

Effects of ozone exposure on growth and photosynthesis of the seedlings of *Liriodendron chinense* (Hemsl.) Sarg, a native tree species of subtropical China

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Abstract

Little is known about the response of trees to elevated ozone (O_3) in the subtropical region of China, where ambient O_3 concentrations are high enough to damage plants. In this study, pigment content, gas exchange and chlorophyll (Chl) *a* fluorescence in leaves of *Liriodendron chinense* (Hemsl.) Sarg seedlings, a deciduous broadleaf tree species native in subtropical regions, were investigated at 15, 40, and 58 days after O_3 fumigation (DAF) at a concentration of $150 \text{ mm}^3 \text{ m}^{-3}$ ($E-O_3$). At the end of experiment, seedlings were harvested for biomass measurement. $E-O_3$ caused visible injuries on the mature leaves *e.g.* necrotic patches and accelerated early defoliation. Relative to the charcoal-filtered air (CF) treatment, $E-O_3$ significantly decreased shoot and root biomass, pigment content, light-saturated net photosynthesis (P_{Nsat}), stomatal conductance (g_s), maximum rate of carboxylation (V_{cmax}), photochemical quenching coefficient (q_p) and effective quantum yield of PSII photochemistry (Φ_{PSII}), and also caused a slight reduction in relative increase of basal diameter. Therefore, *L. chinense* can be assumed to be an O_3 -sensitive tree species, which will be threatened by increasing ambient O_3 concentrations in China.

Additional key words: gas exchange; growth; *Liriodendron chinense*; ozone.

Introduction

Tropospheric O_3 is assumed to be the most important air pollutant in many parts of the world due to its potential threat to crops, (semi)natural grassland and forest ecosystems (Ashmore 2005, Feng *et al.* 2009, Mills *et al.* 2011). The concentration of O_3 has increased globally by about 36% since preindustrial times (IPCC 2007) and is currently increasing at an annual rate of 0.5–2.0% on a

global scale (Vingarzan 2004). From a regional scale, the increasing trend in $[O_3]$ has leveled off or slightly reversed in North America and Europe (IPCC 2007); however, it has been continuing in East Asia and South-East Asia (The Royal Society 2008).

Chronic exposure to elevated $[O_3]$ significantly decreased net photosynthetic rates, accelerated leaf

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Abbreviations: AOT40 – the cumulative O_3 exposure over a threshold of the 1-h average $[O_3]$ of $40 \text{ mm}^3 \text{ m}^{-3}$ during daytime; Car – carotenoids; CF – charcoal-filtered air; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DAF – days after fumigation; $E-O_3$ – elevated $[O_3]$ treatment; F_m' – maximum fluorescence yield of light-adapted state; F_o' – minimum fluorescence yield of light-adapted state; F_s – steady-state fluorescence yield; F_v'/F_m' – actual photochemical efficiency of PSII in the saturated light; g_s – stomatal conductance; J_{max} – maximum rate of electron transport contributing to RuBP regeneration; O_3 – ozone; P_{Nsat} – light-saturated photosynthesis; PPFD – photosynthetic photon flux density; q_p – photochemical quenching coefficient; V_{cmax} – maximum rate of carboxylation; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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senescence and increased dark respiration in many tree species, such as deciduous temperate tree species, evergreen Mediterranean trees (reviews: Matyssek and Sandermann 2003, Karnosky *et al.* 2007, Wittig *et al.* 2007, Paoletti 2009). O₃-induced reduction in photosynthesis rate has been attributed to two factors: stomatal and nonstomatal limitation. A reduction in V_{cmax} is considered to be the primary effect of O₃ on photosynthesis in trees, which induces stomatal closure *via* an increase in intercellular CO₂ concentration (C_i) (Bortier *et al.* 2000, Noormets *et al.* 2001, Yamaguchi *et al.* 2007), although stomatal regulation is considered as a very important factor in controlling the ozone sensitivity of plants (Novak *et al.* 2005) and O₃ uptake (Paoletti and Grulke 2005). O₃ can also alter photosynthetic process at the level of the electron transport, such as decreases in leaf Chl content, reduction in the efficiency of excitation capture in plants, reduced numbers of intact or open photosystem II (PSII) reaction centers, and increases in dissipation of energy through alternative means such as heat (Bortier *et al.* 2000, Ribas *et al.* 2005, Ryang *et al.* 2009). Notably, almost all studies focused on temperate and boreal species (review by Wittig *et al.* 2007). However, there is very little information on the effects of O₃ on subtropical tree species (Feng *et al.* 2008).

