Changes of net photosynthesis, antioxidant enzyme activities, and antioxidant contents of *Liriodendron tulipifera* under elevated ozone

S.Z. RYANG[*], S.Y. WOO[*,+], S.Y. KWON[**], S.H. KIM[*], S.H. LEE[*], K.N. KIM[*], and D.K. LEE[***]

Department of Environmental Horticulture, University of Seoul, Seoul, *130-743, Republic of Korea*[*]
Plant Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea[**]
Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, 151-921, Republic of Korea[***]

Abstract

Liriodendron tulipifera was exposed to gradually elevated ozone concentrations of 100−300 μg kg^{-1} in the naturally irradiated environment chamber. During 15 d of exposure to O_3, net photosynthetic rate (P_N) decreased and there was large difference between the control (C) and treatment with ozone (OT), while there was no significant difference in water use efficiency. Total chlorophyll content as well as the value of fluorescence parameter F_v/F_m decreased, while antioxidant enzyme activities related to ascorbate-glutathione cycle increased after 15 d of OT. Unchanged contents of ascorbate and glutathione indirectly suggest that the species hastened the antioxidant's oxidization/reduction cycle using enzymes instead of expanding their pool against oxidative stress.

Additional key words: ascorbate; catalase; chlorophyll content and fluorescence; glutathione reductase; irradiance; peroxidase; O_3; superoxide dismutase; water use efficiency.

Introduction

Stratospheric ozone is a noticeable air pollutant that harms the ecosystems. The Intergovernmental Panel on Climate Change (Oksanen *et al*. 2004) predicted a continuous 1−2 % annual increase in O_3 concentration due to global change. The ozone alters basic metabolic processes of plants (Bortier *et al*. 2000, He *et al*. 2007, Xu *et al*. 2007) and induces physiological changes including visible injuries, decline in net photosynthetic rate (P_N) and chlorophyll (Chl) content, and growth reduction in tree species distributed in urban areas. Previously using scanning electron microscopy, we observed unique changes in stomatal characteristics (shape, density, and length) of *Liriodendron tulipifera* in its abaxial leaf surfaces under elevated ozone concentration (OT): stomatal shape was not degraded, but epidermis around stomata swelled and seemed to cover the stomata. In addition, stomatal conductance decreased after ozone exposure and in this way *L. tulipifera* defended itself against ozone stress.

Cross *et al*. (1998) noticed the importance of stomata as a primary barrier to protect plants, because all absorption of O_3 occurs practically within the leaves. However, such absorption does not only result in physiological and morphological changes but also in biochemical changes, namely in the activities of antioxidant enzymes and contents of antioxidants.

After entering through the stomata, O_3 decomposes spontaneously into aqueous solution of the apoplast into a number of reactive oxygen species (ROS) (Moldau

Received 4 June 2008, *accepted* 6 October 2008.
[+]Corresponding author; fax: (+82) 2 2210 2838, e-mail; wsy@uos.ac.kr
Abbreviations: AOT40 − the ozone dose above a concentration threshold of 40 nmol mol^{-1}; APX − ascorbate peroxide; As − reduced form of ascorbate; AsA – ascorbate; CAT − catalase; Chl − chlorophyll; DAsA − dehydroascorbate, oxidized form of ascorbate; DHAR − dehydroascorbate reductase; DTT − dithiothreitol; DTNB − 5,5'-dithio-bis(2-nitrobenzoic acid); EDTA − ethylenediamine tetraacetic acid; F_m − maximal fluorescence; F_v − variable fluorescence; GR − glutathione reductase; GSH − tripeptide thiol glutathione; GSSG − glutathione disulphide; H_2O_2 − hydrogen peroxide; MDA − malondialdehyde; MDHAR − monodehydroascorbate reductase; $O_2^{\cdot-}$ − superoxide; $^{\cdot}OH$ − hydroxyl radical; $O_2H^{\cdot}$ − hydroperoxide; OT – ozone treatment; P_N − net photosynthetic rate; PCD − programmed cell death; POD − peroxidase; PPFD − photosynthetic photon flux density; ROS − reactive oxygen species; SOD − superoxide dismutase; TCA − trichloroacetic acid; WUE − water use efficiency.
Acknowledgement: This study was carried out with the support of 'Forest Science & Technology Projects' provided by Korea Forest Service (project No. S210707L1010) and 'The Korea Research Foundation Grant' funded by the Korean Government (KRF-2006-C00118).

