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Effects of SO_2 fumigations on photosynthetic CO_2 gas exchange, chlorophyll *a* fluorescence emission and antioxidant enzymes in the lichens *Evernia prunastri* and *Ramalina farinacea*

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The effects of elevated gaseous SO_2 concentrations in the lichens *Evernia prunastri* (L.) Ach. and *Ramalina farinacea* (L.) Ach. were investigated by means of gas exchange, modulated chlorophyll fluorescence analysis and antioxidant enzyme assays. The response to SO_2 of the studied species differed markedly. Net photosynthetic rates were more adversely affected in *E. prunastri* than in *R. farinacea*. In addition, processes dependent on thylakoid membrane integrity such as PSII-mediated electron flow and nonphotochemical quenching were reduced to a greater extent by exposure to SO_2 in *E. prunastri*. Moreover, the ability to reoxidize the quinone pool was lower in this species. Finally, the activity of chloroplastidic and cytoplasmic antioxidant enzymes was decreased in *E. prunastri* in response to fumigations but increased in *R. farinacea*. The results suggest that the ability to process and deal with the SO_2 once it has been absorbed must play a role in determining the sensitivity of these lichen species to this air pollutant.

Introduction

The sensitivity of lichens to SO_2 has been documented in a large number of laboratory and field studies. Exposure to SO_2 has been proven to cause membrane injury (Fields and St. Clair 1984, Garty et al. 1997a,b), ultrastructural alterations (Eversman and Sigal 1987, Modenesi 1993, Balaguer et al. 1997), pigment degradation (Balaguer and Manrique 1991, Garty et al. 1993, Egger et al. 1994) and impairment of photosynthetic function (Moser et al. 1983, Huebert et al. 1985, Coxson 1988, Sanz et al. 1992, Egger et al. 1994, Gries et al. 1995, 1997).

Because the photosynthetic mechanism of lichens is extremely sensitive to SO_2, net photosynthetic rate has been widely used as a response variable. However, the majority of studies have been mostly descriptive in nature, detailing the loss of CO_2 fixation (see references above) but not the underlying causes. The effects of SO_2 on lichens have not been extensively studied so far by chlorophyll (Chl) fluorescence, except for rapid fluorescence induction kinetics and the fluorescence ratio F_v/F_m (Gries et al. 1995). Chlorophyll fluorescence serves as a noninvasive indicator of the status of photosynthetic reaction centers in the chloroplasts of green plants (Krause and Weis 1991, Govindjee 1995) and has been applied in stress detection in lichens (Demmig-Adams et al. 1990, Valladares et al. 1995, Calatayud et al. 1996, 1997). A first objective of the present work was to characterize SO_2 effects on the potential PSII photochemical efficiency, photochemical quenching (fluorescence quenching associated with Q_A reduction), nonphotochemical quenching (a second collective source of quenching caused by diversion of excitation from PSII to PSI, photo-inhibition and heat conversion of absorbed quanta) and the electron transport rate in the species *Ramalina farinacea* and *Evernia prunastri*.

Abbreviations – AP: ascorbate peroxidase; CAT: catalase; Chl: chlorophyll; F_m: maximal fluorescence yield obtained with dark-adapted sample; F'_m: maximal fluorescence yield in illuminated samples; F_o: minimum fluorescence yield in dark-adapted state; F'_o: level of modulated fluorescence during a brief interruption of actinic illumination in the presence of far-red illumination; F_s: chlorophyll fluorescence yield during illumination; F_v: $(F_m - F_o)$ variable fluorescence in dark-adapted thalli; F'_v: $(F'_m - F'_o)$ variable fluorescence in illuminated thalli; GR: glutathione reductase; NP: net photosynthesis; NPQ: nonphotochemical quenching from Stern-Volmer equation; POD: peroxidase; q_p: photochemical fluorescence quenching coefficient; SOD: superoxide dismutase; Φ_{PSII}: quantum efficiency of PSII.

