Planta (1993) 191:365–376

Planta

Inhibition of photosynthesis, acidification and stimulation of zeaxanthin formation in leaves by sulfur dioxide and reversal of these effects

Sonja Veljovic-Jovanovic*, Wolfgang Bilger, Ulrich Heber

Julius-von-Sachs Institut für Biowissenschaften, Universität Würzburg, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Germany

Received: 19 March 1993 / Accepted: 24 April 1993

Abstract. Leaves of *Pelargonium zonale* L. and *Spinacia oleracea* L. were fumigated with high concentrations of SO_2 for very short periods of time with the aim of first producing acute symptoms of damage and then observing repair. The response of different photosynthetic parameters to SO_2 was monitored during and after fumigation. The following results were obtained: (1) Inhibition of CO_2 assimilation in the light was accompanied by increased reduction of the quinone acceptor, Q_A, of photosystem II and by increased oxidation of the electron-donor pigment P_{700} of photosystem I. Increased control of photosystem II activity in the SO_2-inhibited state was also indicated by increased light scattering and by increased non-photochemical quenching of chlorophyll fluorescence. Both are indicators of chloroplast energization. Apparently, SO_2 did not decrease but rather increased energization of the chloroplast thylakoid system by light. (2) Accumulation of dihydroxyacetone phosphate, fructose-1,6-phosphate and ribulose-1,5-phosphate and a decrease of 3-phosphoglycerate and hexosephosphate indicated that SO_2 inhibited enzymes of the Calvin cycle. (3) Stimulated postillumination CO_2 evolution suggested that when photosynthesis declined respiration increased to provide energy for repair reactions. (4) Increased leaf absorbance at 505 nm indicated increased stimulation of zeaxanthin formation in thylakoid membranes under the influence of SO_2. A similar increase in 505-nm absorbance could be induced by high concentrations of CO_2. In darkened leaves, SO_2 did not produce changes in 505-nm absorbance. (5) While zeaxanthin formation was stimulated, changes in the fluorescence of the pH-indicating dye pyranine, which had been fed to the leaves, indicated acidification of the cytoplasm of leaf cells by SO_2. Maximum acid production by SO_2 required light. In contrast, cytoplasmic acidification of leaf cells by CO_2 was similar in the light and in the dark. (6) Since zeaxanthin formation is known to depend on the acidification of the thylakoid lumen, SO_2-dependent zeaxanthin formation indicated SO_2-dependent acidification of the thylakoid lumen as the indirect result of cytoplasmic acidification by SO_2. (7) Inhibition of photosynthesis and other effects of SO_2 were fully reversible in the light. Detoxification of SO_2 and reactivation of the photosynthetic apparatus were slow or absent in the dark. Light had a dual effect on the action of SO_2. Transiently, it first increased the extent of inhibition of assimilation, but, finally, it reversed inhibition. Sulfur dioxide was inhibitory as a consequence of the chemical reactivity of its hydration products rather than as a result of cellular acidification by the produced acid. The initial acidification was followed by an appreciable alkalisation demonstrating the action of the pH-stat mechanism. (8) The data are discussed in relation to SO_2 toxicity under field conditions when plants are chronically exposed to polluted air.

Key words: Acidification – Chlorophyll fluorescence – Photosynthesis (SO_2 effect) – Xanthophyll cycle – *Spinacia* – *Pelargonium*

Dedicated to Professor O.L. Lange on the occasion of his 65th birthday

* On leave from the Centre for Multidisciplinary Sciences, University of Belgrade, Yugoslavia

Abbreviations: Chl = chlorophyll; DHAP = dihydroxyacetone phosphate; FBP = fructose-1,6-bisphosphate; F6P = fructoce-6-phosphate; F, Fm, Fm′, Fo, Fo′ = chlorophyll fluorescence levels; PGA = 3-phosphoglycerate; P_{700} = primary donor of photosystem I; Q_A = primary quinone acceptor of photosystem II; q_p = photochemical quenching of chlorophyll fluorescence; NPQ = non-photochemical quenching of chlorophyll fluorescence; RuBP = ribulose-1,5-bisphosphate

Correspondence to: U. Heber

Introduction

Sulfur dioxide has long been known to damage plants when present in the air at excessive concentrations. The gas diffuses into leaves through open stomata. It is highly soluble in aqueous phases where it can react with water

to form sulfurous acid. The acid (pK values 1.79 and 6.99) is trapped in alkaline leaf compartments. Both SO_2 and the anions or radicals formed from it can undergo different reactions with cellular constituents thereby interfering with metabolism. Anionic products formed from SO_2 are toxic. Detoxification must outcompete damaging reactions to be effective, and is possible either by reduction which finally yields sulfur-containing amino acids, or by oxidation which results in sulfate formation. Reductive detoxification does not burden cytoplasmic pH-stat mechanisms which maintain pH within physiological limits, whereas formation of sulfurous or sulfuric acid necessitates either mobilization of bases or export of protons to maintain proper control of the cytoplasmic pH. An extensive literature exists on the effects of SO_2, or the sulfite formed from SO_2, on individual cellular reactions (Winner et al. 1985). Nonetheless, little is known about the integrated response of intact leaves to doses of SO_2 which do not cause permanent damage. In the following, we report on experiments performed with the aim to obtain information on SO_2 effects on leaves and the possible repair of SO_2 damage.

Material and methods

Plant material. Spinach (*Spinacia oleracea* L. cv. Yates) was grown in a greenhouse with additional illumination in 10 h light/14 h dark cycles. Seeds were obtained from Fa. Wagner, Würzburg, FRG. *Pelargonium zonale* L. cv. Kardinal, also from a greenhouse, was propagated from cuttings and grown in normal day/night cycles. Leaves were cut from the plants and were used for experimentation with their petioles immersed in water.

Gas exchange measurements and illumination. During measurements, segments of the leaves were enclosed in a thermostatted sandwich-type cuvette which permitted controlled gas flow over their lower surface. The gas flow rate was 30 $l \cdot h^{-1}$. The upper surface was usually attached with a transparent glue made from soluble starch and water (1:10, w/v) to the thermostatted window of the cuvette to minimize an increase in leaf temperature during illumination. Leaf temperature was controlled by a thermocouple and kept close to 20° C. The composition of the gas stream passing over the leaf was adjusted by mass-flow controllers (Tylan, Eching, FRG). The relative humidity of the gas entering the cuvette was 60%. Transpirational water loss and CO_2 exchange were recorded by an infra-red gas analyzer (Binos, from Leybold-Heraeus, Hanau, FRG) in the differential mode. Stomatal and boundary-layer resistance to water and SO_2 diffusion were calculated according to Nobel (1983) and Taylor and Tingey (1983). Short-wavelength red actinic illumination from a 250-W halogen lamp was provided by a red cutoff filter (RG 630; Schott, Mainz, FRG) and K 65 and Calflex X filters (Balzers, Liechtenstein). An additional water layer (4 cm) was used as a heat filter. The half-bandwidth of red light was from 625 to 663 nm. To obtain far-red light from the same light source, the filters RG 724 and RG 610 from Schott were combined with Calflex X, and the half-bandwidth was from 720 to 760 nm. The light was brought to the upper surface of the cuvette-enclosed leaf segment by means of fiber optics which simultaneously transmitted optical signals back from the leaf to receiving devices.

Fluorescence and absorbance measurements. Modulated chlorophyll (Chl) fluorescence was measured by a PAM fluorometer (Walz, Effeltrich, FRG). For fluorescence analysis of the energy state of the thylakoid system and of the redox state of the primary quinone acceptor (Q_A) in the reaction center of PSII, the saturation-pulse method was employed (Bradbury and Baker 1981; Schreiber et al. 1986). In parallel to Chl fluorescence, leaf absorption of modulated 820-nm light was measured by a second PAM fluorometer (Schreiber et al. 1988) to obtain information on the redox state of P_{700}, the primary electron donor in the reaction center of PSI. The fast decrease in 820-nm absorption seen when illumination was interrupted by brief darkening periods (6–10 s) was interpreted to indicate full reduction of P_{700} and of plastocyanin which changes its redox state in parallel with P_{700} (Klughammer and Schreiber 1992). Simultaneously, the decrease in Chl fluorescence permitted measuring the so-called Fo' level of Chl fluorescence. This is needed for the evaluation of photochemical quenching (q_p) (van Kooten and Snel 1990), which is defined as (Fm'–F)/(Fm'–Fo'). The term $1\text{-}q_p$ was taken as a measure for the reduction state of the "primary" acceptor Q_A (Dietz et al. 1985). The quantum yield of PSII was estimated from fluorescence parameters, (Fm'–F)/Fm', according to Genty et al. (1989). Non-photochemical fluorescence quenching was expressed as NPQ=(Fm–Fm')/Fm' (Bilger and Björkman 1990). Fm is the maximum modulated fluorescence observed after a light-saturating flash in a leaf which had been predarkened for a prolonged period of time, and Fm' the maximum flash-induced modulated fluorescence of the leaf in the presence of actinic illumination. Fo' is the minimum modulated fluorescence observed immediately after turning actinic illumination off; F is fluorescence in the presence of actinic illumination. Absorption of a non-modulated, weak green measuring beam was measured by a photomultiplier which was equipped with a BG 18 filter from Schott and filters 9782 and 9780 from Corning Glass Works (Corning, N.Y., USA) to protect it against actinic light and the modulated red and far-red beams. The green measuring beam was produced by a monochromator and was directed to the lower surface of the leaf. Transmitted green light was collected from the upper surface by the multi-branched light guide. One of the branches was connected to the multiplier. Wavelengths mainly used were 505 nm to record changes in zeaxanthin concentration and 540 nm to record changes in light scattering by the leaf which indicate changes in thylakoid energization (Heber 1969; Bilger et al. 1988). To record changes in cellular pH, a fluorescent pH-indicating dye, pyranine, which enters mesophyll cells even though it is a trisulfonate anion, was fed to leaves though the petiole and its fluorescence was excited and measured as described by Yin et al. (1990).

