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Responses of the anti-oxidative system in leaves of *Ginkgo biloba* to elevated ozone concentration in an urban area

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ABSTRACT. To study the effects of elevated tropospheric ozone concentrations on the anti-oxidative systems of trees, the $O_2^{\cdot-}$ generating rate, H_2O_2 content, activities of SOD and all enzymes in the Halliwell-Asada pathway and ascorbic acid content were periodically analyzed in leaves of *Ginkgo biloba* grown in open-top chambers at either ambient (≈45 nmol mol^{-1}) or elevated (80 nmol mol^{-1}) ozone concentrations in an urban area for a growing season. The results show that elevated ozone exposure induced a greater superoxide anion ($O_2^{\cdot-}$) generating rate and higher hydrogen peroxide (H_2O_2) content. Malondialdehyde (MDA) content as an index of lipid peroxidation also increased. The activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) were enhanced by high ozone exposure in the first half of the season before falling to levels lower than those of control. The ascorbic acid content was always lower in the high-ozone exposed leaves. We conclude that the antioxidant system in *Ginkgo biloba* did respond by acclimating in the early season. However, the constant higher level of reactive oxygen species and declining enzyme activities late in the season indicate that the system could not withstand the long-term exposure although no visible injury was observed.

Keywords: Anti-oxidantive system; Elevated ozone concentration; Lipid peroxidation; Reactive oxygen species.

Abbreviations: **ASA**, ascorbate; **SOD**, superoxide dismutase; **APX**, ascorbate peroxidase; **MDAR**, monodehydroascorbate reductase; **GR**, glutathione reductase; **DHAR,** dehydroascorbate reductase; **DHA,** dehydroascorbate; **ROS**, reactive oxygen species; **$O_2^{\cdot-}$,** superoxide anion; **H_2O_2**, hydrogen peroxide; **MDA**, malondialdehyde; **DW**, dry weight.

INTRODUCTION

The total amount of O_3 in the troposphere is estimated to have increased by 36% since pre-industrial times, due primarily to anthropogenic emissions of several O_3-forming gases (IPCC, 2001). Elevated concentrations of ozone at ground-level are known to have negative impacts on human health, ecosystems, and materials. The biological response of plants to ozone stress is dependent on a number of factors, including the species involved, its development stage, and environmental conditions (Heck and Miller, 1994).

Ozone is one of the most powerful oxidants known. In plants, primary damage is largely confined to the leaf mesophyll, where ozone dissolves into the wet surface of the exposed cell walls (Kangasjärvi et al., 1994). Reactions of ozone with water and solutes in the apoplasm lead to the formation of other reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), and singlet oxygen (Oksanen et al., 2003). To scavenge the reactive oxygen species, a relevant defense system is represented by enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR), and glutathione reductase (GR) (van Montagu and Inzé, 1992), together with small molecular antioxidants like ascorbate (ASA), carotenoids, polyamines and glutathione, SOD dismutase $O_2^{\cdot-}$ to O_2, and H_2O_2. The ascorbate-glutathione cycle is responsible for the removal of H_2O_2. Strong induction of these Halliwell-Asada pathway enzymes has been reported in various stress situations (Bowler et al., 1992; Kangasjärvi et al., 1994). SOD cooperates with this cycle in scavenging reactive oxygen species.

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Urban air contains high concentrations of many gaseous, particulate, and photochemical pollutants (such as NOx, O_3). Trees in urban areas can sequestrate CO_2 (McPherson, 1998; Nowak, 1994b), and reduce ozone (Nowak, 1994a; McPherson and Simpson, 1998). Until now, the few studies done on urban forests, however, have mainly dealt with ecological functions (Akbari and Taha, 1992; Simpson, 1998) while biochemical and physiological changes have received scant attention. Therefore, in order to better understand how the main species of urban forests respond to increased ozone concentration, *Ginkgo biloba* grown in an urban area was exposed to 80 nmol mol^{-1} ozone for a growing season, and changes in the activities of related enzymes and antioxidant contents were followed.

