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Growth and Stomatal Conductance of *Prosopis cineraria* (Ghaff Tree) Exposed to Sulphur Dioxide

Salim H. Al-Rawahy, Haitham Al-Amri, Abdulrahman Al-Hinai and Neelam Sherwani
Department of Biology, College of Science, Sultan Qaboos University,
P.O. Box 36, Alkhod 123, Sultanate of Oman

Abstract: The present study aimed to investigate the sensitivity of an indigenous leguminous plant species of Oman, *Prosopis cineraria* (ghaff tree), to SO_2 pollutant. Plants were exposed to 0, 25, 50, 100 and 150 ppb SO_2 for 30 min daily for the period of ten weeks, under light and dark conditions. The formation of marginal necrotic areas on leaflets was seen as the first symptom of SO_2 injury in *P. cineraria* plants. Leaf senescence was highly significant ($p<0.01$) in plants exposed to SO_2 in light conditions and significant ($p<0.05$) in plants exposed to SO_2 in dark conditions compared with control plants. There was significant ($p<0.05$) decrease in Relative Growth Rate per week in plants exposed to SO_2 in both light and dark treatments compared with control plants, but more pronounced reduction in light conditions. Stomatal conductance was significantly ($p<0.01$) reduced after SO_2 exposure in both light and dark treatments. These results became the first record of Oman indigenous plant species showing confirmed injuries as a result of exposure of SO_2 concentrations.

Key words: Growth, stomatal conductance, SO_2, *Prosopis cineraria*

INTRODUCTION

Sulphur dioxide (SO_2) is an industrial air pollutant associated with fossil fuel combustion and refining. It is one of the several pollutants released in automobile exhaust fumes, industrial smokestacks and metal smelting effluent. Together, natural and anthropogenic global SO_2 sources emit an estimated 194 million tones annually, of which about 83% is anthropogenic from fossil fuel combustion (Botkin and Keller, 2005). Once emitted, SO_2 is deposited onto surfaces by diffusion at various rates according to meteorological conditions, or may undergo a number of chemical reactions before dry or wet deposition. Among S gases, SO_2 is considered to be the most important phytotoxic molecule (Jacobson, 2002) and its adverse effects on plants were recognized long before the effects of the other air pollutants (Winner *et al*., 1985). Recently, Swanepoel *et al*. (2007) reported that increased uptake of SO_2 causes toxicity and reduced growth and productivity in plants due to accumulation of sulphite and sulphate within cells.

In some areas of Oman, especially in the vicinity of industrial areas, the visible foliar injury was observed in some indigenous plant species, e.g., *Prosopis cineraria*. Plants growing within these areas are exposed to greater SO_2 concentrations as well as other pollutants, such as ozone (O_3) and nitrogen dioxide (NO_2). Hence there is considerable concern about the interactive and synergistic effects of exposure to mixtures of SO_2 and other pollutants (Ashmoor, 2002). However, it is important as a first step that the effects of each pollutant be understood independently, that can help identify similarities in the ways in which environmental pollutants effect vegetation and contribute to our general understanding of plant responses to combination of pollutants. To evaluate the impact of contamination in an ecosystem, it is necessary to first establish the background level of the contaminants. The background level may be interpreted as a natural level, that is the average conditions of an area where there may be human activity, but which is in a good state of conservation (Conti and Cecchetti, 2001). From continuous air quality monitoring in Oman during the past 5 years (unpublished data), the background concentration of SO_2 in rural area has been recorded as 11 ppb, while the average background concentration in cities is 22 ppb. Frequently (average once per week), however, the SO_2 concentration in Muscat (capital) area exceeds 50 ppm and occasionally (average once per two months) exceeds 100 ppb. Once the background level had been established, the contamination factor may be used to evaluate the state of conservation or degradation (Conti and Cecchetti, 2001). The majority of past researches on the effects of SO_2 on plants were focused on plants of economic value. Recent concern

Corresponding Author: Salim H. Al-Rawahy, Department of Biology, College of Science, Sultan Qaboos University, P.O. Box 36, Alkhod 123, Sultanate of Oman

about the natural environment, however, has focused more interest on the ecological value of ecosystems. This study aimed to investigate the effects of SO_2 exposure with special attention to growth and stomatal conductance in indigenous leguminous plant species of Oman, *Prosospis cineraria*. The species is an excellent multi-purpose tree for local people, particularly in providing fodder, fuel-wood and shade protection, as well as creating microenvironment that supports various wildlife (Brown, 1991).

