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Stimulatory effects of SO_2 on growth of *Eucalyptus rudis* Endl.

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SUMMARY

Eucalyptus rudis Endl. saplings were exposed to either 274 $\mu g\ m^{-3}$ SO_2 or 132 $\mu g\ m^{-3}$ SO_2 for 8 h d^{-1}, over 123 d, in open-top chambers. Exposure to 132 $\mu g\ m^{-3}$ SO_2 increased the height of the plants and increased the average area and dry weight of leaves. Exposure to 274 $\mu g\ m^{-3}$ SO_2 increased the concentration of total sulphur in the leaves and increased the number of leaves abscised. Sulphur accumulation in the leaves decreased as the duration of exposure increased.

Key words: *Eucalyptus rudis*, growth stimulation, open-top chamber, SO_2, abscission.

INTRODUCTION

Of several air pollutants emitted in appreciable quantities in Australia, the most significant for the natural environment, based upon mass emissions, area impacted and toxicity, is SO_2. Annual mass emission of SO_2 in Australia is currently about 2·2 megatonnes and increasing rapidly as a result of major expansions in mineral roasting and smelting operations (Murray & Wilson, 1989). The area potentially affected by these emissions is large, although much of this is often in remote and sparsely populated areas, and of small current value in terms of economic production. The effects of these emissions on native vegetation are not well understood.

The objective of this experiment was to determine the effects of SO_2 on growth and development of a native tree species, *Eucalyptus rudis* Endl., which is of significant ecological and landscape value and grows in areas adjacent to the major heavy industrial area at Kwinana, the second most significant source of SO_2 in the state of Western Australia. Open-top chambers were selected as the exposure system as they permit growth responses to be studied over long periods of time and under near-ambient climatic conditions. Monitoring of SO_2 in the Kwinana area showed that the 24 h average concentrations during an intensive study in 1979 exceeded 200 $\mu g\ m^{-3}$ on 8 occasions and 100 $\mu g\ m^{-3}$ on 49 occasions (Department of Conservation and Environment, 1982). As exposures are rarely continuous, intermittent fumigations were used. Plants were exposed to ambient air ($< 13\ \mu g\ m^{-3}$) or to 274 or 132 $\mu g\ m^{-3}$ of SO_2 for 8 h d^{-1}. Ambient concentrations of NO_x at the experimental site are very low (annual mean $< 10\ \mu g\ m^{-3}$) and consist predominantly of NO. Concentrations of O_3 at the site are unknown.

MATERIALS AND METHODS

Fumigation chambers

Each open-top chamber (Heagle, Body & Heck, 1973) was 3 m in diameter and 2·4 m tall, consisting of a rigid aluminium frame covered by u.v.-treated PVC plastic. The upper half of the frame was covered by a single layer of PVC plastic and the lower half was covered by a double thickness of the PVC envelope with the inner layer perforated by holes 25 mm in diameter. Air was drawn by a fan through a dust filter and then forced along a duct, into the chamber through the holes in the lower envelope and then out through the open top. The output of the fan was 1 $m^3\ s^{-1}$, enabling an air exchange rate of about 3·5 air changes per min. Dry air was mixed with bottled anhydrous SO_2 from a temperature-controlled cylinder and passed through a regulator and series of needle valves to the inlets of the fumigated chambers. The concentration of SO_2 was measured in each chamber for 12 min every 132 min using a timer-controlled electrical sequencer in conjunction with solenoid valves. The concentration of SO_2 was monitored using a Thermo Electron, Series 43 pulsed fluorescent ambient SO_2 analyser, calibrated with a Thermo Electron, Model

145 calibrator, with NBS traceable certified permeation tubes.

The mean (±SD) SO_2 concentrations recorded were 274 (±74) $\mu g\ m^{-3}$ and 132 (±45) $\mu g\ m^{-3}$ for the 8 h fumigation period from 08.00 to 16.00 h. Plants in the control chambers were exposed only to ambient air with the SO_2 concentration below the detectable limits of the analyser of 13 $\mu g\ m^{-3}$. Each treatment was duplicated. The experiment was conducted from April to August.