1999, Bernardi *et al*. 2004). Reactions of O_3 with water and solutes in the apoplasm lead to the formation of ROS including hydrogen peroxide (H_2O_2), hydroperoxide ($O_2H^{\cdot}$), superoxide ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen (Foyer *et al*. 1994, Kangasjärvi *et al*. 1994, Wohlgemuth *et al*. 2002, Oksanen *et al*. 2004). ROSs are continuously produced during normal physiological processes, such as photosynthesis, and plants are able to scavenge them (Foyer and Noctor 2000). Generally, there are mechanisms to prevent or minimize the damage induced by ROS in each cellular organelle. Plants having higher activities of antioxidant enzymes overcome such stress faster. Therefore, the degree of antioxidant enzyme activities can indicate the present condition of plants. Under environmental stress, the production of excess H_2O_2 has been observed in chloroplasts (Asada 1999), mitochondria (Maxwell *et al*. 1999), peroxisomes, plasma membrane, and in the apoplast (Vanacker *et al*. 1998). Thereafter, excess H_2O_2 is converted to water and molecular oxygen spontaneously or by catalase (CAT) or is detoxified through the action of various antioxidant processes (Noctor and Foyer 1998). Various antioxidant enzymes such as superoxide dismutase (SOD; cf. Scandalios 1993), CAT (Willekens *et al*. 1997), and peroxidase (POD; cf. Kronfuss *et al*. 1996) are involved in the plant's defensive mechanism. Also, several antioxidant enzymes within the ascorbate-glutathione pathway act as a substrate for oxidizing or reducing antioxidants (ascorbate and glutathione) and perform a major role in defence against oxidative stress (Hansen *et al*. 2002).

In a previous study (Ryang and Woo 2008), we firstly devised the stepped-increase ozone protocol and compared the degree of injuries by the O_3 dose above a concentration threshold (calculated AOT40). In the present study, we planned another stepped-increase protocol with higher O_3 concentrations and shorter treatment duration. The AOT40 was also used to determine whether visible injuries appear without any overlapping effect through altered O_3 concentration or duration of exposure. There were three objectives in the present study: (*1*) to determine relevance of ozone exposure; (*2*) to investigate the induced changes in photosynthetic mechanism of *L. tulipifera* by measuring P_N, total Chl content, F_v/F_m, and water use efficiency (WUE), and (*3*) to investigate the antioxidant defence mechanism by observing the changes in antioxidant enzyme activities and contents of antioxidants.

Materials and methods

Plants and ozone treatment: A 2-year-old *L. tulipifera* was acclimated in the greenhouse for two months prior to O_3 fumigation (Table 1) in the natural environment chamber (25±1.0/22±1.0 ºC day/night, 65±5 % relative humidity). During the experiment, the mean day light irradiance was about 1 500 μmol m^{-2} s^{-1} PPFD. Ozone was fumigated from oxygen by ozone generator (*400E, Teledyne Instruments,* San Diego, CA, USA). Its concentration was gradually increased from 100 to 300 μg kg^{-1} for 14 d (July 17 to July 30 – cf. Table 1) and monitored by UV absorption ozone analyzer (*400E, Teledyne Instruments,* San Diego, CA, USA). During O_3 fumigation, the position of each plant was changed daily in order to avoid measurement errors induced by plant position. In both previous and present research, the concentration of ozone was compared to AOT40 accumulated O_3 concentration of 40 μg kg^{-1}.

Visible injuries were observed following the criteria of Pääkkönen *et al.* (1998), which involve small, light-green, yellowish, or brown dots, necrotic flecks, and chlorotic leaves.

Gas exchange was measured on the fully expanded, mature leaf number 4 (counted from shoot apex) between 09:00 and 11:00 h every 5 d. The P_N and WUE were measured using a portable photosynthesis system (*LI-6400, Li-Cor*, Lincoln, NE, USA). Artificial irradiation was made by red-blue light-emitting diodes on the top of the cuvette of the *LI-6400* photosynthetic system. Ambient CO_2 partial pressure was supplied by a CO_2 mixer. The irradiance-response curves were measured beginning at the highest irradiance and decreasing to 0 μmol m^{-2} s^{-1} PPFD (2 000, 1 500, 1 000, 800, 500, 300, 100, 50, 30, 0 μmol m^{-2} s^{-1} PPFD). Temperature was 25 ºC and humidity was 65±5 %, which is related to averages during growing season in Korea. WUE was calculated as the ratio of photosynthetic to transpiration rates (Al-Amoudi *et al*. 2002) measured with *LI-6400*.

