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Effects of Elevated Ozone on Photosynthetic CO_2 Exchange and Chlorophyll *a* Fluorescence in Leaves of *Quercus mongolica* Grown in Urban Area

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Abstract The aim of this study was to determine the responses of photosynthesis in leaves of *Quercus mongolica* to elevated O_3 exposure in an urban area. The results showed that the photosynthesis parameters were all decreased by the elevated O_3 during the whole season. Especially, light-saturated net photosynthetic rate (A) was reduced about 72% after 45 days exposure, and over the whole growing season, the differences were significant ($p < 0.05$) compared with control. The reduction is related to both stomatal and non-stomatal factors, including a decrease (about 9%) in the maximum quantum yield of PSII photochemistry (F_v/F_m) detected after 90 days O_3 exposure.

Keywords Elevated O_3 · Photosynthetic CO_2 exchange · Chlorophyll *a* fluorescence · Leaf growth

Tropospheric ozone (O_3) is not only an important greenhouse gas, but also a strong phytotoxic pollutant (Percy et al. 2002). The concentration of O_3 has been increasing rapidly because of the anthropogenic emissions of several O_3-forming gases (Katri et al. 2006). This increase is expected to continue unabated for the foreseeable future (Luis et al. 1996). Many researchers have focused on the effects of O_3 on plants and done work on the mechanism of O_3 induced injury and O_3 resistance of trees (Gian et al. 1998; Chantal et al. 1999; Paoletti et al. 2006). The most important and direct effect of elevated O_3 on crops and trees is the changes in photosynthesis. The study of chlorophyll *a* fluorescence provides information about light reaction of photosynthesis and serves as a non-invasive indicator of the status of photosynthetic reaction centers (Calatayud et al. 2006). It can be combined with gas exchange measurements to obtain a full picture of the responses of photosynthesis to environment. Several articles have used chlorophyll *a* fluorescence and gas exchange characteristics to look for the primary site of photosynthesis limitations exerted by O_3 (Seppo and Wang 1997; Angeles et al. 2002), but the inherent mechanism of the limitation is still not very clear. In addition, high [O_3] occurs mainly around urban regions (Thompson et al. 1990). The trees grown in urban regions are more likely affected by elevated O_3, so the open top chambers (OTCs) of this experiment are built in the center of Shenyang city to simulate the high [O_3] effect on *Quercus mongolica*, so as to have an in-depth understanding on the effects of high [O_3] on photosynthesis of plants.

Materials and Methods

The experiment site is located in Shenyang Arboretum of Chinese Academy of Sciences which is located in the metropolitan area of Shenyang (41°46′ N, 123°26′ E). The factorial design of the OTCs has already been reported (He et al. 2006). Three OTCs with ambient air were used as control, other three were treated by elevated O_3 (80 nmol mol^{-1}, from 08:00−17:00 per day). O_3 was produced from bottled pure oxygen with an ozone generator (GP-5J, China). In order to monitor O_3 concentration,

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an O_3 transducer (S-900, Aeroqual, New Zealand) was used. The soil in the OTCs was loamy, weeds were removed regularly. In April 2007, the uniform and healthy four-year-old *Q. mongolica* trees were brought from a local nursery and randomly planted in the soil of six OTCs, 20 trees per chamber. The O_3 treatment lasted from 18 June to 10 October 2007.

The leaf fresh weight, dry weight, leaf size were measured on 20th day and 90th day of O_3 fumigation. Leaf size was determined with an area-meter (LI-COR 3000, Lincoln, Nebraska, USA).

All photosynthetic parameters were measured by a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln NE, USA), once every 15 days. Gas exchange was measured under saturated light between 10:00 and 11:00 on mature leaves on the middle parts of lateral branches. All measurements were done at a constant air flow rate of 500 μmol s^{-1} and environmental conditions did not show statistical differences between every measurements during the study.

