Planta (1989) 177:91–97

Planta

Light and the maintenance of photosynthetic competence in leaves of *Populus balsamifera* L. during short-term exposures to high concentrations of sulfur dioxide

William W. Adams III[1] *, Klaus Winter[1], and Andrea Lanzl[2]

[1] Lehrstuhl für Botanik II, and [2] Lehrstuhl für Botanik I, Mittlerer Dallenbergweg 64, D-8700 Würzburg, Federal Republic of Germany

Abstract. Leaves of *Populus balsamifera* grown under full natural sunlight were treated with 0, 1, or 2 μl $SO_2 \cdot l^{-1}$ air under one of four different photon flux densities (PFD). When the SO_2 exposures took place in darkness or at 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$, sulfate accumulated to the levels predicted by measurements of stomatal conductance during SO_2 exposure. Under conditions of higher PFD (750 and 1550 μmol $\cdot m^{-2} \cdot s^{-1}$), however, the predicted levels of accumulated sulfate were substantially higher than those obtained from anion chromatography of the leaf extracts. Light- and CO_2-saturated capacity as well as the photon yield of photosynthetic O_2 evolution were reduced with increasing concentration of SO_2. At 2 μl $SO_2 \cdot l^{-1}$ air, the greatest reductions in both photosynthetic capacity and photon yield occurred when the leaves were exposed to SO_2 in the dark, and increasingly smaller reductions in each occurred with increasing PFD during SO_2 exposure. This indicates that the inhibition of photosynthesis resulting from SO_2 exposure was reduced when the exposure occurred under conditions of higher light. The ratio F_V/F_M (variable/maximum fluorescence emission) for photosystem II (PSII), a measure of the photochemical efficiency of PSII, remained unaffected by exposure of leaves to SO_2 in the dark and exhibited only moderate reductions with increasing PFD during the exposure, indicating that PSII was not a primary site of damage by SO_2. Pretreatment of leaves with SO_2 in the dark, however, increased the susceptibility of PSII to photoinhibition, as such pretreated leaves exhibited much greater reductions in F_V/F_M when transferred to moderate or high light in air than comparable control leaves.

Key words: Chlorophyll fluorescence – Photoinhibition – Photosynthesis – *Populus* – Sulfur dioxide

Introduction

There have been numerous studies documenting the phytotoxic nature of SO_2 (see Ziegler 1975; Winner et al. 1985). The majority of these, however, have ignored the influence of light on SO_2 toxicity beyond its effect on stomatal opening and thus on the rate of SO_2 uptake. In the few studies which have considered light as a factor affecting the toxicity of SO_2, it has been shown that plants which take up SO_2 in the dark experience greater leaf injury (foliar necrosis) and reduced growth relative to plants exposed to SO_2 under low to moderate light conditions (Nielsen 1938; Davies 1980; Jones and Mansfield 1982; Olszyk and Tingey 1984).

Precisely what aspects of plant metabolism are more strongly affected by SO_2 uptake in the dark versus the light have not, however, been identified. Furthermore, very high light intensities combined with SO_2 may lead to photoinhibition. We have conducted a series of experiments to examine the effects of short-term exposure to different partial pressures of SO_2 under different photon flux densities (PFDs) on several photosynthetic properties of poplar leaves. We present evidence that the degree to which photosynthesis is impaired by SO_2

* *Permanent address:* Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, CO 80309-0334, USA

Abbreviations and symbols: A_{1200} = photosynthetic capacity (CO_2-saturated rate of O_2 evolution at 1200 μmol photons $\cdot m^{-2} \cdot s^{-1}$); F_O = instantaneous fluorescence emission; F_M = maximum fluorescence emission; F_V = variable fluorescence emission; PFD = photon flux density (400–700 nm); PSII = photosystem II

uptake is reduced under conditions of higher light and that the detoxification of SO_2 by these leaves may be favored in the light. Pretreatment of leaves with SO_2 in the dark, however, leads to an increased sensitivity to photoinhibition of photosystem II (PSII).

Material and methods

Plant material and treatments. Leaves from four-year-old *Populus balsamifera* L. trees, grown in the Würzburg Botanical Garden under natural sunlight with adequate water and nutrient supply (leaf nitrogen content ranged between 2% and 3% on a dry weight basis) as described previously (Demmig et al. 1987), were used in these studies. All experiments were performed in September with detached leaves (collected prior to dawn) whose petioles were kept in water. The leaves were exposed to different levels of SO_2 and light in a ventilated, water-jacketed chamber made of brass. Leaf temperatures were maintained between 22° C and 25° C and the dew point of the incoming air was approx. 11° C. The dew point of the air entering and leaving the chamber was measured with a dew-point mirror (Walz, Effeltrich, FRG). Illumination of the leaves at a PFD of 1550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was provided by a metal-halide lamp (HQI-T 1000 W/D; Osram, München, FRG) and lower PFDs were obtained with glass neutral-density filters. Light was measured with a Li-Cor quantum sensor (LI-190SB; Li-Cor, Lincoln, Neb. USA). Air was passed through the chamber at a rate of 5 $l \cdot min^{-1}$, and SO_2 was introduced to the chamber after passing through a mass-flow controller (Tylan, Carson, Cal., USA).

