

Effects of Sulfur on the Photosynthesis of Intact Leaves and Isolated Chloroplasts of Sugar Beets¹

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ABSTRACT

Effects of sulfur on photosynthesis in sugar beets (*Beta vulgaris* L. cv. F58-554H1) were studied by inducing sulfur deficiency and determining changes in the photosynthesis of whole attached leaves and of isolated chloroplasts. The rates of photosynthetic CO₂ uptake by intact leaves, photoreduction of ferricyanide, cyclic and noncyclic photophosphorylation of isolated chloroplasts, and the rate of CO₂ assimilation by ribulose diphosphate carboxylase, decreased with decrease in total leaf sulfur from 2500 to about 500 $\mu\text{g g}^{-1}$ dry weight. Sulfur deficiency reduced photosynthesis through an effect on chlorophyll content, which decreased linearly with leaf sulfur, and by decreasing the rate of photosynthesis per unit chlorophyll. There was only a small effect of sulfur deficiency on stomatal diffusion resistance to CO₂ until leaf sulfur decreased below 1000 $\mu\text{g g}^{-1}$ when stomatal resistance became a more significant proportion of the total diffusion resistance to CO₂. Light respiration rates were positively correlated with photosynthesis rates and dark respiration was unchanged as leaf sulfur concentrations declined.

after planting, according to the procedure outlined earlier (11). Sulfur deficiency was induced by withholding the supply of sulfate from the culture solution which contained: in mmole/l, 2.0 Ca(NO₃)₂·4H₂O, 1.0 KH₂PO₄, 2.5 KNO₃, 0.5 Mg(NO₃)₂, and 0.5 NaCl, and, in Mg/l, 0.25 B, 0.25 Mn, 0.025 Zn, 0.01 Cu and 0.005 Mo. Iron (2.5 mg/l) was added as ferric-sodium ethylene diamine tetraacetate complex. The control culture solution contained 1 mmole/l MgSO₄ in place of 0.5 mmole/l Mg(NO₃)₂.

Plants were harvested at 2- or 3-day intervals beginning on the first day of treatment. At each harvest, measurements (see references given in parentheses for descriptions of procedures) were made of: (a) the concentrations of mineral elements in the leaf blade, i.e., Na, K, Mg, Ca, Zn, Cu, Fe and Mn, sulfate S and total S (6, 12); (b) Chl content per unit area of leaf (10); (c) CO₂ and water vapor exchange and surface leaf temperatures of individual attached leaves (11, 14); (d) photoreduction of ferricyanide and the concomitant production of ATP (noncyclic photophosphorylation) by isolated chloroplasts, and cyclic photophosphorylation using PMS as catalyst (10); and (e) ¹⁴CO₂ assimilation by RuDP carboxylase extracts (10).

RESULTS

Sulfur deficiency has pronounced effects on plant growth. In cotton it diminished leaf size and stem elongation, protein and soluble sugars, and caused chlorosis (2). Chloroplasts contain proteins rich in S (4) and chloroplast morphology is considerably affected by S deficiency (3, 8, 16). Pirson (7) concluded that S deficiency upsets photosynthesis in a profound way which, after readdition of external sulfate, can only be corrected slowly through the synthesis of new protein and Chl.

Reports on the effects of S on photosynthesis are conflicting. Spencer and Possingham (9) found that S deficiency decreased the Hill reaction activity of isolated tomato chloroplasts, whereas Baszynski *et al.* (1) found that photosystem II activity in isolated maize chloroplasts was increased by S deficiency and photosystem I activity decreased only slightly. Further investigation on the effects of S appears warranted.

The present investigation explores the effects of S on photosynthesis by following changes in net CO₂ exchange of attached leaves, as well as electron transport and photophosphorylation of isolated chloroplasts, and CO₂ assimilation activity by RuDP² carboxylase. This work is part of a continuing series of investigations to study the role of mineral nutrient supply in the regulation of photosynthesis.

