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Effects of sulfur dioxide on growth, gas exchange rate and leaf sulfur content of masson pine seedlings

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Abstract——The effects of sulfur dioxide (SO_2) on the dry weight growth gas exchange rate and leaf sulfur content of masson pine (*Pinus massoniana* Lamb.) were investigated. The results obtained in this study show that the dry weight growth and net photosynthetic rate of masson pine seedlings are reduced by exposure to SO_2 at $\geqslant$ 100 ppb. From these results, one of the main causes in the dieback of masson pine forest reported in Chongqing, China may be relatively high concentrations of atmospheric SO_2 in the relevant area.

Keywords: sulfur dioxide (SO_2); *Pinus massoniana* Lamb.; dry weight growth; gas exchange rate; leaf sulfur content.

1 Introduction

In China, a main source of energy for industrial use is coal with relatively high contents of sulfur. Large fractions of sulfur dioxide (SO_2) are emitted in China originated from coal burning power stations without a desulfurizer. Therefore, relatively high concentrations of atmospheric SO_2, such as those above 100 ppb in annual mean, is recorded at Chongqing and Guiyang (Sakamoto, 1990; Xu, 1992).

Masson pine (*Pinus massoniana* Lamb.) has been one of the main forested trees in the southwest areas of China (Agriculture College of Anhui, 1980). However, since the beginning of 1980s, the trees have been severely damaged in the suburban areas of Chongqing (Yu, 1979). Chinese environmental scientists suggested that relatively high concentrations of atmospheric SO_2 may be one of the main causes in the dieback of the trees (Sakamota, 1990; Xu, 1992). However, causal analyses of the effects of SO_2 on the dry weight growth and physiological functions such as photosynthesis and transpiration of the trees have not been done under field or experimental conditions.

In the present study, we investigated the growth responses of masson pine seedlings to SO_2. We also investigated the effects of SO_2 on the gas exchange rate, chlorophyll content and leaf sulfur content of the seedlings to clarify the factors relating to the SO_2-induced reduction in the dry weight growth.

2 Materials and methods

2.1 Plant material

Seeds of masson pine (*Pinus massoniana* Lamb.) were collected in 1991 from Sichuan Province, China, and were germinated on a piece of wet sheet in an air-conditioned room at 25℃. Three days after germination, the seeds were sown in a 300 ml plastic pot filled with high humic. Andosol collected from an experimental field of the university (Fuchu, Tokyo). Then, the seedlings were grown for 60 days in a naturally-lit growth cabinet at day/night air temperature of 25/18℃ and relative humidity (R.H.) of 70±10% before starting the repeated exposure to SO_2 in an exposure chamber mentioned in the next section.

2.2 Exposure chamber

A pair of rectangular chambers (70cm×70cm, 110cm in height) was made of transparent acrylic boards. An artificial illumination system placed over the chambers consisted of 14 fluorescent twin lamps (Matsushita Co. Ltd., FPR96EX-N/A). The reflection boards were installed at the outside ceiling of the chambers. An air conditioner (Hitachi Co. Ltd., RAV-1435) was used to regulate air temperature in the chambers. The charcoal-filtered air was introduced into the chambers by a ventilator to exchange the air inside the chambers 2.6 times per minute. An air-stirring fan is installed on the base of the chambers to adjust a wind velocity to 0.5 m/s.

2.3 Growth experiment

Thirty minutes before starting the daily exposure to SO_2 (8:30), the seedlings were transferred into the exposure chamber mentioned above from the naturally-lit growth cabinet. The seedlings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours

(9:00 – 17:00) a day in the exposure chamber. The exposure of the seedlings to SO_2 was conducted 23 days during an exposure period of 30 days from the 60th to the 90th day after sowing. The exposure of the seedlings to SO_2 at 100, 300 and 500 ppb was conducted in May, June, August 1992, respectively and the exposure at 50 ppb in May 1993. The atmospheric concentration of SO_2 in the exposure chamber was continuously monitored during the daily exposure to SO_2 with an UV-fluorescence sulfur dioxide automatic analyzer (Kimoto Co. Ltd., Model-365P). The SO_2 gas with a purity of >99.9% was diluted with charcoal-filtered air to obtain the given concentration of SO_2 in the chamber. Thirty minutes after the end of daily exposure to SO_2 (17:30), all the seedlings were returned to the naturally-lit growth cabinet, where they were kept until the next exposure to SO_2. The observation of SO_2-induced visible foliar injury was performed during and just after the daily exposure to SO_2. The control plants were placed in another acrylic chamber under the same environmental conditions except for the SO_2 treatment. The concentration of SO_2 in the control chamber was below 5 ppb. After the exposure period of 30 days, both control and SO_2-exposed seedlings were harvested for measuring dry weight of each plant organ.