Pigment analysis. For the pigment analyses made in conjunction with measurements of Chl fluorescence and ΔA_{505}, an illuminated part of a leaf was frozen in liquid nitrogen immediately after opening the cuvette and stored at −80° C. Extraction and high-performance liquid chromatography (HPLC) analysis of pigment composition were as described by Gilmore and Yamamoto (1991).

Metabolite assays. To relate the state of photosynthesis to the levels of Calvin-cycle metabolites in leaves, a sandwich-type cuvette designed so as to permit both gas-exchange and Chl fluorescence measurements and rapid freeze-stopping of metabolism was used (Siebke et al. 1990). Freeze-stopping of metabolism was done by driving a copper piston which had been precooled with liquid nitrogen through the upper window of the cuvette onto the leaf. Illumination was provided through the lower window. The apparatus used was similar to that described by Badger et al. (1984). Metabolites were determined enzymically as described in Dietz and Heber (1984). When metabolites were determined in the non-aqueously isolated chloroplast fraction of freeze-dried leaves, another larger cuvette was used which permitted monitoring of Chl fluorescence and CO_2-exchange measurements. Non-aqueous fractionation of the leaves was performed according to the methods of Heber and Willenbrink (1964) and Dietz and Heber (1984).

Chlorophyll was determined in acetone extracts according to Arnon (1949) or, after its conversion to pheophytin, in acid-treated leaf homogenates according to Vernon (1960).

Results and discussion

Inhibition of CO_2 assimilation and zeaxanthin formation by a leaf during and after fumigation with SO_2. Figure 1 shows simultaneous recordings of transpirational water loss, photosynthetic CO_2 uptake and absorbance at 505 nm of a leaf of *Pelargonium* before, during and after a 4-min exposure period to three different concentrations of SO_2 in air. During fumigation with SO_2, CO_2 uptake declined. The extent of photosynthesis inhibition increased with increasing concentration of SO_2. Inhibition was not related to stomatal closure, although stomata closed partially during fumigation (as shown by decreased transpiration), accounting for part of the observed decline in CO_2 uptake. Direct inhibition of photosynthesis by SO_2 is shown by a progressive increase in the intercellular concentration of CO_2, c_i, when photosynthesis was progressively inhibited by increased levels of SO_2 (Fig. 2). Inhibition of assimilation by stomatal closure should be accompanied by decreased c_i, not by increased c_i. The calculation of c_i was based on the measured transpiration rate (Nobel 1983).

After termination of fumigation, photosynthesis continued to decline for a few minutes and then recovered, indicating repair of damage. Simultaneously, zeaxanthin formation, which is indicated by increased absorption of 505-nm light, was stimulated by SO_2 (Fig. 1). After fumigation was terminated, 505-nm absorbance continued to increase, but then declined. Original levels were reestablished while photosynthesis recovered. In parallel with inhibition of CO_2 assimilation, the scattering of a weak green measuring beam (set to 540 nm) by the leaf increased. It decreased as photosynthesis recovered (data not shown). Increased light scattering is known to be an indicator of an increased proton gradient across the thylakoid membrane (Heber 1969; Köster and Heber 1982; Kobayashi et al. 1982; Bilger et al. 1988). An increase in the magnitude of the proton gradient, when light remains unchanged, indicates either SO_2-dependent inhibition of ATP formation by the thylakoid ATPase or decreased consumption of ATP by SO_2-inhibited carbon assimilation. In both cases such a decrease would lead to an increased acidification of the thylakoid lumen. This would stimulate the violaxanthin de-epoxidase, the enzyme responsible for zeaxanthin formation, which is known to be pH-dependent (Hager 1980). It should be noted that, at 505 nm, there is no overlap between the absorption change which indicates zeaxanthin formation, and the light-scattering change which peaks close to 540 nm (Bilger et al. 1989; Bilger and Björkman 1990).

In Fig. 3, Q_A reduction, P_{700} oxidation (plus oxidation of some plastocyanin which donates electrons to P_{700}

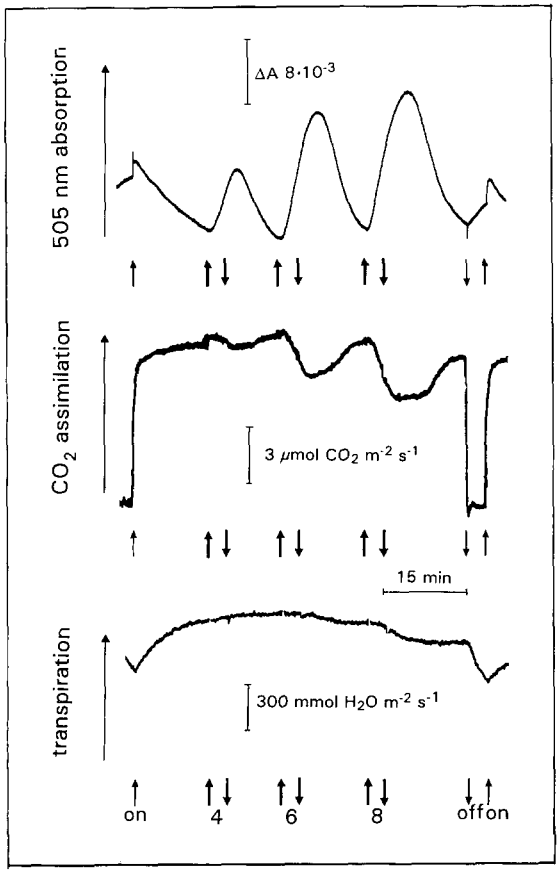

Fig. 1. Effect of three fumigation periods of 4 min each with different concentrations of SO_2 on transpiration, carbon assimilation, and absorption of a weak 505-nm measuring beam by an illuminated *Pelargonium* leaf. *Arrows* indicate addition of SO_2 to and its removal from the gas phase, or illumination and darkening. *Numbers* show concentrations of SO_2 in $\mu l \cdot l^{-1}$. The CO_2 concentration in air was 520 $\mu l \cdot l^{-1}$ and the irradiation 120 $W \cdot m^{-2}$

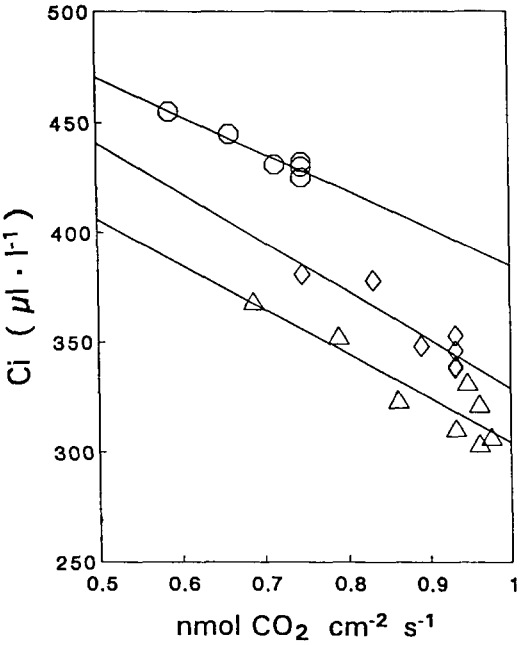

Fig. 2. Measured CO_2 uptake and calculated internal CO_2 concentration (c_i) when inhibition of photosynthesis was maximal (see Fig. 1). Data are from experiments with three different *Pelargonium* leaves (○, Δ, ◇). Each leaf was gassed for 5 min with SO_2. Increasing SO_2 concentrations produced not only a progressive decrease in CO_2 uptake but also an increase in c_i. Between the SO_2 treatments, leaves were illuminated for 40–60 min in the absence of SO_2 to allow recovery from the fumigations. The irradiation was 80 $W \cdot m^{-2}$ and the CO_2 concentration in air 600 $\mu l \cdot l^{-1}$

