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RESPONSE OF TWO TROPICAL TREE SPECIES, *PONGAMIA PINNATA* AND *EUGENIA GRANDIS*, TO O_3 EXPOSURES

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PHILIP, E. & FURUKAWA, A. 2000. Response of two tropical tree species, *Pongamia pinnata* and *Eugenia grandis*, to O_3 exposure. This paper reports a comparative study on the effects of O_3 exposure on stomatal conductance (g_s), photosynthetic rate (A) and intercellular CO_2 concentration (C_i) of two tropical tree species, *Pongamia pinnata* and *Eugenia grandis*. These seedlings were exposed to 0.1, 0.2 and 0.5 μmol mol^{-1} O_3. O_3 reduced both g_s and A after 24 h of fumigation in the two species. g_s decreased rapidly with increase in O_3 concentrations. The decline in A caused by lower concentration of O_3 is assumed to have resulted from stomatal closure. The reduction in C_i indicates that only a slight or no inhibition of photosynthetic CO_2 fixation was induced by O_3. Foliar symptoms were noticed when these seedlings were fumigated with higher concentrations of O_3, 0.2 and 0.5 μmol mol^{-1}. Judging from the degree of the appearance of visible symptoms, of the two species, *E. grandis* was a better O_3 avoider.

Key words: Stomatal conductance - O_3 fumigation - photosynthesis - intercellular CO_2 concentration - *Pongamia pinnata* - *Eugenia grandis*

PHILIP, E. & FURUKAWA, A. 2000. Tindak balas dua spesies pokok tropika, *Pongamia pinnata* dan *Eugenia grandis* terhadap pendedahan O_3. Kertas ini melaporkan keputusan kajian kesan ozon ke atas konduktans stomata (g_s), kadar fotosintesis (A) dan kepekatan CO_2 intersel (C_i) terhadap dua spesies pokok tropika, *Pongamia pinnata* dan *Eugenia grandis*. Anak benih pokok-pokok ini telah didedahkan kepada 0.1, 0.2 dan 0.5 μmol mol^{-1} O_3. O_3 telah mengurangkan kadar g_s dan A, 24 jam selepas fumigasi dibuat ke atas kedua-dua spesies. g_s berkurangan dengan pantas selaras dengan kenaikan kepekatan O_3. Kekurangan A apabila kepekatan O_3 berkurangan disebabkan oleh penutupan stomata manakala kekurangan C_i pula menunjukkan tiada atau hanya sedikit perencatan oleh O_3 berlaku dalam pengikatan CO_2. Kecederaan daun berlaku apabila anak-anak benih ini didedahkan kepada kepekatan O_3 yang tinggi iaitu 0.2 dan 0.5 μmol mol^{-1}. Berdasarkan kepada kecederaan daun, didapati *E. grandis* ialah pengelak O_3 yang lebih baik.

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Introduction

The nationwide haze in Malaysia in 1994 and the recurrent spells ever since indicate that the air quality is on a decline in the Klang Valley (State of Selangor). Klang Valley is the largest urban centre in the country and is currently expanding as part of a merging conurbation stretching from Port Klang on the Straits of Malacca in the west to the foothills of the Main Range in the east (Sham Sani 1994). Emissions of sulphur dioxide and nitrogen dioxide from industries are anticipated to double by the year 2000 (Leong & Lim 1994). The figures for 1991 were 69 000 t y^{-1} and 66 000 t y^{-1} respectively. In addition, the number of vehicles is expected to increase as the country progresses economically. As a result of this, O_3, the photochemical reactant of light and nitrogen dioxide (Heber *et al.* 1993), may also increase in the atmosphere.

O_3 can cause both invisible and visible injuries to plants. Its effect on vegetation may occur at several levels; from the molecular to the organisms, and then to the ecosystem level. The occurrence and the magnitude of the effects on vegetation depend on the concentration of the pollutant, the duration of the exposure, the length of time between exposures, and various environmental and biological factors (USEPA 1984).