Chl: Leaves were collected after O_3 treatment. Chl was extracted with 80 % acetone solution for 7 d at 4 ºC, its absorbance was spectrophotometrically measured, and Chl content was calculated using equations of Arnon (1949).

Chl *a* fluorescence ratio (F_v/F_m) was determined using a portable Chl fluorometer (*PAM-2000, Walz*, Germany). F_v/F_m represents the maximal yield of the photochemical reaction in photosystem 2. After 30 min of adaptation to darkness, five leaves were selected and their minimal fluorescence (F_0) at a 0.1 μmol m^{-2} s^{-1} PPFD and maximal fluorescence (F_m) at saturating 2 400 μmol m^{-2} s^{-1} were measured at room temperature (25 ºC).

Antioxidant enzymes: Leaf material was homogenized with a pestle in an ice-cold mortar with the solution [50 mM K-P_i (pH 7.0), 1 % (m/v) polyvinylpyrrolidone, and 0.2 mM EDTA]. The liquid was centrifuged at 4 ºC for 20 min at 15 000×*g*. The crude enzyme extracts were

Table 1. Protocol of exposure to ozone. O_3 concentrations [μg kg^{-1}] and AOT40 [μg kg^{-1} h^{-1}] are shown. Plants were not exposed to ozone on some days (17, 26, and 31 July).

Date	17	18	19	20	22	23	24	25	27	28	29	30
O_3	100	100	100	100	150	150	200	200	250	250	300	300
AOT40	480	960	1440	1920	800	680	960	240	920	600	680	760

based on supernatants: CAT and POD (Chance and Maehly 1955), SOD (Zhang and Kirkham 1994), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). Absorbances used for determining the activities are given by Nakano and Asada (1981) for APX and DHAR, or Cakmak *et al*. (1993) for GR and MDHAR.

Antioxidants: The supernatant prepared as shown above (1 g leaf material in 10 cm^3 of 5 % *m*-phosphoric acid) was used for analysis of ascorbate and glutathione. For measuring total ascorbate (AsA), a reaction mixture [750 mm^3 of 150 mM P_i buffer (pH 7.4) including 5 mM EDTA, 150 mm^3 of 10 mM dithiothreitol (DTT), and 300 mm^3 of supernatant] was incubated for 10 min at room temperature. Thereafter, 150 mm^3 of 0.5 % N-ethyl-maleimide was added to remove excess DTT. To measure reduced AsA, 300 mm^3 H_2O instead of 150 mm^3 DTT and 150 mm^3 N-ethylmaleimide was added. A colour developing mixture [600 mm^3 of 10 % trichloroacetic acid (TCA), 600 mm^3 of 44 % o-phosphoric acid, 600 mm^3 of 4 % a,a-dipyridyl in 70 % ethanol, and 300 mm^3 of 0.3 % $FeCl_3$ (m/v)] was added to each reaction mixture. After *Voltex*-mixing, the final mixtures were incubated at 40 ºC for 40 min, and absorbances were read at 525 nm. To measure total glutathione and oxidized glutathione, the first step was to prepare each sample solution [total: 1 000 mm^3 of supernatant, 1 500 mm^3 of 0.5 M P_i buffer (pH 7.5), and 50 mm^3 of H_2O; oxidized: H_2O was replaced by 50 mm^3 of 2-vinyl-pyridine]. Afterwards, 100 mm^3 of each sample solution was added to the reaction mixture [100 mM P_i buffer (pH 7.5), 5 mM EDTA, 0.2 mM NADPH, 0.6 mM DTNB, and 3 units GR] and absorbances were read at 412 nm. The GSH content was calculated as total glutathione disulphide (GSSG).

Statistical analysis was done using one-way ANOVA and Duncan's multiple range test. Differences were considered significant at $p<0.05$.

Results

Visible injuries first began after 5-d ozone treatment and its symptoms were similar to those of the previous research (Fig. 1). At first, red stipples came out from the leaf adaxial surface and the overall leaf became reddish and embossed. Leaves finally withered showing early defoliation, which began from old to young leaves. All leaves facing the ozone fumigation fell down prematurely.

Fig. 1. Visible injuries of *L. tulipifera* under elevated ozone.