MATERIALS AND METHODS

Seeds of *P. cineraria* (L.) Druce collected from wild trees when they became available in the summer of 2004 and 2005. Seeds were germinated in pots containing uniform soil compost. Seedlings were grown under growth room conditions (14 h photoperiod, flux density of 110 μmoles m^{-2} sec^{-2} at 28±2°C and 70±5% RH) and irrigated daily with tap water for 4 months. Fifty plants were then selected randomly and exposed to 0 (control) 50, 100 and 150 ppb SO_2 gas (from MEGS Specialty Gases, Inc.) for 30 min daily for 10 weeks in a 150×100×65 cm perforated glass chamber. SO_2 flow rate was manually adjusted using SUPERIOR™ Gas Sulphonator and the concentration within the chamber was measured using a Tetra Crowcon SO_2 gas detector. The experiment was repeated under light and dark conditions. In dark treatment light was switched off 30 min before the exposure to SO_2 and switched on immediately after the exposure. Stomatal conductance of six plants per treatment selected randomly was measured on the first day of the experiment starting 1 h before the exposure to SO_2 and 5 h after the exposure (-60, 0, 60, 120 180, 240 and 300 min) by using LI-COR, LI-6200 Portable Photosynthesis System and LI-6250 Gas Analyser. In order to understand the stomatal behaviour of *P. cineraria* in field conditions, 10 wild plants were randomly selected at various locations between 23°20'N-57°45'E and 22°40'N-58°35'N. Stomatal conductance of each plant was measured at least 2 different days during the month of August 2005 at interval of 1 h between 6.00 and 18.00 h. Experimental plants were observed for any foliar injuries and these were assessed in percentages (No. of injured leaves/total No. of leaves × 100). Observation of minute necrotic spots was made using the light microscope. At the end of the experiment, plants were harvested and the Relative Growth Rate (RGR) per week of each plant was calculated:

$$RGR/week = (\ln(\text{Dry Weight}) - \ln(\Sigma \text{Initial Dry Weight}))/10.$$

Initial dry weight is the average dry weight of 25 plants selected randomly harvested just prior to the first exposure of SO_2 to above experimental plants. Data were analysed using a factorial design Analysis of variance (ANOVA) using SPSS package and presented as means±standard error.

RESULTS

The formation of marginal necrotic areas on leaflets was seen as the first symptom of SO_2 injury (Table 1). These marginal necrotic areas started as dark green color and eventually became dry and changed to brown color. At the end of the experiment, 89 and 41% of the total number of leaves observed had SO_2 damage symptoms under light treatment and dark treatment, respectively. The difference in the number of leaves injured was highly significant ($p<0.01$) between different SO_2 concentrations under light conditions and significant ($p<0.05$) under dark conditions. Minute necrotic spots around or near stomata were observed under the light microscope, which were significantly more numerous in plants exposed to SO_2 under light conditions. There were no observed foliar

Table 1: The effects of fumigated SO_2 on the growth of *P. cineraria* plants

Parameters	Control*	25 ppb	50 ppb	100 ppb	150 ppb	p-value
Light conditions						
RGR/week	1.75±0.06	1.44±0.06	1.19±0.07	0.83±0.04	0.47±0.03	<0.001
Shoot dry wt. (g)	4.36±0.23	4.21±0.20	3.68±0.21	2.67±0.16	1.70±0.14	<0.001
Root dry wt. (g)	3.31±0.22	3.43±0.19	3.24±0.15	3.08±0.15	2.91±0.13	NS
Leaf area (cm^2)	241.60±11.3	193.20±10.6	158.70±7.50	113.30±6.70	64.80±4.60	<0.001
Stem diameter (mm)	7.80±0.30	7.20±0.30	7.10±0.30	6.30±0.30	5.70±0.20	<0.05
Leaf necrosis (%)	0	18.30±1.50	42.50±2.30	76.40±3.70	89.30±4.40	<0.001
Dead plants (%)	0	4	12	28	34	-
Dark conditions						
RGR/week	1.75±0.06	1.71±0.05	1.63±0.06	1.47±0.04	1.03±0.04	<0.05
Shoot dry wt. (g)	4.36±0.23	4.32±0.21	4.01±0.22	3.65±0.18	3.11±0.19	<0.05
Root dry wt. (g)	3.31±0.22	3.29±0.19	3.24±0.20	3.25±0.16	3.02±0.21	NS
Leaf area (cm^2)	241.60±11.3	226.20±12.5	204.20±9.60	173.10±7.70	148.60±7.10	<0.05
Stem diameter (mm)	7.80±0.30	71.00±0.30	6.80±0.20	6.00±0.30	5.90±0.20	<0.05
Leaf necrosis (%)	0	7.20±1.00	15.50±1.70	28.40±2.40	41.40±2.80	<0.05
Dead plants (%)	0	1	3	6	7	-