MATERIALS AND METHODS

Plant material

Six-year-old *Ginkgo biloba* trees were placed in open top chambers (OTCs) in April 2005. Each OTC contained thirty trees. These young trees were exposed to ambient air or elevated (80 nmol/mol) ozone concentration from 10 June to 30 September 2005. Healthy *Ginkgo biloba* leaves, collected at 9 A.M. every ten days, were then analyzed immediately. To calculate dry weight, parallel samples were dried at 80°C for 8 h.

Experimental site and OTCs

The experiment site was established at the Shenyang Arboretum of Chinese Academy of Sciences (41°46' N, 123°26' E) in an urban environment. The factorial design comprising three replicates of elevated O_3 and three of ambient O_3 was used in 6 OTCs. The OTCs imitated the Heagle design (Heagle et al., 1973), were 4 m in diameter and 3 m in height with a 45º sloping frustum, and were placed on 4-m centers (north-south and east-west) to avoid mutual shading. Ozone was produced from pure oxygen with an ozone generator (GP-5J, China). Ozone-enriched air was injected into OTCs through the vertical vent pipes in center of each chamber. Ozone concentrations were continuously monitored by an ozone monitor (S-900, Aeroqual, New Zealand) within the canopy in each OTC. The elevated ozone concentration (80 ± 8 nmol mol^{-1}) was approximately 1.7 times the ambient ozone level (45 nmol mol^{-1}), based on predicted near-future concentrations for the city of Shenyang. The elevated O_3 treatment was maintained and monitored for 8 h (08:00-16:00) everyday.

Growth parameters

Ten *Ginkgo biloba* trees from each treatment were sampled randomly at the 20th and 90th days. Lengths of axial shoot and lateral shoot were measured.

Generating rate of $O_2^{\cdot\cdot}$ and content of H_2O_2

The generating rate of superoxide anion was determined following the method of Ke et al. (2002) with a slight modification. Fresh leaves (0.5 g) were ground with a mortar and pestle in 5 mL of 50 mmol/L (pH 7.8) phosphate buffer. The homogenate was filtered through a 45-μm nylon mesh and centrifuged at 13,000 g for 20 min. One mL Hydroxylammonium chloride (1 mmol/L) was added into 0.5 mL of the above supernatant and incubated for 1 h at 25°C. The color was developed by the addition of 1 mL 4-minobenzenesulfonic acid (17 mmol/L) and 1 mL α-Naphthylamine (7 mmol/L) for 20 min at 25°C. The specific absorption at 530 nm was determined. Sodium nitrite was used as standard solution to calculate the content of $O_2^{\cdot-}$.

Hydrogen peroxide concentrations were estimated by forming a titanium-hydro peroxide complex (Dagmar et al., 2001). Leaves (0.5 g) were ground with 5 mL cooled acetone in an ice bath. This mixture was then filtered through nylon cloth followed by the addition of 4 mL titanium reagent and 5 mL ammonia to precipitate the titanium-hydro peroxide complex. The reaction mixture was centrifuged at 10,000 *g* for 10 min. Precipitate was dissolved in 5 mL 2 M H_2SO_4 and then re-centrifuged. Supernatant was read at 415 nm against a reagent blank in a Shimadzu (UV-1601) spectrophotometer. H_2O_2 concentration was determined using a standard curve plotted with a known concentration of H_2O_2.

Lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to the method of Buege and Aust (1978). MDA is a product of lipid peroxidation. The quantity is determined by thiobarbituric acid reaction. One g leaf was homogenized in 5 mL of 0.6% (v/v) TBA solution in 10% (v/v) trichloroacetic acid. The homogenate was centrifuged at 12,000 *g* for 15 min. and the supernatant was heated in a boiling water bath for 15 min and then cooled quickly in an ice bath. The resulting mixture was centrifuged at 12,000 *g* for 15 min, and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. MDA concentrations were calculated by means of an extinction coefficient of 155 $(mmol/L)^{-1}$ cm^{-1} (Zhangyuan and Bramlage, 1992).