The monitoring of these parameters combined with gas exchange data offers a means to follow changes to the photosynthetic apparatus of these species, whose differential sensitivity to SO_2, has previously been documented (Balaguer and Manrique 1991). It has been proposed that the ability to deal with SO_2 once it has entered the cell, might account for differences in the sensitivity of the species to a particular pollutant (Gries et al. 1997). In this context, antioxidant enzymes must play a crucial role, because they are involved in the regeneration of antioxidants important in the detoxification of reactive oxygen species generated by SO_2 (Foyer et al. 1991, Foyer and Mullineaux 1994). Except for the works of Egger et al. (1994) and Silberstein et al. (1996), there is a paucity of knowledge concerning the effects of pollutants on antioxidant systems in lichens. Therefore, a second objective of the present work was to characterize the activity of cytoplasmic and chloroplastidic antioxidant enzymes following exposure to SO_2 in both species.

Materials and methods

Lichen material

E. prunastri L. (Ach.) and *R. farinacea* L. (Ach.) are fruticose pendulose epiphytic lichen species. The nature of the phycosimbionts in *E. prunastri* is chlorococcoid, whereas in *R. farinacea* it is trebouxioid. Both species occur commonly in Mediterranean sclerophyllous oak forests and were collected in the air-dry state on *Quercus rotundifolia* Lam. at Sierra del Toro (south-western Castellón, Spain). This area is remote from major local industrial and urban sources of pollution and has been used in the past as an unpolluted control site for experiments carried out in the polluted regions (Calatayud et al. 1996). On return to the laboratory, lichen thalli were stored at -18°C until experiments started to prevent deterioration. Prior to the experiments, the lichen thalli were transferred to a climatic chamber and maintained there 4 days (200 μmol m^{-2} s^{-1}, 70% RH, 18/14°C, day/night 12-h photoperiod) to ensure full reactivation. The lichens were sprayed with distilled water once every morning to simulate the daily remoistening cycle.

Fumigation system

The fumigation system is similar to that described by Sanz et al. (1992) except that the fumigations were carried out in the light, using cuvettes fitted with transparent lids. All the connections in contact with SO_2 and the exposure cuvettes were made of teflon, stainless steel or teflon-lined stainless steel. The flow rate was maintained at 1 l min^{-1} with calibrated mass flow controllers (5850 TR-series, Brooks Instruments, Holland). Humidity was regulated to a dew point of 15 ± 0.1°C, using a cold trap (KF 18/2 Walz, Effeltrich, Germany), which resulted in a relative humidity of 97% (± 1%) in the cuvettes measured by a dew point mirror measuring system (TS-2 Walz, Effeltrich, Germany). The temperature was recorded by means of thermopar inserted in the cuvettes. The concentration of SO_2 in nitrogen, from a gas cylinder, was controlled with a micrometering valve and a mass flow controller. Before entering the exposure cuvettes, ambient air and the SO_2 flow were drawn through an injection flask to ensure a uniform gas mixture. The concentration of SO_2 was measured at the outlet of the flask by means of an SO_2 analyser (Model 4108 Dasibi Environ Corp., CA, USA). A metal hallide light source, above and parallel to the cuvettes, provided an even illumination of 500 μmol m^{-2} s^{-1} determined in the position occupied by lichen thalli during experiments.

Fumigation experiments

For the fumigation 1 g air dry material per cuvette was used. Prior to the experiments, the thalli were sprayed with distilled water and maintained 30 min in the climatic chamber. Subsequently, the thalli were shaken to remove extrathalline water and inserted in the cuvettes. Experiments were conducted with 1.7 ppm SO_2 for 5 h daily during 2 consecutive days. Experimental conditions were 15 ± 0.5°C, 97 ± 1% RH and a light intensity of 500 μmol m^{-2} s^{-1}. Precise control of environmental conditions during experiments allowed lichen water status to be maintained within the range of physiological activity. Upon conclusion of fumigation treatments, the lichen material was retrieved from the cuvettes and remoistened prior to fluorescence and gas exchange measurements.