2.4 Measurement of net photosynthesis and dark respiration

To determine net photosynthesis and dark respiration of the seedlings just after the exposure period of SO_2 for 30 days, the aerial parts of the plants (ca. 6cm in height) were enclosed in an assimilation chamber (13cm in diameter, 13.5cm in height). The air inside of the chamber was circulated by a stirring fan to reduce the boundary resistance to CO_2 absorption on leaf surface. The measurements of net photosynthetic rate and dark respiration rate were performed under an artificial illumination of 600 $\mu mol/(m^2 \cdot s)$ PAR and in the dark, respectively. During the measurements, temperature and R.H. of air in the assimilation chamber were maintained at 25±1 ℃ and 65±5%, respectively. The rates were determined by measuring the difference in CO_2 concentrations of air between the plant chamber and the blank chamber using an infrared CO_2 gas analyzer (Fuji Electric Co. Ltd., Japan).

2.5 Measurement of transpiration rate

To determine transpiration rate, a cultivation pot of the seedling was enclosed in a plastic bag just after the exposure period of SO_2 for 30 days. The weight of the pot with the plant was measured repeatedly at 30 min intervals for 4 hours in an air-conditioned room at 25±1 ℃ and 65% R.H. under an artificial illumination of 370 $\mu mol/(m^2 \cdot s)$ PAR. The transpiration rate was determined from the weight reduction per unit time and per unit leaf dry weight.

2.6 Measurements of chlorophyll content and leaf sulfur content

Just after the exposure period of SO_2 for 30 days, needle leaves of the seedlings were sampled, and then chlorophyll was extracted from the leaves with 99% (v/v) ethanol for 48 hours in the dark. The absorbances at 649nm (A_{649}) and 665 nm (A_{665}) of the extract solution were measured with an spectrophotometer (Hitachi Co. Ltd., Model 100−30). The chlorophyll content (Chl. a+b) was determined by the following equation:

$$\text{Chl.a}+\text{b}(\text{mg. L}^{-1})=6.10\ (A_{665})+20.04\ (A_{649}).$$

The leaves of the seedlings were dried in an oven at 80 ℃ for 3 days, and were ground. The leaf sulfur content was determined with a combustion sulfur content analyzer (Horiba Co. Ltd., EMIA-120).

3 Results

Visible injuries of yellow spot were observed on the leaves of masson pine seedlings on the 29th day and on the 20th day after starting the exposure to SO_2 at 300 and 500 ppb, respectively. The visible foliar injuries were divided into two types necrosis at leaf tips and stripped necrosis(ca. 1−5mm in width) at the middle part of leaves. The latter type was more frequently seen that the former one. The boundaries of these visible injuries were clearly recognized. On the other hand, no visible foliar injury was observed in the seedlings exposed to SO_2 at 50 and 100 ppb during and after the exposure period of 30 days.

The effects of SO_2 on the dry weight growth of the seedlings are shown in Table 1. Total dry weight of the seedlings was significantly reduced by the repeated exposure to SO_2 at 100, 300 and 500 ppb. The leaf dry weight of the seedlings exposed to SO_2 at 100, 300 and 500 ppb were significantly reducted to 85%, 83% and 76% of the control values, respectively. The dry weights of trunk and roots were also reduced by the exposure to SO_2 , but the results were not statistically significant. On the other hand, the dry weight growth of the seedlings was not significantly affected by the exposure to SO_2 at 50 ppb.

The results of plant growth analysis are given in Fig.1. The relative growth rates in plant dry weight (RGR) of the seedlings exposed to SO_2 at 100, 300 and 500 ppb were reduced to 89%, 89% and 71% of the control values, respectively. Also, the net assimilation rates (NAR) of the seedlings exposed to SO_2 at 100, 300 and 500 were reduced compared with the control values. However, there were no distinct effects of SO_2 on the leaf weight ratio (LWR) of the seedlings. The RGR, NAR, and LWR of the seedlings were not affected by the exposure to SO_2 at 50ppb.

Table 1 Effects of SO_2 on the dry weight of masson pine seedlings. The seedlings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours (9:00 − 17:00) a day, 23 days during the exposure period of 30 days from the 60th to 90th day after sowing. Each value is the mean of 10 determinations

Treatment	Leaf, mg	Trunk, mg	Root, mg	Total, mg
Control	83	26	50	159
50 ppb SO_2	88	26	49	163
% of control	106	100	98	103
Control	76	17	43	136
100 ppb SO_2	65*	16	37	118*
% of control	86	94	86	86
Control	80	15	43	138
300 ppb SO_2	67*	14	39	120*
% of control	84	93	91	87
Control	76	15	42	133
500 ppb SO_2	58**	14	34	107*
% of control	76	93	81	80

Significance of difference from the control (*t*-test); $^*p<0.05$, $^{**}p<0.01$.