Gas exchange (Fig. 2): *L. tulipifera* under ambient O_3 concentration kept its P_N stable during the study, while P_N under OT decreased with time. The difference was not significant at the first 5 d of OT. WUE showed similar trend to P_N with increasing irradiance (Fig. 2), but the differences were not significant.

Chl content and fluorescence: During the first 5 d, total Chl content was greater after O_3 treatment. However, after 15 d, the situation was reversed (Table 2). F_v/F_m ratio was significantly lower during the O_3 treatment.

Activities of antioxidant enzymes and contents of antioxidants: At 5 d of O_3 treatment, the activities of all enzymes except GR were related to ascorbate-glutathione cycle (Fig. 3). The activities of POD and CAT increased as well (Fig. 4). At 10 d of OT, activities of SOD, APX, and MDAHR, which are connected to AsA, APX and MDAHR had higher activities. POD and CAT kept their higher activities under OT at 10 d, while only CAT

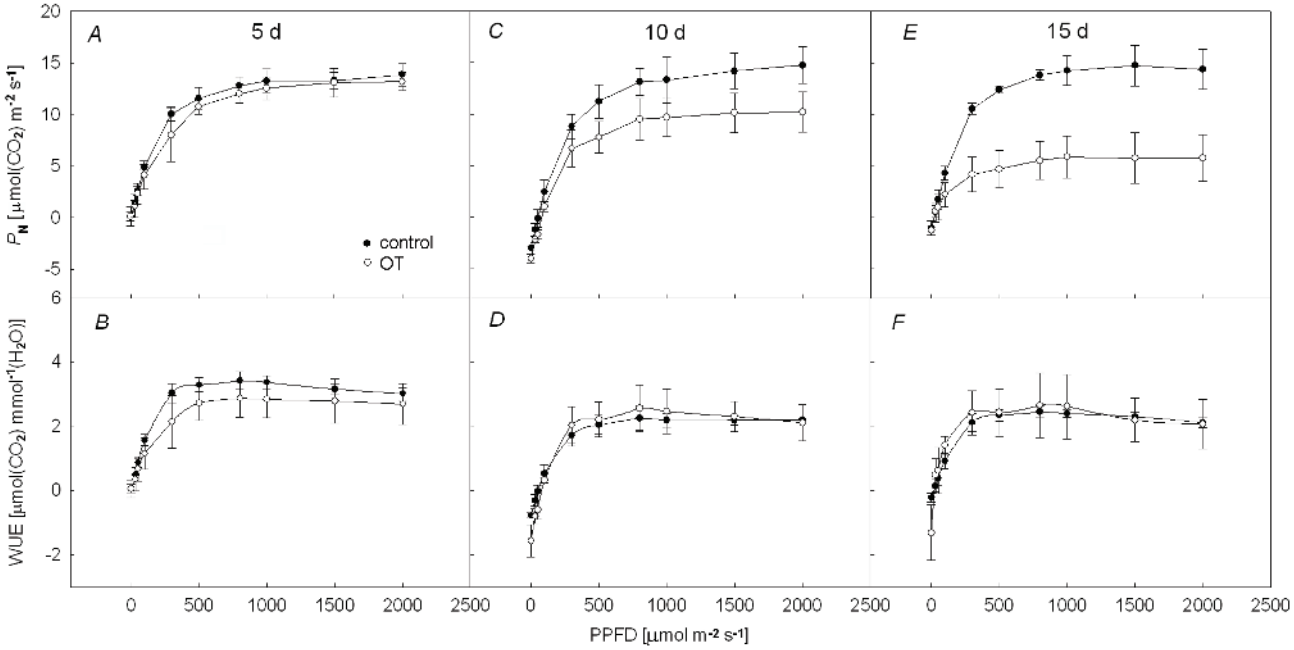

Fig. 2. Irradiance response of net photosynthetic rate (P_N) and water use efficiency (WUE) in *L. tulipifera* exposed to control (●) or elevated (○) O_3 concentration and measured at 5 (*A, B*), 10 (*C, D*), or 15 (*E, F*) d of O_3 treatment (OT). Chamber temperature was 25.00±0.01 ºC, ambient CO_2 pressure 400 μb, and 45−60 % relative humidity. Means ± SD, (n = 3).

Table 2. Changes in total chlorophyll (Chl) content [g kg^{-1}(FM)] and maximal efficiency of photochemistry of photosynthesis (F_v/F_m) of *L. tulipifera* during ozone exposure. C indicates plants under ambient air and OT indicates those under elevated ozone. Ozone concentration was gradually increased from 100 to 300 μg kg^{-1} as shown in Table 1. *Different letters* show significant differences among means at p<0.05. Means ± SD (n = 3).

