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Is Sulfur Accumulation in Sulfur Dioxide-Exposed Plants Related to Biomass Reduction?

G. Lorenzini,[1] A. Panicucci[1,2]

[1]Università, Dipartimento CDSL—Sez. Patologia Vegetale, 56010 San Piero a Grado, Pisa, Italy
[2]Scuola Superiore Studi Universitari e Perfezionamento Sant'Anna, 56124 Pisa, Italy

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Sulfur (S) is an abundant element, there being an average of 520 ppm in the Earth's crust. It is a major plant nutrient and in its various forms is necessary in the metabolism of plants. Normally, S is taken up from soil in the sulphate form and assimilated into various compounds usually after being chemically reduced. Sulfur dioxide (SO_2) absorbed from the air can also supply S for nutrition (Rennenberg 1984). Of all plant organs, the leaves have the highest S content.

SO_2 is an air contaminant arising from various anthropogenic activities, especially as a consequence of burning fossil fuels; there are also significant natural sources. Background levels in the low atmosphere are a few ppb SO_2 (1 ppb = $1*10^{-9}$ parts in vol.). Polluted areas experience levels of some tens of ppb, also for very long periods (Kellogg *et al.* 1972). Plants exposed to excessive SO_2 levels may undergo disturbances, which may, or may, be associated with visible injury and biomass production perturbation.

Two questions may be raised: (*i*) is S accumulation correlated with other responses, such as resistance or tolerance to SO_2? and (*ii*) can S accumulation be a useful tool for diagnostic purposes when SO_2 is suspected of being responsible for altered plants? Unfortunately, these questions are not easily answered, as normal, background S content in plants is rather high and quite variable. In foliage it varies from 0.1% to 1.7% on a dry matter basis (Linzon *et al.* 1979), but wider ranges have been reported (Thomas *et al.* 1950). Seasonal fluctuations are well known (Lauenroth and Preston 1984). It takes a substantial build-up before any increase is significant. Accumulation may also be influenced by the level of S nutrition, as there is full compatibility between the two forms of supply (root for sulphates and leaves for SO_2). To reduce the possible confounding influences of environmental conditions or developmental phases, plants to be compared must be exposed simultaneously, under the same conditions. Therefore, experiments under controlled conditions are essential in order to establish sound correlations between S accumulation and other parameters of intact plants. Many of the experiments performed in the past used rather high SO_2 concentrations and short duration; under such conditions impressive data may result, but the extrapolation to 'natural' situations is difficult. Results here reported refer to experiments carried out in order to get more information on some aspects of sulphur accumulation in plants under realistic, nonmarking, SO_2 pollution conditions, in connection with biomass production.

Correspondence to: G. Lorenzini

MATERIALS AND METHODS

Seed was put in containers filled with an organic compost and plants raised in a cold greenhouse. The material adopted is listed in Table 1. Treatments with SO_2 were performed in fumigation chambers, blown with charcoal-filtered air, in order to provide two complete air changes per minute and maintain a turbulence to cause sufficient leaf flutter. SO_2 was provided by a cylinder and flux checked by electron thermal mass flow controllers, driven by a microprocessor-controlled module (Lorenzini and Panattoni 1986). Air inside the chambers was continually sampled and analyzed by an automatic analyzer operating on the fluorescence method (Monitor Labs 8850). Temperature and light/dark periods were determined by natural conditions. Plants were automatically irrigated via a bed of perlite. The experimental protocol is summarized in Table 1. Charcoal-filtered air controls were included in all the trials. Sample size of control was the same as treatments.

Destructive analyses included dry weight (d. w.) of leaves, stem and roots. Total foliar S content of each sample was determined in triplicate using an automatic analyzer (LECO SC132) (Jones and Isaac 1972). Total S and biometric values are expressed on a d. w. basis. A paired t-test was performed for experiments carried out with a single SO_2 concentration *vs* controls. Data from experiments involving a gradient of SO_2 levels were subjected to regression analysis and linear or polynomial equations were calculated on the basis of the significance of the F ratio. Significance of differences are indicated as: NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Biomass is expressed in g, SO_2 concentrations in ppb.

RESULTS AND DISCUSSION

An aspect common to all the trials was the absence of any visible signs of leaf injury on plants treated with SO_2, with the only exception of a light chlorosis evident on wheat Mec.