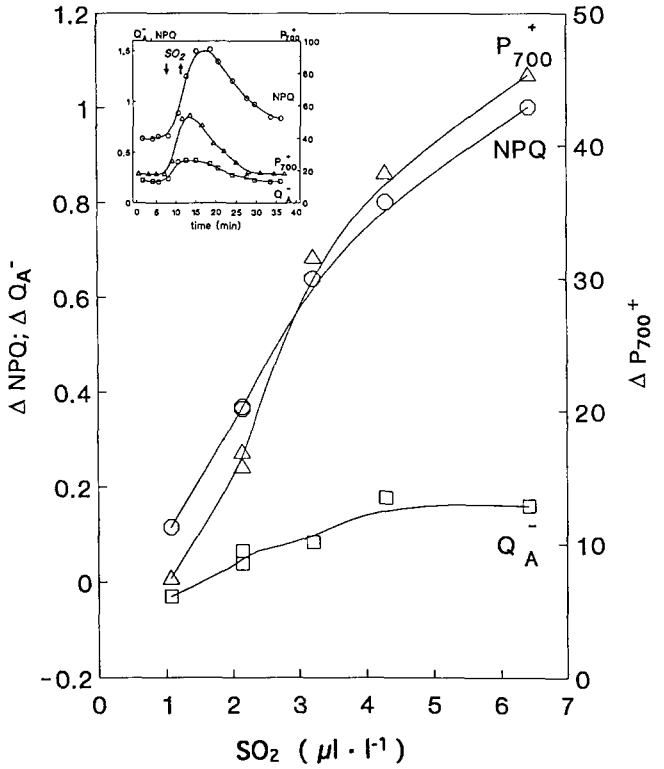

Fig. 3. Increased reduction of Q_A (□), oxidation of P_{700} (Δ) and amount of NPQ (○) of a representative *Pelargonium* leaf as a function of the concentration of SO_2 in air. Values were measured between 3 and 5 min after 3-min fumigation periods when inhibition of photosynthesis was maximal *(inset)*, and are expressed as a difference from the control values measured before fumigation (see *inset*). The control value for CO_2 assimilation was 10.7 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$; P_{700} was 20% oxidized in the control and Q_A was about 40% reduced; NPQ was 0.89 in the control. The irradiation was 80 $W \cdot m^{-2}$, the CO_2 concentration 600 μl · $^{-1}$. Before the experiment, the leaf was kept for 2 h in the light in air with 600 $\mu l \cdot l^{-1}$ CO_2

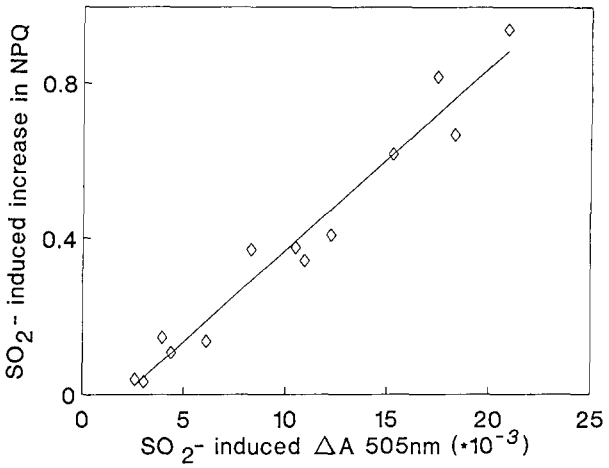

Fig. 4. Representative graph of the correlation between SO_2^- induced increases in NPQ and in 505-nm absorption. *Pelargonium* leaves were illuminated for at least 1 h before fumigation with SO_2 at concentrations ranging from 2 to 8 $\mu l \cdot l^{-1}$. Other conditions were as described in the legend of Fig. 3

and changes its redox state together with P_{700} (Klughammer and Schreiber 1992)) and non-photochemical quenching of chlorophyll fluorescence (NPQ) are compared in their response to a 3-min exposure of a *Pelargonium* leaf to SO_2. The time course of Q_A reduction, of P_{700} oxidation and of NPQ in response to SO_2 is shown in the inset. As photosynthesis inhibition increased due to increased concentrations of SO_2, P_{700} photooxidation, Q_A reduction and NPQ increased also (Fig 3). This shows that control of electron flow from PSII to PSI is increased under the influence of SO_2. The control is exerted both at the level of PSII (as shown by increased NPQ which indicates increased radiationless dissipation of excitation energy) and at the level of plastohydroquinone oxidation. The latter is shown by increased reduction of the quinone acceptor Q_A in the reaction center of PSII, while P_{700} oxidation is increased (Fig. 3). Parallel to the changes in NPQ and Q_A^-, the so-called dark level of chlorophyll fluorescence (Fo′) decreased transiently (not shown). This is another indication of increased conversion of light energy into heat. In leaves, light scattering and NPQ are both indicators of the proton gradient between the chloroplast stroma and the intrathylakoid space (Krause 1973; Köster and Heber 1982; Krause and Weis 1991). A close correlation between the extent of NPQ and zeaxanthin levels has been demonstrated in leaves by Demmig et al. (1987). Figure 4 shows a linear correlation between the SO_2-induced increase in 505-nm absorbance and the simultaneously observed increase in NPQ.

The effect of SO_2 on metabolite levels. In order to answer the question of whether the observed SO_2-dependent inhibition of photosynthesis is caused by inhibition of the thylakoid ATPase (Cerovic et al. 1982), of electron transport (Daniell and Sarojini 1981; Shimazaki et al. 1984) or by inhibition of enzymes of the Calvin cycle (Anderson and Duggan 1977; Tanaka et al. 1983), metabolite levels were measured. A part of a leaf was enclosed in a cuvette which permitted, in addition to measuring gas exchange and Chl fluorescence, fast freeze-stopping of leaf metabolism. After a spinach leaf had reached steady-state photosynthesis in saturating light (after about 40 min), it was fumigated with different concentration of SO_2 for 6 min. When inhibition of photosynthesis was maximal, and before photosynthesis started to recover (see Figs. 1, 3), the part of the leaf which was enclosed in the cuvette was cut out with a precooled (−180° C) copper piston and the metabolites were determined in leaf extracts (Fig. 5). With increasing SO_2 concentration and increasing inhibition of photosynthesis levels of dihydroxyacetone-phosphate (DHAP), fructose-1,6-phosphate (FBP) and ribulose-1,5-bisphosphate (RuBP) increased, whereas levels of 3-phosphoglycerate (PGA) and fructose-6-phosphate (F6P) decreased (Fig. 5).

In Fig. 6, ratios of DHAP to PGA, FBP to F6P and RuBP to PGA from different leaves are compared with the SO_2-dependent decrease in the quantum yield of electron transport in PSII which was measured by Chl fluorescence (Genty et al. 1989). As the concentration of

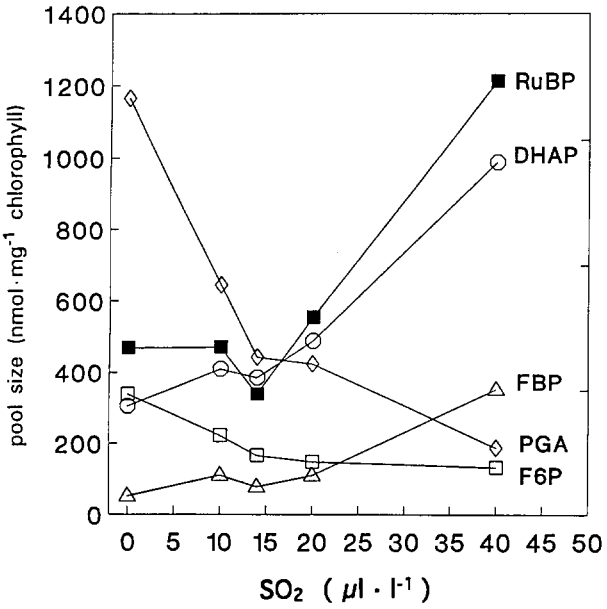

Fig. 5. Representative demonstration of the effect of SO_2 on the levels of DHAP, PGA, FBP, F6P and RuBP in spinach leaves. Leaves were kept for 30 min in the light (200 $W \cdot m^{-2}$) in air with 600 $\mu l \cdot l^{-1}$ CO_2 and were then fumigated with SO_2 for 6 min. When inhibition of photosynthesis was maximal (about 7 min after termination of fumigation) metabolism was arrested by freeze-clamping of the leaves

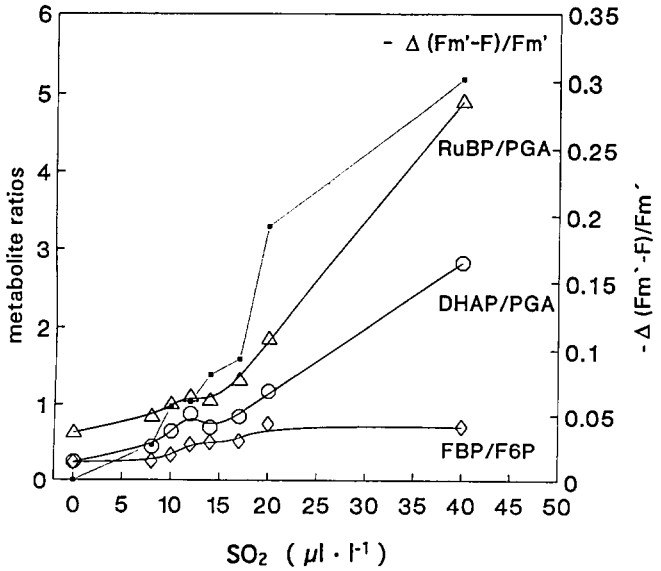

Fig. 6. Effect of SO_2 on ratios of PGA to DHAP (○), FBP to F6P (◇) and RuBP to PGA (△) and on quantum yield of PSII (■). Data are from Fig. 5 and from other experiments. Control values are means of three different leaves. A saturating light pulse had been given 2 min before leaves were frozen. The Δ (Fm′ − F)/Fm′ is the loss of quantum yield of electron flow through PSII caused by SO_2 at the time of freezing the leaves

SO_2 in air was increased, photosynthesis became progressively inhibited. The quantum yield of electron transport in PSII decreased accordingly. This is shown in Fig. 6 as difference between the quantum yield of PSII in the leaf before and after fumigation with SO_2 [$-\Delta$ (Fm′ − F)/Fm′]. All shown metabolite ratios increased while the decreased quantum yield indicated progressive inhibition of photosynthesis.