MATERIALS AND METHODS

Sugar beets (*Beta vulgaris* L. cv. F58-554H1) were cultured hydroponically in growth chambers to the 10-leaf stage, 28 days

Leaf Minerals and Chlorophyll. Seven days after cut-off, the young leaves of S-deficient plants turned yellow-green. During this initial period, total S in the leaf blade decreased by about one-half, eventually reaching about 500 $\mu\text{g g}^{-1}$ after 21 days (Fig. 1A). The proportion of sulfate S of the total S in the leaf blade decreased from about 17% at day 0 to about 5% at day 7; subsequently, sulfate S changed very little but total S levels continued to fall so that the sulfate percentage increased to 11% (Fig. 1A). Other mineral elements did not change significantly in concentration during S deficiency with the exception of Fe, which decreased by about one-half (Table I). The Chl content per unit area was linearly related to the concentration of total S in the leaf blade, and decreased to one-half 21 days after the onset of deficiency (Fig. 1B).

Gas Exchange. The rate of photosynthetic CO₂ uptake was linearly related with total leaf S up to 2500 $\mu\text{g g}^{-1}$ (Fig. 2A); above this concentration it appeared that F was approaching the normal maximal values of 70 to 75 ng CO₂ cm⁻² sec⁻¹. At the lowest leaf S concentration (500 $\mu\text{g g}^{-1}$) F was about 17% of the control, but when photosynthetic CO₂ uptake was expressed per unit Chl rather than per unit area, it was 36% of the control. This was due to the fact that Chl content per unit leaf area decreased by half during S deficiency.

The decrease in photosynthetic rate was associated with increased "mesophyll resistance": very high values of "mesophyll resistance" were obtained, r_m reaching 20 to 25 sec cm⁻¹ (Fig. 2B). Leaf (stomatal) diffusion resistance did not increase appreciably until leaf S dropped below 1000 $\mu\text{g g}^{-1}$, and even then did not reach very high values (Fig. 2D). The effect of leaf S on leaf diffusion resistance in the dark was also investigated. It behaved in a similar way to leaf diffusion resistance in the light and did

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² Abbreviations: RuDP: ribulose 1,5-diphosphate; PMS: phenazine methosulfate; F: rate of photosynthetic CO₂ uptake.

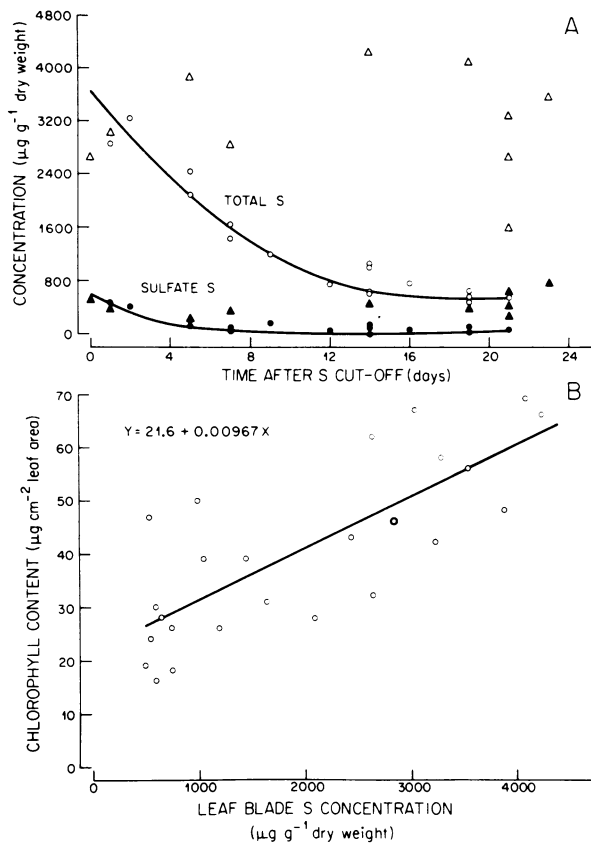


FIG. 1. Effects of S deficiency on leaf S and leaf Chl. A: Changes in sulfate S (\bullet , \blacktriangle) and total S (\circ , \triangle) in the leaf blade with time after cut-off in control (\blacktriangle , \triangle) and S-deficient plants (\bullet , \circ); B: changes in the Chl content per unit area of leaf with blade (total) S concentration.

