Planta (1988) 173:241–252

Planta
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Sulfur-dioxide fluxes into different cellular compartments of leaves photosynthesizing in a polluted atmosphere

II. Consequences of SO_2 uptake as revealed by computer analysis

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Abstract. A computer model is used to analyze fluxes of SO_2 from polluted air into leaves and the intracellular distribution of sulfur species derived from SO_2. The analysis considers only effects of acidification and of anion accumulation. (i) The SO_2 flux into leaves is practically exclusively controlled by the boundary-layer resistance of leaves to gas diffusion and by stomatal opening. At constant stomatal opening, flux is proportional to the concentration of SO_2 in air. (ii) The sink capacity of cellular compartments for SO_2 depends on intracellular pH and the intracellular localization of reactions capable of oxidizing or reducing SO_2. In the mesophyll of illuminated leaves, the chloroplasts possess the highest trapping potential for SO_2. (iii) If intracellular ion transport were insignificant, and if bisulfite and sulfite could not be oxidized or reduced, leaves with opened stomata would rapidly be killed both by the accumulation of sulfites and by acidification of chloroplasts and cytosol even if SO_2 levels in air did not exceed concentrations thought to be permissible. Acidification and sulfite accumulation would remain confined largely to the chloroplasts and to the cytosol under these conditions. (iv) Transport of bisulfite and protons produced by hydration of SO_2 into the vacuole cannot solve the problem of cytoplasmic accumulation of bisulfite and sulfite and of cytoplasmic acidification, because SO_2 generated in the acidic vacuole from the bisulfite anion would diffuse back into the cytoplasm. (v) Oxidation to sulfate which is known to occur mainly in the chloroplasts can solve the problem of cytoplasmic sulfite and bisulfite accumulation, but aggravates the problem of chloroplastic and cytosolic acidification. (vi) A temporary solution to the problem of acidification requires the transfer of H^+ and sulfate into the vacuole. This transport needs to be energized. The storage capacity of the vacuole for protons and sulfate defines the extent to which SO_2 can be detoxified by oxidation and removal of the resulting protons and sulfate anions from the cytoplasm. Calculations show that even at atmospheric levels of SO_2 thought to be tolerable, known vacuolar buffer capacities are insufficient to cope with proton production during oxidation of SO_2 to sulfate within a vegetation period. (vii) A permanent solution to the problem of acidification is the removal of protons. Protons are consumed during the reduction of sulfate to sulfide. Proteins and peptides contain sulfur at the level of sulfide. During photosynthesis in the presence of the permissible concentration of $0.05\ \mu l \cdot l^{-1}$ SO_2, sulfur may be deposited in plants at a ratio not far from 1/500 in relation to carbon. The content of reduced sulfur to carbon is similar to that ratio only in fast-growing, protein-rich plants. Such plants may experience little difficulty in detoxifying SO_2. In contrast, many trees may contain reduced sulfur at a ratio as low as 1/10000 in relation to carbon. Excess sulfur deposited in such trees during photosynthesis in polluted air gives rise to sulfate and protons. If detoxification of SO_2 by reduction is inadequate, and if the storage capacity of the vacuoles for protons and sulfate is exhausted, damage is unavoidable. Calculations indicate that trees with a low ratio of reduced S to C cannot tolerate long-term exposure to concentrations of SO_2 as low as 0.02 or $0.03\ \mu l \cdot l^{-1}$ which so far have been considered to be non-toxic to sensitive plant species.

Key words: Compartmentation – Computer model – Pollution – Proton concentration – Sulfur dioxide – Sulfur metabolism.

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Introduction

In the preceding communication (Laisk et al. 1987), we have described a computer model which permits the analysis of SO_2 fluxes from a polluted atmosphere into the cells of leaves. Depending on the conductances for SO_2 of the boundary layer, the stomata and cellular biomembranes, and on the conductances of biomembranes for sulfur species derived from SO_2, it can describe the intracellular distribution and the time course of the accumulation of sulfur species in different compartments. It can account for oxidation of sulfite to sulfate and for the reduction of the latter to the level of sulfide. Moreover, it is able to predict the time course of intracellular pH changes. Naturally, the model is only as good as its basic assumptions are. Furthermore, to develop the model, simplifications had to be introduced which will limit the predictive value of a computer analysis of SO_2 effects. Still, we are convinced that an experimental analysis of the effects of pollutants on cells must be complemented by computer analysis. A main problem of the experimental treatment of damage by pollutants is the time scale of experiments. In order to observe effects of SO_2 on cells within a reasonable time span and reasonable accuracy, SO_2 concentrations must be used which are well above the concentrations common in polluted air. There is good reason to assume that results obtained under such conditions have little to do with the effects low concentrations will produce when present during prolonged periods of time. Mechanisms of damage may be quite different at different concentrations (Pfanz et al. 1987b). Prolonged observation of the effects of low SO_2 concentrations such as tolerated in moderately polluted air requires considerable financial and analytical investment. Results may be influenced by factors unrelated to SO_2 effects. They may require statistical analysis which is impossible in view of the expenditure involved. To overcome this difficulty, we deduce the effects low concentrations of SO_2 must exert on cells on the basis of the physical and chemical properties of SO_2 and the biophysical and biochemical properties of target cells. Such a deduction, it is hoped, will also reveal the strategies of cells to counter damage or to cope with damage.

Table 1. Maximum permissible concentrations of SO_2 in air which are considered to be non-toxic. Values are taken from the Technische Anleitung Luft (TA Luft) and the guidelines of the Verein Deutscher Ingenieure (VDI), VDI-Handbuch Reinhaltung der Luft, Beuth, Berlin. The VDI has taken steps to define values so that steps can be taken to limit pollution by law. The values of TA Luft have been issued on the basis of the Immissionsschutzgesetz of the Federal Republic of Germany

A: Exposure of humans

	TA-Luft (February 23, 1983)		VDI-guideline 2310/1974	
	IW 1[a]	IW 2[a]	1/2 h MIK[a]	24 h MIK[a]
SO_2 ($mg \cdot m^{-3}$)	0.14	0.4	1.0	0.3
SO_2 ($\mu l \cdot l^{-1}$ approx.)	0.05	0.15	0.37	0.11

B: Exposure of plants

	VDI-guideline 2310/1978 MIK for a vegetation period of 7 months[b]		
	Very sensitive plants	Sensitive plants	Less sensitive plants
SO_2 ($mg \cdot m^{-3}$)	0.05	0.08	0.12
SO_2 ($\mu l \cdot l^{-1}$ approx.)	0.02	0.03	0.045

[a] IW 1 of TA-Luft = average of individual measurements taken during the period of one year; IW 2 of TA-Luft = SO_2 concentration which is surpassed by only 5% of all individual measurements; 1/2 h MIK = average of individual measurements persisting during the period of 30 min; 24 h MIK = average of individual measurements persisting during a 24-h period
[b] MIK = maximum emission concentrations; the average values listed are assumed to guarantee sufficient protection of plants against SO_2 damage even when the toxicity of SO_2 is increased by the simultaneous presence of other pollutants

Results and discussion

In our analysis, we start with a very simple leaf model which will be made more complex as we proceed. Initially, it is supposed to be unable to oxidize sulfite to sulfate or reduce sulfite to the level of sulfide. Such a reduction would consume protons. However, even the most primitive version of the model is capable of hydrating SO_2 so that the species HSO_3^- and SO_3^{2-} are formed together with protons. Parameters of the model are listed in the appendix to the communication of Laisk et al. (1987). Concentrations of SO_2 we use in simulating effects on leaves, which are very difficult to demonstrate experimentally, need to be compared with concentrations thought to be permissible during short or prolonged periods of exposure. Table 1 informs on such concentrations whose limits are defined on the basis of a law of the Federal Republic of Germany (Bundesimmissionsschutzgesetz). The values of Table 1 A refer to human tolerance, those of Table 1 B to plant tolerance. It should be noted that depending on the season of