Metabolites were also measured in chloroplasts which had been isolated non-aqueously from fumigated spinach leaves. Illuminated leaves had been exposed to SO_2 for 5 min. Ten minutes after termination of fumigation, when photosynthesis inhibition was still close to maximum, or 40 min after fumigation, when recovery was extensive, they were frozen in liquid nitrogen and freeze-dried. Chloroplasts were isolated from the dry leaves as described by Dietz and Heber (1984). Fumigation of the leaves had produced a reduction in the quantum yield of PSII by about 50% [(Fm′ − F)/Fm′ was decreased from 0.6 to 0.3]. As in the leaf extracts, the inhibition of photosynthesis by SO_2 was associated with a marked increase in chloroplast DHAP and a decrease in PGA (Table 1). Chloroplast hexose phosphates decreased and FBP and RuBP increased. The ATP/ADP ratio increased from 1.18 in control chloroplasts to 2.38 in chloroplasts of leaves which were inhibited by SO_2 (Table 1).

The data show that inhibition of photosynthesis by SO_2 cannot be explained by inhibition of the thylakoid ATPase. They are consistent with the inhibition by SO_2 of stromal enzymes such as fructose bisphosphatase (Tanaka et al. 1984) and, at higher SO_2 concentrations, ribulose bisphosphate carboxylase (Ziegler 1973). Inhibition of these enzymes should be expected to result in the accumulation of their substrates and in decreased levels of their products. This was observed. In contrast, the level of the substrate of the system phosphoglycerate kinase/glyceraldehyde phosphate dehydrogenase/triose-phosphate isomerase, PGA, declined in the presence of SO_2, whereas its product, DHAP, increased. A decrease of PGA by a factor of about ten has also been observed in SO_2-fumigated spinach leaves by Tanaka et al. (1984). The observations show that the thylakoid system continued to provide ATP and electrons for the reduction of PGA even in the presence of high SO_2 levels, and that the enzymes involved in PGA reduction remained sufficiently active to reduce available PGA.

Importantly, inhibition was reversible, as shown by the recovery of photosynthesis and by the changes in the levels of chloroplast metabolites or in their ratios (Table 1). Of particular interest is the observation that recovery of fructose bisphosphatase activity is indicated only by increased carbon assimilation and an increased level of F6P, not by a decreased ratio of FBP to F6P. Apparently, repair reactions proceed at different rates with different enzymes.

Relationship between SO_2-induced stimulation of zeaxanthin and decreased carbon assimilation. Inhibition of Calvin-cycle enzymes by SO_2, as shown by the experiments of Figs. 5 and 6, decreases photosynthetic energy consumption thereby permitting increased thylakoid energization. An increased transthylakoid proton gradient (or, rather, a decreased intrathylakoid pH) could account for increased zeaxanthin formation which in turn would

Table 1. Metabolite levels (*A*) and metabolite ratios (*B*) in non-aqueously isolated chloroplasts. Leaves of spinach (10 for each treatment) were kept in the dark (control dark) or were illuminated with white light (200 W · m^{-2}) for 1 h in air with 600 µl · l^{-1} CO_2 (control light). In the SO_2 experiment, the leaves were first illuminated without SO_2 in air with 600 µl · l^{-1} CO_2 for 30 min and then fumigated in the light with 20 µl · l^{-1} SO_2 for 6 min, followed by a 10-min illumination period withot SO_2. The recovery experiment was identical to the SO_2 experiment except that the illumination period after the fumigation was not 10 but 40 min. Leaf metabolism was quenched by pouring liquid nitrogen into the leaf chamber either in the dark (control dark) ot in the light. The frozen leaves were freeze-dried and used for non-aqueous fractionation

A	Amounts (nmol · mg^{-1} Chl)							
	DHAP	PGA	RuBP	FBP	F6P	G6P	ATP	ADP
Control (dark)	1	100	0	1	12	41	39	33
Control (light)	13	200	64	18	34	72	36	32
+ SO_2	100	7	200	12	9	28	62	26
Recovery	76	40	100	50	22	44	61	–

B	Metabolite ratios		
	PGA/DHAP	RuBP/PGA	FBP/F6P
Control (light)	14.3	0.32	0.53
+ SO_2	0.07	28.6	1.33
Recovery	0.53	2.5	2.27

lead to increased control of PSII activity and increased dissipation of light energy as heat (Demmig et al. 1987).

The experiment shown in Fig. 7 is intended to reveal whether decreased ATP consumption due to decreased activity of Calvin cycle is the only factor which contributes to increased zeaxanthin formation. The quantum yield of PSII was chosen as a measure for photosynthetic activity. A *Pelargonium* leaf was permitted to photosynthesize initially in the presence of 600 µl · l^{-1} CO_2 and both absorption at 505 nm and Chl fluorescence yield were monitored. At various times the gas phase surrounding the leaf was then changed to reduce photosynthesis by either acceptor limitation (e.g. by removal of CO_2 or O_2) or by application of SO_2 or by high CO_2 partial pressures. In the latter case, high concentrations of CO_2 were used to produce acidification of cytoplasmic compartments of leaf cells. Wagner et al. (1990) have shown that high CO_2 concentrations inhibit photosynthesis of isolated chloroplasts and, transiently, also of mesophyll protoplasts, by decreasing cytoplasmic pH values.

At the start of the experiment the leaf was kept in air containing 600 µl · l^{-1} CO_2 (denoted by 0 at the bottom of Fig. 7). Gassing the leaf with 8 µl · l^{-1} SO_2 for a period of 5 min (Fig. 7; 1) caused a reduction of PSII quantum yield by 25%. Simultaneously NPQ and ΔA_{505} increased appreciably. Upon returning to an SO_2-free atmosphere, all three parameters recovered almost fully within the following 20 min. A reduction of the CO_2 content of the atmosphere from 600 to 200 µl · l^{-1} (Fig. 7; 2) caused a drop in PSII quantum yield of similar magnitude to that during the preceding SO_2 fumigation. This time, however, ΔA_{505} responded to a much smaller extent, whereas the response of NPQ even increased.

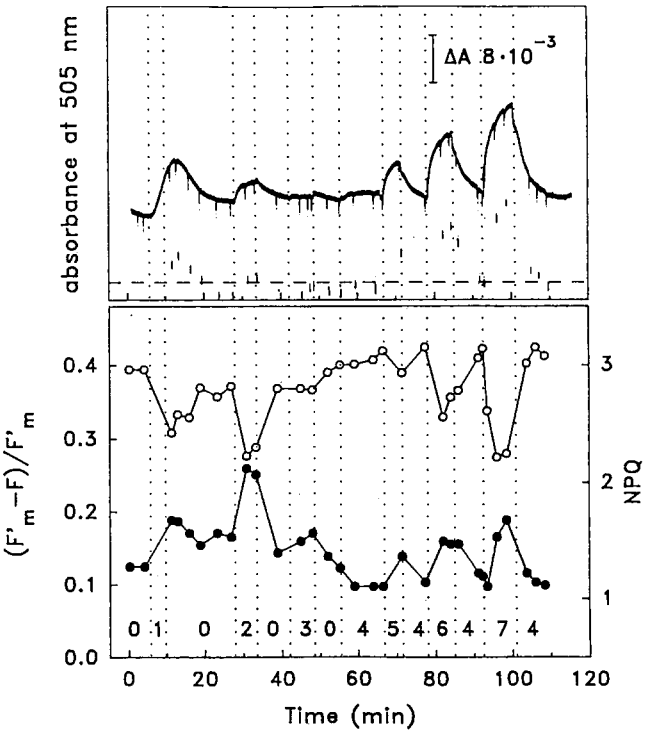

Fig. 7. Absorbance at 505 nm, quantum yield of PSII (Fm′ − F)/Fm′ (○) and NPQ (●) during inhibition of photosynthesis by either acceptor limitation (removal of CO_2) or acidification (high CO_2, SO_2) in a representative leaf of *Pelargonium*. The leaf was kept initially in 21% O_2 and 600 µl · l^{-1} CO_2 (=0). The gas phase was then changed as marked by numbers (*1–7*): (*1*) 21% O_2, 600 µl · l^{-1} CO_2 + 8 µl · l^{-1} SO_2; (*2*) 21% O_2 + 200 µl · l^{-1} CO_2; (*3*) 2.6% O_2 + 600 µl · l^{-1} CO_2; (*4*) 21% O_2 + 0.3% CO_2; (*5*) 21% O_2 + 2% CO_2; (*6*) 21% O_2 + 4% CO_2; (*7*) 21% O_2 + 6% CO_2. The irradiation was 150 W · m^{-2}