Light-saturated net photosynthesis rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), and stomatal conductance (gs) were recorded by Li-6400 on three leaves per plant, and three plants per OTC. The required irradiance for the measurements was a red-blue LED light source from the curette installed in the top of the leaf chamber. Photosynthesis rates were recorded when the coefficient of variation for each measurement was lower than 1%. A preparatory experiment showed the photosynthesis rate was sufficiently saturated at an irradiance 1,500 μmol m^{-2} s^{-1} PPFD and this value was used later on to measure saturated photosynthesis. The temperature and humidity were not controlled but dependent on the ambient weather conditions.

An auto-measurement program was designed and applied to acquire the light response curve of Pn to PPFD of *Q. mongolica* leaves, PPFD was changed from 0 to 2,000 μmol m^{-2} s^{-1} (0, 25, 50, 100, 200, 300, 500, 800, 1,000, 1,500, 2,000 μmol m^{-2} s^{-1}). The steady reference CO_2 concentration was acquired by a CO_2 steel bottle supplied with a CO_2 mixer system. The air temperature of the chamber was maintained at 25°C. Apparent quantum yield (Φa) was calculated from the initial slope dPn/dPPFD of the photosynthesis-light response curve by linear regression using data got with PPFD below 300 μmol m^{-2} s^{-1}. Three replications were performed in each treatment to attain a mean value.

Another auto-measurement program was designed and applied to acquire Pn -CO_2 response curve of *Q. mongolica* leaves. A CO_2 mixer system was used to control the reference CO_2 concentrations, set in steps at 360, 300, 200, 150, 100, 50, 360, 600, 800, 1,000, 1,200, 1,500, and 1,800 μmol mol^{-1}, with PPFD maintained at 1,500 μmol m^{-2} s^{-1}. The air temperature of the chamber was maintained at 25°C. After the measurement, the curve of Pn related to intercellular CO_2 concentration (Ci) was established, the carboxylation efficiency (ΦCO_2) was calculated from the slope dPn/dCi of correlation curve when Ci below 200 μmol mol^{-1}.

Chlorophyll *a* fluorescence was measured (on 10th, 60th, and 90th day) at ambient temperature in the OTC's, using a portable fluorometer (Handy-PEA, Hansatech, England). The environmental conditions were similar to gas exchange measurement. The leaves were dark-adapted for 15 min prior to measurement. The minimum fluorescence F_0 was obtained upon excitation of leaves with a weak beam from a light-emitting diode. The maximum fluorescence (F_m) was determined following an 1,800-ms pulse of saturating white light. The yield of variable fluorescence (F_v) was calculated as $F_m - F_0$.

One-way analysis of variance (ANOVA) was performed using SPSS computer package (SPSS Inc.1999) for all sets of data, and the significance of differences among treatments were estimated by *t*-test ($p < 0.05$). Sample variability is given as the standard deviation (SD) for presentation

Results and Discussion

Mean increments of fresh weight (per leaf), dry weight (per leaf), and leaf size in the period from 20th day to 90th day were reduced 60.2%, 35.8%, and 26.22% by elevated O_3, respectively, compared with control (Table 1).

We have reported that light-saturated net photosynthetic rate in leaves of *Ginkgo biloba* was decreased by elevated O_3 exposure (He et al. 2007). Similar result was got here with *Q. mongolica.* In this experiment, light-saturated net photosynthetic rate (A) of *Q. mongolica* leaves was also significantly ($p < 0.05$) decreased by elevated O_3 exposure over the whole growing season. The maximum difference (about 72%) was recorded after 45 days exposure (Fig. 1). However, in leaves of *G biloba*, the maximum difference of A was about 54.5%, and detected at the end of the season (at 100th day) (He et al. 2007). This revealed that *Q. mongolica* is more sensitive to O_3 stress.