		O_3 exposure [d]		
		5	10	15
Chl	C	1.43±0.10 b	2.28±0.09 a	2.37±0.41 a
	OT	1.76±0.15 a	2.40±0.12 a	1.51±0.09 b
F_v/F_m	C	0.81±0.00 a	0.81±0.01 a	0.81±0.01 a
	OT	0.75±0.03 b	0.78±0.01 b	0.78±0.01 b

Discussion

The change of photosynthetic mechanism under elevated O_3 concentration: WUE was not significantly different between C and OT and this was due to decreased transpiration rate (data not shown). Thus the stomata functioned well. The epidermis around the stomata was swollen and surrounded them like a tower. This is exceptional in other species and therefore needs further study. However, this morphological change probably also contributed to the low stomatal conductance even when the plant had severe visible injuries.

The energy manageability of plants can be estimated by their Chl contents. Also the reduction of photosynthetic efficiency (F_v/F_m) has negative effect in photosynthesis (Meyer *et al*. 1997). The lower photosynthetic efficiency indicates that more electrons are released. At the first 5 d of OT, P_N and total Chl content did not significantly change. At 10 d of OT, however, P_N decreased even though the total Chl content did not show significant difference. Only F_v/F_m significantly decreased from the very beginning. Hence the decreased F_v/F_m was primarily affected by reduced P_N and this effect of F_v/F_m precedes that of Chl content to P_N. In addition to total Chl content, one of the symptoms of stress injury is the decrease in Chl *a/b* ratio, indicating senescence or poor activity increased at 15 d of OT. Activities of DHAR and GR, which are related to the reduction and oxidation of glutathione, significantly increased during 15 d of OT.

Total AsA content did not differ between C and OT except at 5 d (Table 3). The ratio of As to DAsA was lower only at 5 d of OT, while the difference of the ratio between C and OT was not significant at 10 and 15 d. Total glutathione content of OT was significantly lower during treatment compared to that of C, except at 10 d of OT. The ratio of GSSG to GSH did not change at 5 and 10 d of OT; however, the ratio significantly increased at 15 d of OT.