Temperature and relative humidity were recorded throughout the experiment using thermohygrographs in Stevenson screens. The mean daily maximum and minimum temperatures (±SD) were 19·5 (±3·2) and 10·1 (±3·3) °C respectively. The mean daily maximum and minimum relative humidities were 91·4 (±7·5) and 51·6 (±14·6)% respectively. Checks showed that temperature inside the chambers was generally 1 °C higher than that outside and the relative humidity was 1·5% lower inside the chambers than outside.

Plant material

Plants were grown from seed collected from wild populations growing 100 km south of Perth, Western Australia. They were grown in 10 litre pots in a 6:5:4 mix of sawdust, course sand and peat, with complete fertilisers.

Eight plants, selected on the basis of uniform height and growth form, were placed in each chamber. At the beginning of fumigation all of the plants were approx. 0·4 m tall and 4 months old.

Throughout the experiment the plants were given adequate water twice daily by an automated sprinkler system. Endosulfan insecticide (Lane) was applied about once a month or as required to control insect pests. The plants were moved every 2 wk to minimize any positional effects. Two of the youngest fully expanded leaves were collected from each plant at four-weekly intervals and prior to harvesting for sulphur analysis. Surface contamination of the leaves was removed by agitating in deionised water. Samples were dried in a forced draft oven at 80 °C until constant weight. Leaf tissue was ground to pass a 40-mesh sieve in a Wiley hammer mill. Sulphur concentrations were determined using the oxygen flask combustion method for total sulphur (Hunt, 1980).

Harvesting procedure

Plants were harvested from each chamber 17 wk after fumigation commenced. Plants were cut at the soil line and divided into stem, developing leaves and expanded leaves. A developing leaf was considered to be one not fully developed in size, appearance and texture. The separation of developing and expanded leaves is important because of the effect of leaf developmental stage on susceptibility to SO_2 (McCune, 1986).

Leaf number was recorded and leaf area was measured using an area meter (Delta T, Cambridge, UK). Stem diameter was measured using Vernier calipers at a point 0·05 m above the soil line before harvesting. Leaves and stems were dried in a forced draft oven at 80 °C until at constant weight, then dry weights were recorded.

Statistical analysis

The effects of SO_2 on all parameters were determined by one-way analyses of variance (ANOVA). An *a posteriori* Duncan's Multiple Range Test was used to determine which means were significantly different at $P < 0{\cdot}05$. Cochran's C test was used to test for homogeneity of variance. If the variances showed heterogeneity, a $\log_{10}$ transformation was used. The Duncan's test was run at $P < 0{\cdot}01$ if the variances still showed heterogeneity after transformation.

RESULTS

Exposure to 132 $\mu g\ m^{-3}$ SO_2 appeared to have some stimulatory effects, as it increased height growth and the average area and average dry weight of leaves of *E. rudis* (Fig. 1 and Table 1). Specific leaf area (leaf density) was not significantly different between treatments. Exposure to 274 $\mu g\ m^{-3}$ of SO_2 had no significant effect on any of these parameters but increased the number of leaves abscised. Despite the apparent stimulatory effects of 132 $\mu g\ m^{-3}$, SO_2 did not influence stem diameter, stem dry weight, the

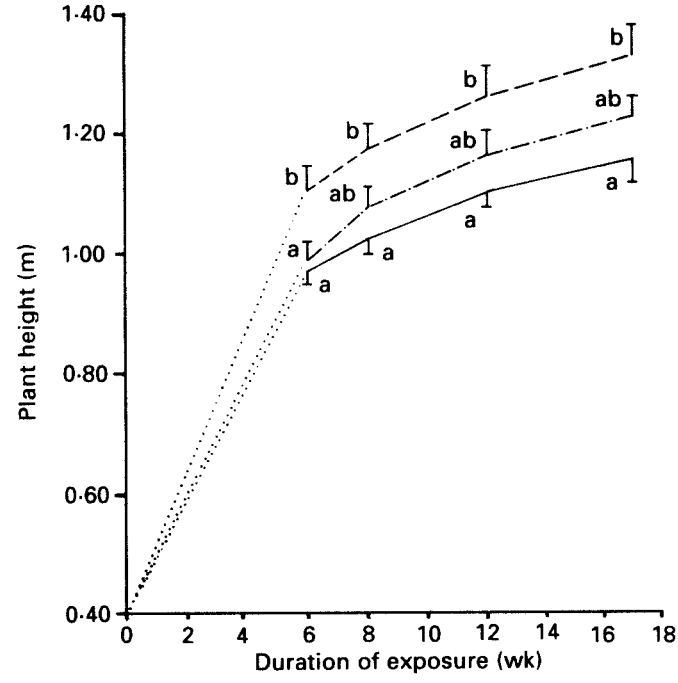

Figure 1. The effect of exposure to SO_2 on height growth in *Eucalyptus rudis*. ——, < 13 $\mu g\ m^{-3}$; – – –, 132 $\mu g\ m^{-3}$; –·–·–, 274 $\mu g\ m^{-3}$. Within each set of values at a given time, values with the same letter are not significantly different (Duncan's Multiple Range Test, $P = 0{\cdot}05$)