The effects of elevated [O₃] on *Liriodendron*

tulipifera L. have been widely investigated in potted seedlings in terms of injury, growth, and photosynthesis (Karnosky *et al.* 2007). *L. tulipifera* has been used as a bioindicator of O₃ in eastern US forests for a long time due to its sensitivity to O₃ (Davis and Skelly 1992). However, no information is available on *L. chinense*, the other species within the genus *Liriodendron*, in response to O₃. *L. chinense* is an ecologically and economically important hardwood species in subtropical China, where O₃ concentrations are high enough to induce significant yield loss in wheat and rice (Feng *et al.* 2003, Wang *et al.* 2007). It is currently widely grown at both sides of main roads in the city or countryside areas of the southern part of China as a greening woody species. Many scientists in China wonder whether this tree species can live long in an environment full of high concentrations of air pollutants due to rapid urbanization. The forest decline and dieback of *L. chinense* has been observed in mountainous areas like Longquan County of Zhejiang Province (Feng *et al.* 1999). In this study, therefore, we investigated effects of O₃ on the growth, gas exchange and Chl *a* fluorescence of the *L. chinense* seedlings. The objective was to determine the sensitivity of *L. chinense* to O₃ and to investigate which factors were responsible for the reduced photosynthetic rate.

Materials and methods

Experimental site: The experimental site was located at the Tiantong National Field Observation and Research Station for Forest Ecosystems (29°48' N, 121°47' E), Ningbo City, Zhejiang province, China. It is typical of the humid subtropical monsoon climate with cold dry winters and warm wet summers. The annual mean temperature is 16.2°C and the warmest month is July with a mean temperature of 28.1°C. Average annual precipitation is 1,375 mm, concentrated from June to August.

Plant material: At April 2008, eight-month-old seedlings of *L. chinense* (Hemsl.) Sarg, provided by Nanjing Agricultural University, were individually planted into plastic pots (13.8 dm³) in a temperature-controlled double-glazed greenhouse (25 ± 2°C, air humidity 70–90%). Pots were filled with native yellowish brown lateritic soil (soil organic C 0.98%, total N 1.36 g kg⁻¹, total P 0.27 g kg⁻¹, total K 1.41%) in mixture with litter collected under firry forest at the ratio of 1:1 (v/v). After one and a half months cultivation in the greenhouse, these seedlings were moved to open-air conditions for field acclimation. By the time of O₃ fumigation, *L. chinense* seedlings (1-year-old) grew to a height of about 35 cm and basal diameter of 8.0 mm with fully expanded leaves. On 1 August, plants with similar height and number of leaves were selected and randomly assigned to six open top chambers (OTCs, octagonal base, 5.5 m² of growth space and 2.6 m in height, covered with transparent

plastic film) with five plants in each chamber. The OTCs were located in an open field and thus shading by other buildings or forest trees did not exist. The position of plants was changed every 3–5 days within each OTC to eliminate positional effects. During the growth of seedlings, average air temperature, relative air humidity, and total radiation density were 25.6°C, 90% and 13.8 MJ m⁻² d⁻¹, respectively. Plants were well watered with tap water to avoid drought stress.

Ozone exposure: Ozone was generated from pure oxygen by an electrical discharge O₃ generator (HY003, Chuangcheng Co., Jinan, China) and then mixed with CF to achieve the target [O₃] in three OTCs. The mean air velocity in the chambers corresponded to approximately two complete air changes per minute. The concentration of O₃ in the OTCs was continuously monitored at approximately 10 cm above plant canopy using a UV absorption O₃ analyzer (Model 49i, Thermo, USA).