The use of physiological bioindication in assessing air pollution injury is particularly important where the naked eye does not perceive any injury at all, and this has been largely neglected (Keller 1982). Furthermore, physiological bioindication is important especially in order to understand how plants and environmental factors affect susceptibility of vegetation to air pollution.

Physiological analysis of the effects of pollution has been focused on suspected direct or indirect alterations of normal plant structure and function (Taylor 1978). Some of the earliest observable physiological effects include altered stomatal responses and decreased CO_2 fixation (Weinstein & McCune 1979). Biochemical changes within the plants are often expressed as visible foliar injury, premature senescence, increased leaf abscission, and reduced plant growth and yield (USEPA 1984).

O_3 damage on temperate plants has been well documented. Darrall (1989) gave a comprehensive review on the effects of O_3 on various temperate plants. By comparison, there are not many reports on tropical species, and hardly any pertaining to Malaysia. Studies on the effect of O_3 are necessary because of its potentially serious impact on tree growth and the terrestrial ecosystem. Today, there is an emphasis on the development of urban forest and *ex situ* conservation programmes within the urban environment. Hence, it is urgent that the impact of O_3 is studied so that threshold levels of different urban tree species can be documented.

This investigation was pursued with the objective of studying the physiological bioindication of O_3 in two forest species, *Eugenia grandis* and *Pongamia pinnata,* that are commonly grown in urban areas. *Eugenia grandis* is gaining popularity among aborists due to its excellent features as a wayside tree. *Pongamia pinnata*

has been planted as a wayside tree for a very long time, but is not preferred nowadays because of its constant leaf shedding habit.

Materials and methods

Plant materials

Eugenia grandis and *P. pinnata* were propagated from seeds in plastic pots (11 cm diameter, 15 cm deep) that were filled with a mixture of 2:2:1:1 (v/v) of vermiculite, perlite, peat moss and gravel and grown in a phytotron greenhouse. They were maintained at day/night temperature regime of 25/25 °C and relative humidity of 70%. Each pot contained 5 g of Magamp K (Hyponex Japan, $N:P_2O_5:K = 6:40:5$) and 15 g of magnesia lime as a base fertiliser. Potted plants were watered twice a day and given Hyponex solution (1 g l^{-1}) once a week.

O_3 fumigation

Six-month-old seedlings of both species were treated with O_3 in a controlled environment cabinet (1.7 × 2.3 × 2.0 m high). Twenty-four metal halide lamps with heat absorbing glass filter provided illumination. Ambient air was passed through activated charcoal filter and through a magnesia filter to remove air pollutants and then led into the cabinet. O_3 was generated by an electrical discharge in dry oxygen and was injected into the air stream quantitatively using a thermal mass flow controller when plants were exposed to O_3. The concentration of O_3 was monitored continuously and regulated by a controlling system based on a chemiluminescent O_3 analyser (Kimoto, Model 806).

Seedlings of both species were subjected to treatment as indicated in Table 1. They were watered twice daily and the stomatal conductance and photosynthetic rates were determined. The stomatal conductance and photosynthesis measurements were made using a LiCOR LI-1600 and LiCOR LI-6200 respectively. These measurements were made before, during and after fumigation for seedlings treated with 0.1μmol mol^{-1} O_3, while for the other two treatments, measurements were made at the termination of fumigation (Table 1). A similar experiment minus the treatment with O_3 was conducted as control. Fully expanded leaves from each seedling were chosen for measurements of photosynthetic and stomatal conductance.

The intercellular CO_2 concentration (C_i) and stomatal limitation to photosynthesis (I) were calculated based on the equations of Farquhar and Sharkey (1982):

$$C_i = C_a - \frac{A \times 1.6}{g_s}$$

where C_a is the ambient CO_2 concentration, g_s is stomatal conductance and A is the rate of photosynthesis. The stomatal limitation to photosynthesis (I) was estimated as follows:

$$I = \frac{(A_o - A)}{A_o}$$

where A_o is the rate of photosynthesis at $C_i = C_a$

Table 1. Treatments given to *Pongamia pinnata* and *Eugenia grandis* seedlings

Treatment ($\mu mol^{-1}mol^{-1}$)	Duration (h)	Light intensity ($\mu mol^{-1}m^{-2}s^{-1}$)	Temperature °C	RH (%)	Sampling interval (h)
0.1	144	700	25	70	8, 24, 48, 72, 96, 120, 144, 168
0.2	8	700	25	70	8
0.5	8	700	25	70	8
Control	144	700	25	70	8, 24, 48, 72, 96, 120, 144, 168

These experiments were conducted with six replicates, each replicate having three samples. Data for stomatal conductances, photosynthetic rates and intercellular CO_2 concentrations were analysed using two-way analysis of variance.