Measurements of Chl *a* fluorescence induction kinetics

Chlorophyll fluorescence at room temperature was measured with a portable pulse-modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) after conclusion of experiments. Samples were kept in the dark for 15 min prior to the measurements. The minimum (dark) fluorescence yield (F_o) was obtained upon excitation of thalli with a weak measuring beam (14 μmol photons m^{-2} s^{-1}) from a light-emitting diode. The maximum fluorescence yield (F_m) was determined with a 600-ms saturating pulse of white light (4000 μmol m^{-2} s^{-1}). Variable fluorescence (F_v) was calculated as $F_m - F_o$. Following 2 min for dark readaptation, actinic white light (260 μmol photons m^{-2} s^{-1}) was switched on, and 600-ms saturating pulses were applied at 60-s intervals for determination of the maximum fluorescence yield during actinic illumination (F'_m), the level of modulated fluorescence during a brief interruption of actinic illumination in the presence of far-red light (F'_o) and the chlorophyll fluorescence yield during actinic illumination (F_s). Calculation of quenching as a result of nonphotochemical dissipation of absorbed light energy (NPQ) was determined at each saturating pulse, according to the equation $NPQ = (F_m - F'_m)/F'_m$ (Bilger and Björkman 1991). The coefficient for photochemical quenching, q_p, was calculated as $(F'_m - F_s)/(F'_m - F'_o)$ (Schreiber et al. 1986). The quantum efficiency of PSII photochemistry, Φ_{PSII}, closely associated with the quantum yield of noncyclic electron transport, was estimated from $(F'_m - F_s)/F'_m$ and the quantum efficiency of excitation capture by oxidized reaction centers of PSII was calculated from F'_v/F'_m (Genty et al. 1989).

Gas exchange measurements

Net photosynthesis (NP) was measured at a light intensity 340 μmol m^{-2} s^{-1} and 20°C, using an LCA-4 type (ADC, Hoddesdon, UK) infrared gas analyzer in small air tight plexiglass cuvettes (of approximately 300 ml). The protocol used is based on the discrete sampling technique (Larson and Kershaw 1975). The cuvettes were flushed with compressed clean ambient air and the opening was sealed. After a 10 min incubation a 1-ml air sample was withdrawn using a syringe and injected into a CO_2-free air stream passing through an infrared gas analyser LCA-4 type (ADC, Hoddesdon, England) set in the differential mode. The peak height was calibrated against known CO_2 concentration by injection of standards. Measurements started with maximally hydrated thalli and proceeded until CO_2 uptake ceased as a result of water deficit. The highest values obtained were considered for each replica. Net photosynthetic response was calculated on a dry weight basis (105°C, 24 h) and is expressed as uptake in mg CO_2 g^{-1} DW h^{-1}.

Enzyme analytical methods

Lichen thalli (1 g dry weight), free from substrate and debris, were homogenized in 10 ml of 100 m*M* potassium phosphate buffer pH 7.5 containing 2 m*M* EDTA and 2% (w/v) PVP. The slurry was centrifuged at 27000 *g* for 20 min. The supernatant was filtered (Millipore, Mitex 0.5 μm) and utilized for enzyme analysis. All operations (until analysis) were carried out at 3–5°C and in a final volume of 3 ml.

Peroxidase (POD, EC 1.11.1.7) was analysed following the protocol described by Astorino et al. (1995) with slight modifications. The assay was performed in a cuvette containing 100 m*M* potassium phosphate buffer (pH 6), 1% (w/v) guaiacol and 6 m*M* H_2O_2. Reaction assays were started by the addition of an appropriate volume of the thallus extract. Activity was determined by the increase in the absorbance at 470 nm as a result of the guaiacol oxidation.

Ascorbate peroxidase (AP, EC 1.11.1.11) was determined by monitoring the decrease in A_{290} for 4 min. The reaction volume contained 100 m*M* potassium phosphate buffer, 0.5 m*M* ascorbate, 0.4 m*M* H_2O_2 and the appropriate volume of thallus extract (Nakano and Asada 1981). Corrections were made for the oxidation of ascorbate in the absence of H_2O_2. The magnitude of the correction ranged between 4 and 6% of the total.