Table 1. *Effects of 17 wk exposure to SO_2 for 8 h d^{-1} on leaf growth in* Eucalyptus rudis (*mean ± SE*). *Within each column, values followed by the same letter are not significantly different (Duncan's Multiple Range Test, $P = 0.05$)*

Exposure concentration ($\mu g\ m^{-3}$) for the 8 h period	Average leaf area ($cm^2\ leaf^{-1}$)	Average leaf dry weight ($g\ leaf^{-1}$)	Specific leaf area ($cm^2\ g^{-1}$)	Total number of leaves abscised
< 13	18·3 ± 0·77 *a*	0·21 ± 0·01 *a*	90·0 ± 3·1	22·3 ± 3·3 *a*
132	25·1 ± 1·46 *b*	0·27 ± 0·02 *b*	97·4 ± 3·2	25·4 ± 3·0 *ab*
274	20·5 ± 1·26 *a*	0·22 ± 0·02 *ab*	95·0 ± 2·7	32·4 ± 3·5 *b*

Table 2. *Effects of 17 wk exposure to SO_2 for 8 h d^{-1} on above-ground plant growth in* Eucalyptus rudis (*mean ± SE*). *No significant differences within columns (Duncan's Multiple Range Test, $P = 0.05$)*

Exposure concentration ($\mu g\ m^{-3}$) for the 8 h period	Leaf number		Leaf area		Dry weight (g)		Stem Diameter (mm)
	Developing leaves	Expanded leaves	Developing leaves	Expanded leaves	Developing leaves	Expanded leaves	
< 13	14 ± 2·3	194 ± 14	34 ± 6	3690 ± 252	0·38 ± 0·06	42 ± 5·0	17·3 ± 0·44
132	16 ± 3·8	192 ± 22	54 ± 11	4963 ± 418	0·56 ± 0·10	51 ± 4·8	17·6 ± 0·61
274	20 ± 3·8	222 ± 30	63 ± 15	4722 ± 536	0·62 ± 0·13	50 ± 5·6	17·4 ± 0·66

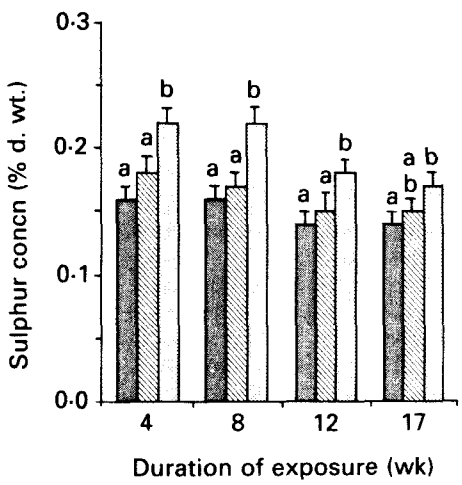

Figure 2. The effect of exposure to SO_2 on leaf sulphur concentration in *Eucalyptus rudis*. ▨, < 13 $\mu g\ m^{-3}$; ▩, 132 $\mu g\ m^{-3}$; ⊡, 274 $\mu g\ m^{-3}$. For significance of letters, see figure 1.

total number, area or dry weight of developing or expanded leaves (Table 2). Variability appeared to be larger in the fumigated treatments.

The increase in the average area and average dry weight of leaves, without a change in specific leaf area, suggests that small leaves were being replaced by leaves of similar density which ultimately grew to a larger size when plants were exposed to 132 $\mu g\ m^{-3}$.

Exposure to 274 $\mu g\ m^{-3}$ SO_2 significantly increased leaf sulphur concentrations although the amount of luxury accumulation decreased as the duration of exposure increased (Fig. 2). Exposure to 132 $\mu g\ m^{-3}$ SO_2 did not increase foliar sulphur concentration at any stage, although at week 17 the mean foliar sulphur concentration due to this treatment was not significantly different from the mean for plants exposed to 274 $\mu g\ m^{-3}$. No symptoms of visible injury developed during the fumigation.