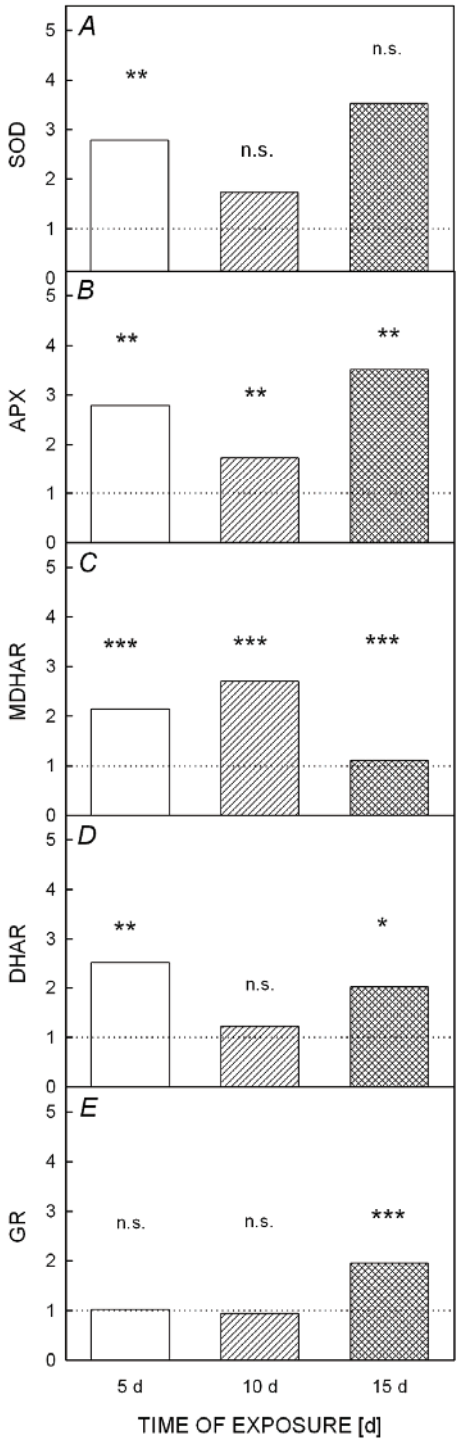

Fig. 3. Antioxidant enzyme activities (APX − ascorbate peroxidase, DHAR − dehydroascorbate reductase, GR − glutathione reductase, MDHAR − monodehydroascorbate reductase, SOD − superoxide dismutase) [%NBT inhibition rate for SOD, nmol kg^{-1}(protein) s^{-1} for others] expressed as ratio of ozone affected to control plants during gradual O_3 exposure from 100 to 300 μg kg^{-1} (cf. Table 1). *Dotted lines* indicate constant value of control. Statistical differences between control and O_3 treatment are shown above bars: $^{***}p<0.001$, $^{**}p<0.001$, $^{*}p<0.001$, n.s. non significant ($n = 3$).

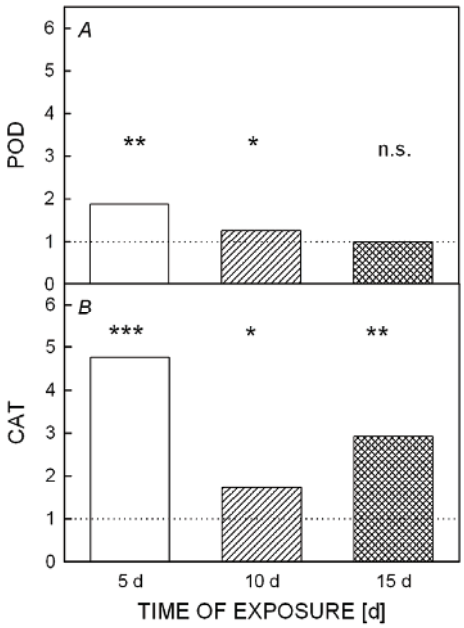

Fig. 4. Peroxidase (POD) and catalase (CAT) activities [nmol kg^{-1}(protein) s^{-1}] expressed as ratio of ozone affected to control plants during gradual O_3 exposure from 100 to 300 μg kg^{-1} (cf. Table 1). *Dotted lines* indicate constant value of control. Statistical differences between control and O_3 treatment are shown above bars: $^{***}p<0.001$, $^{**}p<0.001$, $^{*}p<0.001$, n.s. non significant ($n = 3$).

plant growth (Choi and Chung 2002). The lower Chl *a/b* under O_3 treatment was due to the decrease in Chl *a* content and increase in Chl *b* content (data not shown). This shows early senescence and defoliation affected by O_3 stress.

Change of antioxidant mechanism under O_3^+: The duration of O_3 treatment (15 d) was divided into three 5-d-periods. In the first period, the AsA content as well as the activities of antioxidant enzymes related to ascorbate (SOD, APX, MDHAR, and DHAR) increased, while there was no significant changes in glutathione content and the activity of GR. Thus AsA responded more sensitively than glutathione in *L. tulipifera* under O_3 stress.

Generally, the content of antioxidants increases when plants are under stress. However, the content of AsA did not change during O_3 treatment except on the first period. This plant species has a tendency to overcome O_3 stress by increasing the speed of oxidizing/reducing AsA than enlarging its pool. After O_3 treatment, the content of glutathione was lower than that of the control.

In the second period, activities of SOD, APX, and MDAHR increased although SOD activity did not show any significant difference. There are two ways to reduce oxidized ascorbates, monodehydroascorbate, and dehydroascorbate, using enzymes: one is to reduce monodehydroascorbate using MDHAR and the other is to reduce dehydroascorbate, which was oxidized from

monodehydroascorbate, using DHAR. When *L. tulipifera* leaves in C used both reduction ways almost equally, those in OT used the MDHAR-reduction way more than the DHAR-reduction. This followed the assumption that the plant chose a faster way to reduce AsA for a higher rate of scavenging ROS, and monodehydroascorbate was reduced using MDHAR rather than oxidized to dehydroascorbate and reduced using DHAR. This is probably the plants' early response to more efficiently defend them against the attack of ozone.

In the third period, the activity of all antioxidant enzymes related to ascorbate-glutathione cycle increased, although SOD activity did not show significant difference. Plants that were harshly changed and damaged probably started to defend themselves using all antioxidant enzymes. Also, more GSH was oxidized into GSSG through increased activity of GR and GSSG/GSH, the ratio of oxidized to reduced form of glutathione, statistically increased at this time.