Catalase (CAT, EC 1.11.1.6) was assayed in a reaction mixture containing 100 m*M* phosphate buffer, 6 m*M* H_2O_2 and the appropriate amount of thallus extract. The decomposition of H_2O_2 was monitored at 240 nm as in Cakmak and Marschner (1992).

NADPH-dependent glutathione reductase activity (GR, EC 1.6.4.2) was determined by following the oxidation of NADPH at 340 nm as described by Rao (1992). Corrections were made for NADPH oxidation in the absence of GSSG. The magnitude of the correction ranged between 4 and 6% of the total.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically as described by Beyer and Fridovich (1987). In this assay, 1 U of SOD is defined as the amount required to inhibit the photoreduction of nitroblue tetrazolium by 50%.

Statistical analyses

Pairwise comparison between treatments and controls was performed using Tukey's test calculated at a 95% confidence interval.

Results

SO_2 effects on modulated Chl *a* fluorescence parameters in *E. prunastri* and *R. farinacea*

The photochemical efficiency after dark adaptation (Table 1), estimated by the F_v/F_m ratio, was adversely affected by exposure to SO_2, with percent declines after the second exposure of 46.4 and 8.5% for *E. prunastri* and *R. farinacea*, respectively. The decline in this ratio was mostly as a result of a decrease in the fluorescence parameter F_m. The minimum level of fluorescence F_o was less adversely affected than F_m as a result of SO_2 fumigation.

The changes in modulated fluorescence parameters during actinic illumination in *E. prunastri* and *R. farinacea* are shown in Figs. 1 and 2, respectively. The fraction of open PSII centers, as indicated by q_P (1A and 2A), experienced a slow but gradual increase upon illumination of dark-adapted control samples of both species. This parameter did not experience changes during the experiments in the control samples of the studied species. A single exposure to SO_2 induced a 14.3 and 12.2% decline in q_P in *E. prunastri* and *R. farinacea*, respectively. After the second day of fumigations photochemical quenching was significantly reduced by 40% in *E. prunastri* and by 27% in *R. farinacea*, compared to controls.

Table 1. Changes in dark-adapted F_o, F_m and F_v/F_m in control samples, day 1 [1.7 ppm SO_2 (5 h^{-1})] and day 2 [1.7 ppm SO_2 (10 h^{-1})]. Values are means ± SD of six samples. (* denotes significant values at $P = 0.05$).

Species	Treatment	F_o	F_m	F_v/F_m
E. prunastri	Control	0.373 ± 0.02	1.211 ± 0.05	0.692 ± 0.05
	Day 1	0.371 ± 0.02	0.842 ± 0.04*	0.559 ± 0.05*
	Day 2	0.289 ± 0.03*	0.459 ± 0.04*	0.371 ± 0.10*
R. farinacea	Control	0.390 ± 0.04	1.190 ± 0.06	0.672 ± 0.06
	Day 1	0.320 ± 0.03	0.918 ± 0.04	0.651 ± 0.06
	Day 2	0.302 ± 0.03	0.784 ± 0.04*	0.615 ± 0.06