Sulfate analyses. Leaf discs (3.53 or 10 cm^2) were frozen in liquid nitrogen and ground to a fine powder. Water-soluble components were extracted by boiling the leaf material in 2 or 4 ml of distilled water. The samples were then centrifuged at 3700 · *g* for 20 min. Aliquots of the clear supernatant were diluted to give a final sulfate concentration of about 0.1 $mol \cdot m^{-3}$. Sulfate concentration was measured by isocratic anion chromatography using a chromatograph (IC 1000), fitted with a conductivity (BT 0330) and a UV-detector (BT 3030), automatic sample injector (BT 7041; Biotronik, Maintal, FRG) and integrator (C-R1B; Shimadzu, Kyoto, Japan; Schröppel-Meier and Kaiser 1987).

Oxygen evolution and fluorescence. Measurements of photon yield (on an incident basis) and photosynthetic capacity (rate of O_2 evolution at 1200 μmol photons $\cdot m^{-2} \cdot s^{-1}$, defined as A_{1200}) from leaf discs were made at 25° C with saturating CO_2 (5%) in a leaf-disc O_2 electrode (LD-2 and LS-2 light source; Hansatech, King's Lynn, Norfolk, UK) as described by Björkman and Demmig (1987). Chlorophyll *a* fluorescence (primarily from PSII) was measured at room temperature using a PAM 101 Chlorophyll Fluorometer (Walz; Schreiber et al. 1986). Leaf discs were darkened for 5 min, after which instantaneous fluorescence emission (F_O) and maximum fluorescence emission (F_M) were ascertained using the weak measuring beam (F_O) followed by a 1-s saturating pulse of white light (F_M; Demmig et al. 1987).

Results and discussion

Leaves of *Populus balsamifera* were exposed to either air (0 μl $SO_2 \cdot l^{-1}$ air) or SO_2 in air (1 or 2 μl $SO_2 \cdot l^{-1}$ air; i.e. 2.85 or 5.7 mg $SO_2 \cdot m^{-3}$) in either darkness (0 μmol photons $\cdot m^{-2} \cdot s^{-1}$) or under one of three different PFDs (300, 750, or 1550 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Discs were removed from the leaves for the various analyses prior to the exposure (control discs, 0 h) and after 4 and 8 h of exposure. The water-vapor conductance of leaves exposed in darkness was of a magnitude comparable to that of leaves exposed in the light (Fig. 1 A), indicating that in our experiments the degree of stomatal opening should have imposed little restriction to the uptake of SO_2. This was confirmed by measurements of accumulated sulfate in the leaf tissues after 4 and 8 h of exposure to SO_2 (Fig. 1 B). Exposure of leaves to higher levels of SO_2 resulted in a greater accumulation of sulfate across all light treatments (Fig. 1 B).

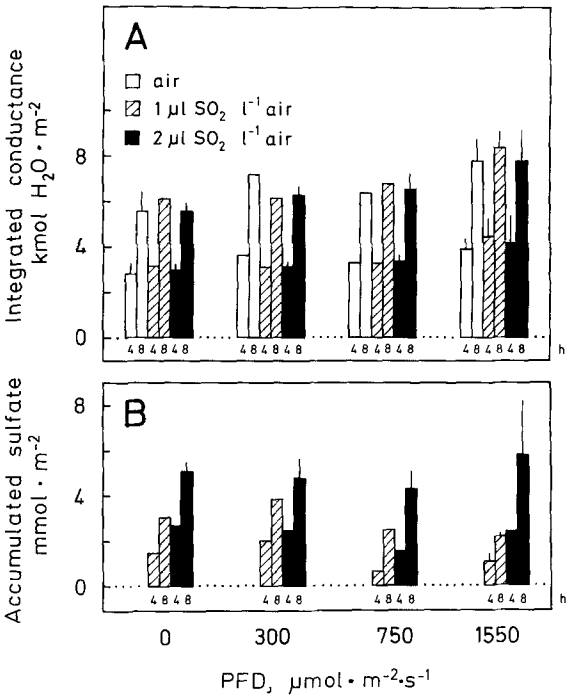

Fig. 1 A, B. Measurements of integrated conductance (**A**) and accumulated sulfate (**B**) during 4 and 8 h exposure of leaves of *Populus balsamifera* to SO_2 under four different PFDs. *Open, hatched,* and *solid bars* refer to 0, 1, and 2 μl $SO_2 \cdot l^{-1}$ air, respectively. Standard deviations shown when $n=3$ (for all treatments at 2 μl $SO_2 \cdot l^{-1}$ air, for a PFD of 1550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1 μl $SO_2 \cdot l^{-1}$ air, and for a PFD of 0 and 1550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 0 μl $SO_2 \cdot l^{-1}$ air). For a PFD of 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1 μl $SO_2 \cdot l^{-1}$ air, $n=2$