In this study, there were two treatments: CF and elevated [O₃] (~150 mm³ m⁻³, E-O₃) with three chambers replicates. Due to a rainy day on 1 August, O₃ exposure started from 2 August and ran until 28 September with a maximum of 8 h (from 08:00 to 16:00) each day, because the timing of O₃ fumigation was dependent on the weather conditions. Ozone was added only in the daytime when there was no rain, fog, mist, or dew. The cumulative O₃ exposure over a threshold of the 1-h

average $[O_3]$ of $40 \text{ mm}^3 \text{ m}^{-3}$ (AOT40) were calculated as the sum of differences between the hourly ozone concentrations in $\text{mm}^3 \text{ m}^{-3}$ and $40 \text{ mm}^3 \text{ m}^{-3}$ for each hour when the concentration exceeded $40 \text{ mm}^3 \text{ m}^{-3}$ during daylight hours over the course the experiment.

Growth measurements: Plant height and stem basal diameter of all plants per chamber were measured at the beginning and at the end of the experiment, respectively. Stem diameters were measured in two directions at a fixed position at 10 cm of stem height. Absolute differences in stem diameter or height increment were calculated from the differences between the final and initial diameters. The relative growth was calculated as the percentage increase of diameter or height compared with the initial diameter or height at the start of the experiment. At the end of experiment, five plants per chamber were harvested and separated into leaves, stems, and roots, respectively. The plant organs were oven-dried at 80°C until a constant mass was reached.

Photosynthetic pigment determination: Two leaf discs (1 cm in diameter) per plant taken from upper fully developed sun leaves (two plants per chamber) were extracted with 4 cm^3 95% ethanol in the dark for 48 h at 4°C . The absorbance of leaf pigment extracts was measured at 470, 646, and 663 nm. Chl and carotenoids (Car) were calculated according to specific absorption coefficients of Lichtenthaler (1987).

Gas exchange and Chl *a* fluorescence: Two upper fully expanded sun leaves per plant and three different plants per chamber were randomly selected. The gas exchange and Chl *a* fluorescence were determined with a portable photosynthesis system fitted with a 6400-40 leaf chamber fluorometer (LCF) (LI-6400, LI-COR Inc., Lincoln, NE, USA). Measurements were first performed by increasing photosynthetic photon flux density (PPFD) to estimate saturating light values for mature leaves of *L. chinense*. During the experiment, all measurements were performed at $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, within the range of saturating PPFD observed in initial experiments. Due to large variability in ambient temperature between mid-August and the end of September, the block temperature was set to the ambient average at each measurement (35 ± 1 , 30 ± 0.5 , $27 \pm 0.5^\circ\text{C}$ from the 1st to the 3rd measurement, respectively). Relative humidity (RH) was controlled at

50–65%. CO_2 was set at $380 \mu\text{mol mol}^{-1}$ with a supply from a pure CO_2 gas cylinder. All gas exchange measurements were conducted during the periods of 09:00–12:00 h. For Chl *a* fluorescence, steady-state fluorescence yield (F_s) was recorded at saturating PPFD of $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The intensity of the saturation pulses of $6,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 0.8 s was then used to produce the maximum fluorescence yield (F_m') by temporarily inhibiting PSII photochemistry. Measurements of minimum fluorescence yield (F_o') were carried out in the presence of far-red light ($5 \mu\text{mol m}^{-2} \text{ s}^{-1}$) in order to fully oxidize the PSII acceptor side. The following Chl *a* fluorescence parameters were calculated: actual photochemical efficiency of PSII in the saturated light [$F_v'/F_m' = (F_m' - F_o')/F_m'$] (Harbinson *et al.* 1989), $q_p = (F_m' - F_s)/(F_m' - F_o')$ (Schreiber *et al.* 1986) and $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ (Genty *et al.* 1989).

