4, in which Rubisco activity in the −S treatment failed to increase after d 2 compared with the +S control. Similar patterns of change were seen with time in leaf chlorophyll content (Fig. 6), which was significantly lower in −S leaf 3 and leaf 4,

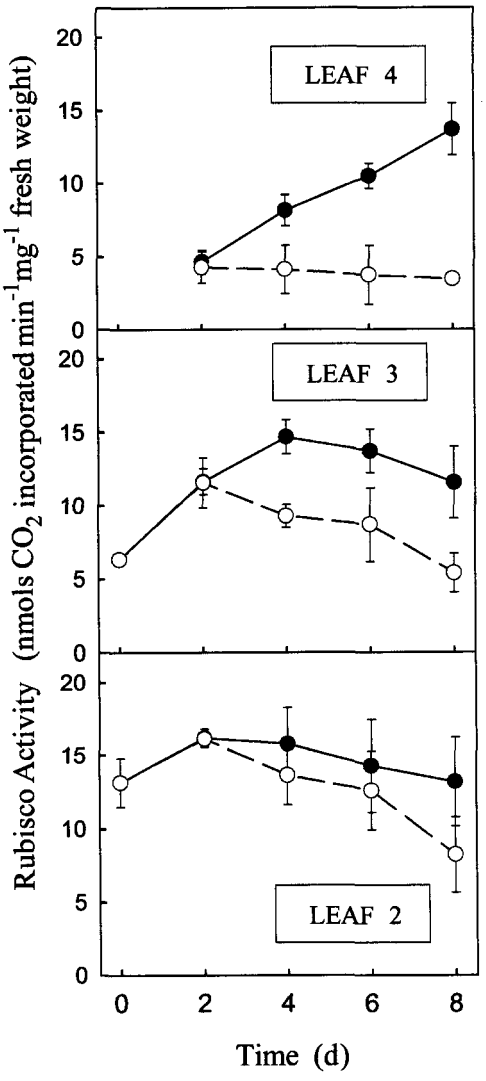


**Figure 5.** Effect of removal of S supply on Rubisco activity of leaves 2, 3, and 4 of wheat. Rubisco activity was determined in soluble protein extracts from control (+S) leaves (●) and in S-deprived leaves (○). Data are means of three independent assays and error bars represent the ± SE. The data represent the activities in 8 mg of fresh-frozen leaf tissue.

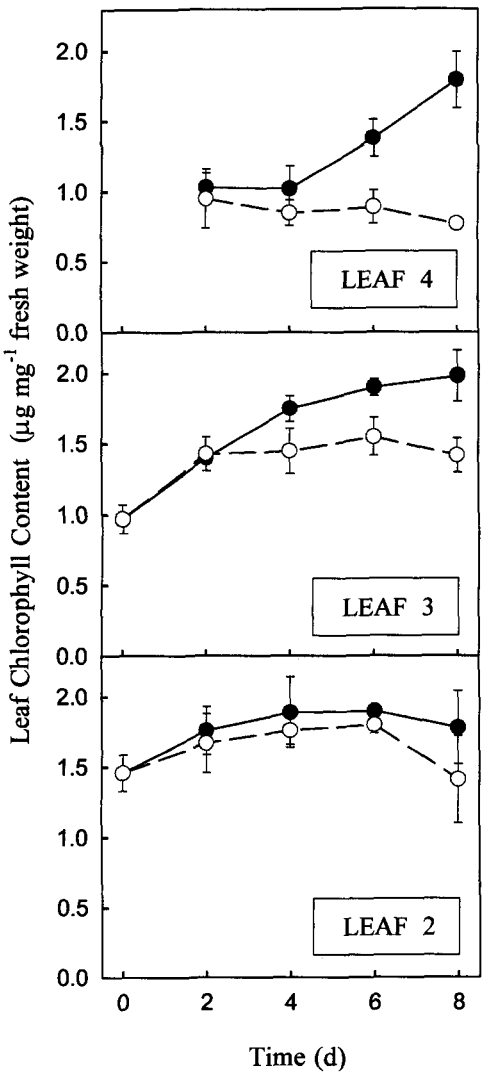


**Figure 6.** Effect of removal of S supply on chlorophyll content of leaves 2, 3, and 4 of wheat for control (+S) plants (●) and S-deprived plants (○). Total chlorophyll was determined from triplicated acetone extracts. Error bars are ± SE.