Suppression of photorespiration by either decreasing the O_2 content of the atmosphere to 2.6% (Fig. 7; 3) or by increasing CO_2 to 3 000 $\mu l \cdot l^{-1}$ (Fig. 7; 4) caused only marginal changes in all three parameters. During the intermediate period in air with 600 $\mu l \cdot l^{-1}$ CO_2, stomata reopened (as monitored by measuring the humidity of the gas stream leaving the cuvette; data not shown) which caused some increase in quantum yield and a concomitant decrease in NPQ. After subjecting the leaf to 3 000 $\mu l \cdot l^{-1}$ CO_2, concentrations of CO_2 were increased first to 2% (Fig. 7; 5), then to 4% (Fig. 7; 6), and finally to 6% (Fig. 7; 7). This mainly reduces the stroma pH owing to the consumption of OH^- according to

$$CO_2 + OH^- \rightarrow HCO_3^-$$

The intrathylakoid pH is not reduced by direct CO_2-dependent acidification, because the first pK of CO_2 is 6.37, whereas the pH of the intrathylakoid space is not far from 5 in illuminated leaves. However, the intrathylakoid pH will be expected to be lowered indirectly if electron transport manages to maintain the magnitude of the transthylakoid proton gradient.

Gassing the leaf with very high CO_2 concentrations caused a strong reduction in the quantum yield of PSII which was approximately proportional to the applied CO_2 concentration. At the same time ΔA_{505} increased strongly, as did NPQ. The change in ΔA_{505} caused by application of high CO_2 contained a component which was rapidly reversible upon returning to 3 000 $\mu l \cdot l^{-1}$ CO_2. This component presumably resulted from the electrochromic shift which can contribute to absorbance changes at 505 nm. The reduction of the quantum yield caused by 4% CO_2 was similar in extent to that caused by fumigation with 8 $\mu l \cdot l^{-1}$ SO_2 and to that caused by reduction of the CO_2 concentration to 200 $\mu l \cdot l^{-1}$. The response of ΔA_{505}, however, to the reduction in quantum yield caused by changing the CO_2 concentration from 600 to 200 $\mu l \cdot l^{-1}$ was much smaller than to the treatments with SO_2 or high CO_2 which also reduced the quantum yield.

The relationship between quantum yield and 505-nm absorbance is explored in Fig. 8. A reduction in PSII quantum yield was produced by reducing the CO_2 and-or O_2 concentration in various combinations. This caused an acceptor limitation which was accompanied by a much smaller increase in ΔA_{505} (triangles) than observed when acidifying gases, SO_2 or CO_2, were used to decrease the quantum yield (circles). In a similar experiment performed with spinach leaves, both 505-nm absorbance changes and levels of xanthophyll cycle components were measured. Figure 9 shows the relationship between the epoxidation state (EPS) of the xanthophylls (Bilger et al. 1989) and the quantum yield of electron flow in PSII. The EPS is defined by [(V+0.5 A)/(V+A+Z); A, antheraxanthin; V, violaxanthin; Z, zeaxanthin)]. The reduction in PSII quantum yield produced by reducing the CO_2 concentration (△) was accompanied by a smaller decrease of the EPS than when SO_2 was used to reduce the quantum yield of PSII (○). The inset shows that the relationship between EPS and 505-nm absorbance changes is linear.

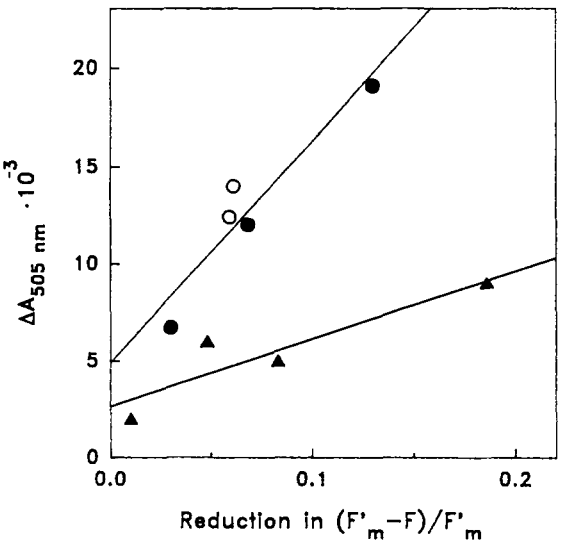

Fig. 8. Comparison between ΔA_{505} and reduction in quantum yield of PSII ($Fm' - F/Fm'$) induced by changes in the gas phase. Data are taken from an experiment similar to that shown in Fig. 5. ▲, decrease of CO_2 or O_2; ○, SO_2-dependent acidification; ●, CO_2-dependent acidification

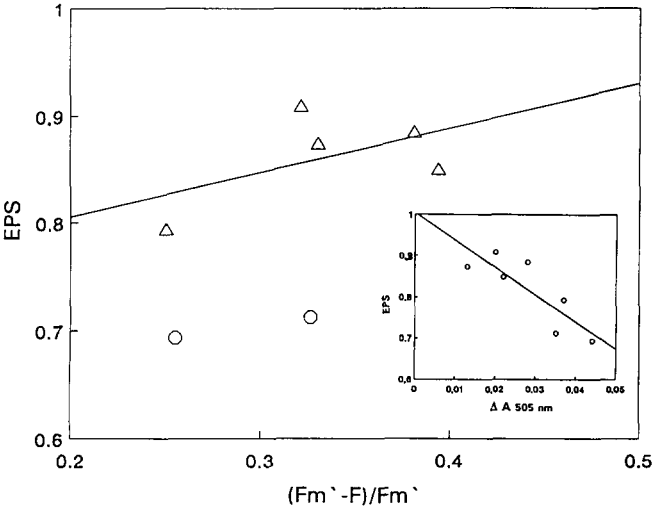

Fig. 9. The relationship between quantum yield of PSII ($Fm' - F/Fm'$) and the epoxidation state, EPS [EPS = (V+0.5A)/V+A+Z); A, antheraxanthin; V, violaxanthin; Z, zeaxanthin], in a representative spinach leaf after imposing an acceptor limitation on electron flow by decreasing the CO_2 level (△) or by inhibiting photosynthesis by SO_2 (○). The *inset* shows the relationship between EPS and 505-nm absorbance changes when darkened leaves were illuminated in the presence of different levels of CO_2 or SO_2 in these experiments. Before the experiments, the leaves were kept for 2 h in low light and then 30 min in the dark to minimize initial zeaxanthin levels

From the data of Figs. 7, 8 and 9 it is concluded that increased zeaxanthin formation under the influence of SO_2 is not only the result of the decrease in intrathylakoid pH caused by the decreased ATP consumption of SO_2-inhibited carbon assimilation but also of another, more indirect, acidifying effect of SO_2.

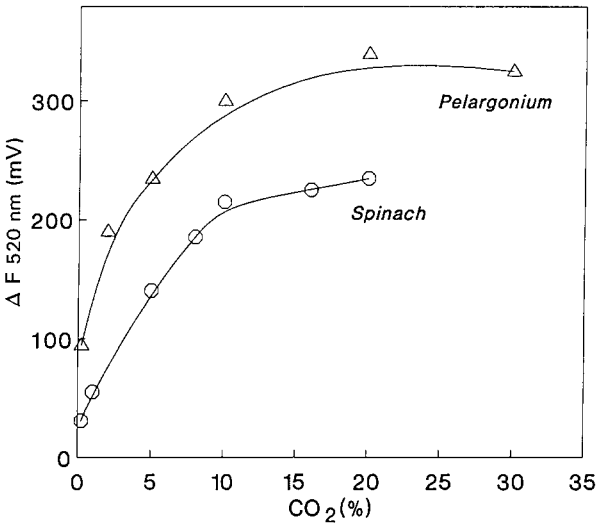

Fig. 10. Demonstration of a typical CO_2-dependent acidification in a spinach leaf and a *Pelargonium* leaf as indicated by the suppression of the fluorescence of pyranine. Leaves were fed for 2 h with pyranine (200 μM in 2 mM Hepes, pH 7.5) in the light. Acidification was caused by 2-min exposure periods to different concentrations of CO_2 in air