The response of stomata to SO_2 exposure is very complex and has been shown to depend on a variety of factors including leaf age, growth conditions (including prior stresses), SO_2 concentration, the time of day in which the exposure is initiated, the duration of the exposure, and the pre-

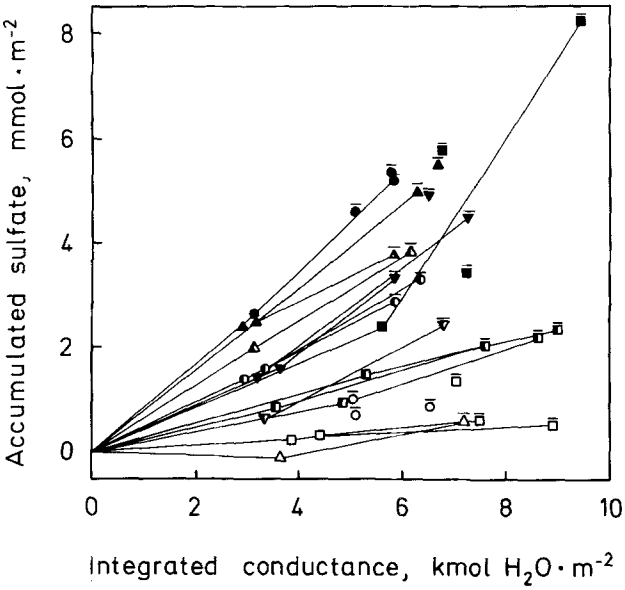

Fig. 2. Relationship between accumulated sulfate and conductance integrated over 4 h (*no bar* above the symbols) and over 8 h (*bar* above the symbols) of SO_2 fumigation (*open, half-closed,* and *solid symbols* refer to 0, 1, and 2 µl $SO_2 \cdot l^{-1}$ air respectively) under 0 (*circles*), 300 (*triangles*), 750 (*inverted triangles*), or 1550 (*squares*) µmol photons $\cdot m^{-2} \cdot s^{-1}$ for leaves of *P. balsamifera*

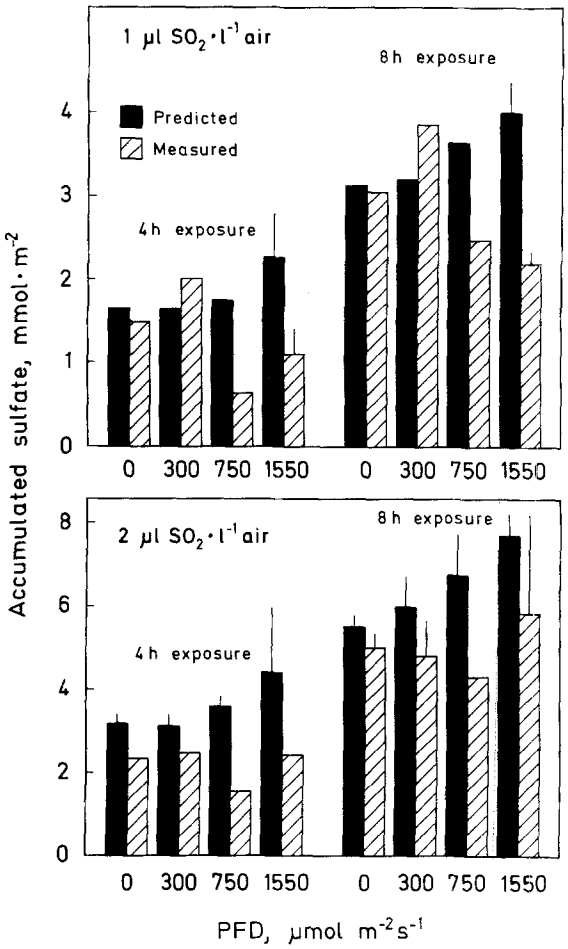

Fig. 3. Predicted (based on measurements of transpiration; *solid bars*) and measured (*hatched bars*) levels of accumulated sulfate after 4 and 8 h of SO_2 fumigation of *P. balsamifera* leaves under different PFDs. See **Fig. 1** legend for further details

vailing environmental conditions during the exposure (Black 1985). The type of response, and its magnitude, is also species-dependent and, in some cases, differences between clones of the same species have been detected (Kimmerer and Kozlowski 1981). Exposure of *P. balsamifera* to 1 and 2 µl $SO_2 \cdot l^{-1}$ air appears to have had only a small effect on stomatal opening which is probably dependent on the amount of SO_2 that was taken up rather than the actual exposure level. As more sulfate was accumulated in the leaves, the conductances decreased, as indicated by the slight negative slope for the population of points arrayed in Fig. 2 after 4 h of exposure and for the population of points after 8 h of exposure.