Ascorbate content

Ascorbate was estimated according to Mukherjee and Choudhuri (1983) with a slight modification. ASA was extracted from 0.2 g fresh leaf tissue with 5 mL of 6% trichloroacetic acid. Two mL of the extract was mixed with 1 mL of 2% dinitrophenylhydrazine (in acidic medium) followed by the addition of one drop 10% thiourea (in 70% ethanol). The mixture was boiled for 20 min in a water bath. After cooling to room temperature, 5 mL of 80% (v/v) H_2SO_4 was added to the mixture at 0°C (in an ice bath). The absorbance was recorded at 530 nm. The concentration of ASA was calculated from a standard curve plotted with a known concentration of ASA.

Enzyme extraction and assays

All enzyme extractions and centrifugations were carried out at 4°C, and the extracts were stored on ice. All assays were made at room temperature and repeated thrice.

Extraction of enzymes: superoxide dismutase (SOD; EC 1.15.1.1) was extracted from 0.5 g leaves (fresh weight) ground with a mortar and pestle in 5 mL of 50 mmol/L phosphate buffer (pH 7.8). The homogenate was filtered through a 45-µm nylon mesh and centrifuged at 13,000 *g* for 20 min.

Ascorbate peroxidase (APX; EC 1.11.1.6), monodehydroascorbate reductase (MDAR; EC 1.6.5.4), dehydroascorbate reductase (DHAR; EC 1.8.5.1), and glutathione reductase (GR; EC 1.6.4.2) were extracted together from 0.5 g of leaves (fresh weight) that had been ground in a mortar. Three and a half mL phosphate buffer (pH 7.6) containing 4% polyvinylprolidone (PVP), EDTA 1 mmol/L and ascorbate (sodium salt, freshly prepared) 1 mmol/L were added. Then 1.5 mL of saturated amonium sulfate in the same buffer was added and stirred well to minimize the interference of resin that would otherwise inhibit APX activity (Krivosheeva et al., 1996). After filtering through nylon cloth, the filtrate was centrifuged, transferred into vials, and then kept on ice. The enzyme activities were assayed immediately.

Assay of enzyme activity: SOD activity was estimated by the inhibition of nitroblueterazolium (NBT) reduction (Beyer and Fridovich 1987). The reaction mixture contained 0.3 ml each of 13 µmol/L riboflavin, 130 mmol/L L-methionine, and 630 µmol/L NBT and enzyme extracts of 0.01, 0.02, 0.04 or 0.08 ml. The phosphate buffer (pH 7.8) was added to give a final volume of 3 ml. The reaction was started by adding riboflavin and carried out for 20 min under the light of two 27-w fluorescent lamps. The absorbance at 560 nm was determined regularly, and the extract volume causing a 50% inhibition of NBT reduction was taken as one unit of activity.

For the APX assay, a 1-mL reaction mixture containing 0.83 mL of 0.5 mmol/L ASA in phosphate buffer (pH 7.0), 0.13 mL of 2 mmol/L H_2O_2 (both were made fresh) and 0.04 mL of crude enzyme was used. The ASA consumption was monitored by the reduction of absorbance at 290 nm taking 2.8 $(mmol/L)^{-1}$ cm^{-1} as the absorption coefficient (Nakano and Asada, 1981). For the MDAR assay, the reaction mixture containing 0.9 mL of 2 mmol/L ASA in phosphate buffer (pH 7.0), 0.04 mL of ascorbate oxidase (2 units) in phosphate buffer (pH 5.6), 0.03 mL of 2 mmol/L NADPH in phosphate buffer (pH 7.6), and 0.03 mL crude enzyme was used. The consumption of NADPH was monitored by the reduction of absorbance at 340 nm taking 6.2 $(mmol/L)^{-1}$ cm^{-1} as the absorbance coefficient (Krivosheeva et al., 1996). For the DHAR assay, a reaction mixture containing phosphate buffer (pH 7.0) 0.7 mL, reduced glutathione (GSH) 20 mmol/L 0.1 ml in the phosphate buffer (pH 7.0), 2 mmol/L DHA 0.1 mL, and crude enzyme 0.1 mL was used. DHA was freshly prepared and kept on ice until it was added to the reaction mixture in the cuvette to prevent its fast oxidation at room temperature. The reduction of DHA to ASA was monitored by the increase in absorbance at 290 nm, taking 2.8 $(mmol/L)^{-1}$ cm^{-1} as the absorbance coefficient (Krivosheeva et al., 1996). For GR assay, the reaction mixture containing 0.86 mL of 1 mmol/L oxidized glutathione (GSSG), 0.1 mL of 2 mmol/L NADPH in phosphate buffer (pH 7.6), and 0.04 mL crude enzyme was used. The consumption of NADPH was monitored for GR assay (Krivosheeva et al., 1996).