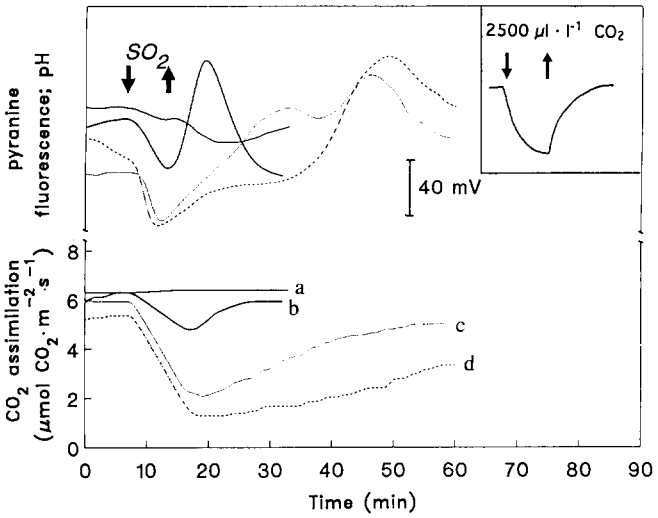

Fig. 11. Changes in the fluorescence of pyranine and CO_2 assimilation of a *Pelargonium* leaf during and after 6-min fumigation periods with SO_2. Concentrations of SO_2 were 1.3 (*a*), 2.6 (*b*), 5.14 (*c*), and 7.7 $\mu l \cdot l^{-1}$ (*d*). The irradiation was 120 $W \cdot m^{-2}$ and the CO_2 concentration 500 $\mu l \cdot l^{-1}$ in air. The *inset* shows acidification after gassing the same leaf with 2500 $\mu l \cdot l^{-1}$ CO_2. The decrease in cytosolic pH is about 0.05 pH units (Yin et al. 1990)

Sulfur-dioxide-dependent acidification in leaves and its reversal. Direct measurements of pH in the chloroplasts of intact leaves are difficult. However, it is possible to introduce pH-indicating fluorescent dyes into mesophyll cells of intact leaves by feeding them to the petiole of cut leaves (Yin et al. 1990). After feeding of pyranine which has a pK of 7.3, a light-dependent increase in fluorescence emission at about 520 nm has been shown to originate mainly from the cytosol of green leaf cells, not from the chloroplasts, the apoplast or from the large vacuoles (Yin et al. 1990).

Figure 10 shows the relationship between the concentration of CO_2 in air and the decrease in cytosolic pH values in leaves of *Pelargonium* and spinach in the dark as shown by the CO_2-dependent quenching of pyranine fluorescence. In the light a similar relationship was obtained (data not shown). The pH response is as expected on the basis of the Henderson-Hasselbalch equation, if a weak acid is added to a buffered solution.

In Fig. 11 the time courses of pyranine fluorescence and of CO_2 assimilation in response to gassing with different concentrations of SO_2 are shown. As the concentration of SO_2 in air was increased, inhibition of assimilation increased. Quenching of pyranine fluorescence followed the photosynthesis inhibition, although it was not proportional to the SO_2 concentration (Fig. 11). Characteristically, acidification did not increase immediately when SO_2 was added to the gas stream which passed over the leaf. There was a lag period of about 1 min before slow acidification started. A similar lag phase was usually seen also in the SO_2-dependent increase in ΔA_{505}. The acidification of the cytosol caused by a few $\mu l \cdot l^{-1}$ SO_2 within a few minutes of fumigation was similar in extent to the corresponding changes produced by 2 500 $\mu l \cdot l^{-1}$ CO_2 (Fig. 11). It is known that oxidative detoxification of SO_2 yields sulfuric acid which, as a strong divalent acid, does not add to cytosolic buffering, whereas CO_2, as a weak acid, increases the buffering capacity of the cytosol.

Some time after termination of fumigation, pyranine fluorescence returned to initial values. Recovery of the original pH was characterized by overshoot or even oscillatory phenomena (Fig. 11). The observations show that fumigation with SO_2 causes the formation of acid which decreases the cytosolic pH in leaves, and that leaves can compensate for acidification even during fumigation either by mobilizing base or by exporting acid to a compartment which does not contain much pyranine, or whose pH is so far from the pK of pyranine that pH changes resulting from acid import do not cause much fluorescence change (Yin et al. 1990). Recovery of pH was always somewhat faster than recovery of photosynthesis.

Role of light and darkness in the effect of SO_2 on the photosynthetic apparatus. When an illuminated leaf of *Pelargonium* photosynthesizing in the absence of SO_2 was darkened, a slow increase in 505-nm absorbance was observed even when no SO_2 was added in the dark (Fig. 12). Its origin is unknown. The typical increase in 505-nm absorbance induced by SO_2 in the light (see Fig. 1) was absent in the dark. However, it appeared when, after short fumigation with SO_2 in the dark, the leaf was illuminated in the absence of atmospheric SO_2 (Fig. 12). Inhibition of CO_2 assimilation by the leaf which had been fumigated with SO_2 during the preceding dark period indicated that SO_2 had already interacted with components of the photosynthetic apparatus in the dark. This interaction proceeded slowly and progressively. Inhibition of the photosynthetic apparatus in the dark

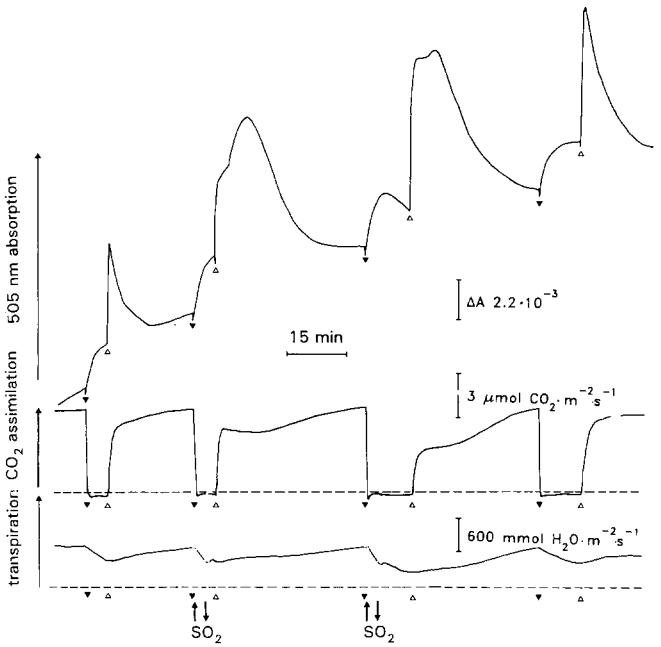

Fig. 12. Effects of fumigation with SO_2 in the dark on a representative *Pelargonium* leaf. Simultaneous recording of CO_2 exchange, transpiration and 505-nm absorbance. An illuminated leaf was first briefly darkened and then reilluminated (control). *Closed triangles* indicate light off, *open triangles* light on. During a second darkening period, the leaf was fumigated for 3 min with 7 μl · l^{-1} SO_2, and the light was turned on after a further 2 min period in the dark. After CO_2 assimilation had recovered, the leaf was again darkened and SO_2 (7 μl · l^{-1}) was added for 3 min to the air stream passing over the leaf. Eight minutes after termination of fumigation, the light was turned on. A fourth dark period without SO_2 served as an additional control. The irradiance was 120 W · m^{-2} and the CO_2 concentration in air 600 μl · l^{-1}

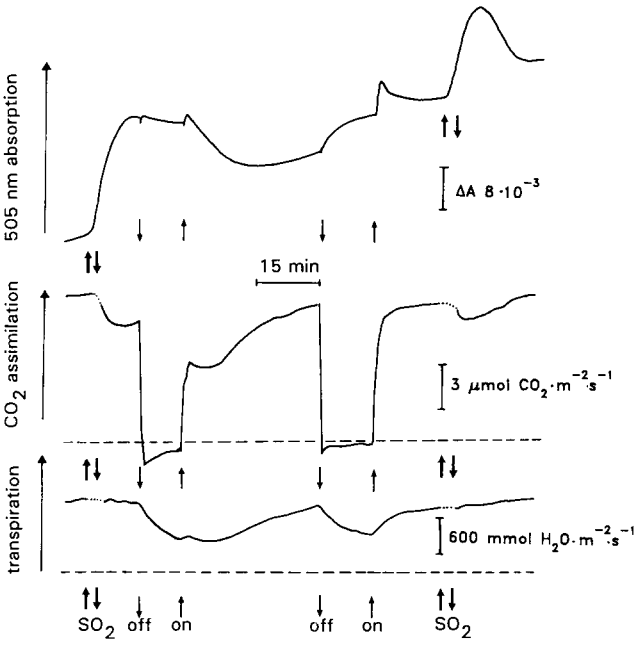

Fig. 13. Effects of illumination and an intermittent dark period (12 min) on inhibition of assimilation by SO_2 and on the 505-nm absorbance change. A leaf of *Pelargonium* was fumigated for two 3-min periods with 3.85 μl · l^{-1} SO_2. Simultaneous recording of CO_2 and H_2O exchange and of 505-nm absorbance. The irradiance was 120 W · m^{-2} and the CO_2 concentration 600 μl · l^{-1}

becomes visible as inhibition of photosynthesis when a leaf which has been exposed to SO_2 only during a dark period is subsequently illuminated. Interestingly, pyranine fluorescence failed to indicate acidification of leaves when SO_2 was added in the dark, whereas CO_2 caused the same acidification in the dark and in the light (data not shown).