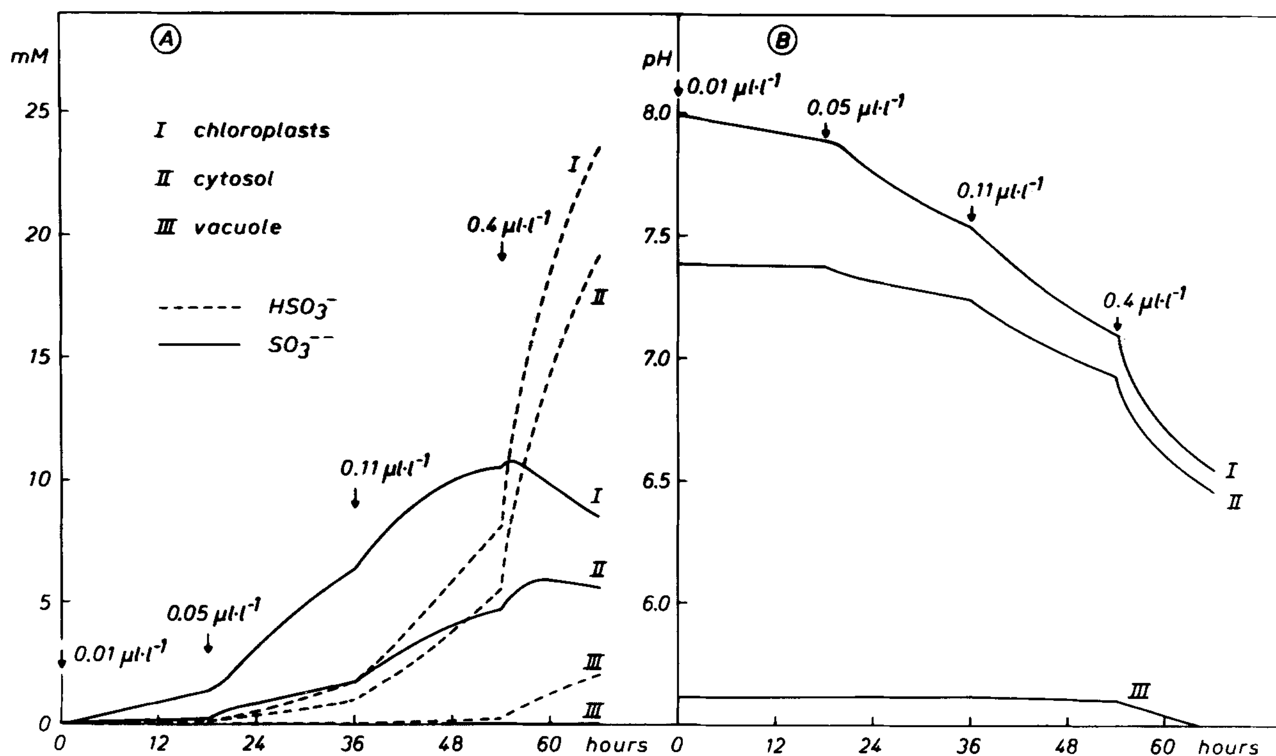
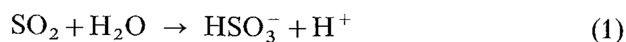


Fig. 1 A, B. Accumulation of bisulfite and sulfite (A) and acidification of chloroplasts, cytosol and vacuole of mesophyll cells (B) during influx of SO_2 into a model leaf. SO_2 concentrations in air were 0.01, 0.05, 0.11 and 0.4 $\mu l \cdot l^{-1}$ (*arrows*). The assumption was that SO_2 is the only molecular species capable of penetrating the plasmalemma, the chloroplast envelope and the tonoplast. The conductance of these biomembranes for SO_2 was assumed to be 0.0125 $cm \cdot s^{-1}$. The boundary layer and stomatal conductance for H_2O was 0.1 $cm \cdot s^{-1}$. Initial influx rates immediately after the transfer from a lower to a higher SO_2 concentration were 2.1 (0.01 $\mu l \cdot l^{-1}$), 10.6 (0.05 $\mu l \cdot l^{-1}$), 22.9 (0.11 $\mu l \cdot l^{-1}$) and 75.5 (0.4 $\mu l \cdot l^{-1}$)$\cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$

the year and on weather conditions, SO_2 concentrations may be far higher in some areas than the maximum concentrations listed in Table 1. The guide lines of the Verein Deutscher Ingenieure (VDI) of August 1978 contain information on sensitive plants. Walnut (*Juglans regia*) and spruce (*Picea sp.*) are classified as very sensitive, beech (*Fagus silvatica*) and pine (*Pinus silvestris*) as sensitive and oak (*Quercus sp.*) and juniper (*Juniperus sp.*) as less sensitive.

Relationship between SO_2 concentration in air, SO_2 fluxes into a model leaf and cellular acidification under the assumption that SO_2 is the only penetrating species, and that it is neither oxidized nor reduced. Figure 1 A shows the accumulation of HSO_3^- and SO_3^{2-} in the chloroplasts, the cytosol and the vacuole of a model leaf. The sum of the boundary-layer and stomatal conductances of the leaf for water is assumed to be $MH_2O = 0.1$ $cm \cdot s^{-1}$. In many plant species, stomata are not fully open at this conductance. In consequence, calculated SO_2 fluxes are below maximum fluxes. Biomembrane conductances for SO_2 (plasmalemma, tonoplast and chloroplast envelope) are set to 0.0125 $cm \cdot s^{-1}$. This is close to experimentally observed values (Pfanz et al. 1987a). To simplify matters, conductances for SO_3^{2-} and HSO_3^- are assumed to be zero. We realize that this is an unphysiological condition. At t_0, SO_2 is added to the air at a concentration of 0.01 $\mu l \cdot l^{-1}$. Eighteen hours later, the concentration is increased to 0.05 $\mu l \cdot l^{-1}$ and later to 0.11 and 0.4 $\mu l \cdot l^{-1}$ (see maximum permissible concentrations listed in Table 1). Initial SO_2 fluxes into the leaf calculated in the model experiment of Fig. 1 are $2.1 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$ at 0.01 $\mu l \cdot l^{-1}$, $10.6 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$ at 0.05 $\mu l \cdot l^{-1}$, $22.9 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$ at 0.11 $\mu l \cdot l^{-1}$ and $75.5 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$ at 0.4 $\mu l \cdot l^{-1}$ SO_2. As long as internal SO_2 concentrations in the leaf had not substantially increased as a consequence of influx of SO_2 (cf. Fig. 2 in the preceding publication), fluxes were proportional to the external SO_2 concentration. After SO_2 permeates cellular biomembranes, it reacts with water inside the cells according to