Table I. Leaf Mineral Concentrations and Their Relations with Leaf Sulfur

Linear regressions were used to test whether the concentrations of various mineral elements changed significantly with leaf S during sulfur deficiency. Presented below are the mean concentrations with standard deviations of the mineral elements in the blades of leaves of different sulfur concentrations and the regression coefficients with their standard errors. The mean sulfur concentration was $1829 \pm 1183 \mu\text{g g}^{-1}$.

Element	Mean Concentration	Regression Coefficient	Element	Mean Concentration	Regression Coefficient
	mg g^{-1} dry wt			$\mu\text{g g}^{-1}$ dry wt	
K	57.3 ± 19.2	3.6 ± 5.3	Cu	30 ± 13	0.0013 ± 0.0035
Na	3.10 ± 0.89	0.55 ± 0.25	Fe	158 ± 39	$0.0304 \pm 0.0112^*$
Ca	7.20 ± 2.65	-0.19 ± 0.72	Mn	199 ± 28	-0.0023 ± 0.0082
Mg	6.40 ± 1.73	0.74 ± 0.37	Zn	54 ± 10	0.0031 ± 0.0034

* Significant at $P = 0.05$.

not increase appreciably until leaf S dropped below $1000 \mu\text{g g}^{-1}$ (Fig. 2F). Respiration in the light followed a curvilinear relationship with leaf S similar to that for photosynthesis (Fig. 2C). There was no indication that respiration in the dark changed significantly with leaf S (Fig. 2E).

Photosynthetic Activities of Isolated Chloroplasts and of Extracted RuDP Carboxylase. With high concentrations of leaf S the rate of ferricyanide reduction by isolated chloroplasts reached about $400 \mu\text{moles mg Chl}^{-1} \text{hr}^{-1}$ whereas for noncyclic

ATP formation (ferricyanide as electron acceptor) it reached $200 \mu\text{moles mg Chl}^{-1} \text{hr}^{-1}$ (Fig. 3A, 3B). This implies a P/2e ratio (*i.e.*, the ratio of ATP molecules formed per pair of electrons transferred) in these experiments of about 1 for chloroplasts of the high-S plants. The curves of Figure 3A and 3B also suggest a P/2e ratio of 1 for low-S plants so that there appeared to be no uncoupling between the processes of photophosphorylation and ferricyanide reduction with S deficiency. The three different types of photosynthetic activity of isolated chloroplasts, *i.e.*, ferricyanide reduction, noncyclic and cyclic ATP formation, and CO_2 assimilation by RuDP carboxylase extracts, were each related in a similar way with leaf S (Fig. 3). As leaf S decreased to $2500 \mu\text{g g}^{-1}$ the photosynthetic activities of the chloroplasts and RuDP carboxylase extract remained constant, although somewhat variable, but at $1000 \mu\text{g g}^{-1}$ the photosynthetic activities had decreased by a half or more.

DISCUSSION

The critical concentration for sugar beet growth, according to Ulrich (15), is about $250 \mu\text{g S g}^{-1}$ dry weight. This value refers to the concentration of sulfate S in the leaf blade at which there is a 10% reduction in growth rate from the optimum. In the present work the critical level was reached only 2 or 3 days after S was withheld from the culture solution and corresponded to a total S (leaf blade) concentration of $2500 \mu\text{g g}^{-1}$. Photosynthetic activity was not reduced with decrease in leaf S until the critical level was reached; this is shown for photosynthetic CO_2 uptake in Figure 4. Similarly, photosynthetic activities of isolated chloroplasts and