At the end of the experiment, P_{Nsat} vs. C_i curves were measured using the automatic program in the LI-6400 photosynthesis system. Before measurement, each sample leaf was illuminated with saturated PPFD provided by the light-emitting diodes light source of the equipment for 10–30 min to achieve fully photosynthetic induction. Measurements were taken by changing $[\text{CO}_2]$ in LCF in 10 steps (380, 200, 100, 150, 50, 400, 600, 900, 1,200, and $1,500 \mu\text{mol mol}^{-1}$ under a constant PPFD of $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, block temperature of $27 \pm 0.5^\circ\text{C}$ and relative humidity of 50–70%. Measurements were performed on upper leaves of two different plants per chamber with three chambers replicates. V_{cmax} and the maximum of electron transport (J_{max}) were derived by iteratively fitting curves (using nonlinear least square regression methods) to P_{Nsat}/C_i response data according to the program of Sharkey *et al.* (2007), as described by Long and Bernacchi (2003).

Statistical analysis: In this study, the open-top chamber was treated as an independent experimental unit for either treatment, which means that we first obtained the average of each chamber from observations of 3–4 plants per chamber and then analyzed the data. ANOVA was performed to test the effects of O_3 , exposure time and interaction between them with the SPSS statistical package (Ver. 16, SPSS, Chicago, IL, USA). Treatment effects between CF and E- O_3 on each date were evaluated by Duncan's HSD test, and differences between treatments were considered significant if $P \leq 0.05$.

Results

Ozone exposure: Due to rainy and cloudy days, there was a total of 25 days for effective O_3 fumigation during the experiment. Mean daily 8-h $[O_3]$ in the CF and E- O_3 were $16.8 \text{ mm}^3 \text{ m}^{-3}$ and $79.5 \text{ mm}^3 \text{ m}^{-3}$, respectively, calculated from the start until the end of fumigation (totally 58 days). Fig. 1 shows the daily O_3 exposure as

expressed by AOT40 in detail. The AOT40 value in E- O_3 was 12.3, 16.6 and $20.3 \text{ cm}^3 \text{ m}^{-3} \text{ h}$ at 15, 40 and 58 DAF, respectively. For those days with no AOT40 value, it meant that the O_3 fumigation was stopped due to rainy, cloudy, or foggy days.

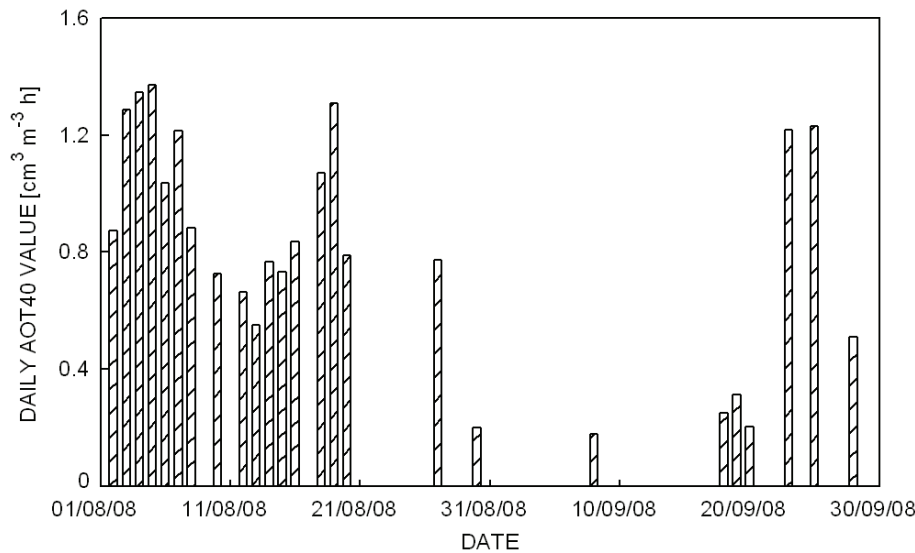


Fig. 1. Daily AOT40 value in the elevated ozone treatment (E-O₃) from 1 August to 28 September, 2008.

Table 1. The growth change in *Liriodendron chinense* seedlings after exposure to different ozone concentrations [charcoal-filtered air (CF) and elevated ozone (E-O₃)]. Mean \pm SD ($n = 3$). * and ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. DM – dry mass.