When a *Pelargonium* leaf photosynthesizing in air was fumigated for 3 min with SO_2 and the light was turned off when photosynthesis inhibition and zeaxanthin formation were maximal, a 10-min dark period increased inactivation of the photosynthetic apparatus beyond that observed in the light (Fig. 13). During darkening, the zeaxanthin content remained high. It decreased as a subsequent illumination period resulted in the slow recovery of photosynthesis.

The observations show that SO_2 is particularly inhibitory in the dark, and that light has a dual role. In combination with SO_2, it may increase inhibition of assimilation as shown by the transient decrease of photosynthesis after the initial increase when the light was turned on after a dark period (Figs. 12, 13), but light also helps in reversal of inhibition and detoxification of SO_2.

The different pH responses of leaves to SO_2 in darkness and in light as monitored by pyranine fluorescence closely correspond to the observations made with 505-nm absorption which monitors zeaxanthin formation in the thylakoid membranes. In contrast to pyranine fluorescence which records cytosolic pH events, zeaxanthin formation responds to acidification of the thylakoid interior. Apparently, membrane barriers do not prevent

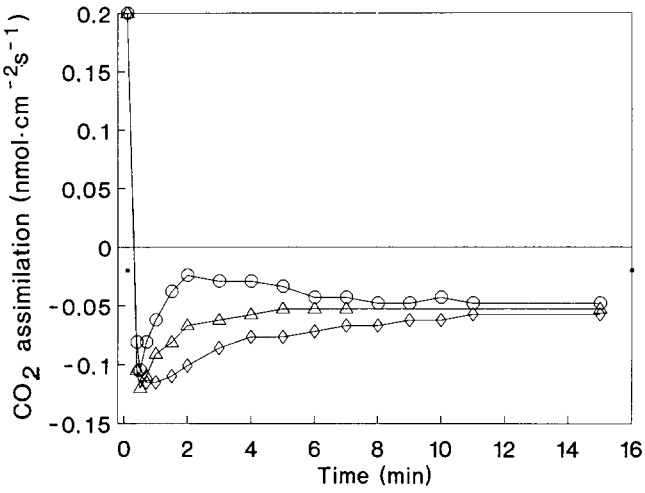

Fig. 14. Stimulation of post-illumination CO_2 production of a *Pelargonium* leaf by SO_2. A control leaf was illuminated for 30 min and then darkened (○). After another 20 min in the light, the same leaf was fumigated with either 4 (△) or 6 (◇) μl · l^{-1} SO_2 for 5 min. Illumination was continued for another 10 min

pH communication between the different cellular compartments (Laisk et al. 1989; Yin et al. 1990; Thaler et al. 1992).

Post-illumination CO_2 evolution after fumigation of an illuminated leaf in air with SO_2. Figure 14 shows post-illumination CO_2 evolution after an assimilating *Pelargonium* leaf had been fumigated for 5 min in air with SO_2 (4 and 6 $\mu l \cdot l^{-1}$) and then kept for another 5 min in the light before it was darkened. In the control leaf, a transient CO_2 burst is attributed to CO_2 evolution from the pool of photorespiratory intermediates. After illumination in the presence of SO_2, CO_2 evolution was increased, and the increase was larger after fumigation with 6 $\mu l \cdot l^{-1}$ SO_2 than after fumigation with 4 $\mu l \cdot l^{-1}$ SO_2.

Discussion

Our results show that SO_2 inhibits photosynthesis reversibly. Loss of photosynthesis in the presence of high concentrations of SO_2 is due to inhibition of Calvin-cycle enzymes such as fructose bisphosphatase and ribulose bisphosphate carboxylase, not to inhibition of thylakoid reactions (Ziegler 1973; Tanaka et al. 1984; Figs. 2, 5, 6, Table 1). Formation of zeaxanthin by the thylakoid enzyme violaxanthin de-epoxidase is even stimulated by SO_2. After inhibitory reactions have resulted in appreciable loss of photosynthesis during and after a short period of fumigation with high SO_2 concentrations, detoxification is indicated by the recovery of photosynthesis. It was fast in the light and slow or absent in the dark. Obviously, chloroplast reactions are mainly responsible for detoxification. Removal of bisulfite and sulfite by reduction and incorporation of reduced sulfur into amino acids or by oxidation to sulfuric acid, the main detoxification pathways for SO_2 (Dittrich et al. 1992), appear to facilitate dissociation of addition compounds between enzymes and SO_2 or its hydration products. This can explain the observed reactivation of the enzymes. Oxidative detoxification of SO_2 burdens the cells with sulfuric acid. Indeed, during and after fumigation with SO_2, cytoplasmic acidification was indicated in the light by a transiently decreased fluorescence of the pH-indicating dye pyranine (Fig. 10). Decreases in the cytosolic pH were accompanied by increases in the level of zeaxanthin (Figs. 1, 4, 7, 9) which are attributed to a lowering of the intrathylakoid pH (Fig. 8).

Importantly, SO_2 failed to produce an appreciable acidification response in darkened leaves, but was even more inhibitory to photosynthetic enzymes in the dark than in the light (Figs. 12, 13). In darkened leaves, SO_2 neither caused appreciable quenching of pyranine fluorescence nor zeaxanthin formation, whereas high concentrations of CO_2 both quenched pyranine fluorescence in the dark (Yin et al. 1990) and induced zeaxanthin formation (O. Björkman, Department of Plant Biology, Carnegie Institution of Washington, Stanford, USA; personal communication) and increased absorbance at 505 nm (data not shown).

Hydration of SO_2 must result in the formation of divalent sulfurous acid which liberates at least 1.5 protons per SO_2 at cytosolic pH values. The failure to observe appreciable acidification during short-term fumigation of leaves with SO_2 in the dark then either means that SO_2 can be absorbed in a form which does not act as an acid, or that cytoplasmic buffering and the action of cytoplasmic pH-stat mechanisms effectively prevented acidification in the dark, but not in the light. At any rate inactivation of the photosynthetic apparatus in the dark cannot be attributed to cellular acidification but must result from the chemical reactivity of SO_2 or of the reactive species derived from SO_2.

In isolated spinach chloroplasts, both reductive and oxidative detoxification of SO_2 proceed at comparable rates, but only when the production of oxygen radicals, which promotes sulfite oxidation in a fast chain reaction, is curtailed by the addition of radical scavengers such as superoxide dismutase and ascorbate, and when reduction is stimulated by the addition of O-acetylserine which permits formation of the reduction product cysteine (Ghisi et al. 1990; Dittrich et al. 1992). Cells of intact leaves are usually well equipped with radical-scavenging systems, but may, in the absence of rapid growth, lack sinks for sulfur-containing amino acids. During fumigation of young spruce trees with SO_2 over a period of weeks, essentially all absorbed SO_2 was recovered as sulfate (Kaiser et al. 1991).

Under normal conditions of plant exposure to a polluted atmosphere, SO_2 concentrations are unlikely to ever increase to the concentrations which were used in the present work, and which caused photosynthesis inhibition within a very short time span. However, whereas exposure times were very short in our experiments, plant exposure in the field to a polluted atmosphere may be chronic, even though maximum concentrations of SO_2 will rarely rise beyond 0.1 $\mu l \cdot l^{-1}$. Our data suggest that oxidative and, when protein demand is high, reductive detoxification will normally be able to cope with influx of SO_2 in the field so that reactive anions are very unlikely to accumulate under field conditions. However, oxidative detoxification produces sulfuric acid. Sulfate accumulates in the vacuoles. The protons of sulfuric acid must be neutralized. Sulfate concentrations in needles of spruce from the Ore Mountains at the border between Germany and Czechoslovakia may contain more than 100 mM sulfate (Kaiser et al. 1991). The equivalent protons (200 mM) cannot be stored in the cells. If, owing to cation deficiency of the soil or other reasons, effective cation/proton exchange is not possible first at the root/soil interface and then inside the organism, acidification or, in other words, cation deficiency will be a more important cause of eventual damage than reactions between reactive anions and enzymes (Slovik et al. 1992). Cation deficiency is indeed indicated by known needle symptoms such as needle yellowing in Norway spruce (Beyschlag et al. 1987), but its linkage to acidification has not yet been generally recognized.

As a main result of the present work, it has become clear that the observed inhibition of photosynthesis in leaves by high concentrations of SO_2 is not caused by cellular acidification but by the chemical reactivity of

SO_2 or the anions formed during its hydration, and that such inhibition is reversible. Efficient cellular detoxification of SO_2 requires light. It causes cellular acidification. Obviously, neutralization of the acid formed requires a base which must become available if damage is to be avoided in the long term.