Foliar injury was assessed based on the number of leaves with symptoms. Changes in the colour were noted when fumigation was terminated.

Results

O_3 fumigation resulted in a rapid decline in A (photosynthesis) for both *P. pinnata* and *E. grandis* at a concentration well below that required to induce visible leaf injury (Figure 1). When these plants were exposed continuously to 0.1 µmol mol^{-1} O_3 for 7 days, A for these species declined significantly ($p < 0.01$) within the first 24 h. No significant decline in A was observed in either species beyond 24 h exposure.

Exposure to O_3 reduced g_s from control values (Figure 2), which in turn reduced A (Figure1). The changes in A could be due to stomatal behaviour, direct O_3 effects on the photosynthetic apparatus or both.

Stomatal conductance (g_s) also decreased for both species during the first 24 h fumigation with 0.1 µmol mol^{-1} O_3 to about 30% of the initial values (Figure 2). The decline in g_s caused by continuous fumigation with 0.1 µmol mol^{-1} O_3 showed a similar pattern with photosynthesis.

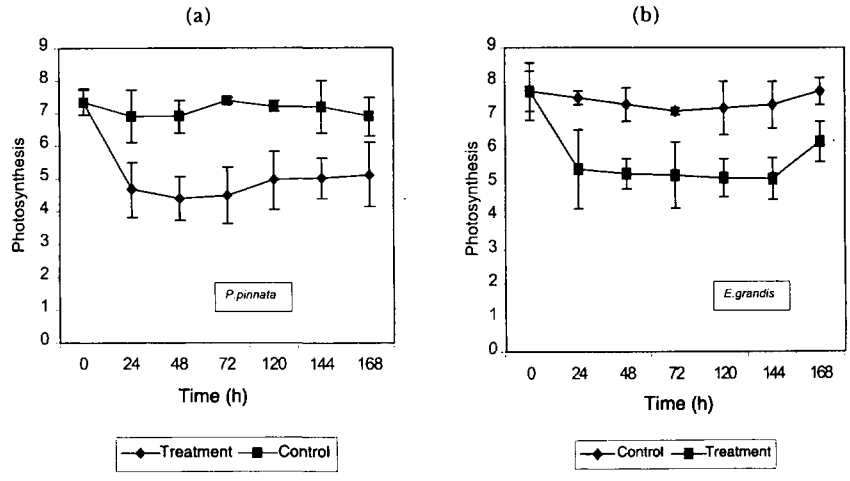

Figure 1. Time course of O_3 induced decrease of photosynthesis in (a) *Pongamia pinnata* and (b) *Eugenia grandis*. Plants were exposed to 0.1 μmol mol^{-1} O_3 for 7 days. The exposure to O_3 and measurements of photosynthesis were made at 25 °C and 70% RH. Each value is the mean of 6 replicates ± s.d.