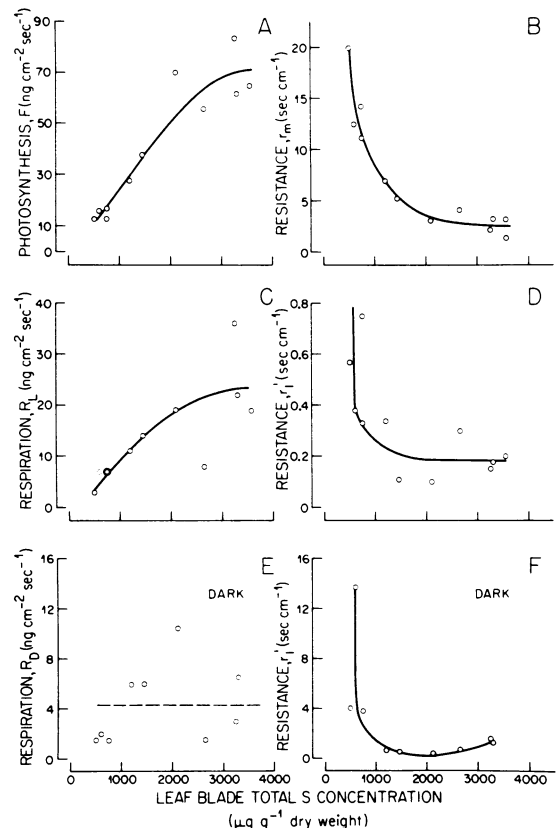


FIG. 2. Effects of S deficiency on various leaf gas exchange parameters A: Changes in F; B: changes in CO_2 mesophyll resistance, r_m ; C: changes in rate of respiratory CO_2 evolution in the light, R_L ; D: changes in leaf diffusion resistance for water vapor, r_1' , in the light; E: changes in rate of respiratory CO_2 evolution in the dark, R_D ; F: changes in leaf diffusion resistance for water vapor, r_1' , in the dark. The data for F and r_1' (in the light) were determined at 35 mw cm^{-2} visible radiation, 25°C , and at an ambient CO_2 concentration of 300 ng cm^{-3} .

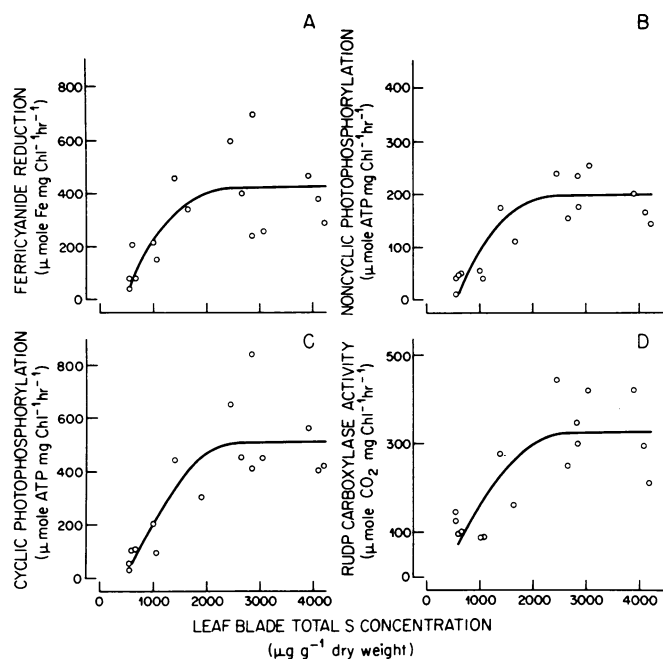


FIG. 3. Effects of S deficiency on the photosynthetic activity of isolated chloroplasts and of RuDP carboxylase extract. A: Changes in rate of photoreduction of ferricyanide; B: changes in rate of noncyclic ATP production (with ferricyanide as electron acceptor); C: changes in rate of cyclic ATP production (with PMS as catalyst); D: changes in rate of CO_2 assimilation by RuDP carboxylase chloroplast extract.