All the ascorbate-glutathione cycle enzyme reactions were monitored for 1 min in the cuvette of a Shimazu UV-1601 spectrophotometer. NADPH was bought from Merk and DHA, GSSG, GSH, AsA (sodium salt), PVP and ascorbate oxidase were bought from Sigma.

Statistical analysis

The results presented are the means (n=3-8) of all the measurements. One-way analysis of variance (ANOVA) was performed using the SPSS computer package (SPSS Inc. 1999) for all sets of data, and the mean differences were compared by t-test ($P < 0.05$). Sample variability is given as the standard deviation (S. D.) for presentation with line diagram.

RESULTS

Growth parameters

The increment of axial shoot and lateral shoot decreased 66% and 47%, respectively, from 20 d to 90 d of elevated O_3 exposure, compared to plants grown under ambient O_3 (Table 1).

Generating rate of superoxide anion (O_2^{-}) and content of hydrogen peroxide (H_2O_2)

Elevated ozone induced $O_2^{\cdot-}$ and H_2O_2 accumulation (Figure 1). The generating rate of O_2^{-} and content of H_2O_2 increased by 30.7% and 31.7%, respectively, during the first 10 days (on 21 June), compared to those of plants grown under ambient O_3. However, prolonged exposure to elevated O_3 resulted in a gradual decrease in the $O_2^{\cdot-}$ generating rate, which bottomed out after 40 days of exposure and then increased rapidly after 60 days (Figure 1A). H_2O_2 content always increased in the growing season in response to elevated ozone (Figure 1B).

Lipid peroxidation

MDA content increased under elevated O_3, compared to plants grown under ambient O_3 in the growing season (Figure 2). The fluctuation of MDA content was parallel to the $O_2^{\cdot-}$ generating rate.

Ascorbate content

Ascorbate is an integral weapon in the defense against ROS generated by ozone (Conklin and Barth, 2004). Under elevated O_3, ascorbate content fell significantly

Table 1. Effects of elevated O_3 exposure during 20 and 90 days on growth parameters (axial shoot and lateral shoot lengths) in *Ginkgo biloba*.

		Axial shoot (cm)	Lateral shoot (cm)
Ambient O_3	20 day	25.10 ± 5.39	19.59 ± 3.75
	90 day	33.67 ± 5.69	25.63 ± 3.46
	Increment (90 day-20 day)	8.57 ± 1.01	6.04 ± 2.23
Elevated O_3	20 day	31.07 ± 4.74	24.08 ± 3.29
	90 day	34.00 ± 5.29	27.25 ± 3.77
	Increment (90 day-20 day)	2.93 ± 0.67	3.18 ± 1.70

Means ± S.D. (n=8) for length of axial shoot and lateral shoot.

after 20 days exposure (Figure 3) compared to plants grown under ambient O_3.

Activity of antioxidative enzymes

A single peak appeared after 50 days of exposure (on 31 July). Elevated O_3 exposure enhanced SOD activity significantly in the first 40 days. However, prolonged O_3 exposure gradually decreased SOD activity compared to plants grown under ambient O_3 (Figure 4).