*Only one set of control treatment, grown under growth room conditions, NS: Non significant

Table 2: Stomata conductance (mmol m^{-2} sec^{-1}) of *P. cineraria* as affected by exposure of different concentration of fumigated SO_2 for 30 min. Stomata conductance measurements of control plants under light condition were made only once

	Stomata conductance (mmol m^{-2} sec^{-1})					
Time (min)	Control	25 ppb	50 ppb	100 ppb	150 ppb	p-value
Light conditions						
-60*	213	206	217	212	208	NS
0^+	213	181	134	86	67	<0.01
60	213	196	125	95	58	<0.01
120	213	187	129	115	84	<0.01
180	213	204	140	118	87	<0.01
240	213	210	157	122	113	<0.01
300	213	209	203	191	156	<0.05
p-value	-	NS	<0.05	<0.01	<0.01	-
Dark conditions						
-60*	213	212	215	209	216	NS
0^+	126	124	108	95	81	<0.05
60	218	163	167	154	147	<0.05
120	211	215	206	210	190	NS
180	221	206	198	211	206	NS
240	215	217	207	216	210	NS
300	219	214	212	210	217	NS
p-value	NS	NS	<0.05	<0.05	<0.05	-

*60 min before SO_2 exposure, $^+$Measurements start immediately after SO_2 exposure and last for 30 min, NS: Non significant

Table 3: Stomata conductance of 10 wild *P. cineraria* plants measured during the month of August 2005 at interval of 2 h

	Mean* stomata conductance (mmol m^{-2} sec^{-1})										
	Wild plant										
Time	1	2	3	4	5	6	7	8	9	10	Average±SE
6.00	151	164	136	124	140	129	156	144	162	128	143.4±4.6
8.00	221	205	231	213	210	194	217	208	215	225	213.9±3.3
10.00	124	152	117	162	142	97	136	146	150	127	135.3±6.1
12.00	0	2	5	0	12	0	7	0	0	4	3.0±1.3
14.00	0	0	0	4	2	0	14	0	0	3	2.3±1.4
16.00	7	0	18	24	7	13	32	1	0	5	10.7±3.4
18.00	216	199	242	219	204	187	222	211	222	216	213.8±4.7

*n = 2

injuries in control plants. Leaf senescence was mostly observed in plants exposed to SO_2 under light conditions. Compared to the control plants, there was a highly significant ($p<0.01$) decrease in RGR/week in plants exposed to SO_2 under light treatment and a significant ($p<0.05$) decrease under the dark treatment compared with control plants. Stem diameter was significantly ($p<0.05$) reduced in exposed plants under both light and dark conditions. Stomatal conductance was substantially reduced after SO_2 exposure under light conditions compared to SO_2 exposure under dark conditions (Table 2). These responses were transient in dark treatment and maintained for several hours after SO_2 exposure under light treatment. Stomatal conductance in wild plants was greater during the early and late hours of the day (Table 3).

DISCUSSION

In this study, the symptoms of SO_2 injury were first seen on the leaves of *P. cineraria*. Almost 75% of the overall population exposed to SO_2 has showed visible symptoms and incidence of injury was greater for plants exposed to SO_2 under light condition. Descriptions of visible injuries and susceptibility of many plant species to SO_2 have been reported by a number of investigators (Bell and Mudd, 1976; Ayazloo and Bell, 1981; Keller, 1981; Black, 1982; Krupa, 1996; Legge and Krupa, 2002; Moraes *et al*., 2002; Raziuddin *et al*., 1999). Chlorosis and marginal necrotic areas on leaves are the prominent phenomenon of SO_2 phytotoxicity and are derived from the breakdown of photosynthetic pigments in mesophyll tissues (Garsed, 1985). The necrotic areas in this study ranged in color from dark green to reddish-brown to brown. This is supported by SO_2-induced damage reported by Legge and Krupa (2002). Dry deposition of SO_2 involves the transfer of SO_2 from the air stream to the canopy (Rennenberg and Polle, 1994). Once in the canopy, SO_2 may penetrate the boundary layer by a diffusion process. SO_2 molecules that move through boundary layer of *P. cineraria* canopy will most probably enter the leaves through stomatal openings. Garsed (1985)