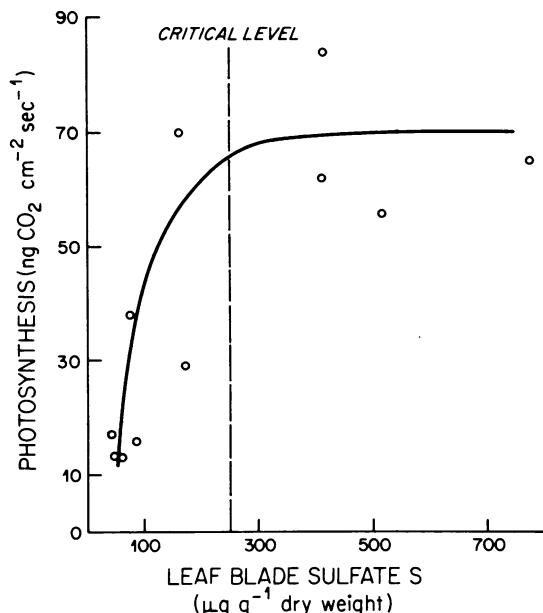


FIG. 4. Net photosynthetic CO_2 uptake by attached leaves decreased only when leaf S dropped below the independently determined critical level for growth, $250 \mu\text{g g}^{-1}$.

RuDP carboxylase extracts did not change until S concentrations decreased below the critical level ($2500 \mu\text{g g}^{-1}$ total leaf blade S).

Most of the decrease in the rate of photosynthetic CO_2 uptake with S deficiency was attributable to an increase in "mesophyll resistance." Leaf diffusion resistance was a relatively small component of the total resistance to CO_2 transfer from the atmosphere to the chloroplasts until leaf S decreased to about $1000 \mu\text{g g}^{-1}$. At this concentration the total diffusion resistance to CO_2 ($r_a + r_l + r_m$, where r_a is the boundary layer resistance) had in-

creased by 5 sec cm^{-1} ; of this increase, 80% was due to an increase in r_m and only 20% to an increase in r_l . Below $1000 \mu\text{g g}^{-1}$, when leaves were severely deficient, leaf diffusion resistance increased sharply and became a more significant proportion of the total resistance to photosynthetic CO_2 uptake.

The increase in "mesophyll resistance" with S deficiency was in part due to a decrease in the Chl/unit area, and to a decrease in the photosynthetic activities/unit Chl, *i.e.*, the photoreduction of ferricyanide, noncyclic ATP formation, cyclic ATP formation using PMS, and CO_2 assimilation by RuDP carboxylase. None of these chloroplast activities was apparently affected any more than the other by S deficiency and each activity changed in a similar way with leaf S concentration. This suggests that S deficiency affected some factor which secondarily affected the photosynthetic activities within the chloroplast. Chloroplasts have been shown to contain a large amount of high-S protein (4), and diminished protein synthesis resulting from S deficiency would probably impair all types of photosynthetic activity.

The increase in leaf diffusion resistance in the dark which occurred below leaf S levels of $1000 \mu\text{g g}^{-1}$ was probably due to a greater degree of stomatal closure. Leaf diffusion resistance, r_l , is related to stomatal diffusion resistance, r_s , and cuticular diffusion resistance, r_c , by the relation $1/r_l = 1/r_s + 1/r_c$. In bright light and low ambient CO_2 concentration, r_l closely approximates r_s (13), but at lower light levels and in darkness stomata tend to close so that cuticular resistance becomes a more significant component of r_l . Other work (5) has indicated that stomata may not close fully in darkness so that conductance of water vapor may still occur through them. In sugar beet, r_l' may be as low as 0.2 sec cm^{-1} in control plants in the light, increasing to 2 to 4 sec cm^{-1} in darkness. In the S-deficient beets, r_l' increased to 14 sec cm^{-1} in darkness, while in N-deficient beets (Terry, unpublished) r_l' increased to as much as 100 sec cm^{-1} . The increase in r_l' which occurred in S- (or N-)deficient beets in darkness is attributed to an increase in r_s rather than r_c and suggests that stomata do not fully shut at night in leaves plentifully supplied with mineral nutrients.

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