whereas there was little difference between control and −S in leaf 2 until the last sampling on d 8. Expressing the Rubisco activity on a chlorophyll basis (Fig. 7) rather than on fresh weight revealed that the effect of S deprivation on Rubisco activity was greater than that on chlorophyll content, particularly in leaves 3 and 4.

Coomassie blue R-250-stained gels of solubilized proteins from leaves allowed the visualization of the LSU of Rubisco, the most abundant polypeptide in the extract (Fig. 8). The relative abundances of the LSU polypeptide on these gels were quantified by image analysis of replicate SDS-PAGE gels (Fig. 9). The patterns of change in abundance of the LSU closely match the observed changes in Rubisco activity (Fig. 5). This confirmed that the lower Rubisco activities in S-deprived plants did indeed correspond to lower contents of both LSU Rubisco polypeptides (Figs. 8 and 9). Similar results were obtained for the small subunit polypeptide (data not shown).

In plants supplied with sufficient S it was clear that the increase in the observed total Rubisco activity in leaf 4 (Fig. 5) corresponded to the increased abundance of the LSU as observed by SDS-PAGE (Figs. 8 and 9). In leaf 4 of the −S plants, where no increase in activity of Rubisco was observed, no increase in LSU abundance was observed either, and even a modest decrease in abundance was detected. However, as leaf 4 was expanding in the S-deprivation treatment (Fig. 2), to maintain the Rubisco content at this nearly constant level (equal fresh weights were extracted and loaded on the gels), some synthesis of Rubisco must have occurred, which was dependent upon available mobilized S.

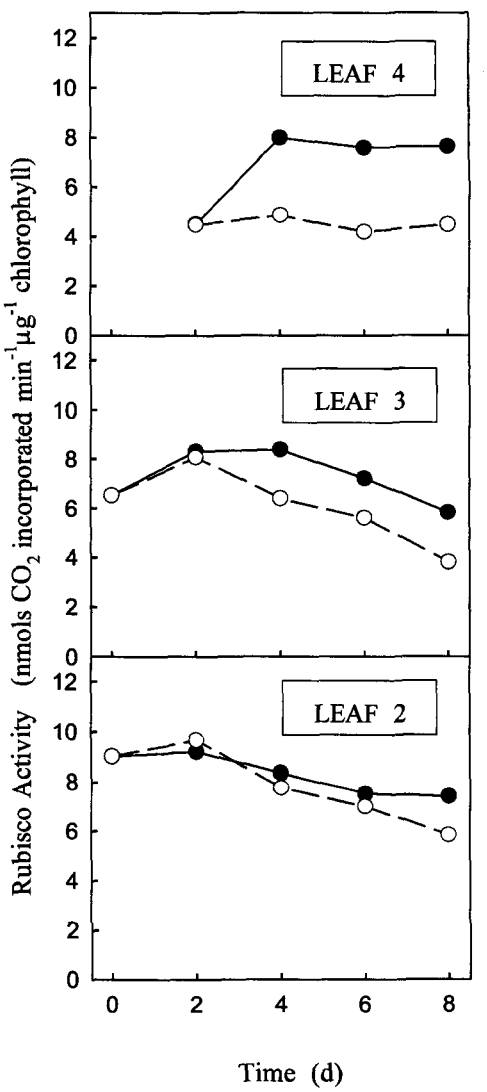


**Figure 7.** Effect of removal of S supply on Rubisco carboxylase activity: total chlorophyll ratios of leaves 2, 3, and 4 of wheat. ●, Rubisco activity in control leaves from control (+S) plants; ○, Rubisco activity in S-deprived leaves. Data are means of three separate leaf samples ± SE.