At the end of the experiment all the cultivars of wheat exhibited large increases in total S concentration (Tab. 2). In Aurelio it ranged from a 0.325% (control) to 1.485% (SO_2-treated); in Chiarano the figures are 0.338% (control) and 1.260 (SO_2); in Manital S passes from 0.370% to 1.400%, and in Mec from 0.393% to 1.850%. Comparing these results with growth and biomass production data (Lorenzini *et al.* 1990), we observe that plants of cv. Mec treated with SO_2 suffer a significant reduction in biomass in connection with the most relevant S accumulation; Chiarano shows a large S accumulation even if biomass is identical to controls. Aurelio and Manital show an intermediate response.

S increases in all SO_2-fumigated barley plants and their total dry weight is significantly reduced by the treatment (Tab. 2). In Panda, the S increase (0.297% *vs* 0.372%) is associated with a 62.4% reduction in biomass; in Gerbel S passes from 0.368% to 0.621% and biomass is reduced by 45.5%; in Barberousse, S raises from 0.414% to 0.624% while biomass is reduced by 49.3% (Lorenzini *et al.* 1990).

A non significant biomass reduction in Italian ryegrass is associated with a significant increase in S content (from 0.225% to 0.846%); a 38.5% depletion in d. w. is coupled with a large S build-up (0.279% *vs* 0.811%) in perennial ryegrass (Lorenzini *et al.* 1990) (Tab. 2).

Table 1. Plant species and cultivar, age of plants at the beginning of the experiment, duration of exposure, concentrations of sulphur dioxide and number of plants for each cultivar for each experiment.

Expt No	Plants, cultivars	Age (days)	Length of fumigation (days)	$[SO_2]$ (ppb)	*n*
1	Wheat *Triticum aestivum* cvs Aurelio, Chiarano Manital, Mec	19	37	74	5
2	Barley *Hordeum vulgare* cvs Barberousse, Gerbel, Panda	17	60	90	9
3	Ryegrasses *Lolium perenne* cv. Tetralite *Lolium multiflorum* cv. Turbo Pajbjerg	13	117	68	15
4	Maize *Zea mays* cvs Lenor G4441, Pardus G4480	10	28	79	10
5	Broad bean *Vicia faba* cv Aquadulce	15	41	40,70,130	10
6	Barley *Hordeum vulgare* cvs Barberousse, Gerbel, Panda	20	49	47,81,117	9
7	Maize *Zea mays* cvs Lenor G4441, Pardus G4480	11	19, 40	48,68,128	10
8	Spinach *Spinacia oleracaea* cvs Matador, Riccio	25	90	43,81,118	10

Total d. w. reduction of 17.2% is associated to a 15.6% S increase in maize Pardus (0.244% *vs* 0.281%); in Lenor S increases from 0.172% to 0.476% but biomass is unaffected by the treatement (Panicucci 1988) (Tab. 2).

Table 2. Effects of SO_2 treatments on the total S foliar concentration and biomass production of some cultivars of different agricultural plants.

Plants, cultivars	Increase in S content %	P	Reduction in biomass %	P
Wheat				
cv Aurelio	355	*	35.4	NS[a]
cv Chiarano	270	*	-0.6	NS
cv Manital	270	*	23.5	NS
cv Mec	370	**	44.0	*
Barley				
cv Barberousse	51	*	49.3	**
cv Gerbel	69	*	45.5	***
cv Panda	25	*	62.4	***
Perennial ryegrass				
cv Tetralite	191	**	38.5	*
Italian ryegrass				
cv Turbo Pajbjerg	276	**	24.7	NS
Maize				
cv Lenor	177	*	-0.3	NS
cv Pardus	16	NS	17.2	NS

[a] Significance of P is: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

S in broad bean passes from 0.253% (filtered air) to 0.506% (40 ppb SO_2) (100% increase) (*), to 0.812% (70 ppb SO_2) (+220%) (**), to 0.845% (130 ppb SO2) (+234%) (**) (Tab. 3); similarly, d. w. does not significantly differ from controls (Farina 1987). This implies that relevant S content increases are not related to biomass production.

The following regression equations indicated the relationship between SO_2 concentrations and total d. w. for barley Panda, Y=1.86-0.001X (***), Gerbel, Y=1.65-0.009X (***) and Barberousse, Y=1.3-0.007X (***) (Lorenzini *et al.* 1990; Schenone and Lorenzini 1992). S accumulation as a function of SO_2 is represented in Table 3. Fig. 1 (*upper*) shows that in Barberousse and Gerbel there is no significant connection between S accumulation and biomass reduction, while in Panda the relationship is expressed by a 2nd order function.