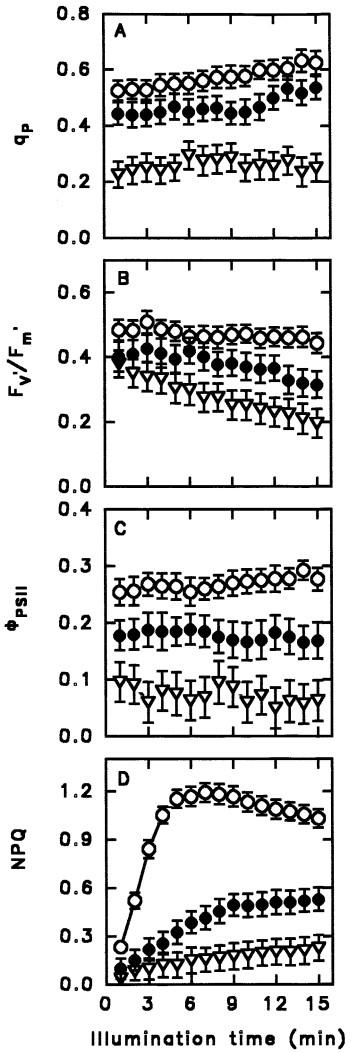

Fig. 1. Effects of SO_2 fumigations [1.7 ppm (5 h)$^{-1}$] on the kinetics of fluorescence parameters q_p (A), F_v'/F_m' (B), Φ_{PSII} (C) and NPQ (D) in thallus from *E. prunastri*. Data are mean values and SD for n = 6. Symbols denote control (○), one fumigation event (●) and two fumigation events (▽).

The excitation capture efficiency of PSII, F_v'/F_m' (Figs. 1B and 2B), declined from the onset of actinic illumination in control samples of both species. This parameter did not undergo changes as a result of experimental manipulation in control samples. Exposure to SO_2 did not alter the induction kinetics of F_v'/F_m', although it induced a significant decline of the parameter in *E. prunastri* and a nonsignificant decrease in *R. farinacea* after the first exposure. After the second exposure significant effects on this parameter were recorded in both species, although F_v'/F_m' was more adversely affected in *E. prunastri*, which displayed the greatest decline (56%) compared to *R. farinacea* (20%).

The actual PSII photochemical efficiency (Φ_{PSII}) (Figs. 1C and 2C) attained almost steady-state values very rapidly upon illumination of dark-adapted control samples of *E. prunastri*, whereas it increased continuously throughout the kinetic in *R. farinacea*. This parameter did not exhibit changes during the experiments in control samples. Exposure to SO_2 resulted in a decline of Φ_{PSII} from day 1 in both species, although significant differences were only found in *E. prunastri*. The decline was more conspicuous after the second day of fumigation. Again, Φ_{PSII} was less affected in *R. farinacea* compared to *E. prunastri* with significant decreases of 25 and 77.2%, respectively.

Immediately following actinic illumination NPQ (Figs. 1D and 2D) experienced a quick rise and reached maximum values within 6 min in control samples of *E. prunastri*. Thereafter, NPQ progressively declined until it reached steady-state values after approximately 12 min of actinic illumination. Control samples of *R. farinacea* exhibited lower rates of NPQ development and did not reach steady-state values in the time scale used in experiments. Exposure to SO_2 significantly decreased the rates of NPQ formation in both lichens as well as the maximal values after a single 5-h fumigation. Subsequent exposure practically abolished the capacity for NPQ formation in *E. prunastri* (decline of 78%)

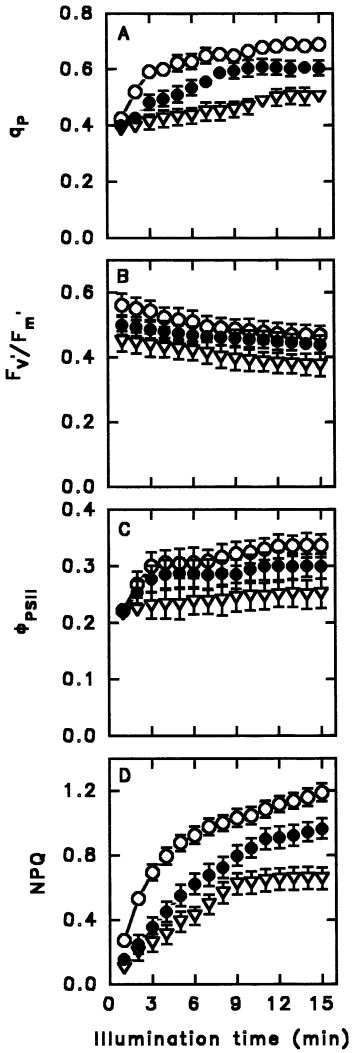

Fig. 2. Effects of SO_2 exposure [1.7 ppm (5 h)$^{-1}$] on the kinetics of fluorescence parameters q_p (A), F_v'/F_m' (B), Φ_{PSII} (C) and NPQ (D) in thallus from *R. farinacea*. Data are mean values and SD for n = 6. Symbols denote control (○), 1-day exposure (●) and 2-day exposure (▽) to SO_2.