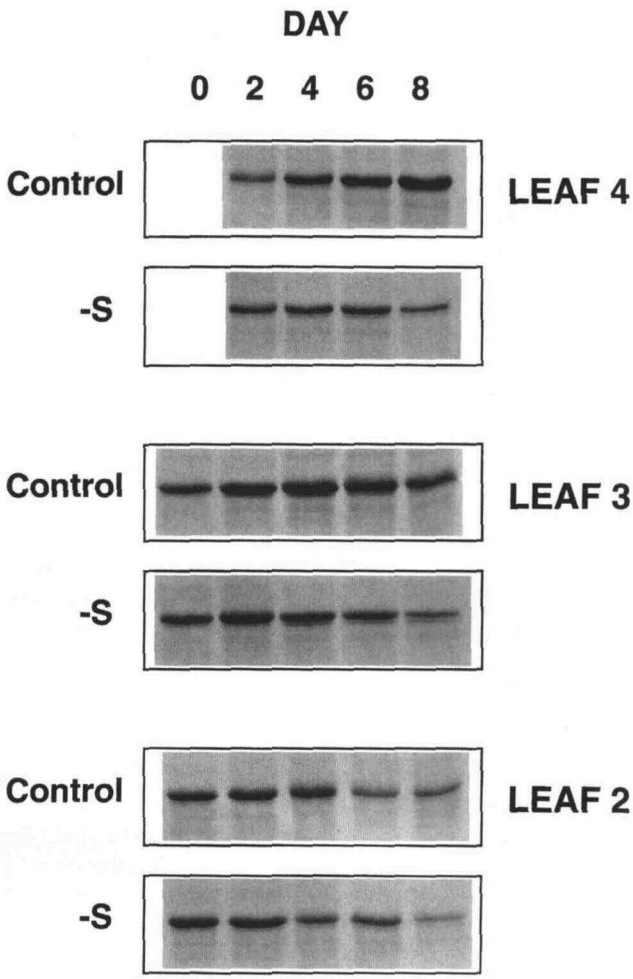


**Figure 8.** SDS-PAGE of soluble protein from extracts of leaves 2, 3, and 4 of control (+S) and S-deprived wheat. The abundance of the LSU of Rubisco was visualized by staining with Coomassie brilliant blue R-250. All lanes were loaded with equal equivalent fresh weights of leaf tissue.

### Nutrient Composition of Tissues

There was a significant increase in the $[NO_3^-]$ in leaf 3 of −S plants during the first 6 d of S deprivation; this trend may also be seen from d 2 to 4 in leaf 4 but there was no effect in leaf 2. As expected, the $[SO_4^{2-}]$ fell sharply in leaves 2 and 3 during the first 2 d of S deprivation, but stabilized between d 2 and 6 before falling to very low concentrations on d 8 (Fig. 10). In leaf 4, which expanded during the period of S deprivation, the tissue $[SO_4^{2-}]$ increased significantly between d 4 and 6, presumably due to re-allocation of $SO_4^{2-}$ from other parts of the plant (Sunarpi and Anderson, 1996b). Evidently, this reallocation was not adequate to allow the development of the photosynthetic capacity of the leaf or its chlorophyll and Rubisco content. The reduction of $NO_3^-$ by $NO_3^-$ reductase has been seen to be an early target for S deficiency in our own work with spinach (Clarkson et al., 1993; Prosser et al., 1997) and in other species (Reuveny et al., 1980), and S deficiency has long been recognized to result in accumulation of nonprotein N, especially $NO_3^-$, in leaves of various species (e.g. Stewart and Porter, 1969).