This work was supported by the Deutsche Forschungsgemeinschaft within the Sonderforschungsbereich 251 of the University of Würzburg. S. V.-J. acknowledges support by the Leibniz program of the Deutsche Forschungsgemeinschaft and by the Fonds for Science of the Republic of Serbia (contract no. 8604). We are grateful to Drs. Z.-H. Yin, U. Takahama and K.-J. Dietz (Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, FRG) for cooperation and helpful discussions.

References

Anderson, L.E., Duggan, J.X. (1977) Inhibition of light modulation of chloroplast enzyme activity by sulfite. Oecologia **28**, 147–151

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

Badger, M.R., Sharkey, T.D., v. Caemmerer, S. (1984) The relationship between steady state gas exchange of bean leaves and the levels of carbon-reduction-cycle intermediates. Planta **160**, 305–313

Bradbury, M., Baker, N.R. (1981) Analysis of the slow phases of the in vivo chlorophyll fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystems I and II. Biochim. Biophys. Acta **635**, 542–551

Beyschlag, W., Wedler, M., Lange, O.L., Heber, U. (1987) Einfluß einer Magnesiumdüngung auf Photosynthese und Transpiration von Fichten an einem Magnesium-Mangelstandort im Fichtelgebirge. Allg. Forst. Z. **27/28/29**, 738–741

Bilger, W., Björkman, O. (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth. Res. **25**, 173–185

Bilger, W., Heber, U., Schreiber, U. (1988) Kinetic relationships between energy-dependent fluorescence quenching, light scattering, chlorophyll luminescence and proton pumping in intact leaves. Z. Naturforsch. **43c**, 877–887

Bilger, W., Björkman, O., Thayer, S.S. (1989) Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. Plant Physiol. **91**, 542–551

Cerovic, Z.G., Kalezic, R., Plesnicar, M. (1982) The role of photophosphorylation in SO_2 and SO_3^{2-} inhibition of photosynthesis in isolated chloroplasts. Planta **156**, 249–254

Daniell, H., Sarojini, G. (1981) On the possible site of sulfite action in the photosynthetic electron transport chain and the light modulation of enzyme activity. Photobiochem. Photobiophys. **2**, 61–68

Demmig, B., Winter, K., Krüger, A., Czygan, F.-C. (1987) Photoinhibition and zeaxanthin formation in intact leaves. A possible role of the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol. **84**, 218–224

Dietz, K.-J., Heber, U. (1984) Rate limiting factors in photosynthesis. I. Carbon fluxes in the Calvin cycle. Biochim. Biophys. Acta **767**, 432–443

Dietz, K.-J., Schreiber, U., Heber, U. (1985) The relationship between the redox state of Q_A and photosynthesis in leaves at various carbon-dioxide, oxygen and light regimes. Planta **166**, 219–226

Dittrich, A.P.M., Pfanz, H., Heber, U. (1992) Oxidation and reduction of SO_2 by chloroplasts and formation of sulfite addition compounds. Plant Physiol **98**, 738–744

Genty, B., Briantais, J.-M., Baker, N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and photochemical quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87–92

Ghisi, R., Dittrich, A.P.M., Heber, U. (1990) Oxidation versus reductive detoxification of SO_2 by chloroplasts. Plant Physiol. **92**, 846–849

Gilmore, A.M., Yamamoto, H.Y. (1991) Resolution of lutein and zeaxanthin using a non-endcapped lightly carbon-loaded C_{18} high-performance liquid chromatographic column. J. Chromatogr. **543**, 137–145

Hager, A. (1980) The reversible, light induced conversions of xanthophylls in the chloroplasts. In: Pigments in plants, pp. 57–79, Czygan, F.-C., ed. Fischer, Stuttgart

Heber, U. (1969) Conformational changes of chloroplasts induced by illumination of leaves in vivo. Biochim. Biophys. Acta **180**, 302–319

Heber, U., Willenbrink, J. (1964) Sites of synthesis and transport of photosynthetic products within the leaf cell. Biochim. Biophys. Acta **82**, 313–324

Kaiser, W.M., Dittrich, A.P.M., Heber, U. (1991) Sulfatakkumulation in Fichtennadeln als Folge von SO_2-Belastung. In: Proceedings. 2. Statuseminar der PBWU zum Forschungsschwerpunkt "Waldschäden". GSF Bericht 26/91, pp. 425–438, GSF Forschungszentrum für Umwelt und Gesundheit GmbH., Neuherberg

Klughammer, C., Schreiber, U. (1991) Analysis of light-induced absorbance changes in the near-infrared spectral region. I. Characterization of various components in isolated chloroplasts. Z. Naturforsch. **46c**, 233–244

Kobayashi, V., Köster, S., Heber, U. (1982) Light scattering, chlorophyll fluorescence and state of adenylate system in illuminated spinach leaves. Biochim. Biophys. Acta **682**, 44–52

Köster, S., Heber, U. (1982) Light scattering and quenching of 9-aminoacridine fluorescence as indicators of the phosphorylation state of the adenylate system in intact chloroplasts. Biochim. Biophys. Acta **680**, 88–94

Krause, G.H. (1973) The high-energy state of the thylakoid system as indicated by chlorophyll fluorescence and chloroplast shrinkage. Biochim. Biophys. Acta **292**, 715–728

Krause, G.H., Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant. Mol. Biol. **42**, 313–349

Laisk, A., Oja, V., Kiirats, O., Raschke, K., Heber, U. (1989) The state of the photosynthetic apparatus in leaves as analyzed by rapid gas exchange and optical methods: the pH of the chloroplast stroma, and activation of enzymes in vivo. Planta **177**, 350–358

Nobel, P.S. (1983) Biophysical plant physiology and ecology. W.H. Freeman, New York

Schreiber, U., Schliwa, U., Bilger, W. (1986) Continuous recording of photochemical and non-photochemical fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. **10**, 51–62

Schreiber, U., Klughammer, C., Neubauer, C. (1988) Measuring P_{700} absorbance changes around 830 nm with a new type of pulse modulation system. Z. Naturforsch. **43c**, 686–698

Siebke, K., Laisk, A., Oja, V., Kiirats, O., Raschke, K., Heber, U. (1990) Control of photosynthesis in leaves as revealed by rapid gas exchange and measurements of the assimilatory force F_A. Planta **182**, 513–522

Shimazaki, K., Nakamachi, K., Kondo, N., Sugahara, K. (1984) Sulfite inhibition of photosystem II in illuminated spinach leaves. Plant Cell Physiol. **25**, 337–341

Slovik, S., Kaiser, W.M., Körner, Ch., Kindermann, G., Heber, U. (1992) Quantifizierung der physiologischen Kausalkette von SO_2-Immissionsschäden für Rotfichten (*Picea abies* (L.) Karst.). II. Ableitung von SO_2-Immissionsgrenzwerten für chronische Schäden. Allg. Forst Z. **17**, 913–920

Tanaka, K., Otsubo, T., Kondo, N. (1983) Participation of hydrogen peroxide in the inactivation of Calvin cycle enzymes in SO_2-fumigated leaves. Plant Cell Physiol. **23**, 1009–1018

Tanaka, K., Mitsuhashi, H., Kondo, N., Sugahara, K. (1984) Further evidence for inactivation of fructose-1,6-bisphosphatase at the begining of SO_2 fumigation. Increase in fructose-1,6-bisphosphate and decrease in fructose-6-phosphate in SO_2-fumigated spinach leaves. Res. Report Natl. Inst. Environ. Stud. Jpn. **65**, 213–216

Taylor, G.E. jr, Tingey, D.T. (1983) Sulfur dioxide flux into leaves of *Geranium carolinianum* L. Plant Physiol. **72**, 237–244

Thaler, M., Simonis, W., Schönknecht, G. (1992) Light-dependent changes of the cytoplasmic H^+ and Cl^- activity in the green alga *Eremosphaera viridis*. Plant Physiol. **99**, 103–110

Vernon, L.P. (1960) Spectrophotometric determination of chlorophylls and phaeophytins in plant extracts. Anal. Chem. **32**, 1144–1150

van Kooten, O., Snel, J.F.H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. **25**, 147–150

Wagner, U., Kolbowski, J, Oja, V., Laisk, A., Heber, U. (1990) pH homeostasis of the chloroplast stroma can protect photosynthesis of leaves during the influx of potentially acidic gases. Biochim. Biophys. Acta **1016**, 115–120

Winner, W.E., Mooney, H.A., Goldstein, R.A. (1985) Sulfur dioxide and Vegetation, Stanford University Press, Stanford

Ziegler, I. (1973) The effect of SO_3 on the activity of ribulose-1,5-diphosphate carboxylase in isolated spinach chloroplasts. Planta **103**, 155–163

Yin, Z.H., Neimanis, S., Wagner, U., Heber, U. (1990) Light-dependent pH changes in leaves of C_3 plants. I. Recording pH changes in different cellular compartments by fluorescent probes. Planta **182**, 244–252