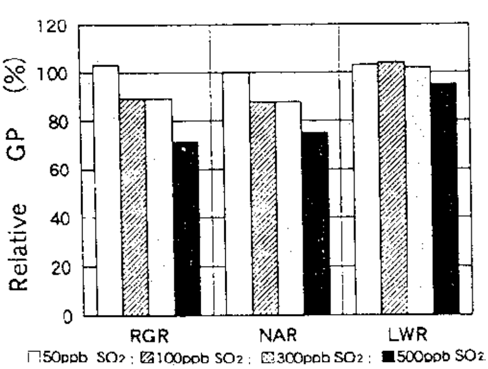

Fig.1 Effects of SO_2 on the relative growth rate of plant dry weight (RGR), net assimilation rate (NAR) and leaf weight ratio (LWR) of masson pine seedlings. The seedlings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours (9:00 − 17:00) a day, 23 days during the exposure period of 30 days from the 60th to 90th day after sowing. The growth parameters (GP) are expressed as percentage of the control values.

As shown in Fig.2, net photosynthetic rates of the seedlings exposed to SO_2 at 100 and 500 ppb were significantly reduced compared with the control values. However, the dark respiration rate of the seedlings was not significantly affected by the exposure to SO_2 (Fig.2). On the other hand, the exposure of the seedlings to SO_2 at 50 ppb had no significant effect on the net photosyntheitc rate.

The effect of SO_2 on transpiration rate of the seedlings are shown in Table 2. The transpiration rates of the seedlings exposed to SO_2 at 100, 300 and 500 ppb were significantly increased

Table 2 Effects of SO_2 on the transpiration rate of masson pine seedlings. The seedlings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours (9:00-17:00) a day, 23 days during the exposure period of 30 days from the 60th to 90th day after sowing. The transpiration rate was measured just after the exposure period of 30 days. Each value is the mean of 5 determinations

Treatment	Transpiration rate, $g \cdot g^{-1} D.W. \cdot h^{-1}$	% of control
Control	1.03	
50 ppb SO_2	0.98	95
Control	1.16	
100 ppb SO_2	1.43*	123
Control	0.91	
300 ppb SO_2	1.05*	115
Control	0.91	
500 ppb SO_2	1.32*	145

Significance of difference from the control (t-test) ; $*p<0.05$

to 123%, 115% and 145% of the control values, respectively. However, the exposure of the seedlings to SO_2 at 50 ppb had no significant effect on the transpiration rate.

The chlorophyll content was not significantly affected by the repeated exposure to SO_2 at 100, 300 and 500 ppb, as shown in Table 3. On the other hand, in the seedlings exposed to SO_2 at 100, 300 and 500 ppb, the foliar sulfur contents were apparently increased during the exposure period of 30 days compared with contrl values at each time (Fig.3). However, the chlorophyll content and leaf sulfur content of the seedlings were not significantly affected by the exposure to SO_2 at 50 ppb.

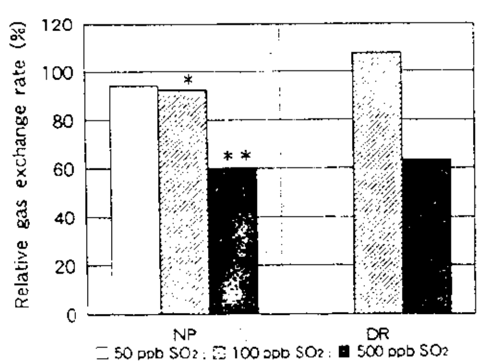

Fig.2 Effects of SO_2 on the rate of net photosynthesis (NP) and dark respiration (DR) of masson pine seedlings. The seedlings were exposed to SO_2 at 50, 100 or 500 ppb for 8 hours (9:00-17:00) a day, 23 days during the exposure period of 30 days from the 60th to 90th day after sowing. The measurement of gas exchange rate was conduced just after the exposure period of 30 days. The gas exchange rates are expressed as percentage of the control values. Singnificance of difference from the control (t-test): $*p<0.05$; $**p<0.01$

Table 3 Effects of SO_2 on the leaf chlorophyll content of masson pine seedlings. The seedings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours (9:00 – 17:00) a day, 23 days during the exposure period of 30 days from the 60th to 90th day after sowing. The chlorophyll content was measured just after the exposure period of 30 days. Each value is the mean of 3 determinations.