$$SO_2 + H_2O \rightarrow HSO_3^- + H^+ \quad (1)$$

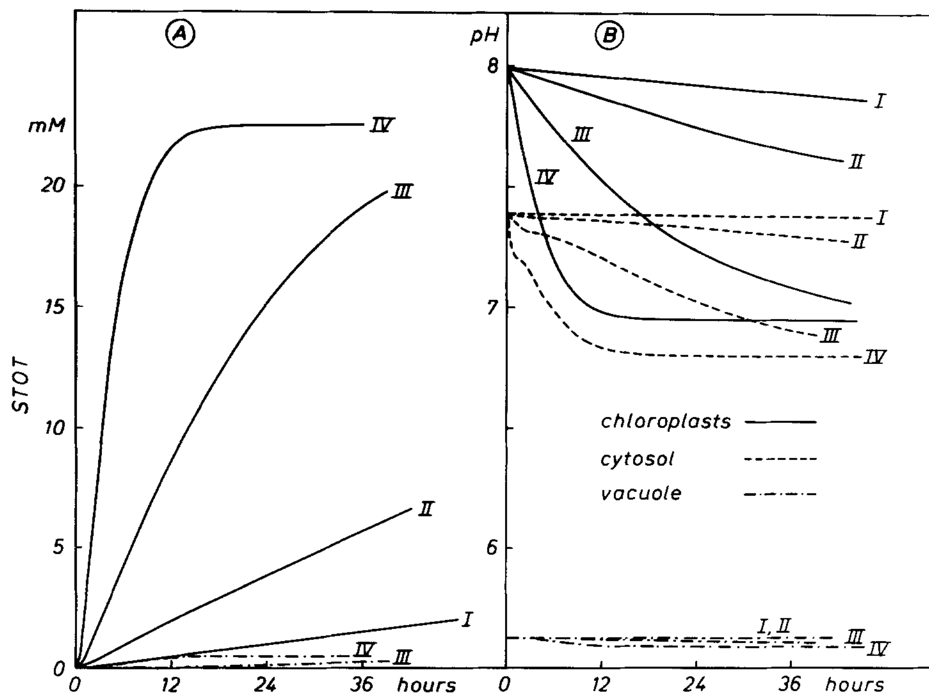


Fig. 2A, B. Accumulation of bisulfite plus sulfite (=STOT, in mM) in the chloroplasts and the vacuole (**A**) and acidification of chloroplasts, cytosol and the vacuole (**B**) during the influx of SO_2 (0.05 $\mu l \cdot l^{-1}$ in air) into a model leaf. Biomembrane conductances for SO_2 were 0.0125 $cm \cdot s^{-1}$. Boundary-layer and stomatal conductances for H_2O were 0.01 (I), 0.04 (II), 0.2 (III) and 1 $cm \cdot s^{-1}$ (IV). Initial influx rates of SO_2 into the leaf were $11 \cdot 10^{-15}$ (0.01 $cm \cdot s^{-1}$), $43 \cdot 10^{-15}$ (0.04 $cm \cdot s^{-1}$), $210 \cdot 10^{-15}$ (0.2 $cm \cdot s^{-1}$) and $932 \cdot 10^{-15}$ (1 $cm \cdot s^{-1}$) $mol \cdot cm^{-2} \cdot s^{-1}$

and

$$HSO_3^- \rightarrow SO_3^{2-} + H^+ \quad (2)$$

The distribution between HSO_3^- and SO_3^{2-} depends on pH. In the complete absence of SO_2, the pH of the stroma was assumed to be 8, in the cytosol 7.4 and in the vacuole 5.6 (Oja et al. 1986; Raven and Smith 1981). Since the alkaline stroma has the highest trapping potential for SO_2, chloroplasts show the largest accumulation of the sulfur anions. Very little sulfur was trapped in the acidic vacuole. The cytosol occupied an intermediate position. Figure 1B shows the pH of the stroma, the cytosol and the vacuole as a function of time during the influx of SO_2. Data for buffer capacities which are needed for the calculation of pH were taken from Pfanz and Heber (1986). As should be expected from the accumulation of sulfur in the chloroplast stroma, this compartment suffers the largest decrease in pH. During the time span of the experiment, the vacuolar pH responded only to the highest SO_2 concentration (Fig. 1B).

In order to judge effects of a decrease in cellular pH on metabolism and, indeed, on cellular survival, it is necessary to know that many enzymes are catalytically active within a narrow span of pH values. For instance, photosynthesis depends on the activity level of enzymes such as fructose bisphosphatase and sedoheptulose bisphosphatase. These enzymes are light-regulated (Buchanan 1980). Both light-activation and activity of the activated enzymes are strictly pH-dependent (Leegood et al. 1982). Below pH 7, even the activated enzymes are incapable of catalyzing substrate hydrolysis (Garnier and Latzko 1972). Extrapolation of the data of Fig. 1B shows that SO_2 at a concentration of 0.05 $\mu l \cdot l^{-1}$ which is thought to be tolerated by less-sensitive plants for a period as long as a vegetation period of 7 months (Table 1B) would decrease the chloroplast pH below 7 within less than 100 h of exposure. In the simulation experiment of Fig. 1, the decrease in chloroplast and cytosolic pH is practically proportional to the external SO_2 concentration as long as influx of SO_2 has not increased the internal SO_2 concentration.

Relationship between boundary-layer and stomatal conductances of a model leaf, rate of SO_2 entry, internal accumulation of sulfur species and cellular acidification under the assumption that SO_2 is the only penetrating species, and that it is neither oxidized nor reduced. Figure 2 explores the relationship between the accumulation of sulfur species in the chloroplasts and the vacuole, the pH of the

cellular compartments chloroplast, cytosol and vacuole and stomatal opening, when the SO_2 concentration in air is increased from zero to 0.05 $\mu l \cdot l^{-1}$ SO_2. Initial rates of sulfur accumulation in the chloroplasts and the vacuole (and in the cytosol, not shown) and of acidification are practically proportional to stomatal opening. This is a consequence of the high solubility of SO_2 in water (Hocking and Hocking 1977) which results in the rapid absorption of SO_2 by the tissue once it has entered the intercellular space (Weigl and Ziegler 1962). When the stomata are open ($MH_2O = 1$ cm $\cdot$ s^{-1}), initial sulfur influx is high ($93.2 \cdot 10^{-14}$ mol $\cdot$ $cm^{-2} \cdot s^{-1}$) and leads to rapid acidification and sulfur accumulation. Equilibration between external and internal SO_2 is reasonably fast. This explains observed saturation kinetics. Both acidification and sulfur accumulation would be highly injurious to a leaf if they were allowed to proceed in vivo as simulated in the model. Obviously, both must be prevented if the cells are to survive. Figure 2 shows that the easiest way to prevent damage is to close the stomata. When the stomatal conductance is as low as 0.01 $cm \cdot s^{-1}$, the rate of SO_2 influx through the stomata is $1.1 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$. It should be noted that we neglect here the uptake of SO_2 through the cuticle of the epidermis, a process which is very slow but not completely negligible especially when the stomata are largely closed (Olszyk and Tingey 1985). Closure of stomata is the rule for many plants at night. During the winter, the stomata of conifer needles may remain closed also during the daytime.

Effect of biomembrane conductance for SO_2 on rates of SO_2 entry into a model leaf. We have measured the conductance of the plasmalemma, the tonoplast and the chloroplast envelope of barley mesophyll cells for SO_2. Conductances of the different biomembranes were comparable and close to 0.0125 $cm \cdot s^{-1}$ (Pfanz et al. 1987a). Table 2 shows that if the SO_2 permeability of the biomembranes of a model leaf for SO_2 is increased by a factor of 10 beyond this value, influx of SO_2 remains essentially unchanged, indicating that biomembrane resistance to SO_2 flux is so small compared to stomatal resistance (see also Fig. 2) that it can be neglected. However, when conductance is decreased by a factor of 100 ($MSO_2 = 1.25 \cdot 10^{-4}$ $cm \cdot s^{-1}$ in Table 2), influx of SO_2 is significantly decreased. When the biomembrane conductance for SO_2 is very low, variation of stomatal resistance values shows that there is also a considerable contribution to flux control by the biomembranes (data not shown). However, this is not a physiological condition.