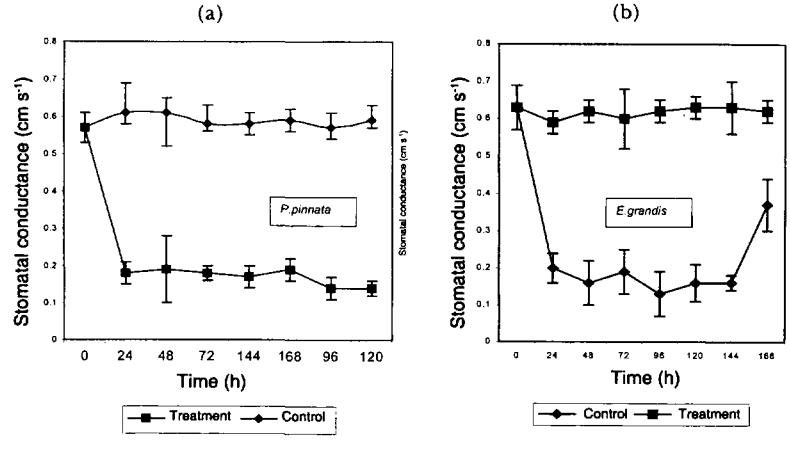

Figure 2. Time course of O_3 induced decrease of stomatal conductance (g_s) in (a) *P. pinnata* and (b) *E. grandis*. Plants were exposed to 0.1 μmol mol^{-1} O_3 for 7 days. The exposure to O_3 and measurements of stomatal conductance were made at 25 °C and 70% RH. Each value is the mean of 6 replicates ± s.d.

Figure 3 shows apparent inhibition of A and g_s for the two species as a function of O_3 concentrations. The photosynthetic decline at the end of 8 h fumigation increased with an increase in the O_3 concentration. O_3 concentrations more than 0.2 μmol mol^{-1} resulted in similar relative declines in A for both species. When fumigated with 0.2 μmol mol^{-1} O_3, A decreased to about 65% in *E. grandis* and *P. pinnata*. Following the most extreme exposure, both species had A of about 5 μmol m^{-2} s^{-1}.

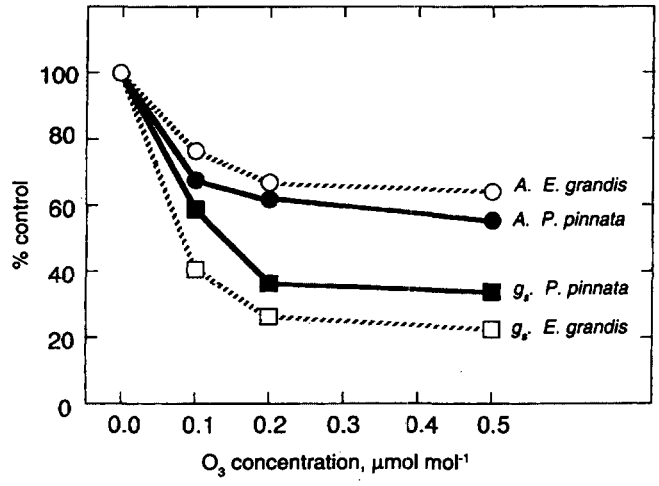

Figure 3. Effects of various concentration of O_3 on photosynthetic rate (A) and stomatal conductance (g_s) of *P. pinnata* and *E. grandis*. O_3 was exposed for 8 h. Measurements were made at 25 °C and 70% RH. Each value is the mean of 6 replicates.

As with photosynthesis, the degree of decline in g_s was related to the concentration of O_3. Following the exposure to 0.2 and 0.5 μmol mol^{-1} O_3, g_s was reduced to an average of about 0.20 cm s^{-1} for both the species. At any concentration of O_3, *P. pinnata* maintained higher values of g_s than *E. grandis*. During the exposure to O_3, the relative decline in g_s was greater than the relative decline in A.

O_3 fumigation did not significantly decrease the initial slope of the A/C_i curve which is defined as the rate of photosynthesis at an ambient CO_2 concentration (A at C_a) (Table 2). However, stomatal limitations (I) were higher in O_3 treated plants than the control. Stomatal limitations doubled to about 30%, when fumigated with O_3. These results suggested that the restriction in CO_2 supply to the photosynthesising organs might have influenced the decline in photosynthesis.

Table 2. Estimations of stomatal limitations for photosynthesis. All measurements were made at 25 °C and 70% RH. O_3 concentration was 0.1μmol mol^{-1}. A = photosynthetic rate at light saturation. C_i = mole fraction of CO_2 in the intercellular air space, C_a = mole fraction of CO_2 in air surrounding the leaf; I = stomatal limitation; PpCon = *P. pinnata* control treatment; PpO_3 = *P. pinnata* treated with 0.1μmol mol^{-1} O_3; EgCon = *E. grandis* control treatment; EgO_3 = *E. grandis* treated with 0.1μmol mol^{-1} O_3.