The target of O_3 stress in the cell bringing about the decrease in light-saturated photosynthesis has not been clearly identified. Stomatal conductance is a measure accepted today for quantifying plant response to ozone. Therefore, stomatal regulation must be an important factor in controlling ozone sensitivity of plants (Reich 1987). Our results showed that stomatal conductance of *Q. mongolica* leaves was significantly ($p < 0.05$) decreased by high O_3 at the beginning of the experiment, while the similar decrease was appeared in transpiration rate of leaves (Fig. 2). These results indicate that the partial closure of stomata can be

Table 1 Effects of elevated O_3 exposure on leaf growth of *Q. mongolica*

		Fresh weight per leaf (g)	Dry weight per leaf (g)	Leaf size (cm^2)
Ambient O_3	20 day	0.462 ± 0.065	0.147 ± 0.017	35.80 ± 3.66
	90 day	0.819 ± 0.082	0.375 ± 0.036	41.00 ± 6.33
	Increment in 70 days	0.357 ± 0.072	0.228 ± 0.031	5.20 ± 1.16
Elevated O_3	20 day	0.339 ± 0.061	0.113 ± 0.015	29.11 ± 3.42
	90 day	0.481 ± 0.101	0.259 ± 0.062	32.94 ± 7.49
	Increment in 70 days	0.142 ± 0.031	0.146 ± 0.025	3.83 ± 0.95

Data are means ±SD (n = 30)

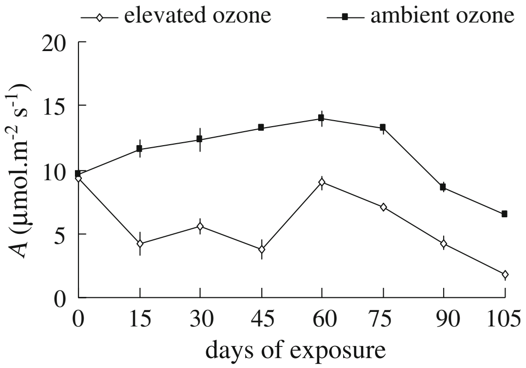

Fig. 1 Effects of elevated O_3 concentration on light-saturated net photosynthetic rate (A) of *Q. mongolica* leaves

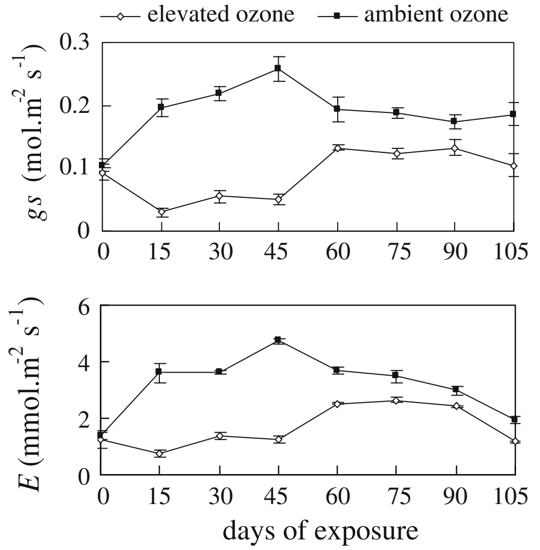

Fig. 2 Effects of elevated O_3 concentration on stomata conductance (gs) and transpiration rate (E) of *Q. mongolica* leaves

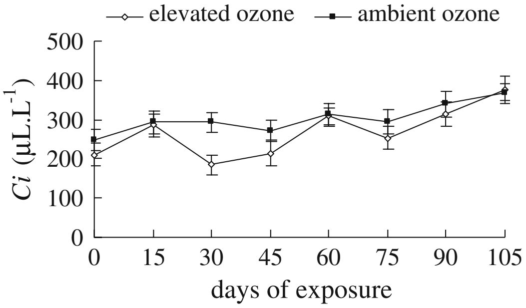

Fig. 3 Effects of elevated O_3 concentration on intercellular CO_2 concentration (Ci) of *Q. mongolica* leaves

induced by elevated O_3 to reduce further ozone uptake. Many researches suggested that stomata closure is not a direct response to O_3 insult, but a reaction to an increased internal CO_2 concentration resulted from the inhibition of carbon assimilation (Reich 1987; Weber et al. 1993). However, in our study, there was nearly no increase but decrease, some times significantly decrease in intercellular CO_2 concentration of leaves detected in the O_3 elevated environment compared with control when stomata conductance (gs) of leaves had decreased to minimum (Figs. 2, 3), so in our case, the stomata closure maybe directly associated with the effect of high O_3 on guard cells of *Q. mongolica* leaves.