Parameter	CF	E-O ₃
Relative increment in stem height [%]	59.6 \pm 13.6	59.1 \pm 13.0
Relative increment in basal diameter [%]	72.5 \pm 10.2	66.3 \pm 13.6
Absolute increment in stem height [cm]	21.5 \pm 6.3	16.1 \pm 2.9
Absolute increment in basal diameter [mm]	5.72 \pm 0.74	5.06 \pm 0.77
Stem biomass [g(DM)]	28.2 \pm 1.6	21.9 \pm 2.3*
Root biomass [g(DM)]	26.1 \pm 3.0	17.8 \pm 1.5*
Foliage biomass [g(DM)]	15.2 \pm 1.8	11.6 \pm 2.3
Total biomass [g(DM)]	69.5 \pm 3.4	51.3 \pm 6.1*
Shoot/root ratio	1.70 \pm 0.2	1.96 \pm 0.2

Visible injuries first appeared on the edges of fully expanded leaves with necrotic spots on the upper surface of mature leaves and a light-brown coloration on the younger leaves. With the increase in AOT40, the light-brown coloration turned dark brown and necrotic spots formed necrotic patches. Finally, the mature leaves turned yellow and withered, resulting in early defoliation.

Growth response: E-O₃ had significant negative effects on the several growth parameters of *L. chinense* seedlings in comparison with CF (Table 1). Relative increases in main stem height and basal diameter did not show a significant difference between O₃ treatments, although absolute increments of seedlings grown in E-O₃ were reduced by 25.6% and 14.0% in comparison with CF, respectively. Relative to CF, total biomass, stem, and root dry mass in E-O₃ were significantly reduced by 26.2%, 23.0% and 31.8%, respectively (Table 1).

Leaf pigments: The differences between E-O₃ and CF were significant at the first sampling date (15 DAF). The ANOVA results indicated a significant interaction between O₃ and DAF for Chl and Car, which was due to

O₃-induced significant decreases at 15 and 58 DAF and no difference at 40 DAF (Fig. 2A,B). Across three measurements, E-O₃, as compared with CF, significantly decreased Chl and Car contents by 40.6% and 35.9%, respectively. Chl *a/b* ratio was not affected by E-O₃ during O₃ fumigation (Fig. 2C), indicating similar response to E-O₃ between Chl *a* and Chl *b*.

Gas exchange parameters: ANOVA results indicated that E-O₃ significantly decreased P_{Nsat} and g_s but had no effect on C_i (Fig. 3). Due to similar response to O₃ at each measurement, interactions between O₃ and DAF were not observed in P_{Nsat} and g_s . Relative to CF, E-O₃ significantly decreased P_{Nsat} by 27.0% and 41.6% at 40 and 58 DAF, respectively. However, significant reduction (34.7%) in g_s was only observed at 40 DAF. In comparison with CF, E-O₃ significantly decreased by 20% in V_{cmax} , whereas had no significant effect on J_{max} or J_{max}/V_{cmax} despite a 12% decrease in J_{max} (Table 2).

Chl *a* fluorescence: Across three measurements, E-O₃ significantly decreased q_p and Φ_{PSII} by 30.6% and 35.6%, respectively, but there was no effect on F_v'/F_m' .