The activities of APX, MDAR and GR increased by 21%, 21%, and 22%, respectively, in the first 10 days, compared to plants grown under ambient O_3 (Figure 5). With prolonged exposure under high O_3, APX, and MDAR activities decreased after 40 days of exposure while GR activity underwent no significant difference from 30 day to 80 day. An evident increase appeared in the last 10 day of exposure. Under high O_3 exposure, DHAR activity was 20.8% lower than under ambient O_3 in the first 10 day. Then, higher activities were shown in the high O_3 exposed leaves. However, after prolonged exposure, DHAR activity became again lower than that under ambient O_3 in the last 20 day. APX activity was consistently much higher than the sum of DHAR and MDAR activities in this growing season.

DISCUSSION

Tropospheric ozone is a major component of a photochemical air pollutant responsible for significant damage in both natural and cultivated plants. In this study, elevated O_3 caused a significant decrease in the growth of axial and lateral shoots in *Ginkgo biloba* trees. However, no visible foliar injury symptom was observed on *Ginkgo biloba* leaves exposed to high ozone concentration.

The phytotoxicity of O_3 is due to its ability to generate other ROS such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Mudd, 1997; Runeckles and Vaarthou, 1997). Growth under elevated O_3 significantly increased $O_2^{\cdot-}$ generating rate and H_2O_2 content compared to those of plants grown under ambient O_3 (Figure 1). Superoxide anion is relatively unstable and is readily dismuted to

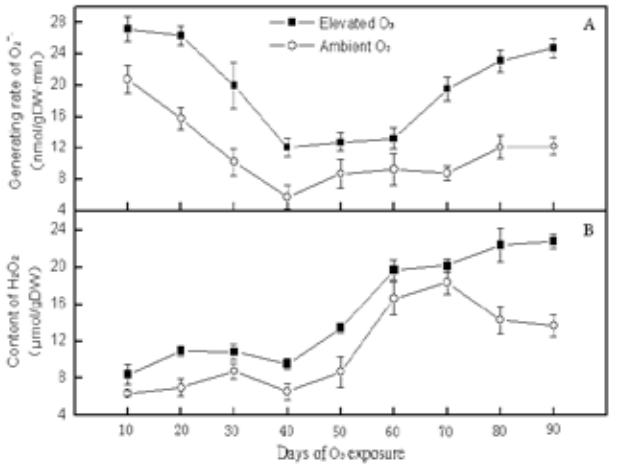

Figure 1. Generating rate of $O_2^{\cdot-}$ (A) and content of H_2O_2 (B) in *Ginkgo biloba* leaves related to time of elevated O_3 exposure. Elevated O_3: the trees were grown under elevated (80 nmol/mol) O_3; Ambient O_3: the trees were grown under ambient O_3; means on n=3-6. The same for all the following figures.

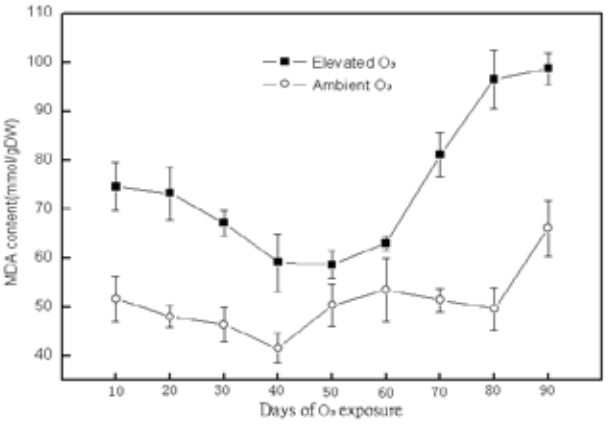

Figure 2. MDA content in *Ginkgo biloba* leaves related to time of elevated O_3 exposure.