Moreover, reduction in carboxylation efficiency and apparent quantum yield was observed in parallel with reduction of net photosynthetic rate (A) of leaves under elevated O_3 exposure (Fig. 4). This indicates that there was also non-stomatal limitation in photosynthesis in *Q. mongolica* leaves. The decrease in carboxylation efficiency and apparent quantum yield may be attributed to the direct oxidative stress on the Calvin cycle or to indirect effect caused by membrane disorders (Gian et al. 1998).

The ratio between variable and maximal fluorescence, F_v/F_m, is widely used as an estimation of the maximum quantum yield of PSll photochemistry (Guidi and Soldatini 2002), while the ratio between variable and minimum fluorescence, F_v/F_0 reflect the potential activity of PSII. At the end of elevated O_3 exposure (90 days), there were 9% and 28% decreases in F_v/F_m and F_v/F_0 (Fig. 5) indicating that O_3 can limit the capability of the plant to use photon energy and thus alter photosynthetic processes (Calatayud et al. 2006). This result was supported by Angeles (Angeles et al. 2002). Just in the late season (75–105 days), prolonged O_3 exposure gradually increased

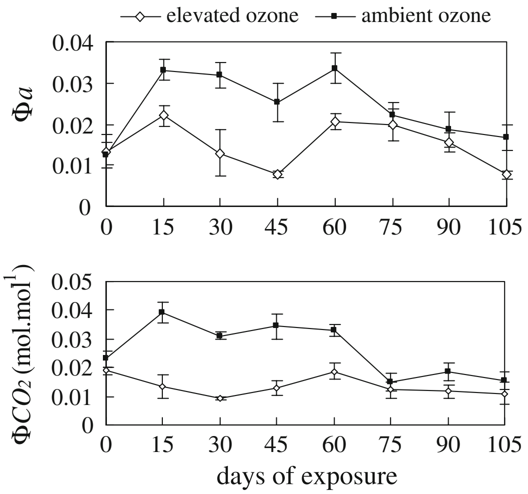

Fig. 4 Effects of elevated O_3 concentration on apparent quantum yield (Φa) and carboxylation efficiency (ΦCO_2) of *Q. mongolica* leaves

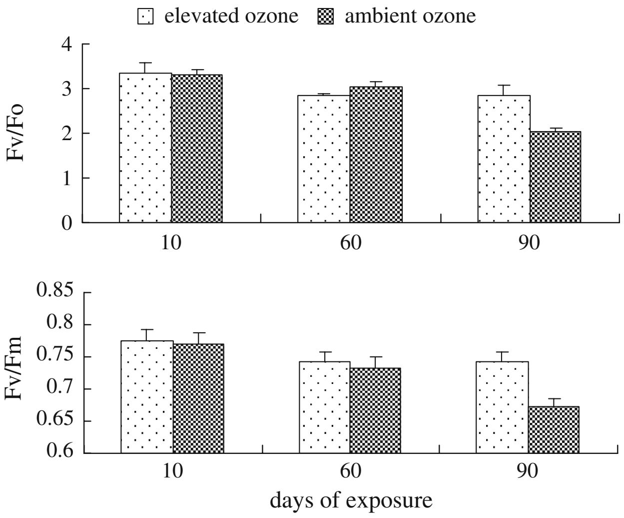

Fig. 5 Effects of elevated O_3 concentration on F_v/F_0 and F_v/F_m of *Q. mongolica* leaves

intercellular CO_2 concentration. That made the difference declined gradually, and eventually there was no significant difference between high O_3 treatment and control. That may be caused by the low CO_2 assimilation efficiency induced by the lower efficiency of light conversion of PSII.

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