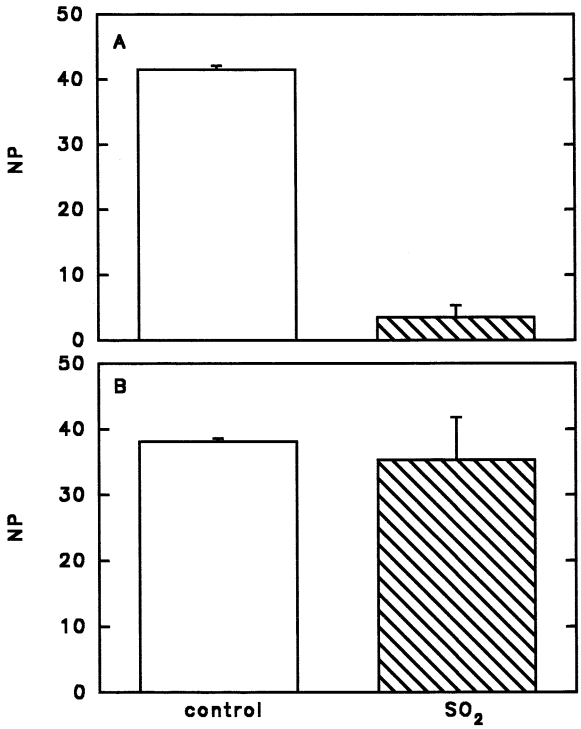

Fig. 3. Changes in net photosynthesis (NP, mg CO_2 g^{-1} DW h^{-1}) after 2 days fumigation with SO_2 in *E. prunastri* (A) and *R. farinacea* (B). Data are mean values and SD for n = 3.

and caused a further decline of the maximal values in *R. farinacea* (decline of 44.8%). Thus, as in the previously described parameters, NPQ was more severely affected by fumigations in *E. prunastri* than in *R. farinacea*.

Gas exchange

Fig. 3A shows NP data of control and fumigated material of *E. prunastri*. The detrimental effect of exposure to 1.7 ppm SO_2 after 2 days can be seen in the significant 91.5% decrease in NP of fumigated samples compared to controls. Nevertheless, *E. prunastri* was able to maintain some carbon fixation activity above dark respiration. *R. farinacea* (Fig. 3B) was more resistant to SO_2 and did not experience significant changes in NP as a result of fumigations.

SO_2-induced changes in antioxidant enzyme activities

All the antioxidant enzyme activities assayed were significantly decreased by exposure to SO_2 in *E. prunastri* (Fig. 4) except for CAT and GR that displayed nonsignificant declines. Contrarily, SO_2 fumigation significantly stimulated the activity of CAT, GR and SOD and induced nonsignificant increases of POD and AP at a significant level of 0.05 in *R. farinacea*.

Discussion

High SO_2 concentrations for short exposure times affected photosynthesis performance more adversely in *E. prunastri* compared to *R. farinacea*. This could be seen as higher percent decreases in the maximal PSII photochemical efficiency, the PSII-mediated electron flow, the capacity for photoprotective processes and carbon fixation rates, in addition to higher degree of closure of PSII centers in *E. prunastri* compared to *R. farinacea*, at a given light intensity.