	PpCon	PpO_3	EgCon	EgO_3
C_i (μmol mol^{-1})	319.0	288.3*	320.2	290.0*
C_a (μmol mol^{-1})	350.0	350.0	350.0	350.0
A at C_i (μmol m^{-2} s^{-1})	7.20	4.88*	7.70	5.80*
A at C_a (μmol m^{-2} s^{-1})	8.40	7.40	9.00	8.10
I (%)	14.2	34.1*	14.4	28.4*

* Significant over control at 5% level.

The effect of O_3 on intercellular CO_2 concentration (C_i) was examined to discriminate the stomatal influence (Figure 4). C_i was affected by O_3 exposure for both species. C_i of the exposed plants declined in the first 24 h and then remained fairly constant until the end of the fumigation for *E. grandis* and *P. pinnata*.

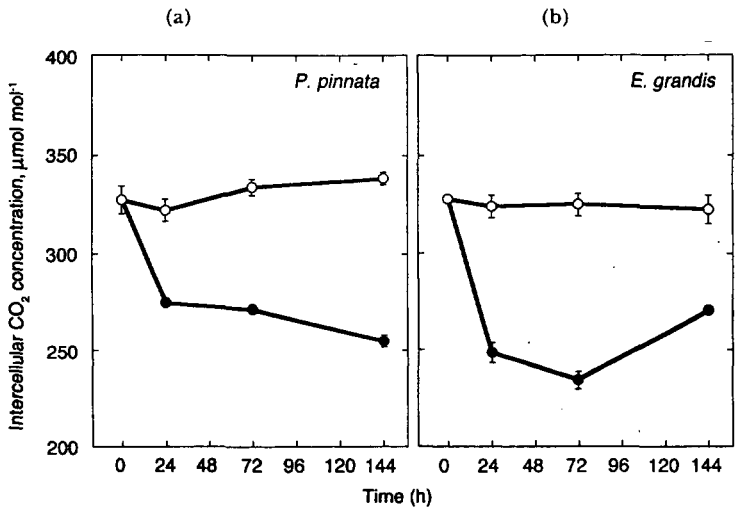

Figure 4. Effects of O_3 on intercellular CO_2 concentrations (C_i) in (a) *P. pinnata* and (b) *E. grandis*. Plants were exposed to 0.1 µmol mol^{-1} O_3 for 7 days. The exposure to O_3 and measurements of C_i were made at 25 °C and 70% RH. Each value is the mean of 6 replicates ± s.d. • = O_3 treated plants; o = control plants.

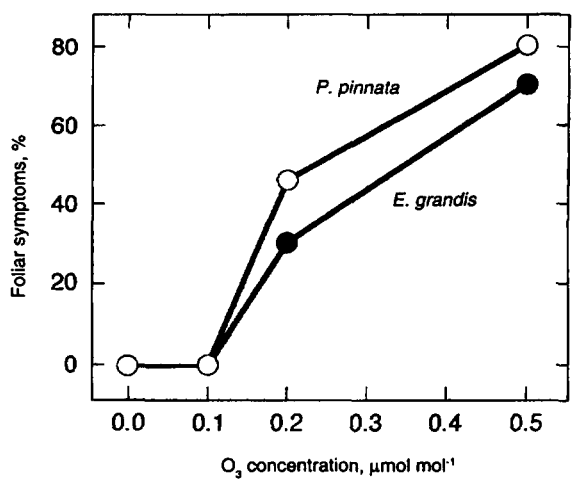

Figure 5. Visible leaf injury induced by O_3 fumigation. Percentage foliar symptoms were estimated on the basis of number of leaves damaged. The exposure to O_3 and estimation of visible injury were made at 25 °C and 70% RH.