From measurements of transpiration it is possible to estimate the total flux of SO_2 into a leaf (Taylor and Tingey 1983). Using the model elaborated by Pfanz et al. (1987) and Laisk et al. (1988), the total sulfate expected to have been accumulated in the leaves during the various exposures was calculated. These estimations were based on measurements of transpiration made during the exposures, with the assumption that all of the SO_2 taken up was accumulated as sulfate. Naturally, this is at best an approximation (see below). Detached leaves were used in these experiments, and thus any possible export of sulfur metabolites has been ignored. The calculated values are plotted beside the measured levels of accumulated sulfate in Fig. 3. As expected, the measured levels of sulfate were very close to those estimated from measurements of transpiration when leaves were exposed to SO_2 in darkness. With increasing PFD during the exposure to SO_2, however, the discrepancy between the estimated and measured values became greater such that at the highest PFDs (750 and 1550 µmol $\cdot m^{-2} \cdot s^{-1}$), the actual levels of accumulated sulfate were, in several instances, less than 50% of the estimated values.

These results indicate that when SO_2 entered the leaf during darkness it accumulated primarily as sulfate, whereas with increasing irradiance during SO_2 exposure, a greater proportion was metabolized to other products. Accumulation of sulfite, one of the main forms SO_2 takes upon hydration in the aqueous phase of leaves, seems unlikely, as Garsed and Read (1977) have shown that there

was no sulfite present in leaves of *Glycine max* following exposure to SO_2 in the light. They, and others, have found that sulfate is the major form in which sulfur accumulates in the leaf after exposure to SO_2 (Thomas et al. 1944; Weigl and Ziegler 1962; Garsed and Read 1977; Sekiya et al. 1982). The incorporation of sulfur into organic compounds is one route by which SO_2 that has entered the leaf could be metabolized (detoxified; Dijkshoorn and van Wijk 1967). It appears, however, that this is relatively minor compared to the reduction of SO_2 to H_2S and its emission to the atmosphere (DeCormis 1968; Rennenberg 1984; Garsed 1985). The emission of H_2S from leaves is, in fact, dependent on light (Wilson et al. 1978; Hällgren and Fredricksson 1982), and greater resistance to injury from SO_2 fumigation is also associated with greater levels of H_2S emission (Sekiya et al. 1982).

Measurements of photosynthetic O_2 evolution before SO_2 fumigation, and after 4 and 8 h of fumigation (Fig. 4A, B) indicated that less SO_2-induced inhibition of photosynthesis occurred if the leaves were exposed to SO_2 under higher PFD. There was some leaf-to-leaf variability in the control (0 h) levels of photosynthetic capacity (47.3 ± 7.6 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$; $n=27$), and therefore changes in A_{1200} in response to SO_2 and light have been expressed as percentages of the initial, prefumigation rates of O_2 evolution. Exposure of the leaves to the various PFDs without SO_2 resulted in little or no change in photosynthetic capacity and photon yield, except at the highest PFD where there was a small reduction in both. This slight reduction is probably reflective of a small degree of photoinhibition caused by the high light exposure alone. Moderate reductions in photosynthetic capacity and photon yield occurred under all PFDs during exposure to 1 μl $SO_2 \cdot l^{-1}$ air, without any clear differences in the magnitude of the reductions between PFD treatments. With exposure to 2 μl $SO_2 \cdot l^{-1}$ air, however, clear differences in the reductions in photosynthetic O_2 evolution between PFD exposures became apparent. The greatest reductions in both photosynthetic capacity and photon yield occurred in leaves exposed to 2 μl $SO_2 \cdot l^{-1}$ air in darkness, and smaller reductions occurred in the light, with the smallest reductions in both resulting from SO_2 fumigation at the highest PFD. These findings provide some explanation for the previous observations that exposure of plants to SO_2 in the dark or low light results in greater foliar injury and reduced growth than SO_2 exposure under higher PFD (Nielsen 1938; Davies 1980; Jones and Mansfield 1982; Olszyk and Tingey 1984).