## DISCUSSION

The results obtained indicate that photosynthesis in wheat leaves is quickly upset when an external supply of S is withdrawn. Mobilization of S from other parts of the plant is too little or too slow to sustain the assembly of an efficient $CO_2$-fixation apparatus in expanding leaves. This emphasizes the need for a continuous supply of S to growing plants. The effects of S deprivation on *A* were detected before any decrease in dry matter gain was recorded (com-

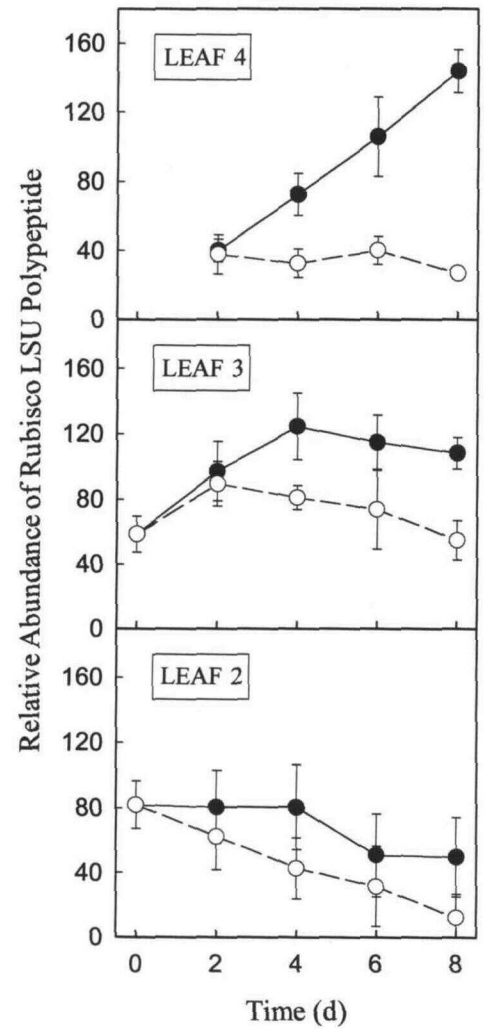


**Figure 9.** Effect of removal of S supply on relative abundance of the LSU of Rubisco as visualized by image analysis of SDS-PAGE. Relative abundance of the LSU detected in soluble protein from separate triplicate extracts of leaves 2, 3, and 4 of control (●) and S-deprived (○) wheat after electrophoresis on three separate SDS-PAGE gels. All gels were stained identically with Coomassie brilliant blue R-250 and were independently analyzed with local background correction. The means of the determined volumes (with error bars representing ± SD) of the bands corresponding to the LSU are presented. All lanes were loaded with equal equivalent fresh weights of leaf tissue.