Table 2. Effect of biomembrane conductance for SO_2 on fluxes of SO_2 from air into a model leaf. The boundary layer and stomatal conductance for water is 0.2 $cm \cdot s^{-1}$ and the SO_2 concentration in air 0.05 $\mu l \cdot l^{-1}$. Plasmalemma, chloroplast envelope and tonoplast have identical conductances for SO_2

Biomembrane conductance for SO_2 ($cm \cdot s^{-1}$)			
$1.25 \cdot 10^{-4}$	$1.25 \cdot 10^{-3}$	$1.25 \cdot 10^{-2}$	$1.25 \cdot 10^{-1}$
SO_2 flux, 10^{-14} $mol \cdot cm^{-2} \cdot s^{-1}$			
7.1	17.4	20.3	20.6

Influence of the permeability of the chloroplast envelope and of the tonoplast to HSO_3^- and SO_3^{2-} on SO_2 fluxes. Figure 2 shows that very little HSO_3^- and SO_3^{2-} accumulates in the vacuole when internal SO_2 is permitted to equilibrate with external SO_2. This is a consequence of the low vacuolar pH. In the absence of ion fluxes across the chloroplast envelope and tonoplast, large ion gradients exist between chloroplasts, cytosol and vacuole (Fig. 1B). In reality, however, ion fluxes are not absent. Both the chloroplast envelope and the tonoplast contain carrier systems capable of catalyzing ion transport (Hampp and Ziegler 1977; Ziegler and Hampp 1977; Hampp et al. 1980; Heber and Heldt 1981; Martinoia et al. 1986). Figure 3 compares SO_2 influx into a model leaf (A), sulfur accumulation (C) and acidification (D) in the absence and in the presence of a high permeability of the chloroplast envelope and the tonoplast for SO_3^{2-} and HSO_3^-. It is assumed that transport of these species is accompanied by stoichiometric proton transport. The increased SO_2 influx shown in Fig. 3A shows that the introduction of ion transfer increases the sink capacity of leaf cells for external SO_2. Ion flux in response to concentration gradients decreases the concentration of sulfur species in the chloroplasts and increases concentrations in the vacuole (Fig. 3C). As the latter is acidic, protonation of HSO_3^- and SO_3^{2-} will liberate SO_2 in the vacuole (Fig. 3B). This introduces a concentration gradient between vacuole and cytosol and leads to SO_2 diffusion into the cytosol, thereby establishing a cyclic flux system. The ion fluxes towards the vacuole decrease acidification of the chloroplast stroma and the cytosol. Increased acidification is observed in the vacuole (Fig. 3D).

Effects of the oxidation of sulfite to sulfate. In the preceding sections it was assumed that SO_2 is neither oxidized nor reduced. In reality, oxidation oc-

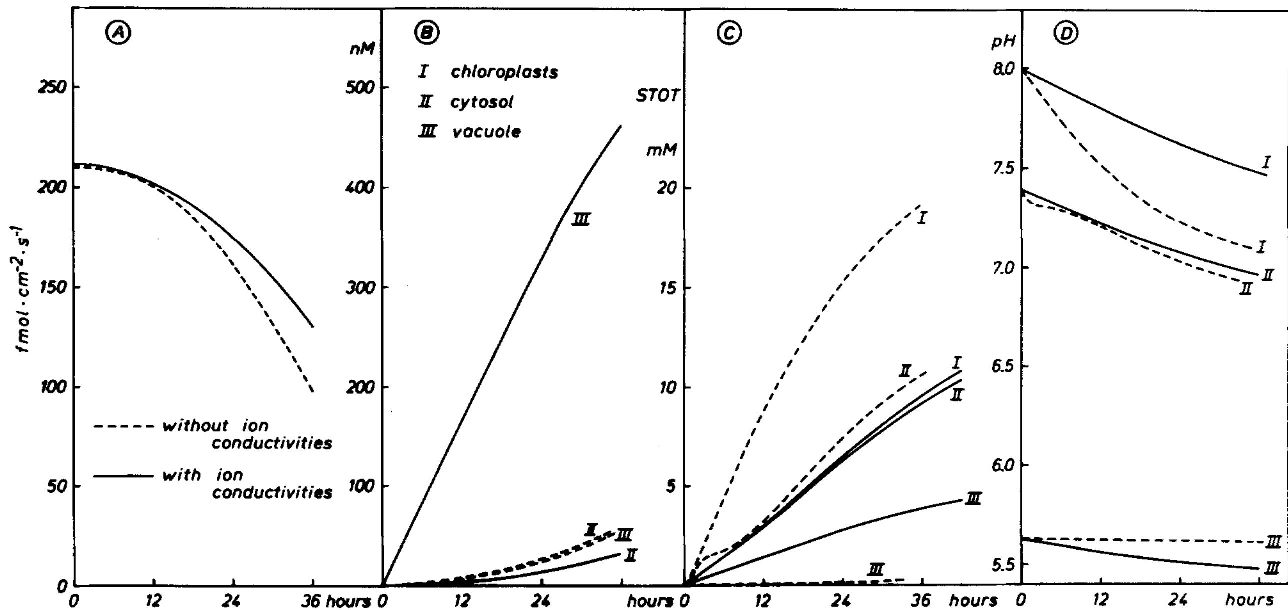


Fig. 3A–D. Effect of anion transport across the chloroplast envelope and the tonoplast on the rates of SO_2 influx (**A**) and SO_2 concentrations in cytosol and vacuole (**B**), accumulation of bisulfite and sulfite (HSO_3^- plus SO_3^{2-} = STOT; **C**) and acidification of the chloroplast stroma, the cytosol and the vacuole (**D**). The SO_2 concentration in air is 0.05 μl·l^{-1}. The boundary layer and stomatal conductance for H_2O is 0.2 cm·s^{-1}. In the control (-----), SO_2 is the only species capable of traversing biomembranes (MSO_2 = 0.0125 cm·s^{-1}). Introduction of high conductances of the chloroplast envelope and the tonoplast for HSO_3^- and SO_3^{2-} (——) results in transfer of anion and equivalent amounts of H^+ into the vacuole ($MHSO_3^-$, MSO_3^{2-} = $8 \cdot 10^{-7}$ cm·s^{-1}). This decreases ion accumulation and acidification in chloroplasts and cytosol and increases the sink capacity of the mesophyll for SO_2, but liberates SO_2 in the vacuole which diffuses back into the cytosol establishing a cyclic sulfur-transfer system

curs and reduction is also possible so that the intracellular concentrations of sulfite and bisulfite are kept low (Asada and Kiso 1973; Brimblecombe and Spedding 1974; Beilke and Gravenhorst 1978; Hällgren and Fredriksson 1982; Rennenberg and Filner 1982; Sekiya et al. 1982; Rennenberg 1984). In Fig. 4 we compare SO_2 fluxes into a model leaf, sulfur accumulation in chloroplasts and chloroplast acidification under two different flux conditions. (a) SO_2 diffuses readily across all biomembranes. HSO_3^- and SO_3^{2-} can penetrate the chloroplast envelope and the tonoplast and oxidation does not take place. (b) is similar to (a), but oxidation occurs in chloroplasts and, at a reduced rate, also in the cytosol. Sulfate (SO_4^{2-}) is not transported across biomembranes in these simulation experiments. Fig. 4B shows that oxidation effectively decreases the chloroplast concentration of sulfite and bisulfite. An increased sink function of the compartments where oxidation takes place is reflected by increased influx of SO_2 (Fig. 4A). Sulfate accumulates in the chloroplasts (Fig. 4B) and in the cytosol (not shown). Because oxidation of the weak acid sulfurous acid produces the strong acid sulfuric acid which, under the assumptions of the simulation experiment, is retained in the chloroplasts and the cytosol, acidification is dramatically increased in these compartments and decreased in the vacuole (Fig. 4C). Removal of the toxic species SO_3^{2-} and HSO_3^- thus results in increased production of H^+ in the cytoplasm. In terms of cellular survival, oxidation replaces one evil (accumulation of reactive sulfite) by another one (acidification of metabolic compartments).