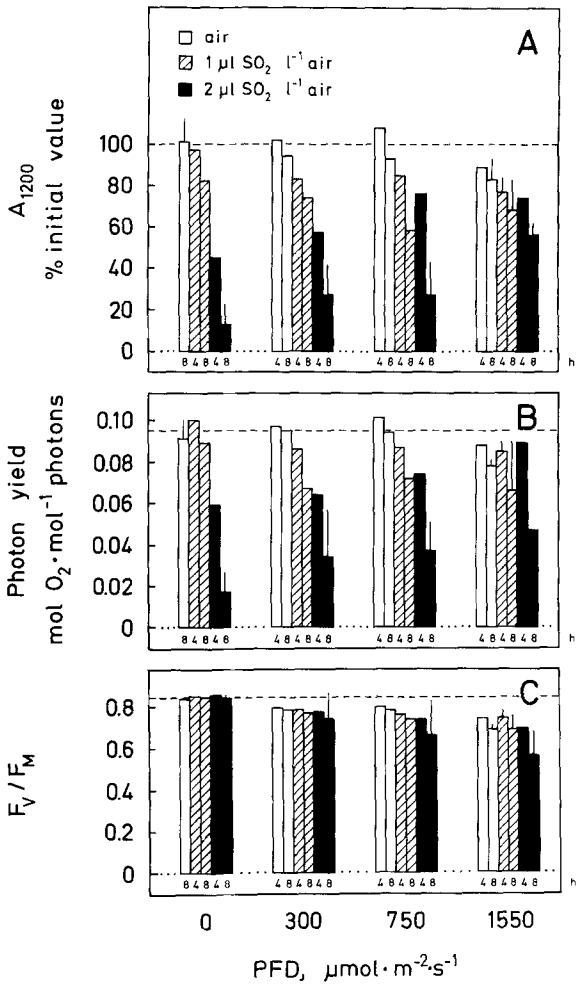

Fig. 4A–C. Measurements of photosynthetic capacity (A_{1200}) at a PFD of 1200 μmol $\cdot m^{-2} \cdot s^{-1}$ (**A**), photon yield (**B**), and F_V/F_M fluorescence from PSII (**C**) following 4 and 8 h of exposure of *P. balsamifera* leaves to SO_2 under different PFDs. See **Fig. 1** legend for further explanation. The control values of A_{1200}, obtained from the same leaves prior to SO_2 fumigation, had a mean value of 47.3 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ with an SD of 7.6 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ ($n=27$). The *dashed line* in each panel represents the mean of the control values obtained from each of the leaves prior to exposure ($n=27$). See **Fig. 5** for the SDs of the control values

When comparing the mean reductions in A_{1200} with the mean reductions in photon yield after 4 and 8 h of fumigation with 2 μl $SO_2 \cdot l^{-1}$ air (Fig. 4A, B), in almost every case A_{1200} was reduced to a slightly greater degree than was photon yield. This is also evident when the individual data for all treatments are plotted against one another (Fig. 5A), although these differences are rather small. During exposure to lower concentrations of SO_2 than those used in the present study, several species experienced reductions in photosynthetic

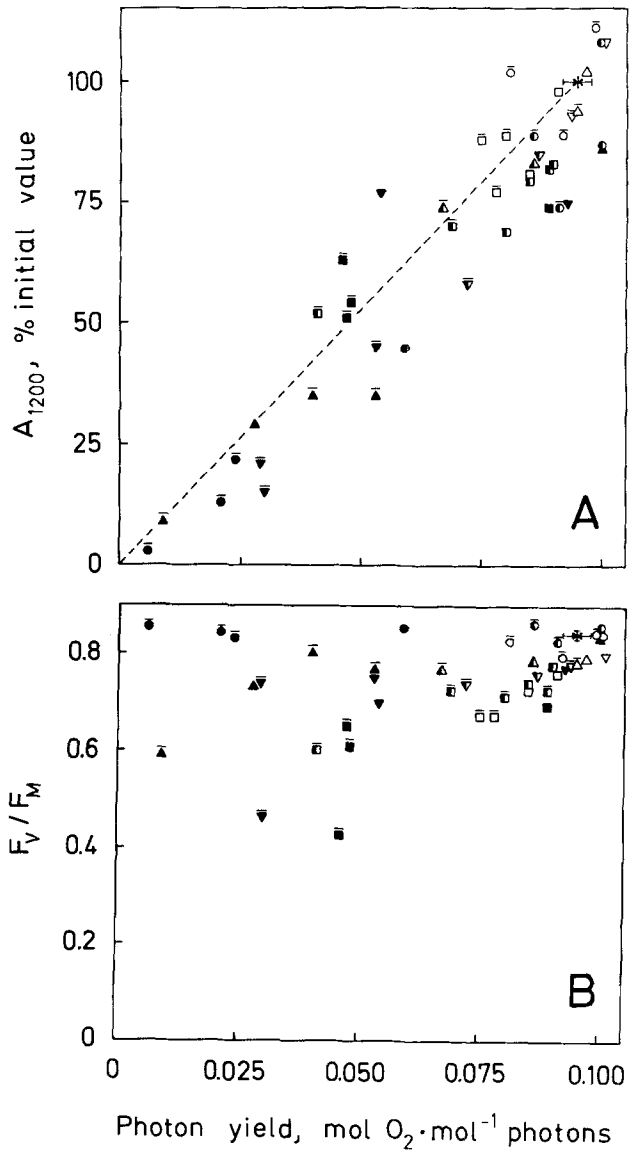

Fig. 5A, B. Relationship between photosynthetic capacity at 1200 μmol photons $\cdot m^{-2} \cdot s^{-1}$ and photon yield (**A**) and F_V/F_M fluorescence from PSII and photon yield (**B**) following 4 and 8 h of SO_2 fumigation of *P. balsamifera* leaves under different PFDs. See **Fig. 2** legend for an explanation of the symbols. * = mean of the control values obtained from each of the leaves prior to exposure ($n = 27$) with SDs indicated

capacity with no effect on photon yield (Black and Unsworth 1979; Hällgren and Gezelius 1982). Hällgren and Gezelius (1982) found ribulose-1,5-bisphosphate-carboxylase activity was reduced as well under these conditions (see also Ziegler 1972). Under the high SO_2 concentrations employed in the current study, it seems likely that appreciable acidification of the stroma would take place, leading to the inactivation of several key enzymes necessary for CO_2 reduction (see Pfanz and Heber 1986; Pfanz et al. 1987). Although such an inactivation is likely to inhibit photosynthetic capacity first, if great enough it could also lead to reductions in photon yield.