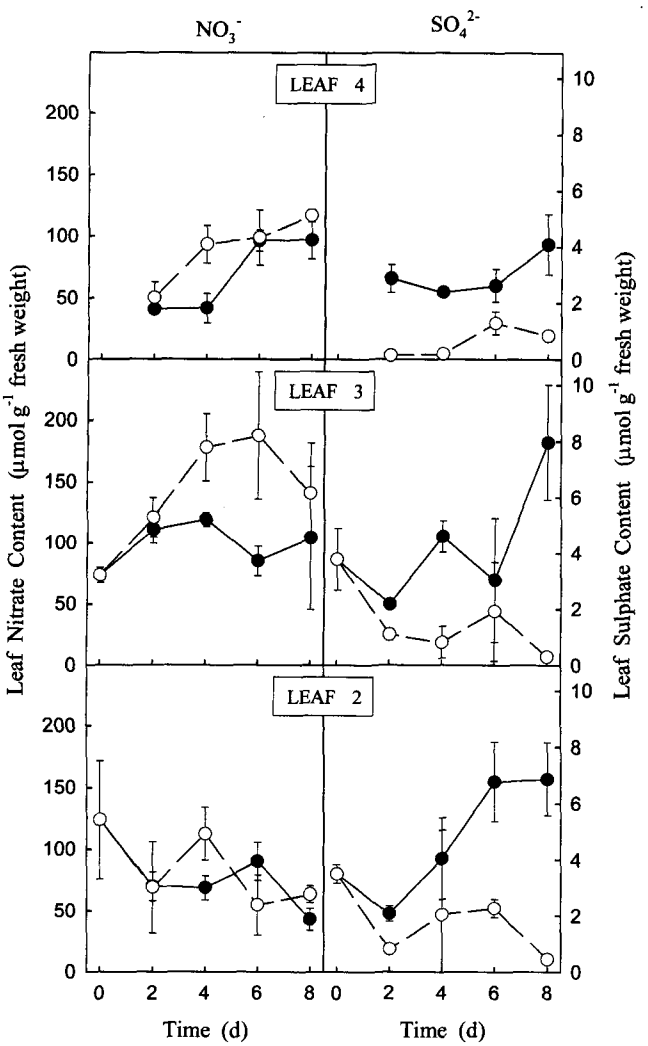


**Figure 10.** Effect of removal of S supply on $NO_3^-$ and $SO_4^{2-}$ contents of leaves 2, 3, and 4 of wheat. Anions were determined by HPLC from hot (80°C) aqueous extracts from triplicate samples of fresh-frozen leaf material from control (+S) plants (●) or from S-deprived leaves (○). Error bars are ± SE.

pare Figs. 1 and 4), and appeared first (and were most marked) in leaves that were expanding during the period of S deprivation. In this instance, presumably, the limitation in the S supply prevents the de novo synthesis of components of the photosynthetic apparatus; for example, as shown here for the Rubisco LSU protein. In the fully expanded leaf 2, in which a complete photosynthetic system had developed before S deprivation began, effects on $A$, Rubisco content, and chlorophyll were slight, becoming significant only at the end of the experimental period. In this case, the observed decrease in the photosynthetic apparatus may reflect natural turnover of proteins, especially Rubisco LSU protein, and inefficient reutilization of available S for protein synthesis within these leaves.

The effect on photosynthesis was unrelated to any effect of S deprivation on $g_s$ or $[C]_i$. Analysis of the initial slope of the $A/[C]_i$ curves indicated that lower assimilation in S-deprived leaves at saturating light intensity was a consequence of the lower efficiency of the carboxylase. The independence of effects of nutrient deprivation and $g_s$ was shown in maize (*Zea mays*) (Wong et al., 1979). The lower efficiency seems to be related specifically to lower amounts of Rubisco in S-deprived leaves, the severity of the effects on net assimilation being broadly correlated with the extent to which Rubisco activity and Rubisco protein abundance were lowered (compare Figs. 4 and 8).

The control on photosynthesis exerted by the amount of Rubisco is known to vary with light intensity. In both *Nicotiana tabacum* (Stitt et al., 1991; Hudson et al., 1992; Krapp et al., 1994) and *Arabidopsis thaliana* (Eckhardt et al., 1997), any lowering of the amount or activity of Rubisco by the expression of antisense mRNA to the Rubisco small subunit reduced net assimilation and growth. At low light intensities (200–300 $\mu$mol $m^{-2}$ $s^{-1}$) the C-flux control by Rubisco was much lower, even negligible. Stitt et al. (1991) concluded that only marginal control was exerted by Rubisco until over 50% of the available capacity was being utilized. The light conditions in the RUU growth room (approximately 500 $\mu$mol $m^{-1}$ $s^{-1}$) were less than light saturating for photosynthesis in leaf 4 of the +S plants, but observations on −S leaves showed no increase in $A$ above 500 $\mu$mol $m^{-1}$ $s^{-1}$. It is not easy to interpret this result simply in terms of the amounts of Rubisco in the −S leaves since their chlorophyll content was also lower. Terry (1976) observed that several major components of the photosynthetic system in sugar beet chloroplasts declined at similar rates as the S content of the leaf tissue declined.

The sensitivity of photosynthesis to Rubisco amount under natural light conditions calls into question the notion that plants are oversupplied with enzyme and that it might be regarded as being analogous to a vegetative storage protein (Millard, 1988; Millard and Catt, 1988). In N deficiency protein is broken down and its amino acids reallocated to growing tissues. It is hard to decide whether this is a specific enhancement of the turnover of Rubisco or merely the most obvious manifestation of the engagement of the leaf senescence program; Rubisco dominates the leaf protein in most species. S deficiency does not appear to initiate the senescence program in higher plants, and characteristic symptoms in both monocotyledonous and dicotyledonous plants are pale yellow young leaves and apparently healthy older ones. Sequence analyses revealed that the Rubisco LSU from various sources contains 14 to 21 S-amino acids. With 8 LSU per functional molecule, there would be 120 to 168 Cys and Met in total (Miziorko and Lorimer, 1988), which would make it an effective store of amino-S when it is degraded.