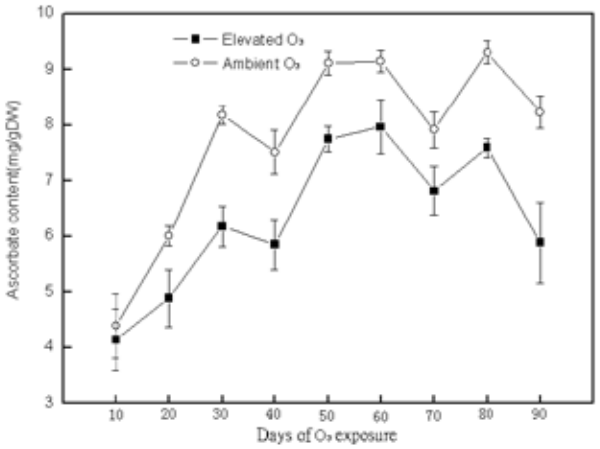

Figure 3. Ascorbate content in *Ginkgo biloba* leaves related to time of elevated O_3 exposure.

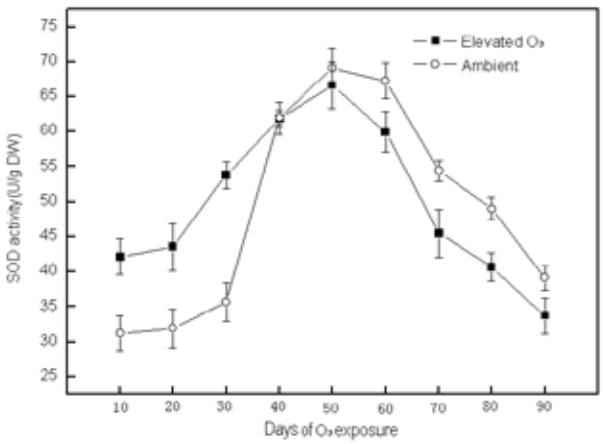

Figure 4. SOD activity in *Ginkgo biloba* leaves related to time of elevated O_3 exposure.

H_2O_2 spontaneously and/or enzymatically by SOD (Rao and Davis, 1999). H_2O_2 is a toxic ROS with deleterious effects in plant tissue. Ozone-induced H_2O_2 accumulation within leaf mesophyll cells led to structural injuries (Oksanen et al., 2003). Much accumulation of H_2O_2 and O_2^- caused oxidative stress in *Ginkgo biloba* leaves under high O_3 exposure. Accumulation of H_2O_2 in response to high ozone concentration has been reported in tobacco (Schraudner et al., 1998), birch (Pellinen et al., 1999), and aspen (Oksanen et al., 2003) while the accumulation of both $O_2^{\cdot-}$ and H_2O_2 has been observed in *Arabidopsis* (Rao and Davis, 1999). Injury caused by these ROS is one of the major damaging factors in plants exposed to environmental stresses.

ROS generated by O_3 might induce lipid peroxidation, therefore affecting the structure of cell membranes (Calatayud et al., 2003). It has been suggested that decreases in cell membrane stability reflect the extent of lipid peroxidation caused by ROS (Sairam and Srivastava, 2002). In this study, MDA value was higher in *Ginkgo blioba* leaves exposed to elevated O_3 than in those exposed only to ambient O_3, confirming an oxidative stress (Figure 2). MDA concentration, which represents the state of membrane lipid peroxidation, has been shown to be correlated with the degree of elevated O_3 exposure to plants (Prince et al., 1990; Yoshida et al., 1994; Ranieri et al., 1996). Data obtained by MDA analysis confirmed the occurrence of lipid peroxidation in leaves exposed to elevated O_3. Elevated ozone-induced membrane lipid peroxidation has been reported in pumpkin (Ranieri et al., 1996), sunflower (Cagno et al., 2001), lettuce (Calatayud et al., 2002), and spinach (Calatayud et al., 2003).