DISCUSSION

Growth stimulations due to exposure to low concentrations of SO_2 have been reported previously in several species. A number of mechanisms have been proposed and it is evident that different types of growth stimulation exist which are produced by different mechanisms.

Plant growth stimulation can be real or apparent. Real growth may result from a fertilization response (Cowling & Lockyer, 1976; Lockyer & Cowling, 1981) whereby atmospheric sulphur dioxide supplements a plant's sulphur requirements and enhances growth beyond the previously sulphur-deficient rate of growth. However, once nutrient supply is in the adequate range no further growth enhancement is afforded (Smith, 1986).

A fertilization effect is not likely to have occurred in this experiment as the *E. rudis* plants were grown with a more than adequate supply of nutrients. Leaf sulphur concentrations in control plants were substantially higher than those reported for healthy eucalypt trees of the region (Hingston, Dimmock & Turton, 1981; Hingston, Turton & Dimmock, 1979; Grove & Malajczuk, 1985). Plants with high nitrogen fertilization can also be sulphur deficient and show growth stimulation when exposed to SO_2 (Cowling & Lockyer, 1978; Milchunas *et al.*, 1981). However, the nitrogen levels in the *E. rudis* plants were also within the range of average foliar concentrations recorded in healthy eucalypt forests of the region.

Apparent growth may result from changes in resource allocation (McLaughlin & McConathy, 1983; Milchunas, Lauenroth & Dodd, 1982) such as an increase in specific leaf area to compensate for the impairment of photosynthesis (Bell, 1982) or an increase in shoot growth at the expense of root growth (Darrall, 1989). Other stimulatory effects may be associated with SO_2 reaction products interfering with meristematic activity or hormone metabolism (Garsed, Farrar & Rutter, 1979), for example changes to indole-3-acetic acid activity, affecting apical dominance (Zwoch, Muller & Schaub, 1985).

E. rudis plants exposed to 132 $\mu g\ m^{-3}$ SO_2 in this experiment appeared to replace small leaves with ones which ultimately grew to a larger size than leaves on plants exposed to other treatments. In addition, these plants grew taller.

The mechanism for these effects is not clear. Morphogenic changes are a possible explanation. It is known that metabolic products of SO_2 are translocated to areas of high metabolic activity and follow similar movement patterns to $^{14}CO_2$ assimilate (Garsed & Read, 1974). As it has been proposed that cell division is controlled by the balance between oxidised and reduced sulphur radicals (Hammett, 1930; Bleasdale, 1973), this may explain how SO_2 could affect leaf ontogeny and apical growth. It is also possible that a change in resource allocation between roots and above ground plant parts led to larger leaves and increased height growth, although it would be expected that other parameters would also show significant increases if this were the case.

Accelerated rates of senescence and abscission are often observed as SO_2 derivatives accumulate and impair metabolic function (Bell, 1982). In *E. rudis* exposed to 274 $\mu g\ m^{-3}$ of SO_2 leaf sulphur concentration and the rates of leaf abscission were increased. However, the total number of leaves was maintained. This has been recorded in several other *Eucalyptus* species where leaf loss is initially offset by an increased rate of leaf production to maintain leaf area despite an increased leaf turnover rate (Murray & Wilson, 1988 *a*, *b*). A species closely related to *E. rudis*, *E. camaldulensis*, has also shown an increased rate of leaf abscission when exposed to 532 and 931 $\mu g\ m^{-3}$ SO_2 for 30 h (Norby & Kozlowski, 1981).

The decrease in the amount of excess sulphur accumulated in *E. rudis* leaves as the duration of exposure to 274 $\mu g\ m^{-3}$ SO_2 increased, has been reported previously in other species (Murray, 1984). This does not appear to have been a growth dilution effect, but may have been part of an adaptation process. Stomatal conductance may have adapted to reduce uptake of SO_2 (Black, 1982). Excessive sulphur accumulated in leaves may have been emitted as H_2S (Filner *et al.*, 1984), redistributed to other organs, lost through abscission or lost through leakage from the roots into the soil, as occurs in crop plants (Garsed, 1985).

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