Several sites in the photosynthetic electron-transport chain have also been implicated as being susceptible to disruption by SO_2 fumigation (Cerović et al. 1982; Shimazaki et al. 1984; Wellburn 1985), including PSII (Shimazaki and Sugahara 1979; Shimazaki et al. 1984). Our data, however, indicate that the PSII complex is not a primary site of damage by SO_2. Measurements of variable (F_V) over maximum fluorescence (F_M) at room temperature (a measure of PSII photochemical efficiency; see Kitajima and Butler 1975) were made from tissue samples adjacent to those used for measurements of O_2 evolution. Unlike A_{1200} and photon yield, which both experienced greater reductions with decreasing PFD during SO_2 fumigation at 2 μl $\cdot l^{-1}$ air (Fig. 4A, B), F_V/F_M remained unaffected by exposure to SO_2 in the dark and exhibited small but increasing reductions with increasing PFD during the exposure (Fig. 4C). When excessive light was the only factor responsible for reductions in photosynthesis (photoinhibition), comparable reductions in photon yield and F_V/F_M from PSII have been observed (Demmig and Björkman 1987; Demmig et al. 1987). With exposure to SO_2, however, severe reductions in the photon yield of O_2 evolution could be induced with no reduction in F_V/F_M (Fig. 5B). Such differences are likely to arise whenever an environmental factor, such as water stress (Ben et al. 1987), is responsible for inhibiting photosynthesis at some point beyond primary photochemistry.

Although photon yields were slightly less reduced than photosynthetic capacities following exposure of *P. balsamifera* leaves to the various PFDs and SO_2 concentrations (Fig. 5A), the fact that both of these decreased almost in parallel does lead one to suspect that at least one of the sites of photosynthetic inhibition by SO_2 is somewhere in the electron-transport chain. Since F_V/F_M from PSII was relatively unaffected in these experiments, the site of inhibition must be at some point beyond PSII.

A light requirement for PSII inactivation with SO_2 exposure was confirmed in several experiments in which leaves were fumigated in the dark with air or 2 μl $SO_2 \cdot l^{-1}$ air and then exposed to light in air without SO_2 (Fig. 6). Following 8 h of darkness in air, the photon yield was unaffected (see Fig. 6 legend) and F_V/F_M was also high (Fig. 6A; see also Fig. 4C). During a subsequent 2-h exposure to high PFD (1550 μmol $\cdot m^{-2} \cdot s^{-1}$), F_V/F_M declined only slightly (by 13%; Fig. 6A).

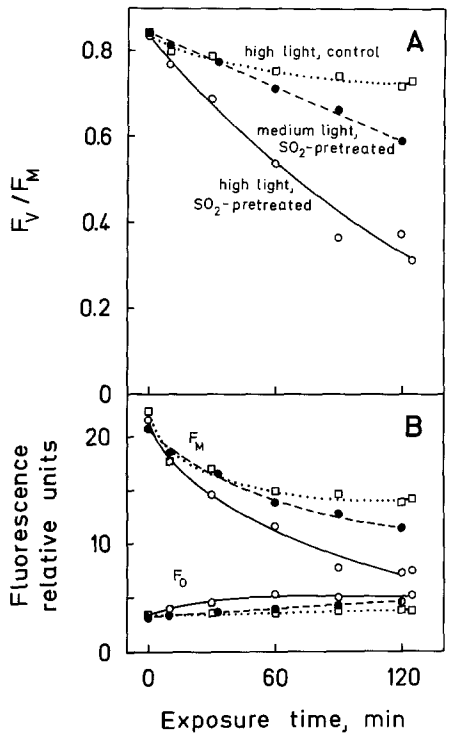

Fig. 6A, B. Changes in PSII fluorescence from leaves of *P. balsamifera* during exposure to 1550 μmol photons·m^{-2}·s^{-1} (*open symbols*) or 400 μmol photons·m^{-2}·s^{-1} (*closed symbols*) in air following a pretreatment of either 8 h darkness in air (□·····□, photon yield of 0.099 mol O_2·mol^{-1} photons) or 8 h darkness in 2 μl SO_2·l^{-1} air (●——●, photon yield of 0.021 mol O_2·mol^{-1} photons; and ○——○, photon yield of 0.024 mol O_2·mol^{-1} photons)

Similar treatments, in which the leaves were subjected to 2 μl SO_2·l^{-1} air during the 8 h of darkness prior to light exposure, yielded quite different results. After the 8 h of SO_2 fumigation in the dark, photon yields were reduced by approx. 75% (see Fig. 6 legend), but F_V/F_M values were as high as those from the leaves exposed to air for 8 h in the dark (Fig. 6A; see also Fig. 4B, C). A severe reduction in PSII photochemical efficiency during a subsequent 2-h treatment with 1550 μmol photons·m^{-2}·s^{-1} in air was indicated by a reduction in F_V/F_M to 38% of its value prior to the exposure of the leaf to light, whereas F_V/F_M was reduced by 30% with exposure of the leaf to a moderately low PFD (400 μmol·m^{-2}·s^{-1}; Fig. 6A).