It has been established that plant tolerance to O_3 depends largely on the ability to detoxify ROS (Rao et al.,

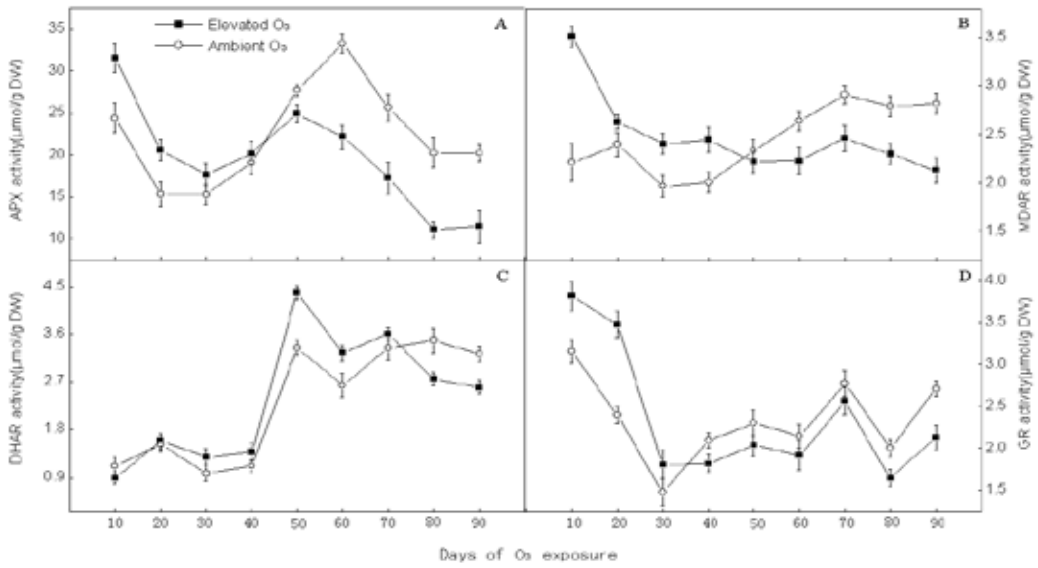

Figure 5. Activity of APX (A); activity of MDAR (B); activity of DHAR(C) and activity of GR (D) in *Ginkgo biloba* leaves related to time of elevated O_3 exposure.

1995). ASA is an integral weapon in the defense against ROS generated by ozone, able to donate electrons to the reactive oxygen intermediates produced by O_3 (Tanaka et al., 1985) and to react with O_3 directly (Chameides, 1989; Van Hove et al., 2001; D'Haese et al., 2005). In this experiment, ASA content in *Ginkgo biloba* leaves decreased significantly under O_3 exposure, apparently as a consequence of substantial oxidative stress. ASA acting as an ozone scavenger was sensitive to ozone stress. It was not able to resist the elevated O_3 exposure, as documented by other authors in the course of pollutant stress (Nouchi, 1993; Wellburn and Wellburen, 1996; Calatayud et al., 2002). The minimum ASA concentration of *Ginkgo biloba* required to protect against ozone stress is not known. In this study, elevated O_3 exposure enhanced the activity of APX in the first 20 days. The results show that the ascorbate content is still sufficient to maintain APX activity in the early stages of O_3 exposure. However, under prolonged exposure, APX activity fell below that under ambient O_3. This may be associated with the even lower ASA content. APX activity could be used as a sensitive indicator for stress tolerance in tree species, for acclimation, and for the injury caused by environmental stresses (Jin et al., 2003). APX activity in consuming ASA is much higher than the sum of MDAR and DHAR activity in reproducing ASA (Figure 5). APX was very sensitive to the environmental changes whether the change led to acclimation or injury, but MDAR and DHAR always responded more slowly (Jin et al., 2003). MDAR activity was enhanced, but DHAR activity declined or showed no significant change after 40 day exposure to elevated O_3. This result suggests that, from this moment on, the regeneration of ASA is mostly catalyzed by MDAR, and not by DHAR. The increase of MDAR and decrease of DHAR activity mentioned above agree with the findings reported for other types of stress (Anderson et al., 1991; Krivosheeva et al., 1996; Gupta et al., 1999). With prolonged O_3 exposure MDAR activity decreased, but dehydroascorbate (DHA) content increased, which stimulated the activity of DHAR. The pathway through which DHAR catalyzed the regeneration of ASA became dominant, however, not sufficiently to prevent the decrease of ASA content. On the other hand, GR plays an important role in the detoxification of oxygen radicals by converting the oxidized glutathione into its reduced form in ascorbate-glutathione cycle (Tanaka et al., 1985; 1990). The GR activity does not usually increase to more than double under stress conditions and sometimes fails to increase at all (Pasqualini et al., 2001). In this study, GR activity was enhanced in the first 20 days in response to elevated O_3 exposure. After prolonged exposure, an apparent decrease appeared in the last 10 days. Rao et al. (1995) reported O_3 exposure for 2 weeks enhanced GR activity in wheat leaves but prolonged exposure decreased it.