Treatment	Chlorophyll a+b, $mg \cdot g^{-1}$ F.W.	% of control
Control	0.72	
50 ppb SO_2	0.74 NS	103
Control	0.68	
100 ppb SO_2	0.63 NS	93
Control	0.44	
300 ppb SO_2	0.39 NS	87
Control	0.44	
500 ppb SO_2	0.43 NS	98

NS: Not significant

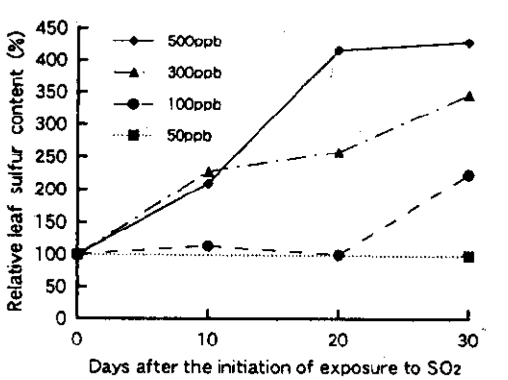

Fig.3 Effects of SO_2 on the leaf sulfur content of masson pine seedlings. The seedlings were exposed to SO_2 at 50, 100, 300 or 500 ppb for 8 hours (9:00 – 17:00) a day, 23 days during the exposure period of 30 days from teh 60th to 90th day after sowing. The leaf sulfur content is expressed as percentage of the control value at each time

4 Discussion

It has been reported that the exposure to SO_2 caused the reductions in dry weight growth and physiological activity of several plants without the appearance of visible foliar injury induced by this pollutant (Sugahand, 1979a, b; Shimazaki, 1979). In the present study, the dry weight growth of the seedlings exposed to SO_2 at 100 ppb was reduced (Table 1), but no visible foliar injury was observed during and after the exposure period of 30 days. In masson pine, therefore, the threshold concentration of SO_2 for the reduction in the dry weight growth is lower than that for the appearance of visible foliar injury.

When the exposure of the seedlings to SO_2 at 100, 300 or 500 ppb caused the reduction in the dry weight growth (Table 1), the NAR of the seedlings was also reduced (Fig.1). This result suggests that the efficiency of dry matter production was reduced by the repeated exposure to SO_2 . Results reported before have suggested that the net photosynthetic rate of plants is reduced by exposure to SO_2 (Furukawa, 1979; Matsuoka, 1978). As shown in Fig.2, the exposure of the seedlings to SO_2 at 100 or 500 ppb induced the reduction in the net photosynthetic rate. Therefore, it is considered that the SO_2-induced reduction in the efficiency of dry matter production of masson pine seedlings is mainly due to the inhibition of net photosynthesis.

Frank *et al.* (Frank, 1987) reported that the exposure of spinach plants to SO_2 at 250 ppb for 2 weeks induces a significant yield reduction with an increase in the shoot sulfur content (Maas, 1987). Furthermore, Fujiwara (Fujiwara, 1975) suggested that leaf sulfur content of plants exposed to SO_2 is closely correlated with the extent of visible foliar injury induced by this pollutant (Fujiwara, 1975) . In the present study, the leaf sulfur content of masson pine seedlings was significantly increased by the repeated exposure to SO_2 (Fig.3). These results suggested that the SO_2-induced increase in the leaf sulfur content may be one of the factors relating to the inhibition of net photosynthesis of the seedlings.

In general, transpiration rate of many plants is reduced by exposure to SO_2 (Kondo, 1979; Omasa, 1979; Natori, 1985; Kondo, 1979) . However, Majernik *et al.* (Majernik, 1970) and Unsworth *et al.* (Unsworth, 1972) reported that the transpiration rates of legume and corn plants are increased by exposure to SO_2 (Majernik, 1980; Unsworth, 1972). In the present study, the repeated exposure of masson pine seedlings to SO_2 at 100, 300 or 500 ppb induced the increase of transpiration rate (Table 2) . When the increased transpiration of the seedlings exposed to SO_2 is continued for a longer time, the water balance in the seedlings is disordered, which may cause the reduction in the net photosynthetic rate and dry weight growth.

In conclusion, it can be said that the dry weight growth and net photosynthetic rate of masson pine seedlings are reduced by exposure to SO_2 at $\geqslant$100 ppb. Therefore, one of the main causes in the dieback of masson pine forest reported in Chongqing, China may be relatively high concentration of atmospheric SO_2 in the relevant area.

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