BRIEF COMMUNICATION

Sensitivity of *Metasequoia glyptostroboides* to ozone stress

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*State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, P.R. China***Abstract**

2-year-old seedlings of *Metasequoia glyptostroboides* were grown in open top chambers and exposed to four ozone concentrations [O_3] (charcoal-filtered air, CF; 50, 100, and 200 $mm^3\ m^{-3}$) for 25 d. Measurements of growth, leaf chlorophyll (Chl) content, and gas exchange parameters were made before and/or after O_3 exposure. Leaf length, crown width, Chl *a/b*, net photosynthetic rate, stomatal conductance, and transpiration rate were significantly reduced at 100 and 200 $mm^3(O_3)\ m^{-3}$. A remarkable decrease in stomatal conductance also occurred at 50 $mm^3(O_3)\ m^{-3}$.

Additional key words: air pollution; chlorophyll; crown width; leaf length; net photosynthetic rate; stem height; stomatal conductance; transpiration rate; urban greening tree species.

Tropospheric ozone (O_3) is the most important air pollutant contributing to forest decline and tree dieback in North America and Western Europe (Ashmore 2005). Although information on the effects of O_3 on Asian forests is limited, researchers in Japan suggested that ambient [O_3] reduced growth and net photosynthetic rate (P_N) of *Betula ermanii* and *Fagus crenata* (Feng *et al.* 2005, Takeda and Aihara 2007). In China, relatively high [O_3] above 100 $mm^3\ m^{-3}$ have been frequently observed between spring and autumn in mountain and urban areas (Wang *et al.* 2005, Yin *et al.* 2005). Mean [O_3] of 40 $mm^3\ m^{-3}$ can induce visible injury symptoms and physiological change in sensitive plant species (Ashmore 2005, Wittig *et al.* 2007). However, little information is available regarding effects of O_3 on trees in China (He *et al.* 2007).

Metasequoia glyptostroboides, known as a living fossil, is a rare deciduous conifer of the redwood family (*Taxodiaceae*). Because it is adaptable to a wide range of climate regimes, *M. glyptostroboides* has been selected as a preferred species for both urban greening and wood production in many countries (Williams *et al.* 2003). *M. glyptostroboides* is sensitive to air pollutants such as SO_2 and acid rain (Feng *et al.* 2002). Tropospheric [O_3] negatively affects the growth of plants and global background [O_3] are rising at a rate of 0.5–2 % per year

(Vingarzan 2004, Ashmore 2005). To our knowledge, however, no investigation has been conducted on the response of *M. glyptostroboides* to [O_3].

The experiment was conducted at Shuangqiao Farm (31°53'N, 121°18'E) at Jiading City, about 100 km far from Shanghai. 2-year-old seedlings of *M. glyptostroboides* were planted in pots (*ca.* 7 500 cm^3), 2 plants per pot before April. After 60 d of growth, 16 seedlings (8 pots) were assigned randomly to one of four open top chambers. In each chamber, the seedlings were exposed to either charcoal-filtered air (CF) with O_3 at <15 $mm^3\ m^{-3}$, or elevated [O_3] at: (1) 45–55 $mm^3\ m^{-3}$ (50); (2) 90–110 $mm^3\ m^{-3}$ (100); or (3) 175–225 $mm^3\ m^{-3}$ (200) for 25 d for 8 h per day between 09:00 to 17:00. Ozone was generated from pure O_2 by O_3 generator (QHGI-1, Yuyao, China) and then mixed with CF air to achieve the target [O_3] in each OTC. [O_3] within the chambers was measured continuously on a 5-min interval by ozone analyzer (ML9810B, Monitor Labs, USA).

For every seedling, the stem diameter at 1 cm from soil surface, main stem height, crown width, and the length of 5 leaves were measured before and just after O_3 exposure, respectively. The difference of measurements of two times determined the growth increment of seedlings under different O_3 treatments. After 25 d of O_3 exposure, three upper leaves of seedlings were sampled

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Abbreviations: CF – carbon-filtered air; *E* – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate.

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Table 1. The change of stem diameter at 1 cm from soil surface (TD), stem height (SH), crown diameter (CD), and leaf length (LL) of 2-year-old *Metasequoia glyptostroboides* seedlings after exposure to different ozone concentrations [charcoal-filtered air (CF); $50 \pm 5 \text{ mm}^3 \text{ m}^{-3}$ (50); $100 \pm 10 \text{ mm}^3 \text{ m}^{-3}$ (100); $200 \pm 25 \text{ mm}^3 \text{ m}^{-3}$ (200)] for 25 d. Mean $\pm$ SE ($n = 8$). Different letters in columns indicate significant difference between treatments ($p < 0.05$).