Transport of sulfate and H^+ across the chloroplast envelope and the tonoplast. The only cellular compartment which should be expected to tolerate acidification is the vacuole, which in mesophyll cells may occupy as much as 80% or even more of the cellular volume. Its capacity for proton storage exceeds that of the cytoplasm by a large factor (Pfanz and Heber 1985, 1986). Obviously, damage by proton accumulation in the cytoplasm can be prevented or at least postponed, if protons are transported from chloroplasts and cytosol into the vacuole. The transport of protons across biomembranes is possible only on the basis of cation exchange or anion cotransport. In the model experiment of Fig. 5, it is assumed that protons transferred into the vacuole are accompanied by sulfate anions. To facilitate transport from the chloroplasts into the vacuole, the chloroplast envelope must be permeable to sulfate. It is believed that

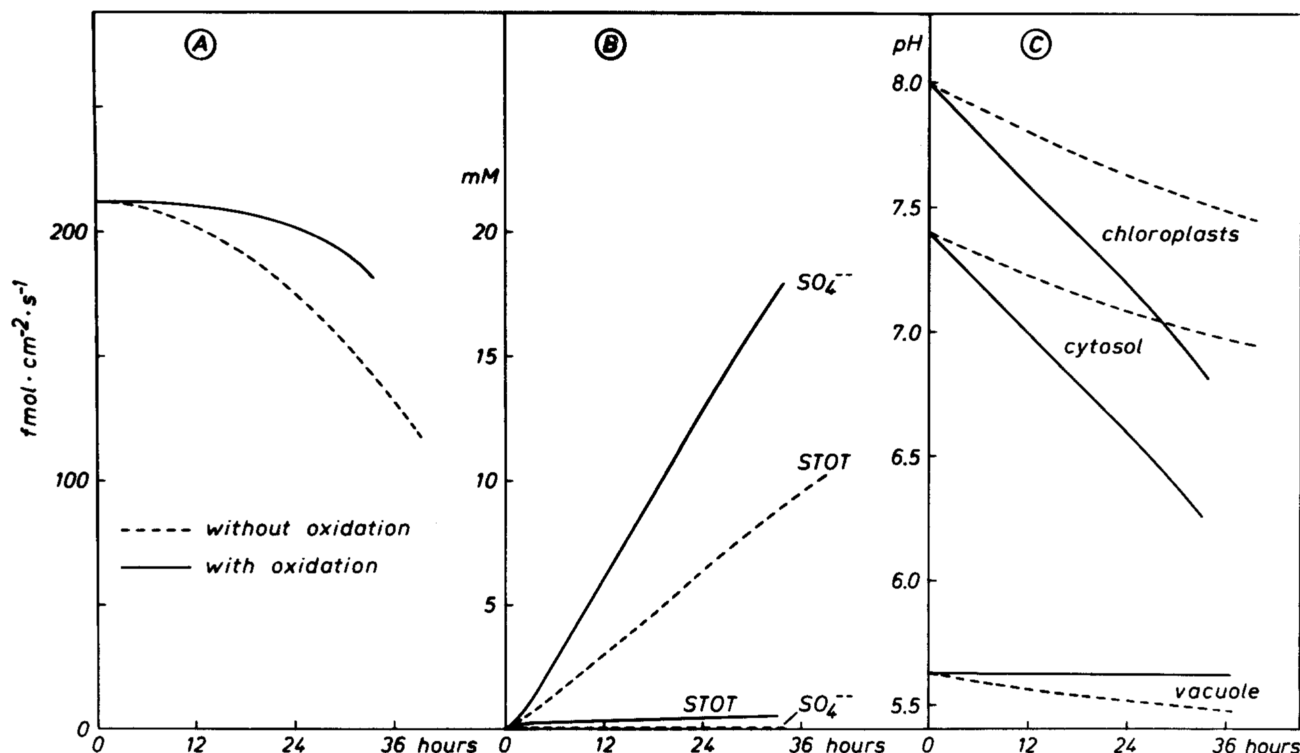


Fig. 4A–C. Effect of oxidation of sulfite and bisulfite on the rates of influx of SO_2 (**A**), levels of bisulfite and sulfite (actually, the sum of both, STOT) and accumulation of sulfate in the chloroplasts (**B**) and acidification of the chloroplast stroma (**C**). The boundary layer and stomatal conductance for H_2O is 0.2 $cm \cdot s^{-1}$. Oxidation is not possible in the control (-----), but occurs in (——). The biomembrane conductance for SO_2 is 0.0125 $cm \cdot s^{-1}$. Other conductances are $MSO_3^{2-}{}_{pc,pv}$ and $MHSO_3^{-}{}_{pc,pv} = 8 \cdot 10^{-7}$ $cm \cdot s^{-1}$. The rate constant of sulfite and bisulfite oxidation is $8 \cdot 10^{-5}$ $l \cdot s^{-1}$ in the cytosol and 10^{-3} $l \cdot s^{-1}$ in the chloroplast stroma. The SO_2 concentration in air is 0.05 $\mu l \cdot l^{-1}$

the phosphate translocator of the envelope can exchange not only phosphate and some phosphate esters, but also sulfate (Hampp and Ziegler 1977; Ziegler and Hampp 1977; Mourioux and Douce 1979). In the computer model, a permeability coefficient for sulfate is assigned to the chloroplast envelope, and protons are assumed to cross the envelope together with the sulfate anion. Transport of protons and sulfate across the tonoplast occurs not only downhill. Uphill transport must be energized. In the computer model, it is unidirectional. Figure 5 shows the time course of the pH of the chloroplast stroma and of the vacuole, when a model leaf is transferred from zero to 0.05 $\mu l \cdot l^{-1}$ SO_2 at the following stomatal conductances: 0.04, 0.12, 0.36 and 1.08 $cm \cdot s^{-1}$. The envelope conductance for sulfate is assumed to be 10^{-9} $cm \cdot s^{-1}$ and the V_{max} of active sulfate transfer across the tonoplast $5 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$. The Figure shows that sulfate and H^+ transfer into the vacuole can preserve cytoplasmic pH values at the expense of the vacuolar pH as long as the sulfate and H^+-transport system of the tonoplast can cope with the protons and the sulfate produced in the cytoplasm during the influx of SO_2. If this system is overtaxed, it cannot prevent acidification of the cytosol and the organelles embedded in it. In Fig. 5, H^+ and sulfate transport into the vacuole is proportional to SO_2 influx into the leaf (which, in turn, is proportional to stomatal opening) only at low stomatal conductances (0.04 and 0.12 $cm \cdot s^{-1}$). Owing to a limited transfer capacity of the tonoplast for H^+ and sulfate, acidification of chloroplast stroma and cytosol occurs in the model experiment when the stomata are widely open.

