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BRIEF COMMUNICATION

Sensitivity of *Metasequoia glyptostroboides* to ozone stress

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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 crenanta* (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 Jiaxing 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 (*QHG-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±5 $mm^3\ m^{-3}$ (50); 100±10 $mm^3\ m^{-3}$ (100); 200±25 $mm^3\ m^{-3}$ (200)] for 25 d. Mean±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±0.24 ab	3.61±0.65 a	8.41±0.67 a	8.15±0.44 a
50	1.38±0.13 a	4.84±0.43 a	7.89±0.59 a	6.86±0.53 ab
100	1.07±0.18 ab	3.55±0.81 a	5.45±1.04 b	6.74±0.35 b
200	0.83±0.14 b	3.54±0.54 a	5.23±0.81 b	6.06±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±5 $mm^3\ m^{-3}$ (50); 100±10 $mm^3\ m^{-3}$ (100); 200±25 $mm^3\ m^{-3}$ (200)] for 25 d. Mean±SE (n = 3 or 8). *Different letters in columns* indicate significant difference between treatments (p<0.05).

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

randomly and used to measure chlorophyll (Chl) content (Arnon 1949) with three replicates. On the 24th d of O_3 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±1.0 ºC and 42.3±2.1 %, respectively. CO_2 concentration in the leaf chamber was maintained at 380 $\mu mol\ mol^{-1}$ and the leaf was irradiated with a photosynthetic photon flux density of 1 000 $\mu mol\ m^{-2}\ s^{-1}$ 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 [O_3] increased, stem diameter, main stem height, crown width, and leaf length decreased, with few exceptions (Table 1). Relative to CF, 100 $mm^3(O_3)\ m^{-3}$ 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 $mm^3(O_3)\ m^{-3}$ treatments. However, no significant difference was observed between CF and 50 $mm^3(O_3)\ m^{-3}$ (Table 1). This differs from previous data that other coniferous species showed sensitivity to low [O_3]. 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× ambient [O_3] 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 $mm^3(O_3)\ m^{-3}$, respectively (Table 2). Elevated [O_3] of 50 $mm^3\ m^{-3}$ 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 [O_3] of 100 $mm^3\ m^{-3}$, suggesting g_s possibly restrained the CO_2 uptake. But the decrease in P_N could also have been a result of less efficient carboxylation, due to the depression caused by O_3 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 $mm^3(O_3)\ m^{-3}$, respectively (Table 2), indicating water use efficiency was greatly improved at 50 $mm^3(O_3)\ m^{-3}$ compared with other treatments (Table 2).

We conclude that *M. glyptostroboides* is sensitive to O_3. Further studies are needed on the effects of chronic and low-level O_3 exposure on this plant species.

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