According to the model developed by Kitajima and Butler (1975), a decrease in F_O fluorescence reflects an increase in radiationless dissipation of energy in the antenna chlorophyll, while an increase in F_O may be indicative of PSII inactivation. In the experiments described in the previous paragraph, F_O remained relatively stable during the high-light exposure of the leaf pretreated with air in darkness, whereas it increased in the leaves pretreated with 2 μl SO_2·l^{-1} air for 8 h in darkness during the subsequent exposure to light (Fig. 6B). Thus it is probable that some inactivation of PSII occurred in the leaves pretreated with SO_2 upon exposure to light, whereas the situation in the leaf which received no SO_2 prior to exposure to high light was less clear. Given the reduction in F_M (Fig. 6B), one would anticipate a reduction in F_O as well if an increase in radiationless energy dissipation had been the only effect of the exposure of the control leaf to high light. As F_O remained relatively constant during the high-light treatment, it seems possible that some degree of PSII inactivation also occurred in the control leaf.

These experiments (Fig. 6) indicate that the foliar uptake of SO_2 resulted in a decrease in photosynthetic efficiency which then predisposed PSII to photoinhibition by exposure to light. Fumigation with SO_2 obviously reduces photosynthetic electron transport at some point beyond PSII. This results in a reduction in the capacity for orderly dissipation of excitation energy, thereby increasing the susceptibility of PSII to inactivation by light.

Conclusions

We have shown that less sulfate was accumulated during exposure to SO_2 than expected from measurements of transpiration under conditions of increased irradiance, and that the inhibition of photosynthesis resulting from foliar SO_2 uptake was greater when the SO_2 fumigation occurred in darkness or under low PFD than under higher PFD. This SO_2-induced inhibition of photosynthesis was not associated with a decrease in PSII photochemical efficiency, and therefore the site of inhibition must be at some point beyond PSII. Photoinhibition of PSII does, however, occur with exposure to SO_2, but only as a secondary effect. Following fumigation of leaves with 2 μl SO_2·l^{-1} air in the dark, photosynthetic capacity and photon yields were reduced, and the susceptibility of PSII to photoinhibition by high light was markedly increased, presumably because of a reduced capacity to dissipate excitation energy through photosynthesis. The concentrations of SO_2 used in this investigation are higher than those normally encountered in polluted areas, and it should therefore be instructive to carry out similar studies with lower SO_2 concentrations over longer exposure periods.

This work was supported by the Deutsche Forschungsgemeinschaft. We are grateful to Barbara Demmig-Adams for stimulating discussions and comments on the manuscript, and Hardy Pfanz and Werner Kaiser for reviewing the paper. One of us

(W.W.A.) was supported by the North Atlantic Treaty Organization under a grant awarded in 1987.

References

Ben, G.-Y., Osmond, C.B., Sharkey, T.D. (1987) Comparisons of photosynthetic responses of *Xanthium strumarium* and *Helianthus annuus* to chronic and acute water stress in sun and shade. Plant Physiol. **84**, 476–482

Björkman, O., Demmig, B. (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta **170**, 489–504

Black, V.J. (1985) SO_2 effects on stomatal behavior. In: Sulfur dioxide and vegetation, pp. 96–117, Winner, W.E., Mooney, H.A., Goldstein, R.A., eds. Stanford University Press, Stanford

Black, V.J., Unsworth, M.H. (1979) Effects of low concentrations of sulphur dioxide on net photosynthesis and dark respiration of *Vicia faba*. J. Exp. Bot. **30**, 473–483

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

Davies, T. (1980) Grasses more sensitive to SO_2 pollution in conditions of low irradiance and short days. Nature **284**, 483–485

DeCormis, L. (1968) Dégagement d'hydrogene sulfuré par des plantes soumises à une atmosphère contenant de l'anhydride sulfureaux. C. R. Acad. Sci. **266C**, 683–685

Demmig, B., Björkman, O. (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. Planta **171**, 171–184

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

Dijkshoorn, W., van Wijk, A.L. (1967) The sulphur requirements of plants as evidenced by the sulphur-nitrogen ratio in the organic matter. Plant Soil **26**, 129–157