Linearity between S accumulation and SO_2 concentration, for both the cultivars of maize at both the sampling times (19 and 40 days) is reported in Table 3. D. w. did not turn out to be significantly influenced by the exposure to the gas, apart from the case of Lenor at 40 days of treatment (Y=4.02-0.01X [***]); in this case at 128 ppb SO_2 we observed a 29.8% depletion in dry matter production, associated to a 100% increase in S content (Panicucci, 1988; Lorenzini *et al.* 1990). As shown in Fig. 1 (*middle*), the only significant relationship between S accumulation and biomass is related to Lenor and is a 2nd order function.

Table 3. Regression equations for the effects of a range of SO_2 concentrations (0 to 130 ppb) on the total S foliar content on some cultivars of different agricultural plants.

Equation	*n*	F	r
Broad bean cv Aquadulce			
$Y = 0.30 + 0.005X$ [(a)]	24	** [(b)]	0.91
Barley cv Panda			
$Y = 0.52 + 0.004X$	12	***	0.86
Barley cv Gerbel			
$Y = 0.42 + 0.005X$	12	**	0.98
Barley cv Barberousse			
$Y = 0.34 + 0.01X - 0.0001X^2$	12	**	0.96
Maize cv Pardus			
$Y_{19days} = 0.25 + 0.003X$	12	***	0.97
$Y_{40days} = 0.18 + 0.005X$	12	***	0.95
Maize cv Lenor			
$Y_{19days} = 0.42 + 0.003X$	12	***	0.99
$Y_{40days} = 0.24 + 0.002X$	12	*	0.87
Spinach cv Riccio			
$Y = 0.22 + 0.010X$	12	**	0.89
Spinach cv Matador			
$Y = 0.33 + 0.004X$	12	***	0.94

[(a)] $X = [SO_2]$, ppb; Y = S concentration, % d.w.
[(b)] Significance of F is: *, $F<0.05$; **, $F<0.01$; ***, $F<0.001$.

A linear function was found between S accumulation and SO_2 levels for both the spinach cultivars (Table 3). In Riccio we observed a reduction of total d. m. as a function of SO_2: $Y=5.80-0.013X$ (*), while in Matador this was not found. As a consequence, in Matador there is no correlation between S accumulation and biomass reduction, while in Riccio the relationship is curvilinear (Fig. 1, *lower*).

SO_2 is known to be rapidly absorbed by leaves and over a wide range its flux is nearly linear with concentration and duration of the exposure. Absorbed SO_2 is rapidly hydrated and forms phytotoxic sulphite and bisulphite (Thomas *et al.* 1950); possible fates of SO_2-derived sulphite include: (*i*) an 'expulsion route', based on a photoreductive detoxification to H_2S, which is emitted into the atmosphere, and (*ii*) a 'storage route', based on oxidation to sulphate (Alscher 1984). According to this second pathway, sulphate content in SO_2-exposed plants tends to rise, especially in the cell vacuole. The accumulated sulphate may be translocated and metabolically converted to organic forms. Plants may modulate