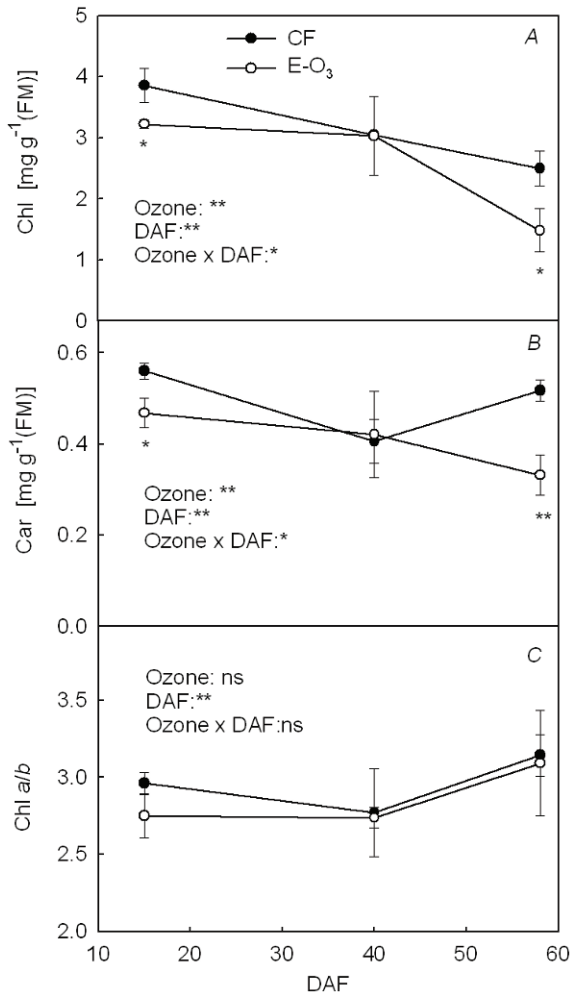


Fig. 2. The change of chlorophyll (Chl), carotenoids (Car) contents, and Chl *a/b* in *Liriodendron chinense* seedlings after exposure to different ozone concentrations (CF – charcoal-filtered air; E-O₃ – elevated ozone). Mean \pm SD ($n = 3$). * and ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. ns – not significant; DAF – days after fumigation.

Individual measurements indicated that O₃ treatment induced a significant decrease in q_p and Φ_{PSII} at 40 DAF with AOT40 of $16.6 \text{ cm}^3 \text{ m}^{-3} \text{ h}$ (Fig. 4). Similar to P_{Nsat}

Discussion

To the best of our knowledge, this is the first study to investigate the effects of elevated [O₃] on native deciduous broadleaf tree species in subtropical regions, where the typical climate is a humid and hot summer with more than 70% of annual precipitation, accompanied with typhoons.

Growth parameters are the major criterion considered when critical ozone levels are discussed; their change can reflect the damage or disruption to metabolic and physiological processes (Broadmeadow 1998). In this study, there was no significant difference between O₃

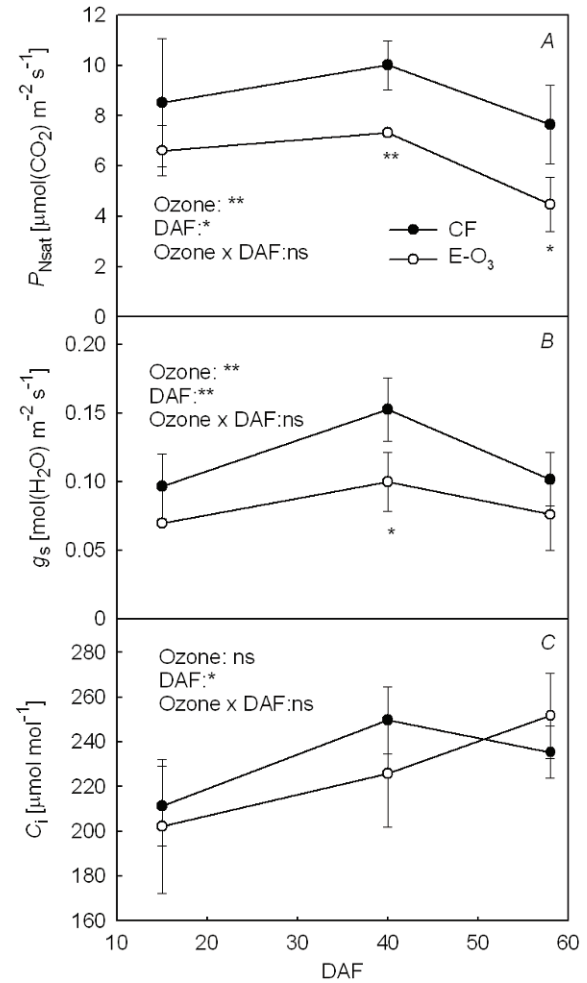


Fig. 3. The change of light-saturated rates of photosynthesis (P_{Nsat}), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) in *Liriodendron chinense* seedlings after exposure to different ozone concentrations (CF – charcoal-filtered air; E-O₃ – elevated ozone). Mean \pm SD ($n = 3$). * and ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. ns – not significant; DAF – days after fumigation.