CAT is mainly found in sub-cellular organelles and an essential antioxidant enzyme of metabolism (del Rio *et al*. 2002). Although H_2O_2 is removed through the activity of APX *via* Mehler reaction prior to that of CAT (Willekens *et al*. 1997), the real situation is more complex because CAT usually diffuses through membranes (Willekens *et al*. 1997). There was a significantly higher activity of CAT during OT, which might be one of the defence responses against O_3 stress. Also, POD showed high activity after OT. Kronfuss *et al.* (1996) reported that induction of POD is an important component of O_3-eliciting adaptive mechanisms. In addition, POD is involved in several cellular processes, including the control of development, lignification, pathogen defence, and the catabolism of growth regulators (van Huystee 1987, Lewis *et al*. 1999).

Table 3. Changes in total ascorbate content, AsA/DAsA, total glutathione content, and GSSG/GSH of *L. tulipifera* under elevated ozone. C indicates plants under ambient air and OT indicates those under elevated ozone. Ozone concentration was gradually increased from 100 to 300 μg kg^{-1} as shown in Table 1. *Different letters* show significant differences among means at $p<0.05$. Means ± SD, ($n = 3$).

		23 July	28 July	2 August
AsA	C	123.06±5.81 a	171.13±18.91 a	155.98±12.22 a
	OT	142.86±12.19 a	178.15±28.01 a	165.93±20.30 a
AsA/DAsA	C	12.29±4.49 a	1.48±0.34 a	1.86±0.55 a
	OT	4.06±1.84 b	1.83±9,51 a	1.82±0.32 a
Total glutathione	C	14.96±0.51 a	17.40±0.14 a	13.58±0.37 a
	OT	12.28±0.78 b	15.85±0.98 a	10.73±1.27 b
GSSG/GSH	C	3.23±0.86 a	2.71±0.67 a	1.01±0.76 b
	OT	5.29±1.94 a	6.14±5.25 a	6.97±3.14 a

In conclusion, *L. tulipifera* showed the most radical visible injuries in comparson with other plant species and impaired photosynthetic mechanism including P_N and F_v/F_m. In spite of severe injuries, unaffected WUE indirectly gave information that this species controlled its stomata to defend against elevated O_3 and the morphological changes on leaf abaxial surfaces observed previously also acted as defence against elevated ozone. The activities of antioxidant enzymes increased and made the oxidation/reduction cycle of antioxidants faster to scavenge extra produced ROS under environmental stress.

References

Al-Amoudi, A., Dubochet, J. Studer, D.: Amorphous solid water produced by cryosectioning of crystalline ice at 113. − Korean J. Microsc. **207**: 146-153, 2002.

Arnon, D.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. − Plant Physiol. **24**: 1-15, 1949.

Asada, K.: The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. − Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Bernardi, R., Nali, C., Ginestri, P., Pugliesi, C., Lorenzini, G., Durante, M.: Antioxidant enzyme isoforms on gels in two poplar clones differing in sensitivity after exposure to ozone. − Biol. Plant. **48**: 41-48, 2004.

Bortier, K., Ceulemans, R., de Temmerman, L.: Effect of ozone exposure in growth and photosynthesis of beech seedlings (*Fagus sylvatica*). − New Phytol. **146**: 271-280, 2000.

Cakmak, I., Strbac, D., Marchner, H.: Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. − J. exp. Bot. **44**: 127-132, 1993.

Chance, B., Maehly, A.C.: Assay of catalases and peroxidases. − In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. **2**. Pp. 764-775. Academic Press, New York 1955.

Choi, J.H., Chung, J.C.: Chlorophyll content changes of some *Hibiscus syriacus* cultivars in growth period. − J. nat. Resource Life Sci. **24**: 28-34, 2002.

Cross, C.E., van der Vliet, A., Louie, S., Thiele, J.J., Halliwell, B.: Oxidative stress and antioxidants at biosurfaces: Plants, skin, and respiratory tract surfaces. − Environ. Health Perspectives **106**: 1241-1251, 1998.

del Rio, L.A., Corpas, J., Sandalio, L.M., Palma, J.M., Gómez, M., Barroso, J.B.: Reactive oxygen species. antioxidant systems and nitric oxide in peroxisomes. − J. exp. Bot. **53**: 1255-1272, 2002.

Foyer, C.H., Lelandais, M., Kunert, K.J.: Photooxidative stress in plants. – Physiol. Plant. **92**: 696-717, 1994.