There is the question how many protons the vacuole can store. In barley mesophyll cells, the buffer capacity of vacuolar contents has been measured. Fourteen μequivalents $H^+ \cdot mg^{-1}$ chlorophyll or 45 μequivalents $\cdot dm^{-2}$ leaf area would decrease the vacuolar pH from 6 to 3 (Pfanz and Heber 1986). We realize that such a decrease is unlikely to be tolerated by the plant, but use it to gain an impression of a maximum storage capacity for H^+ in relation to H^+ production from SO_2. In Table 3, SO_2 influx and H^+ production during oxidation to sulfate are calculated for a summer period (May to the end of September)

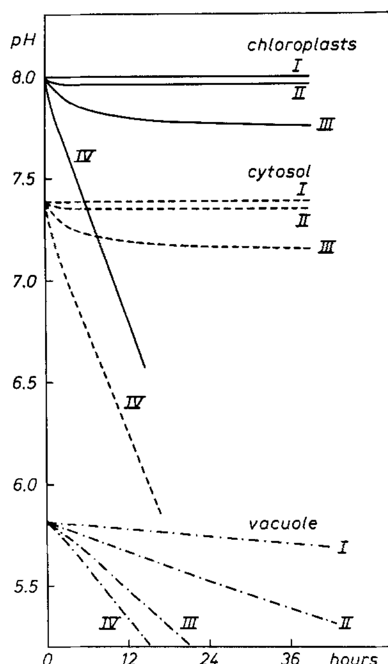


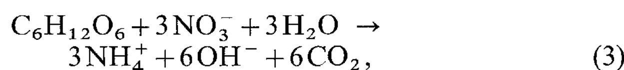
Fig. 5. Acidification of chloroplasts and vacuole of mesophyll cells during the influx of SO_2 (0.05 $\mu l \cdot l^{-1}$ in air) into a model leaf at different boundary-layer and stomatal conductances for water (*I*: $MH_2O = 0.04$; *II*: $MH_2O = 0.12$; *III*: $MH_2O = 0.36$; *IV*: $MH_2O = 1.08$ $cm \cdot s^{-1}$). The biomembrane conductance for SO_2 is 0.0125 $cm \cdot s^{-1}$. Other conductances are: chloroplast envelope: $MHSO_3^-$ and $MSO_3^{2-} = 10^{-7}$ $cm \cdot s^{-1}$; $MSO_4^{2-} = 10^{-9}$ $cm \cdot s^{-1}$; tonoplast: $MHSO_3^-$ and $MSO_3^{2-} = 10^{-9}$ $cm \cdot s^{-1}$. The V_{max} of active sulfate and H^+ transport across the tonoplast is $5 \cdot 10^{-14}$ $mol \cdot l^{-1} \cdot s^{-1}$ and the K_m is 0.5 mM. The rate constant of sulfite and bisulfite oxidation is $5 \cdot 10^{-5}$ $l \cdot s^{-1}$ in the cytosol and $2 \cdot 10^{-3}$ $l \cdot s^{-1}$ in the chloroplast stroma. Initial rates of SO_2 influx are $43 \cdot 10^{-15}$, $128 \cdot 10^{-15}$, $375 \cdot 10^{-15}$ and $104 \cdot 10^{-14}$ mol cm^{-2} s^{-1}. They increase from the lower to the higher stomatal conductance

Table 3. Influx of SO_2 into leaves and intracellular formation of H^+ during oxidation to sulfate at different stomatal conductances for water. The period of exposure to 0.05 $\mu l \cdot l^{-1}$ SO_2 is considered to be 2000 h which is equivalent to a growth period from May to September

	Conductance ($cm \cdot s^{-1}$)		
	0.04	0.1	0.25
SO_2 influx, 10^{-7} $mol \cdot cm^{-2}$ leaf surface × 2000 h	3.0	7.2	18.0
H^+ production from SO_2, 10^{-7} $equivalents \cdot cm^{-2}$ × 2000 h	6.0	14.4	36.0

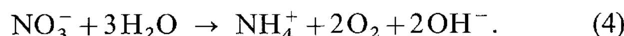
under the assumption that leaves of different stomatal conductances are exposed to 0.05 $\mu l \cdot l^{-1}$ SO_2 in air (see also Table 1). The time during which the stomata were open as indicated is assumed to be 2000 h. Table 1 shows that even when stomata are largely closed ($MH_2O = 0.04$ $cm \cdot s^{-1}$), H^+ production from SO_2 exceeded the maximum storage capacity of vacuoles as determined for barley mesophyll cells. The difference between storage capacity and storage requirement was large for a stomatal conductance of 0.1 $cm \cdot s^{-1}$ which may represent a conductance typical for conifer needles. Dicots such as spinach exhibit conductances usually higher than 0.25 $cm \cdot s^{-1}$. It is thus obvious that the vacuolar buffering capacity is insufficient to cope with intracellular H^+ formation during prolonged exposure of leaves to SO_2 levels which are considered to be still tolerable.

Metabolization of sulfite and-or sulfate. Removal of H^+ from cells is possible by exchange against another cation, by excretion together with an anion or by neutralization. The latter process requires a base. The reduction of nitrate produces hydroxyl ions. In the dark, carbohydrate may be the electron donor for the reduction of nitrate



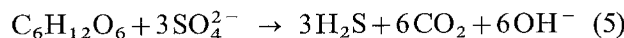
$$C_6H_{12}O_6 + 3NO_3^- + 3H_2O \rightarrow 3NH_4^+ + 6OH^- + 6CO_2, \quad (3)$$

whereas in the light water can assume this function



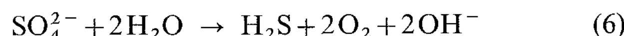
$$NO_3^- + 3H_2O \rightarrow NH_4^+ + 2O_2 + 2OH^-. \quad (4)$$

However, the availability of nitrate is limited in nitrate-deficient forest soils. Another possible source of hydroxyl ions is sulfate itself. Its reduction may be written as



$$C_6H_{12}O_6 + 3SO_4^{2-} \rightarrow 3H_2S + 6CO_2 + 6OH^- \quad (5)$$

or, in the light,



$$SO_4^{2-} + 2H_2O \rightarrow H_2S + 2O_2 + 2OH^- \quad (6)$$

The hydroxyl ions formed will remove protons. A small part of the sulfide produced during the reduction of sulfate is known to be released by the leaves (Hällgren and Fredriksson 1982; Sekiya et al. 1982; Rennenberg and Filner 1982, 1983), but most of it is incorporated into organic compounds (Faller et al. 1970; Guderian 1970, 1977; Garsed and Read 1977a, b; Grill et al. 1980; Garsed 1985). Thus, metabolization of sulfate (or sulfite) results in detoxification of SO_2. It should be noted that, for the purpose of this presentation, metabolization of sulfate is equivalent to the excretion of H^+ and stoichiometric amounts of sulfate from the cells.