A decline in the ratio F_v/F_m can result from an increase in protective nonradiative energy dissipation, to photodamage of the PSII center, or both (Osmond 1994). Inasmuch as NPQ is believed to indicate the capacity for photoprotective processes (Osmond et al. 1993), the results suggest that the decline in F_v/F_m in fumigated thalli was attributable to severe stress to PSII, because NPQ was adversely affected by SO_2. The low F_v/F_m values observed were mostly as a result of a decline in the level of F_m, which indicated that the capacity for Q_A reduction of the living photobiont cells was impaired by exposure to SO_2. In addition, the lower q_P levels exhibited by fumigated thalli compared to controls

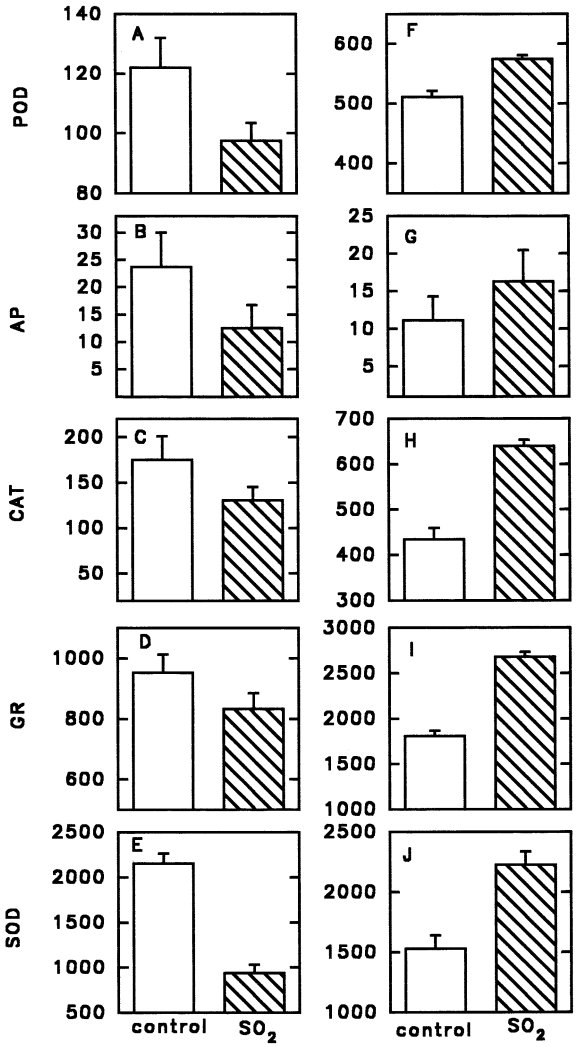

Fig. 4. Changes in the antioxidant enzyme activities after a 2-day exposure to SO_2 (5 h day^{-1}, 1.7 ppm SO_2) in *E. prunastri* (A–E) and *R. farinacea* (F–J): POD, CAT and GR, nmol g^{-1} DW min^{-1}; AP μmol g^{-1} DW min^{-1}; SOD, U g^{-1} DW. Each data point represents the mean of three samples ± SD.

indicated that SO_2 exposure decreased the capacity for reoxidizing Q_A during actinic illumination. This means that SO_2 increased excitation pressure on PSII and contributed to the closure of PSII reaction centers. Closed PSII centers have Q_A fully reduced and deny the possibility of electron transport to PSI and beyond (Seaton and Walker 1990). In accordance with this, the PSII quantum yield (Φ_{PSII}), closely correlated with the quantum yield of noncyclic electron transport (Genty et al. 1989), was markedly reduced by SO_2. It seems reasonable that the rate of production of ATP and reducing power in the form of NADPH might be considerably reduced in thalli exposed to SO_2. In this respect, Kardish et al. (1987) found an inverse correlation between ATP contents and field pollution in *Ramalina duriaei*.