Treatment	TD [mm]	SH [cm]	CD [cm]	LL [cm]
Control	1.23 $\pm$ 0.24 ab	3.61 $\pm$ 0.65 a	8.41 $\pm$ 0.67 a	8.15 $\pm$ 0.44 a
50	1.38 $\pm$ 0.13 a	4.84 $\pm$ 0.43 a	7.89 $\pm$ 0.59 a	6.86 $\pm$ 0.53 ab
100	1.07 $\pm$ 0.18 ab	3.55 $\pm$ 0.81 a	5.45 $\pm$ 1.04 b	6.74 $\pm$ 0.35 b
200	0.83 $\pm$ 0.14 b	3.54 $\pm$ 0.54 a	5.23 $\pm$ 0.81 b	6.06 $\pm$ 0.49 b

Table 2. Chlorophyll (Chl) content, Chl *a/b*, stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), and water use efficiency (WUE) of upper leaves of main stems in 2-year-old seedlings of *Metasequoia glyptostroboides* after exposure to different ozone concentrations [charcoal-filtered air (CF); $50 \pm 5 \text{ mm}^3 \text{ m}^{-3}$ (50); $100 \pm 10 \text{ mm}^3 \text{ m}^{-3}$ (100); $200 \pm 25 \text{ mm}^3 \text{ m}^{-3}$ (200)] for 25 d. Mean $\pm$ SE ($n = 3$ or 8). Different letters in columns indicate significant difference between treatments ($p < 0.05$).

Treatment	Chl [g kg ⁻¹]	Chl <i>a/b</i>	g_s [mmol m ⁻² s ⁻¹]	P_N [μmol m ⁻² s ⁻¹]	E [mol m ⁻² s ⁻¹]	WUE [μmol mol ⁻¹]
CF	1.120 $\pm$ 0.007 a	4.52 $\pm$ 0.14 a	111.0 $\pm$ 6.2 a	8.88 $\pm$ 0.22 a	2.45 $\pm$ 0.12 a	3.69 $\pm$ 0.14 b
50	1.090 $\pm$ 0.035 a	4.33 $\pm$ 0.12 ab	74.2 $\pm$ 13.0 b	8.90 $\pm$ 0.25 a	1.85 $\pm$ 0.24 b	5.66 $\pm$ 0.95 a
100	1.090 $\pm$ 0.040 a	3.84 $\pm$ 0.06 c	73.1 $\pm$ 11.0 b	5.25 $\pm$ 0.39 b	1.98 $\pm$ 0.27 ab	3.24 $\pm$ 0.56 b
200	1.070 $\pm$ 0.004 a	3.96 $\pm$ 0.14 bc	53.1 $\pm$ 4.7 b	4.46 $\pm$ 0.21 b	1.55 $\pm$ 0.13 b	3.06 $\pm$ 0.34 b

randomly and used to measure chlorophyll (Chl) content (Arnon 1949) with three replicates. On the 24th d of O₃ fumigation, P_N , transpiration rate (E), and stomatal conductance (g_s) were measured with a portable photosynthetic system (CIRAS-1, PP Systems, UK) on one upper leaf from the main stem of 8 seedlings, with a total of 8 replications per chamber. During these measurements of leaf gas exchange, environmental temperature and relative humidity averaged 32.4 $\pm$ 1.0 °C and 42.3 $\pm$ 2.1 %, respectively. CO₂ concentration in the leaf chamber was maintained at 380 μmol mol⁻¹ and the leaf was irradiated with a photosynthetic photon flux density of 1 000 μmol m⁻² s⁻¹ with the leaf chamber LED source.

Analysis of variance (ANOVA) was performed on experimental data, and the results were analyzed by SPSS 14.0 for Windows. The least significance differences between the means were estimated at 95 % confidence level. Significant differences among treatments are given at $p < 0.05$.

As [O₃] increased, stem diameter, main stem height, crown width, and leaf length decreased, with few exceptions (Table 1). Relative to CF, 100 mm³(O₃) m⁻³ significantly reduced the crown width by 35.2 % and by 17.3 % the leaf length. During the experiment, most leaves showed extensive visible injury in the 100 and 200 mm³(O₃) m⁻³ treatments. However, no significant difference was observed between CF and 50 mm³(O₃) m⁻³ (Table 1). This differs from previous data that other

coniferous species showed sensitivity to low [O₃]. For example, total biomass and relative growth rate were reduced by 8.0 and 1.5 % relative to CF, respectively, when Norway spruce saplings (*Picea abies* Karst) were exposed during 4 growth seasons to 1.4 $\times$ ambient [O₃] of averaged 44 ppb (Karlsson *et al.* 2002).

Although Chl content was not affected, Chl *a/b* was reduced significantly by 15 and 13 % when seedlings were exposed to 100 and 200 mm³(O₃) m⁻³, respectively (Table 2). Elevated [O₃] of 50 mm³ m⁻³ significantly reduced g_s by 24.9 % relative to CF (Table 2). This decrease was much higher than the average of a meta-analysis across all trees (Wittig *et al.* 2007). Relative to CF, P_N was reduced by more than 41 % at higher [O₃] of 100 mm³ m⁻³, suggesting g_s possibly restrained the CO₂ uptake. But the decrease in P_N could also have been a result of less efficient carboxylation, due to the depression caused by O₃ in the synthesis and the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase, which can precede the stomatal responses (Farage and Long 1999). Compared with CF, E was reduced by 24.4 and 36.7 % at 50 and 200 mm³(O₃) m⁻³, respectively (Table 2), indicating water use efficiency was greatly improved at 50 mm³(O₃) m⁻³ compared with other treatments (Table 2).

We conclude that *M. glyptostroboides* is sensitive to O₃. Further studies are needed on the effects of chronic and low-level O₃ exposure on this plant species.

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