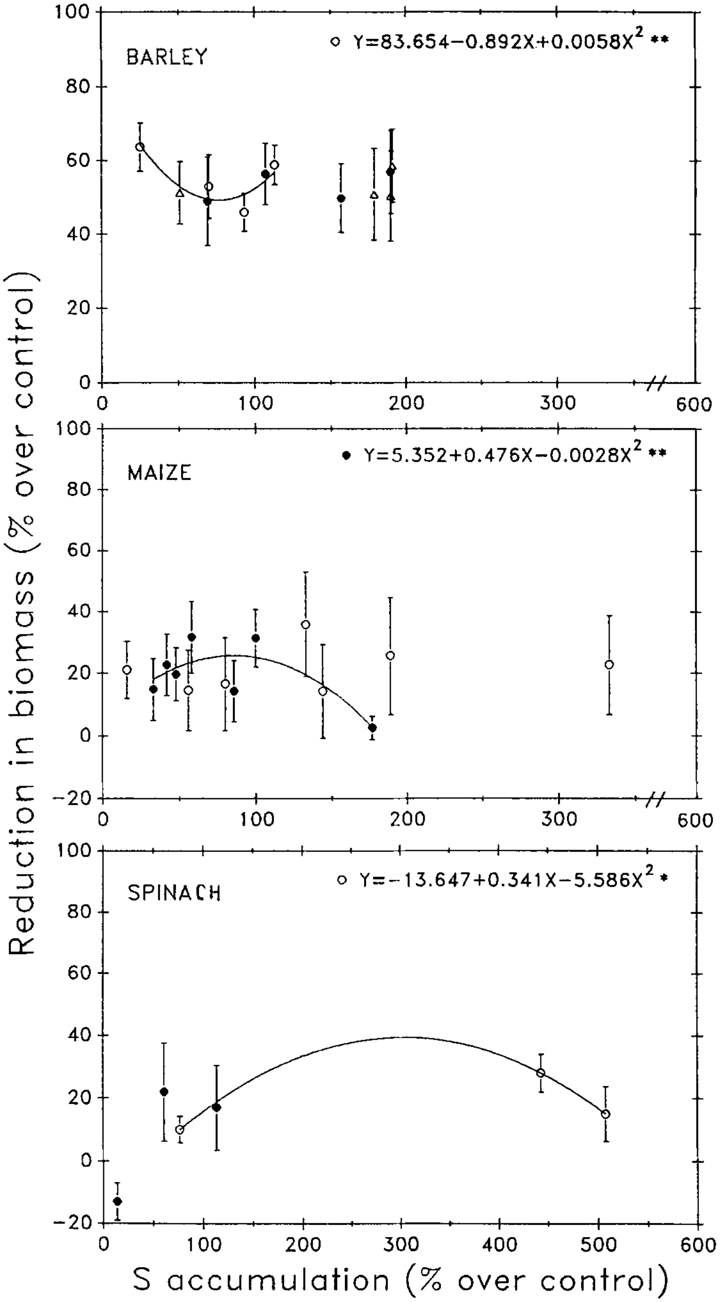

Figure 1. Correlations between total S accumulation in leaves and biomass production in barley (○: cv. Panda;●: cv Gerbel;△: cv. Barberousse), maize (○: cv. Pardus;●: cv. Lenor), and spinach (○: cv. Riccio;●: cv. Matador). Each point represents the average of 7 data. Bars represent standard deviations of the means.

S build-up to a certain extent, and limit sulphate root absorption when in the presence of sufficient SO_2 levels (Rennenberg 1984). Factors which may affect S levels in plants include translocation, dilution by new growth, losses through leaching, gaseous emission or exudation through the roots. Tingey and Olszyk (1985) reviewed factors related to intraspecific variability in responses to SO_2. As far as S enrichment is concerned, the picture that emerges is quite puzzling and comparison is not easy, due also to the great variability of experimental conditions and of parameters taken into account to quantify plant response. There are species in which no correlation between S accumulation and resistance was found; in others there was an inverse correlation, but in some others the opposite was true (more S, more resistance).

In this work total S of foliar tissues following long-term SO_2 exposure has been determined in herbaceous plants grown in a substrate provided with adequate S levels. Our data seem to support the lack of a general trend between S enrichment and plant response. S accumulation varied with the level of SO_2, and also between species and cultivars. The concentrations of S in leaves were strongly, directly and linearly correlated with the SO_2 concentrations, according to previous papers (Cowling and Koziol 1978; Lauenroth *et al.* 1979; Bytnerowicz *et al.* 1987). Unique exception was barley Barberousse, which showed a correlation with a 2nd order polynomial, i.e. a sharp increase of S content associated with the lowest SO_2 level.

S was accumulated in large quantities before visible symptoms occurred. In spinach Riccio, S content can increase to even 6 times normal without any leaf visible symptom; under such conditions biomass was reduced by a mere 15%. A wide range of responses of wheat was observed: in Mec a striking reduction in biomass was associated with a large increase in S content over the controls, but Chiarano treated with SO_2 did not differ from the controls even if plants accumulated much more S than the controls. In broad bean relevant additional S content was not associated with any significant variation in biomass. In two out of three cultivars of barley, S accumulation was not twinned to significant biomass variations; in the other one, the lower S content increase was associated with the highest growth reduction. In maize Lenor, S levels were not linearly correlated to biomass reduction. The two cultivars of spinach responded in a different way to increasing concentrations of SO_2, with the sensitive Riccio accumulating far more S than the resistant Matador.

An increase in the S concentration of foliar tissues can serve as an indication of SO_2 exposure under conditions that soil S supply is constant, but its physiological importance is uncertain. It appears that there is no direct relation between the level of S accumulation and the interference of SO_2 - in terms of biomass production - in most of the plants tested. Therefore, we can concur with De Kok (1989) that '*it is questionable whether a disturbed regulation of S assimilation is a significant factor responsible for the phytotoxicity of SO_2*'.

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