reported that the pathway of SO_2 to unwetted foliage of *Vicia faba* is largely through open stomata and less than 10% of the total flux was actually accounted for by adsorption on the cuticle. The absorption to the surface of the leaves characterized by a thick cuticle layer could be very much reduced. The diffusion flux of SO_2 molecules to the site of assimilation or damage in the leaf mesophyll is determined by the concentration gradient and leaf diffusion resistance (Heldt, 1996). Following the absorption of SO_2 through the stomatal pores the gas is dissolved in mesophyll spaces near the stomata to form hydrated SO_2 ($SO_2.H_2O$), which act as a strong acid, dissociating to HSO_3^- and SO_3^- in proportions determined by the pH of the water films bounding the mesophyll epidermal apoplast (Legge and Krupa, 2002). Peiser and Yang (1985) reported that SO_2 is rapidly hydrated, forming bisulfite and sulphite. These byproducts have been implicated in SO_2 toxicity and if the uptake exceeds the capacity of cells to detoxify sulfites, minute necrotic spots are formed near or around stomata.

In this study the foliar SO_2 injury symptoms were mostly seen on leaves at a full stage of development. Similar SO_2 injury symptoms were observed in other plant species including Alsike clover (*Trifolium hybridum*) and prickly rose (*Rosa acicularis*) (Legge and Krupa, 2002). Various studies showed that there is a strong correlation between leaf senescence in plants and SO_2 exposure (Heldt, 1996).

The results presented here showed highly significant difference in leaf senescence in plants exposed to SO_2 under light conditions and significant in plants exposed to SO_2 in dark conditions compared with control plants. If the rate of leaf senescence becomes faster than the rate at which new leaves grow, the photosynthetic leaves will decrease and this in turn will reduce net assimilation rates and relative growth rates. SO_2 can also affect photosynthesis by altering stomatal conductance (Marshall, 2002) or by changing the metabolic capacity of mesophyll cells (Winner *et al*., 1985). Mansfield and Pearson (1996) reported that short-term exposure to SO_2, particularly at concentration <50 ppb, often causes wider stomatal opening, while long-term exposure with higher concentrations usually causes partial stomatal closure. Present results with *P. cineraria* showed that stomatal closure can occur within minutes after the SO_2 exposure of 50, 100 and 150 ppm under light treatment and this response is maintained for a few hours. In field measurements of 10 wild *P. cineraria* plants, it was found that stomatal conductance was greater during early and late hours of the photoperiod. The morning hour peak and late evening peak of SO_2 levels during most days in the vicinity of industrial areas and urban areas may directly alter natural stomatal behavior in this plant species. Photosynthetic decline in *P. cineraria* in the presence of SO_2 pollution could therefore be directly related to reduced stomatal conductance. Moreover, the proportion of photosynthetic inhibition due to non-stomatal factors, including physiological and biochemical damage, increase as greater quantities of SO_2 are absorbed into the leaf (Krupa, 1996). The advantage of stomatal closure is the reduction or alteration in the quantity of SO_2 that enters the plant and arrives at metabolic sites. The disadvantage, however, is the depression in photosynthetic carbon dioxide uptake (Raziuddin *et al*., 1999). The level of leaf physiological responses of decreased photosynthetic capacity that lead to altered carbon allocation pattern may ultimately influence growth characteristics such as shoot or root growth (Al-Rawahy, 2000). Decreased growth may affect plant's ability to acquire essential resources from the environment (Novak *et al*., 2003).

In response to many stresses, shoots are affected more than roots (Al-Rawahy, 2000). This was also the case of *P. cineraria* seedlings, where there was no significant reduction in root dry weight after SO_2 exposure compared with control seedlings in both light and dark treatments. Cheesman (1993) suggested that increase root/shoot ratio in stressed plants help to reduce the demand for photosynthetic products to shoot while maintain the root size so the absorption of water and mineral is not affected. However, the cost for this change is the reduced ability to supply products of photosynthesis to the growing apices (Al-Rawahy *et al*., 2003). In the long term exposure to stress the growth is likely to be strongly reduced even in roots.

The current study was successful in validating SO_2-induced foliar injury and reduction in stomatal conductance in *P. cineraria* plant species. These results became the first record of Oman indigenous plant species showing confirmed injuries as a result of exposure of SO_2 concentrations. Several genera and species need to be investigated in order to establish which are the more sensitive species that may serve as bioindicators of SO_2 pollutants in the field. Use of native plant species as bioindicators would have a higher significance for the characterization of the air pollution impact on the ecosystem than those from studies with exotic plant species (Moraes *et al*., 2002).

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