The ability of plants to scavenge ROS depends largely on the induction of the activity of SOD and ascorbate-glutathione cycle enzymes. SOD is the only enzyme for the reduction of $O_2^{\cdot-}$ to H_2O_2 or the oxidation of $O_2^{\cdot-}$ to O_2 to have been found to date in either plants or other organisms (Asada, 2000). In the present study, a significant correlation (R=-0.902) between the $O_2^{\cdot-}$ generating rate and SOD activity of *Ginkgo biloba* was shown in the growing season. Although elevated O_3 exposure for 30 days enhanced the activity of SOD, with prolonging exposure SOD activity began declining (Figure 4). This suggests SOD activity might be stimulated by superoxide anion, but could not resist continuous O_3 exposure throughout growing season. The result is similar to the report in wheat by Rao et al. (1995).

In present study, the activities of all antioxidation enzymes showed an evident seasonal fluctuation. The maximum activity of all enzymes appeared between 30 July and 30 August, when weather became warmer, and the daily maximum temperature attained 37°C in the OTCs. High temperature might stimulate anti-oxidative enzyme activities as well, but it did not eliminate the O_3 effect (Figures 4, 5). In the Mediterranean, SOD activity of *Pinus halepensis* was enhanced under O_3 exposure in summer (Elvira et al., 1998). Similar responses by antioxidants to ozone were found by several authors in conifers (Castillo et al., 1987; Tandy et al., 1989) as well.

In conclusion, short term O_3 exposure induced acclimation of the antioxidation defence system in *Ginkgo biloba* leaves. However, the acclimation effect was not sufficient to prevent the accumulation of reactive oxygen species in leaves and, after prolonged exposure, the system itself became affected, and the lowered protection level led to lipid peroxidation of the leave cells.

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城市銀杏葉片抗氧化系統對臭氧濃度升高的回應

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本文以生長在市區開頂箱內的銀杏為試材，對高濃度臭氧（80 nmol mol^{-1}）條件下銀杏葉片內超氧自由基（$O_2^{\cdot-}$）產生速率，過氧化氫（H_2O_2）含量，抗壞血酸（ASA）含量，超氧化物歧化酶（SOD）、抗壞血酸過氧化物酶（APX）、谷胱甘肽還原酶（GR）活性動態變化進行分析，探討大氣臭氧（O_3）濃度升高對樹木抗氧化系統的影響。結果表明，高濃度臭氧使 $O_2^{\cdot-}$ 產生速率提高，H_2O_2 含量增加，膜脂過氧化指標丙二醛（MDA）含量也隨之增加。超氧化物歧化酶（SOD）、抗壞血酸過氧化物酶（APX）、單脫氫抗壞血酸還原酶（MDAR）、谷胱甘肽還原酶（GR）及脫氫抗壞血酸還原酶（DHAR）活性在高濃度臭氧薰蒸的前期升高，但隨著臭氧暴露時間的延長這些抗氧化酶活性均變得低於對照。生長在高濃度臭氧條件下銀杏葉片抗壞血酸（ASA）含量一直低於對照。因此，在高濃度臭氧薰蒸的前期，銀杏抗氧化系統能夠適應環境變化。但在生長季的後期，連續的高濃度臭氧脅迫導致活性氧含量升高，抗氧化酶活性下降，雖然肉眼可見得傷害尚未觀察到，但仍可表明銀杏的抗氧化系統不能抵禦長期臭氧脅迫所帶來的氧化傷害。

關鍵詞：抗氧化系統；活性氧；膜脂過氧化；銀杏；高濃度臭氧。