Foyer, C.H., Noctor, G.: Oxygen processing in photosynthesis: regulation and signalling. − New Phytol. **146**: 359-388, 2000.

Hansen, U., Fiedler, B., Rank, B.: Variation of pigment composition and antioxidative systems along the canopy light gradient in a mixed beech/oak forest: a comparative study on deciduous tree species differing in shade tolerance. − Trees **16**: 354-364, 2002.

He, X.-Y., Fu, S.-L., Chen, W., Zhao, T.-H., Xu, S., Tuba, Z.: Changes in effects of ozone exposure on growth, photosynthesis, and respiration of *Gingko biloba* in Shenyang urban area. – Photosynthetica **45**: 555-561, 2007.

Kangasjärvi, J., Talvinen, J., Utriainen, M., Karjalainen, R.: Plant defense systems induced by ozone. − Plant Cell Environ. **17**: 783-794, 1994.

Kronfuss, G., Wieser, G., Havranek, W.M. Polle, A.: Effects of ozone and mild drought stress on total and apoplastic guaiacol peroxidase and lipid peroxidation in current-year needles of young Norway spruce (*Picea abies* L. Karst.). − Plant Physiol. **148**: 203-206, 1996.

Lewis, N.G., Davin, L.B., Sarkanen, S.: The nature and function of lignins. − In: Pinto. P.M. (ed.): Comprehensive Natural Product Chemistry. Vol. 3. Pp. 617-745. Elsevier, Amsterdam 1999.

Maxwell, D.P., Wang, Y., McIntosh, L.: The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. − Proc. nat. Acad. Sci. USA **96**: 8271-8276, 1999.

Meyer, U., Kollner, B., Willenbrink, J., Krause, G.H.M.: Physiological changes on agricultural crops induced by different ambient ozone exposure regimes. 1. Effects on photosynthesis and assimilate allocation in spring wheat. − New Phytol. **136**: 645-652, 1997.

Moldau, H.: Ozone detoxification in the mesophyll cell wall during a simulated oxidative burst. − Free Radical Res. **31**: S19-S24, 1999.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. − Plant Cell Physiol. **22**: 867-880, 1981.

Noctor, G., Foyer, C.H.: Ascorbate and glutathione: keeping active oxygen under control. − Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 249-279, 1998.

Oksanen, E., Häikiö, E, Sober, J. Karnosky, D.F.: Ozone-induced H_2O_2 accumulation in field-grown aspen and birch is linked to foliar ultrastructure and peroxisomal activity. − New Phytol. **161**: 791-799, 2004.

Pääkkönen, E., Vahala, J., Pohjolai, M., Holopainen, T., Kärenlampi, L.: Physiological, stomatal and ultrastructural ozone responses in birch (*Betula pendula* Roth.) are modified by water stress. − Plant Cell Environ. **21**: 671-684, 1998.

Ryang, S.Z., Woo, S.Y.: Stomata density and size of *Acer palmatum* to the elevated ozone. – J. Kor. For. Soc. **97**: 552-554, 2008.

Scandalios, J.G.: Oxygen stress and superoxide dismutases. − Plant Physiol. **101**: 7-12, 1993.

Vanacker, H., Harbinson, J., Ruisch, J., Carver, T.L.W., Foyer, C.H.: Antioxidant defences of the apoplast. − Protoplasma **205**: 129-140, 1998.

van Huystee, R.B.: Some molecular aspects of plant peroxidase biosynthetic studies. − Annu. Rev. Plant Physiol. **38**: 205-219, 1987.

Wohlgemuth, H., Mittelstrass, K., Kschieschan, S., Bender, J., Weigel, H.-J., Overmyer, K., Kangasjärvi, J., Sandermann, H., Langebartels, C.: Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. − Plant Cell Environ. **25**: 717-726, 2002.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., van Montagu, M., Inzé, D., van Camp, W.: Catalase is a sink for H_2O_2 and is indispensable for stress defence in C_3 plants. − EMBO J. **16**: 4806-4816, 1997.

Xu, H., Biswas, D.K., Li, W.-D., Chen, S.-B., Zhang, L., Jiang, G.-M., Li, Y.-G.: Photosynthesis and yield responses of ozone-polluted winter wheat to drought. – Photosynthetica **45**: 582-588, 2007.

Zhang, J., Kirkham, M.B.: Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. − Plant Cell Physiol. **35**: 785-791, 1994.