Garsed, S.G. (1985) SO_2 uptake and transport. In: Sulfur dioxide and vegetation, pp. 75–95, Winner, W.E., Mooney, H.A., Goldstein, R.A. Stanford University Press, Stanford

Garsed, S.G., Read, D.J. (1977) Sulphur dioxide metabolism in soy-bean, *Glycine max* var. *biloxi*. II. Biochemical distribution of $^{35}SO_2$ products. New Phytol. **79**, 583–592

Hällgren, J.E., Fredricksson, S.A. (1982) Emission of hydrogen sulfide from sulfur dioxide fumigated pine trees. Plant Physiol. **70**, 456–459

Hällgren, J.E., Gezelius, K. (1982) Effects of SO_2 on photosynthesis and ribulose bisphosphate carboxylase in pine tree seedlings. Physiol. Plant. **54**, 153–161

Jones, T., Mansfield, T.A. (1982) The effect of SO_2 on growth and development of seedlings of *Phleum pratense* under different light and temperature environments. Environ. Pollut. **27**, 57–71

Kimmerer, T.W., Kozlowski, T.T. (1981) Stomatal conductance and sulfur uptake of five clones of *Populus tremuloides* exposed to sulfur dioxide. Plant Physiol. **67**, 990–995

Kitajima, M., Butler, W.L. (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim. Biophys. Acta **376**, 105–115

Laisk, A., Pfanz, H., Schramm, M.J., Heber, U. (1988) Sulfur dioxide fluxes into different cellular compartments of leaves photosynthesizing in a polluted atmosphere. I. Computer analysis. Planta **173**, 230–240

Nielsen, J.P. (1938) A study of the action of sulfur dioxide on growing plants. PhD thesis, Stanford University, Stanford, Cal., USA

Olszyk, D.M., Tingey, D.T. (1984) Phytotoxicity of air pollutants. Evidence for the photodetoxification of SO_2 but not O_3. Plant Physiol. **74**, 999–1005

Pfanz, H., Heber, U. (1986) Buffer capacities of leaves, leaf cells, and leaf cell organelles in relation to fluxes of potentially acidic gases. Plant Physiol. **81**, 597–602

Pfanz, H., Martinoia, E., Lange, O.-L., Heber, U. (1987) Flux of SO_2 into leaf cells and cellular acidification by SO_2. Plant Physiol. **85**, 928–933

Rennenberg, H. (1984) The fate of excess sulfur in higher plants. Annu. Rev. Plant Physiol. **35**, 121–153

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

Schröppel-Meier, G., Kaiser, W.M. (1987) Amperometric titration largely overestimates chloride concentrations in chloroplast extracts. Z. Naturforsch. **42c**, 1109–1112

Sekiya, J., Wilson, L.G., Filner, P. (1982) Resistance to injury by sulfur dioxide. Correlation with its reduction to, and emission of, hydrogen sulfide in Cucurbitaceae. Plant Physiol. **70**, 437–441

Shimazaki, K.-I., Ito, K., Kondo, N., Sughara, K. (1984) Reversible inhibition of the photosynthetic water-splitting enzyme system by SO_2-fumigation assayed by chlorophyll fluorescence and EPR signal in vivo. Plant Cell Physiol. **25**, 795–803

Shimazaki, K.-I., Sugahara, K. (1979) Specific inhibition of photosystem II activity in chloroplasts by fumigation of spinach leaves with SO_2. Plant Cell Physiol. **20**, 947–955

Taylor, G.E., Jr., Tingey, D.T. (1983) Sulfur dioxide flux into leaves of *Geranium carolinianum* L. Evidence for a nonstomatal or residual resistance. Plant Physiol. **72**, 237–244

Thomas, M.D., Hendricks, R.H., Bryner, L.C., Hill, G.R. (1944) A study of the sulphur metabolism of wheat, barley and corn using radioactive sulphur. Plant Physiol. **19**, 227–244

Weigl, J., Ziegler, H. (1962) Die räumliche Verteilung von ^{35}S und die Art der markierten Verbindungen in Spinatblättern nach Begasung mit $^{35}SO_2$. Planta **58**, 435–447

Wellburn, A.R. (1985) SO_2 effects on stromal and thylakoid function. In: Sulfur dioxide and vegetation, pp. 133–147, Winner, W.E., Mooney, H.A., Goldstein, R.A., eds. Stanford University Press, Stanford

Wilson, L.G., Bressan, R.A., Filner, P. (1978) Light-dependent emission of hydrogen sulfide from plants. Plant Physiol. **61**, 184–189

Winner, W.E., Mooney, H.A., Goldstein, R.A., eds. (1985) Sulfur dioxide and vegetation. Stanford University Press, Stanford, Cal., USA

Ziegler, I. (1972) The effect of SO_3^- on the activity of ribulose-1,5-diphosphate carboxylase in isolated spinach chloroplasts. Planta **103**, 155–163

Ziegler, I. (1975) The effect of SO_2 pollution on plant metabolism. Residue Rev. **56**, 79–105

Received 6 June; accepted 22 August 1988