The turnover of protein-S in leaves is not accelerated by S-deficiency if the N supply remains optimal (Sunarpi and Anderson, 1996b); only when N is deficient are the S-amino acids of Rubisco likely to be mobilized rapidly. However, there is a report that conflicts with this idea: protein turnover in the fronds of *Lemna minor* was increased during S deficiency, with a strong indication that Rubisco is preferentially degraded (Ferreira and Teixeira, 1992). There was only a slight indication in our results with wheat leaves that the Rubisco of mature leaf 2 was degraded during the

experiment and that there must have been some Rubisco synthesized in leaf 4 from imported sources of S (Figs. 5 and 9), but these effects are small and do not alleviate the S deficiency of the expanding leaf. Evidently, the regulation of Rubisco turnover is very different in *L. minor* and in wheat, a point underlined by the observation that N starvation did not lead to Rubisco degradation in *L. minor* (Ferriera and Davies, 1987).

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## LITERATURE CITED

**Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15

**Bell CI, Clarkson DT, Cram WJ** (1995) Partitioning and redistribution of sulfur during S-stress in *Macroptilium atropurpureum* cv. Sirato. J Exp Bot **46:** 73–81

**Bell CI, Cram WJ, Clarkson DT** (1994) Compartmental analysis of $^{35}SO_4^{2-}$ exchange kinetics in roots and leaves of a tropical legume *Macroptilium atropurpureum* cv. Siratro. J Exp Bot **45:** 879–886

**Clarkson DT, Saker LR, Prosser IM, Purves JV** (1993) Rapid disruption of nitrate assimilation in spinach plants occurs during sulphate-deprivation (Abstract P5.15). J Exp Bot (Suppl) **44:** 27

**Clarkson DT, Smith FW, Vanden Berg PJ** (1983) Regulation of sulfate transport in a tropical legume, *Macroptilium atropurpureum*. J Exp Bot **34:** 1463–1483

**Eckhardt NA, Snyder GW, Portis AR Jr, Ogren WL** (1997) Growth and photosynthesis under high and low irradiance of *Arabidopsis thaliana* antisense mutants with reduced ribulose-1,5-bisphosphate carboxylase/oxygenase activase content. Plant Physiol **113:** 575–586

**Ferreira RMB, Davies DD** (1987) Protein degradation in *Lemna* with particular reference to ribulose bisphosphate carboxylase. II. The effect of nutrient starvation. Plant Physiol **83:** 878–883

**Ferreira RMB, Teixeira ARN** (1992) Sulfur starvation in *Lemna* leads to degradation of ribulose bisphosphate carboxylase without plant death. J Biol Chem **267:** 7253–7257

**Hawkesford MJ, Belcher AR**(1991) Differential protein synthesis in response to sulfate and phosphate deprivation: identification of possible components of plasma-membrane transport systems in cultured tomato roots. Planta **185:** 323–329

**Hudson GS, Evans JR, von Caemmerer S, Arvidsson YBC, Andrews TJ** (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. Plant Physiol **98:** 294–302

**Keys AJ, Parry MAJ** (1990) Ribulose bisphosphate carboxylase/oxygenase and carbonic anhydrase. *In* PJ Lea, ed, Methods in Plant Biochemistry, Vol 3: Enzymes of Primary Metabolism. Academic Press, London, pp 1–14

**Krapp A, Chaves MM, David MM, Rodriques ML, Pereira JS, Stitt M** (1994) Decreased ribulose-1,5-bisphosphate carboxylase/oxygenase in transgenic tobacco transformed with "antisense" *rbc*S.VIII. Impact on photosynthesis and growth in tocacco growing under extreme irradiance and high temperature. Plant Cell Environ **17:** 945–953