and g_s , the Chl *a* fluorescence parameters showed no significant interaction between O₃ and DAF due to the similar response to E-O₃ at each measurement.

treatments in relative increase of both basal diameter and stem height. However, there was a tendency towards a reduction in absolute stem diameter increment (11.5%) and in the stem height (25.6%) after exposure to E-O₃ (AOT40 $20.3 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) compared with CF treatment. Similar responses to O₃ were also reported in other broad-leaved tree species, e.g. beech (*Fagus sylvatica* L.) (Dixon *et al.* 1998, Bortier *et al.* 2000). On the other hand, E-O₃ (AOT40 of $20.3 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) significantly reduced the biomass of different organs in *L. chinense* by 23–32%, with the largest reduction in root. Oksanen and

Table 2. The change of maximum rate of carboxylation (V_{cmax}) and maximum rate of electron transport contributing to RuBP regeneration (J_{max}) and the ratio of $J_{\text{max}}/V_{\text{cmax}}$ in *Liriodendron chinense* seedlings after exposure to different ozone concentrations [charcoal-filtered air (CF) and elevated ozone (E- O_3)]. Mean \pm SD ($n = 3$). * and ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively.

	CF	E- O_3
V_{cmax} [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	62.6 ± 4.94	$50.0 \pm 6.69^*$
J_{max} [$\mu\text{mol}(\text{electron}) \text{ m}^{-2} \text{ s}^{-1}$]	93.4 ± 14.9	82.4 ± 10.6
$J_{\text{max}}/V_{\text{cmax}}$	1.51 ± 0.19	1.68 ± 0.14

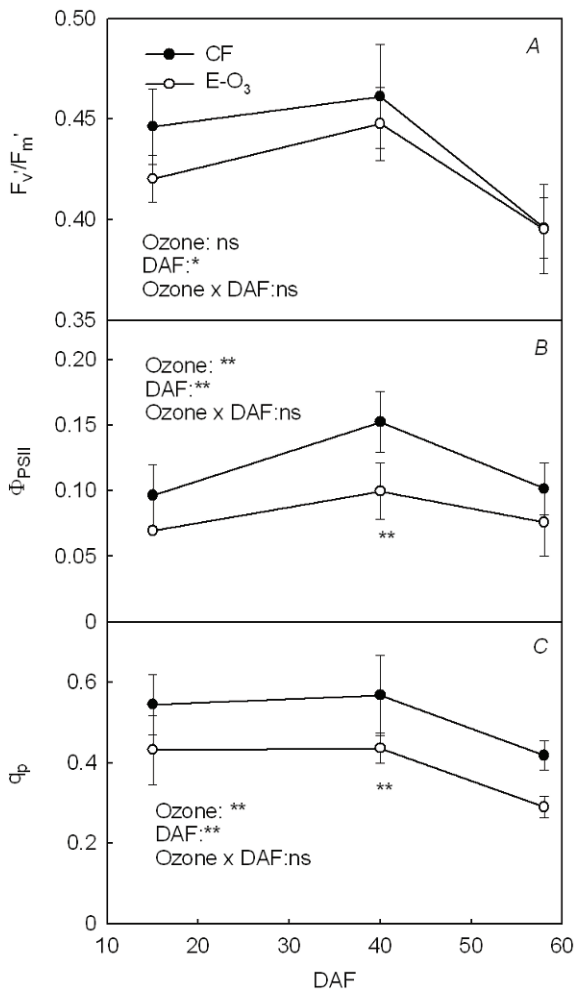


Fig. 4. The change of actual photochemical efficiency of PSII (F_v'/F_m'), photochemical quenching coefficient (q_p), and effective quantum yield of PSII photochemistry (Φ_{PSII}) in *Liriodendron chinense* seedlings after exposure to different ozone concentrations (CF – charcoal-filtered air; E- O_3 – elevated ozone). Mean \pm SD ($n = 3$). * and ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively. ns – not significant; DAF – days after fumigation.