Dissipation of excess absorbed energy requires that the level of excess can be accurately sensed to the light harvesting complex. It is accepted that the level of thylakoid energization is the light sensor (Mohanty and Yamamoto 1995, Ruban and Horton 1995). Thus, dissipative processes are highly dependent on membrane integrity. In fact, NPQ results from the interaction of lumen acidification (Walters and Horton 1991), proton domains within thylakoid membranes (Park et al. 1996) and de-epoxidised xanthophylls (Krause and Weis 1991, Pfündel and Bilger 1994, Demmig-Adams et al. 1995). It is possible that the lower rates of noncyclic electron transport exhibited by fumigated thalli were associated with a decreased ability to establish a proton gradient across thylakoid membranes. However, this could also be as a result of increased membrane leakiness caused by free radical attack. Increases of electrolyte leakage in lichens have been measured after exposure to SO_2 in the laboratory (Pearson and Henriksson 1981, Fields and St. Clair 1984) or in the field (Pearson and Rodgers 1982, Egger et al. 1994, Garty et al. 1997a,b). In any case, a lower proton gradient would interfere with xanthophyll deepoxidation and ultimately with NPQ formation.

Exposure of *E. prunastri* to SO_2 induced a marked reduction of photosynthetic rates. The negative effects of SO_2 on carbon fixation have been widely described in numerous lichen species (Moser et al. 1983, Fields and St. Clair 1984, Huebert et al. 1985, Sanz et al. 1992, Gries et al. 1995, 1997). Considering the adverse effects of SO_2 on the electron transport rate, it is possible that the inhibition of photosynthesis could be a result, at least in part, of a decreased availability of ATP and reducing power. However, the possibility of direct effects of SO_2 on Calvin cycle enzymes (Ziegler 1977) and chloroplast ultrastructure (Holapainen and Kärelampi 1984, Eversman and Sigal 1987) must also be considered. Fluorescence parameters were more sensitive than gas exchange as a response variable to SO_2 in *R. farinacea* and, therefore, should be considered at least as good a means of detecting SO_2 stress in lichens.

The low sensitivity to SO_2 of *R. farinacea* and of its isolated lichen phycobiont has been established in the works of Baddeley et al. (1972) and Marti (1983). In addition, Balaguer and Manrique (1991) have reported on the resistance to SO_2 of *R. farinacea* compared to *E. prunastri*. More recently, Sanz et al. (1992) conducted short-term experiments that included *E. prunastri* and a different *Ramalina* (*R. fraxinea*). Again, *E. prunastri* was more sensitive to SO_2. The results conform with the postulated higher resistance of *R. farinacea* compared with *E. prunastri*. *R. farinacea* must be better protected against SO_2-induced oxidative stress as suggested by the less adversely affected photosynthetic parameters studied. It has recently been proven by Gries et al. (1997) that differences in the sensitivity to SO_2 in several species of nongelatinous lichens cannot be explained in terms of SO_2 uptake rate; the authors suggest that the way a lichen deals with SO_2 once absorbed into the cells must determine the degree of sensitivity or resistance to a particular pollutant. The fact that *R. farinacea* enhanced the activity of antioxidant enzymes following exposure to the pollutant must confer some protection against SO_2-induced formation of oxygen free radicals. In fact, scavenger enzymes are more effective in preventing damage by air pollution than other agents or processes (Perl et al. 1993, Sen-Gupta et al. 1993). An stimulation of the activity of POD and SOD by exposure to pollutants has been documented in *Xanthoria parietina* and *R. farinacea*, respectively (Egger et al. 1994, Silberstein et al. 1996). On the other hand, the activity of all the antioxidant enzymes assayed was decreased by SO_2 exposure in *E. prunastri*. Similarly, Silberstein et al. (1996) found declines in the activity of SOD, POD and CAT in pollution sensitive *R. duriaei*. It is possible that pollutant accumulation exceeded the rate of detoxification processes in *E. prunastri* and that this resulted in H_2O_2 accumulation, a product of the oxidation of SO_2 that has been proven to inhibit AP, GR and Calvin cycle enzymes (Hippeli and Elstner 1996).

To summarize, the present study provides some clues to the mechanisms underlying the differential pollution sensitivity between these fruticose lichen species. In any case, many other mechanisms must be involved in determining the resistance of lichens to air pollution as suggested by Silberstein et al. (1996).

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