**Mackinney G** (1941) Absorption of light by chlorophyll solutions. J Biol Chem **140:** 315–322

**Marschner H** (1995) Mineral Nutrition of Higher Plants. Ed 2. Academic Press, London

**Millard P** (1988) The accumulation and storage of nitrogen by herbaceous plants. Plant Cell Environ **11:** 1–8

**Millard P, Catt JW** (1988) The influence of nitrogen supply on the use of nitrate and ribulose 1,5-*bis*phosphate carboxlase oxygenase as leaf nitrogen stores for growth of potato tubers (*Solanum tuberosum* L.). J Exp Bot **39:** 1–11

**Miziorko HM, Lorimer GM** (1988) Ribulose-1,5-bisphosphate carboxylase-oxygenase. Annu Rev Biochem **52:** 507–535

**Poot P, Pilon J, Pons TL** (1997) Photosynthetic characteristics of leaves of male-sterile and hermaphrodite sex types of *Plantago lanceolata* grown under conditions of contrasting nitrogen and light availability. Physiol Plant **98:** 780–790

**Prosser IM, Schneider A, Hawkesford MJ, Clarkson DT** (1997) Changes in nutrient composition, metabolite concentrations and enzyme activities in spinach in the early stages of S-deprivation. *In* WJ Cram, LJ De Kok, I Stulen, C Brunold, H Rennenberg, eds, Sulphur Metabolism in Higher Plants. Backhuys Publishers, Leiden, The Netherlands, pp 339–342

**Quick WP, Fichtner K, Schultze E-D, Wendler R, Leegood RC, Mooney H, Rodermel SR, Bogorad L, Stitt M** (1992) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbc*S. IV. Impact on photosynthesis in conditions of altered nitrogen supply. Planta **188:** 522–531

**Quick WP, Schurr U, Scheibe R, Schultze E-D, Rodermel SR, Bogorad L, Stitt M** (1991) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbc*S. I. Impact on photosynthesis in ambient growth conditions. Planta **183:** 542–554

**Reuveny Z, Dougall DK, Trinity PM** (1980) Regulatory coupling of nitrate and sulfate assimilation pathways in cultured tobacco cells. Proc Natl Acad Sci USA **77:** 6670–6672

**Robson AD, Pitman MG** (1983) Interactions between nutrients in higher plants. *In* A Lauchli, RL Bieleski, eds, Encyclopedia of Plant Physiology, New Series, Vol 15A. Springer-Verlag, Berlin, pp 147–180

**Stewart BA, Porter LK** (1969) Nitrogen-sulfur relationships in wheat (*Triticum aestivum* L.), corn (*Zea mays*) and beans (*Phaseolus vulgaris*). Agron J **61:** 267–271

**Stitt M, Quick WP, Schurr U, Schulze E-D, Rodermel SR, Bogorad L** (1991) Decreased Rubisco in tobacco transformed with "antisense" *rbc*S. II. Flux control coefficients for photosynthesis in varying light, $CO_2$ and air humidity. Planta **183:** 555–556

**Sunarpi, Anderson JW** (1996a) Distribution and redistribution of sulfur supplied as [$^{35}$S] sulfate to roots during vegetative growth of soybean. Plant Physiol **110:** 1151–1157

**Sunarpi, Anderson JW** (1996b) Effect of sulfur nutrition on the redistribution of sulfur in vegetative soybean plants. Plant Physiol **112:** 623–631

**Sunarpi, Anderson JW** (1997) Effect of nitrogen nutrition on the export of sulphur from leaves in soybean. Plant Soil **188:** 177–187

**Terry N** (1976) Effects of sulfur on the photosynthesis of intact leaves and isolated chloroplasts of sugar beets. Plant Physiol **57:** 477–479

**von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and gas exchange of leaves. Planta **153:** 376–387

**Wong SC, Cowan IR, Farquhar GD** (1979) Stomatal conductance correlates with photosynthetic capacity. Nature **282:** 424–426