Visible injury occurred on leaves of both *E. grandis* and *P. pinnata* when these species were exposed to O_3 concentrations higher than 0.2 μmol mol^{-1} (Figure 5). Acute foliar injury was observed on *P. pinnata* at the end of the 8-h fumigation with 0.5 μmol mol^{-1}. Necrotic leaves were noticed on these trees. On the other hand, treatment with 0.2 μmol mol^{-1} O_3 for 8 h caused chlorotic mottles on the leaves of both the species. However, the number of leaves with foliar injury was less in *E. grandis* than in *P. pinnata.*

Discussion and conclusion

O_3 caused a greater decline in g_s than in A in both *P. pinnata* and *E. grandis.* g_s was reduced by about 70% while A was reduced by about 30% for both species, when they were exposed to 0.1 μmol mol^{-1} O_3 for 7 days. The different responses of g_s and A to O_3 are similar to those of other herbaceous and woody species observed by Darrall (1989). O_3 may have direct or indirect effects on stomatal apertures (Temple 1986, Heath 1994, Farage & Long 1995, Reiling & Davison 1995). Results obtained from this study indicate that stomata closure may be due to the direct effect of O_3 on guard cells. In this case, on exposure to 0.1 μmol mol^{-1} O_3, C_i was reduced from 330 μmol mol^{-1} in the control plants to 270 μmol mol^{-1} and 250 μmol mol^{-1} in *P. pinnata* and *E. grandis* respectively. O_3 may indirectly induce stomatal closure by reducing photosynthesis, thereby increasing C_i (Farquhar & Sharkey 1982).

There are two possibilities that caused the decline in photosynthesis by O_3. One is that O_3 affects photosynthesis directly through the collapse of chloroplasts (Miyake *et al.* 1984) and/or by inhibiting photosynthetic enzymes (Heath 1994). Another possibility is a decrease in CO_2 concentration at the substomatal cavity as observed in the current study. Thus the decline in photosynthetic rates by 0.1 μmol mol^{-1} O_3 was mainly due to limiting supply of CO_2 to the photosynthesising organ as a result of decreasing stomatal aperture. Moldau *et al.* (1990) reported that the inhibition of photosynthesis in *Phaseolus vulgaris* by O_3 was a consequence of reduced stomatal conductance rather than mesophyll conductance. Furthermore, Furukawa *et al.* (1983) reported that mesophyll resistance of poplar cultivars was not influenced by low concentration of O_3, though mesophyll resistance increased by low concentration of O_3 in one poplar cultivar with unresponsive stomata to changes in environmental conditions.

O_3 diffuses through stomata into leaves and induces visible symptoms by altering the permeability of guard cells (Heath 1994, Reiling & Davison 1995). When plants were exposed to 0.2 μmol mol^{-1} O_3 or higher, visible symptoms were detected on leaves of the two species. However, the number of leaves that showed the visible symptoms was higher in *P. pinnata* than in *E. grandis.*

Resistance of plants to O_3 is related to both O_3 tolerance and O_3 avoidance (Levitt 1972). The present experiments show that *E. grandis* is a better O_3 avoider than *P. pinnata.* Thus, during comparable exposures, *E. grandis* absorbed less O_3 than did *P. pinnata,* indicated by the higher percentage drop in g_s. These different

responses of g_s to O_3 between the two species may have resulted in the different degrees of foliar symptoms.

Results obtained indicate that a concentration greater than 0.1 μmol mol^{-1} O_3 would cause visible foliar symptoms on *E. grandis* and *P. pinnata*. Nevertheless, both g_s and A were reduced when treated with 0.1 μmol mol^{-1}. No visible foliar symptoms by 0.1 μmol mol^{-1} O_3 suggest that stomata were not dysfunctional and the effect of O_3 was not irreparable. This suggestion is supported by the findings that g_s and A in *E. grandis* recovered to some extent when treatment was terminated. Thus, leaves may have a detoxifying capability under concentrations well below those required to produce visible foliar injury (Heber *et al.* 1993).

In conclusion, comparison of the two tropical tree species under strictly identical conditions has shown that their physiological response to acute O_3 exposure is very similar. Stomatal conductance has been identified as particularly sensitive to O_3 exposure while the reduction of photosynthesis is secondary in nature. Between the two species, *E. grandis* seems to be a better avoider.

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