Holopainen (2001) found that the dry shoot mass and stem height increment of an ozone-sensitive clone of

white birch (*Betula pendula* Roth) were reduced by up to 17% and 46%, respectively, after exposure to O_3 for 20 days (AOT40 of $14.4 \text{ cm}^3 \text{ m}^{-3} \text{ h}$). Another recent study found that ambient O_3 (AOT40 of $27.3 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) reduced the total biomass by 17.6% in *Fagus crenata* seedlings, a sensitive Japanese tree species relative to CF (Yamaguchi *et al.* 2007).

During our experiment, there were three typhoons, respectively with a 6-day, 8-day and 9-day rain period, resulting in the significantly reduced AOT40 value ($4.8 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) between 21 August and 28 September compared to that ($15.5 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) between 1 August and 20 August. The climate in subtropical regions might curtail the negative effects of ozone on plant growth due to recovery at some degree during the long segments of rainy days (Heath 1996). However, the significant negative impacts of O_3 (AOT40 of $20.3 \text{ cm}^3 \text{ m}^{-3} \text{ h}$) on growth parameters indicated that *L. chinense* is an O_3 -sensitive tree species. This also supports the idea that faster growing species tend to be more sensitive than slower growing species (Bortier *et al.* 2000, Novak *et al.* 2005).

Numerous experiments have demonstrated that O_3 can cause significant reductions in photosynthetic rates (Heath 1996, Bortier *et al.* 2000, Novak *et al.* 2005, Ribas *et al.* 2005, Yamaguchi *et al.* 2007, Wittmann *et al.* 2007, Ryang *et al.* 2009). In the present study, significant reduction in P_{Nsat} (27.0%) and g_s (34.7%) of *L. chinense* was observed after 40 days of O_3 exposure with AOT40 of $16.6 \text{ cm}^3 \text{ m}^{-3} \text{ h}$. E- O_3 induced similar patterns of change in both P_{Nsat} and g_s during the fumigation, suggesting that reduced P_{Nsat} may be due to stomatal closure. However, the two should change in the same direction if CO_2 uptake and photosynthesis were only regulated by stomatal opening. In contrast, our result showed that E- O_3 did not affect C_i across three measurements or at individual measurement, thus P_{Nsat} had no definite pattern of decrease or increase with C_i , which became more evident in the lower correlation coefficient in relationships of P_{Nsat} with C_i ($r = 0.052$, $n = 6$) as compared with that of P_{Nsat} and g_s ($r = 0.839$, $n = 6$). It can be inferred that stomatal closure is not a direct effect of O_3 , but a reaction to an increased internal CO_2 concentration resulting from the inhibition of carbon assimilation (Reich 1987). Significant correlation between P_{Nsat} and q_p ($r = 0.955$, $n = 6$) or P_{Nsat} and Φ_{PSII} ($r = 0.945$, $n = 6$) was found, suggesting that *L. chinense* exposure to E- O_3 failed to keep PSII centers in an open state so that excitation energy can not be fully used for noncyclic electron transport. The results support the notion that the decrease in the quantum yield of PSII electron transport may be a mechanism to down-regulate photosynthetic rate. Moreover, V_{cmax} , the primary target for O_3 injury, was also significantly reduced by the end of experiment. Therefore, the present results indicated that non-stomatal factors, including electron transport and biochemical changes, contributed more to the decreased photosynthesis than stomatal closure.

Admittedly, this experiment was conducted using a relatively high O₃ concentration for a short duration. However, artificial exposure of seedlings was a useful surrogate to confirm the sensitivity of plants to O₃ and to determine the physiological effects of O₃ (Manning 2005). Therefore, this approach of seedlings exposure can help understanding whether ambient O₃ is the cause of visible foliar injury of mature trees. Similar exposure dose experiments were also conducted in other tree species (Oksanen and Holopainen 2001, Wittmann *et al.*

2007, Ryang *et al.* 2009).

In conclusion, our results suggested that E-O₃ caused significant reductions in biomass and net photosynthetic parameters. The main causes of reduced P_{Nsat} were nonstomatal factors, including noncyclic electron transport in light reaction and V_{cmax} . Based on the results obtained from the present study, *L. chinense* can be assumed to be an O₃-sensitive tree species. Current ambient ozone concentrations in Southern China could be an important limiting factor for growth of *L. chinense*.

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