Figure 6 shows that metabolization of sulfate is capable of reducing or, if it is sufficiently fast, of preventing the acidification of chloroplasts, cytosol and the vacuole. In the absence of metabolization, influx of SO_2 (0.05 $\mu l \cdot l^{-1}$ in air) into a model leaf resulted in considerable acidification of

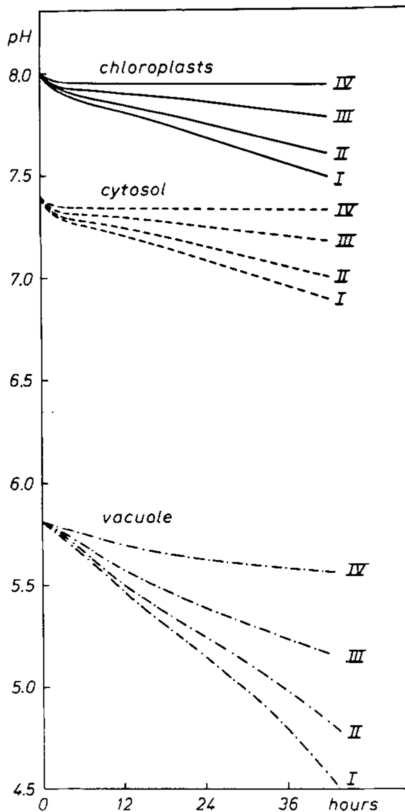


Fig. 6. Effect of sulfate reduction in the chloroplasts on acidification of chloroplasts, cytosol and the vacuole. Conditions are as described for the model experiment shown in **Fig. 5**, but the stomatal conductance is kept constant at $MH_2O = 0.36$ cm·s^{-1}. In addition to active SO_4^{2-} and H^+ transfer into the vacuole, passive SO_4^{2-} and H^+ flux is permitted and the passive conductance of the tonoplast for SO_4^{2-} and H^+ is 10^{-11} cm·s^{-1}. The V_{max} of sulfate reduction in the chloroplasts is varied from zero (I) to $9 \cdot 10^{-7}$ mol·l^{-1}·s^{-1} (IV). Intermediate V_{max} values are 10^{-7} (II) and $3 \cdot 10^{-7}$ mol·l^{-1}·s^{-1} (III). The K_m of the sulfate-reduction system for SO_4^{2-} is 0.5 mM

chloroplasts and the cytosol even though the main part of the protons was transferred into the vacuole which, owing to active transport of sulfate, contained a higher concentration of sulfate than the cytoplasm at least during the later stages of the model experiment. When sulfate reduction was permitted in the chloroplasts, acidification was decreased in all leaf cell compartments, because the model permitted passive back-transfer of sulfate from the vacuole into the cytosol and from there into the chloroplasts. Whereas vacuolar storage of protons can solve the problem of cytoplasmic acidification on a temporary basis, the removal of excess protons, a process which depends on metabolism, can actually prevent cellular acidification.

Capacity for metabolization of airborne sulfur. It is instructive to consider the capacity of cellular systems suitable for the detoxification of SO_2 in relation to SO_2 influx. We will base such a consideration on a comparison of the influx of SO_2 with influx of carbon in photosynthesis, on the loss of carbon in respiration, on the release of H_2S into the atmosphere and on the relationship of reduced (and thereby detoxified) sulfur to reduced carbon in the biomass of a tree. Gas-exchange measurements have provided good information on photosynthesis of spruce trees in an area of the Fichtelgebirge (Frankonia) near the border to Czechoslovakia where forest decline causes considerable concern. At a boundary and stomatal conductance of 0.07 cm·s^{-1} (calculated from Fig. 1 of Beyschlag et al. (1987)) the rates of net photosynthesis were $12.8 \cdot 10^{-11}$ and $9 \cdot 10^{-11}$ mol·cm^{-2}·s^{-1} (the area represents the total surface of the needles, not as is usual for the broad leaves of dicotyledenous species a projection of the upper surface) in a comparatively undamaged and in a damaged tree. Calculations such as performed in this and the previous publication (Laisk et al. 1987) show that at 0.05 μl·l^{-1} SO_2 in air SO_2 influx will be $7.7 \cdot 10^{-14}$ mol·cm^{-2}·s^{-1}. Thus, the ratio of sulfur to carbon influx is 0.8/1000 and 0.6/1000 under these conditions. However, a very considerable part of the assimilated carbon is lost again. Respiration, loss of leaves or needles and death of roots and small branches may account for the loss of as much as 75% of the assimilated carbon from forest trees (Schulze 1970, 1981, 1982). In comparison, loss of reduced sulfur is small. Less than 10% is released into the atmosphere as H_2S (Hällgren 1978; Rennenberg 1984). Loss of sulfur-containing amino acids is also small, as most of the leaf protein is degraded and the resulting amino acids and peptides are imported into surviving tissue before leaves drop or tissue dies. It may be less than another 10%. Thus, at an SO_2 concentration in air of 0.05 μl·l^{-1} a tree may be burdened with airborne sulfur at a ratio of 1 S-atom per 400 or 500 C-atoms which remain in the organism. Since SO_2 influx is, at a constant boundary and stomatal resistance, directly proportional to the SO_2 concentration in air, other S/C ratios can easily be calculated for SO_2 levels different from 0.05 μl·l^{-1}.

Intake ratios must be compared with the ratio of reduced sulfur to carbon in the biomass. Less than 1% of the biomass of grown fir and spruce trees is contained in the needles (Schulze 1982). The protein content of the needles is below 10% of the dry weight and that of wood and other tissue on average not much higher than 1% (Lyr et al. 1967; Schulze 1982). The carbon content of the

dry mass is about 45%. As the sulfur content of plant proteins is about or even below 1% of the protein weight (Dijkshoorn and van Wijk 1967), a simple calculation demonstrates that the average ratio of reduced sulfur to carbon is, on a molar basis, lower than 1/10000 in the trees. Clearly, there is a large discrepancy between the deposition of airborne sulfur in the trees (ratio of deposited sulfur to deposited carbon 1/500) and the content of reduced and thereby detoxified sulfur (S/C=1/10000), when air contains 0.05 $\mu l \cdot l^{-1}$ SO_2. A balance between the deposition of airborne sulfur in the trees and its reduction is obtained only when the SO_2 concentration in air is reduced to about 0.0025 $\mu l \cdot l^{-1}$. This is a simplified calculation. It does not consider influx of SO_2 through the cuticle of the epidermis. Moreover, it does not consider an important difference between reduction of airborne sulfate and of sulfate which has been taken up from the soil in a reaction involving anion exchange or cotransport with potassium ions. Reduction of the latter according to equation (6) produces hydroxyl ions which will be consumed by other reactions. Reduction of airborne sulfate, on the other hand, consumes only the protons produced when SO_2 is oxidized to sulfuric acid. It does not lead to net production of hydroxyl ions. Thus, reduction of sulfate from the soil and of sulfate from the air is not equivalent in terms of the proton balance of metabolism. Cellular pH control mechanisms must balance the cytoplasmic pH even when airborne SO_2 is detoxified by reduction.

What has been derived here for fir and spruce trees is subject to considerable variation. Ratios of reduced sulfur to carbon in the biomass will tend to decrease with tree age. Also, ratios will be different in different species. In a previous publication (Heber et al. 1987), we have drawn attention to differences in the composition of the biomass of different plants. Many crop plants are rich in proteins. In consequence, they have a much higher ratio of reduced sulfur to carbon in their biomass than forest trees. Leaves of spinach contain somewhat less than 20% protein or 0.2% sulfur in the dry weight. As the carbon content of the dry weight is 45%, the molar ratio of reduced sulfur to carbon is about 1/500. Similar ratios can be calculated for maize (Salisbury and Ross 1978). They do not differ much from the ratios of sulfur to carbon deposition observed when leaves photosynthesize in air polluted with 0.05 $\mu l \cdot l^{-1}$ SO_2. Clearly, these plants need more sulfur to satisfy sulfur requirements of protein synthesis and other reactions than trees. They must be capable of reducing more sulfate. In other words, their sulfur-reduction system is likely to have a much greater capacity for SO_2 detoxification than that of forest trees. In consequence, they should suffer less under SO_2 pollution than trees. As a matter of fact, crops do not show appreciable signs of damage in areas where forest decline is thought to be caused mainly by SO_2 pollution.

Temporary storage of H^+ and sulfate in the vacuole in relation to the reduction of sulfate. If, during the influx of SO_2 into a leaf, the capacity of the sulfur-reduction system is overtaxed, cytoplasmic acidification can be avoided if excess protons and sulfate are transported into the vacuole. When, at a decreased stomatal conductance or a decreased SO_2 level in air, influx of SO_2 is decreased below the capacity of the sulfur-reduction system, stored sulfate can be released from the vacuole and reduced in the cytoplasm. Thus, the vacuole can serve as a temporary proton buffer. A model experiment demonstrating these relations is shown in Fig. 7. A concentration of 0.11 $\mu l \cdot l^{-1}$ SO_2 (see also Table 1) produced rapid acidification of the vacuole owing to fast active transport of sulfate and H^+ across the tonoplast. The chloroplast sulfate-reduction system was unable to maintain cytoplasmic pH values under the chosen conditions. Reducing the SO_2 content of air to 0.01 $\mu l \cdot l^{-1}$ partially restored cytoplasmic pH values. The vacuole released H^+ and sulfate and the cytoplasmic pH increased accordingly. These effects were reversed when the SO_2 content of air was increased to 0.05 $\mu l \cdot l^{-1}$. At this concentration, closure of stomata (conductance for water $MH_2O=0.2$ $cm \cdot s^{-1}$ $\rightarrow MH_2O=0.005$ $cm \cdot s^{-1}$) permitted restoration of intracellular pH values. When the stomata were only partially opened ($MH_2O=0.1$ $cm \cdot s^{-1}$), metabolization of sulfate was capable of balancing the acidification produced by the hydration of SO_2 and the oxidation of the hydration products to sulfate, if the maximum rate of sulfate reduction in the chloroplasts was $2 \cdot 10^{-7}$ $mol \cdot l^{-1} \cdot s^{-1}$. Slow acidification still occurred under the same conditions at half that maximum rate. In vivo, the rate of sulfate reduction in the chloroplasts will be negligible during the night (Wilson et al. 1978) which makes SO_2 detoxification practically impossible.

In the experiment of Fig. 7 it is assumed that the vacuole buffers must of the protons which are released during hydration and oxidation of SO_2. The capacity of plant vacuoles for temporary storage of protons and sulfate is unknown. It is likely to vary in different species. If there is a large discrepancy between net SO_2 intake through the sto-

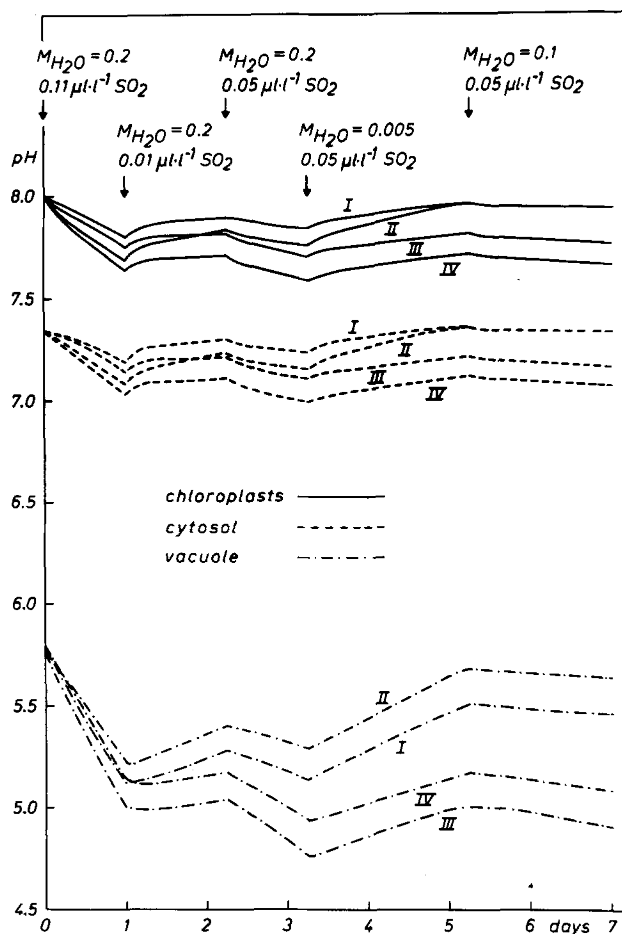


Fig. 7. Simulation of intracellular pH changes when the SO_2 content of air is varied or stomata close. The following conductances are used: plasmalemma: 0.0125 $cm \cdot s^{-1}$ (SO_2); chloroplast envelope: 0.0125 $cm \cdot s^{-1}$ (SO_2), 10^{-7} $cm \cdot s^{-1}$ (HSO_3^-, SO_3^{2-}), 10^{-9} $cm \cdot s^{-1}$ (SO_4^{2-}); tonoplast: 0.0125 $cm \cdot s^{-1}$ (SO_2), 10^{-9} $cm \cdot s^{-1}$ (HSO_3^-, SO_3^{2-}), $5 \cdot 10^{-11}$ $cm \cdot s^{-1}$ (SO_4^{2-}). The rate constant of sulfite and bisulfite oxidation is $5 \cdot 10^{-5}$ $l \cdot s^{-1}$ in the cytosol and $2 \cdot 10^{-3}$ $l \cdot s^{-1}$ in the chloroplasts. The V_{max} of active transport of SO_4^{2-} and H^+ into the vacuole is $2.5 \cdot 10^{-13}$ $mol \cdot cm^{-2} \cdot s^{-1}$ (I, III) and $5 \cdot 10^{-14}$ $mol \cdot cm^{-2} \cdot s^{-1}$ (II, IV), the apparent K_m for sulfate 0.5 mM. The V_{max} of sulfate reduction in the chloroplasts is $2 \cdot 10^{-7}$ $mol \cdot l^{-1} \cdot s^{-1}$ (I, II) and 10^{-7} $mol \cdot l^{-1} \cdot s^{-1}$ (III, IV), the K_m for sulfate 0.5 mM. The calculations show that protection against SO_2 damage is afforded by a high capacity of the leaf for sulfate reduction, a high capacity for active sulfate and H^+ transfer into the vacuole and the capability to close the stomata. A combination of these properties is particularly effective

mata of leaves and the content of reduced (and thereby detoxified) sulfur in the biomass, vacuoles are obvious candidates for storage. Photosynthesis of isolated chloroplasts is known to be sensitive not only to a decrease in pH, but also to millimolar concentrations of sulfate (Baldry et al. 1968). If the vacuolar storage capacity for protons and for sulfate produced during the oxidation of SO_2 is exhausted and sulfate reduction cannot be increased, damage is unavoidable.

Conclusions. If our deductions are correct, and if we have not neglected major areas of SO_2 detoxification, we should not expect sensitive species of forest trees with a low S/C ratio to tolerate exposure to SO_2 concentrations of 0.02 to 0.05 $\mu l \cdot l^{-1}$ in air which are thought to be permissible over long periods of time. If such concentrations are really permitted to act on a forest vegetation, we should be prepared for the disappearance of sensitive forest trees. It is perhaps not a very comforting thought that other, more tolerant, plant species will take their place.

This work has been performed within the Research Group Ecophysiology of the Deutsche Forschungsgemeinschaft. It was supported by the Bayerische Forschungsgruppe Forsttoxikologie, the Projektgruppe Bayern zur Erforschung der Wirkung von Umweltschadstoffen (PBWU) and the Fonds der Chemischen Industrie. We are very grateful to Professor Mengel, Giessen, and Dr. Giersch, Düsseldorf, for stimulating advice.

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Received 29 January; accepted 6 July 1987