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Physiological Damages and Biochemical Alleviation to Ozone Toxicity in Five Species of genus *Acer*

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Abstract : We investigated physiological damages and biochemical alleviation of five species of genus *Acer* under ozone fumigation in order to assess their tolerant ability against ozone toxicity. At the end of 150 ppb O_3 fumigation, photosynthetic characteristics were measured, and chlorophyll contents, malondialdehyde (MDA) and antioxidative enzyme activities were analyzed in the leaves of five maple trees (*Acer buergerianum*, *A. ginnala*, *A. mono*, *A. palmatum*, and *A. palmatum* var. *sanguineum*). The reduction of chlorophyll (*chl*) a in ozone-exposed plants was 16.8% (*A. buergerianum*) to 26.7% (*A. ginnala*) of control plants. For the content of *chl* b, *A. ginnala* and *A. palmatum* var. *sanguineum* represented the high reduction of 26.3% and 23.6%, respectively. The highest reduction on the *chl* a:b ratio was observed in the leaves of *A. palmatum*. The reduction of net photosynthesis in five species varied from 2.4% to 37.6%. Among five species, *A. ginnala* showed remarkable reduction (37.6%) for net photosynthesis in comparison with control. Carboxylation efficiency differed significantly ($P < 0.05$) among species and between control and ozone treatment. The reduction of carboxylation efficiency was the highest in the leaves of *A. ginnala* (44.7%). *A. palmatum* var. *sanguineum* showed the highest increase (41.7%) for MDA content. The highest increase of superoxide dismutase (SOD) activity represented in *A. palmatum* (26.1%) and the increase of ascorbate peroxidase (APX) activity ranged from 16.5% (*A. ginnala*) to 49.1% (*A. palmatum* var. *sanguineum*). *A. mono* showed the highest increase (376.6%) of glutathione reductase (GR) activity under ozone fumigation and A. *buergerianum* also represented high increase (42.3%) of GR activity. Catalse (CAT) activity increased in the leaves of *A. ginnala, A. palmatun* and *A. palmatum* var. *sanguineum* under ozone exposure, whereas *A. buergerianum and A. mono* decreased in comparison with control plants. In conclusion, physiological markers such as chlorophyll content and photosynthesis that responded sensitively to O_3 in maple trees were considered as the very important indicators in order to evaluate the tolerance against O_3 stress, and parameters were closely related with each other. Among antioxidative enzymes, SOD and APX might be contributed to alleviate to O_3 toxicity through the increase of activity in all maple trees. Therefore, these compounds can be used as a biochemical maker to assess the stress tolerance to O_3.

Key words : *maple, chlorophyll content, photosynthesis, MDA, antioxidative enzyme*

Introduction

Ozone (O_3) is one of the most important pollutants covering large areas of the world, and a phytotoxic air pollutant widely considered a risk factor for forest trees (Pye, 1988; Matyssek and Innes, 1999). Despite the increasing environmental awareness and regulations designed to limit industrial and vehicular emissions, ozone levels potentially harmful to human health and vegetation have increased every year in Korea (Ministry of Environment, 2005).

Many results suggest that ozone causes visible leaf injury (Heggestad, 1991; Posthumus. 1991), destruction of photosynthetic pigments (Agrawal *et al.*, 1993; Soldatini *et al.*, 1998) and depression of photosynthetic activity (Barnes *et al.*, 1990; Agrawal *et al.*, 1993; Della Torre *et al.*, 1998; Guidi *et al.*, 1998). Kudson *et al.* (1977) proposed that chlorophyll content could be used as a useful indicator for the evaluation of injury induced by ozone and other pollutants. Since then, the change in chlorophyll content has been used in many studies investigating the effects of ozone on plants (Robinson and Wellburn, 1991; Della Torre *et al.*, 1998). The results, however, of such investigations do not always lead to the same conclusions concerning the pattern of effects. Moreover, the findings on the relative sensitivities of chlorophyll a and b to ozone were often contradictory.

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Toxic effects of O_3 on trees include decreases in photosynthesis (Reich, 1987; Pye, 1988; Dizengremel, 2001; Matyssek and Sandermann, 2003). Photosynthesis has been shown to be particularly sensitive to ozone. Exposure to ozone produces a variety of effects ranging from stomatal closure, a decrease in electron transport rate (Hill and Littlefield, 1969; Moldau *et al.*, 1993; Guidi *et al.*, 1999), to an increase in biochemical limitations, such as a decrease in ribulose-1,5-diphosphate-carboxylase-oxygenase (Rubisco) activity (Ltz *et al.*, 2000; Dizengremel, 2001).

In addition, O_3 toxicity is caused by its redox potential (+2.07 V). Although O_3 is not a radical species in itself, uptake into the internal leaf is believed to result in the generation of acute toxins of a potentially reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-) and hydroxyl radicals (OH·), all of which impair metabolic processes (Wolf *et al.*, 1986; Asada and Takahashi, 1987; Mehlhorn *et al.*, 1990). The high oxidative capacity of O_3 determines, in exposed plants, the induction of reactive oxygen species (ROS) that can initiate multiple oxidation events in cells (Apel and Hirt, 2004). The light and dark reactions of photosynthesis might be impaired by ROS, lipid peroxidation and changes in membrane permeability (Andersen, 2003). As a consequence, plants have evolved physiological and biochemical mechanisms, including increases in the activities of enzymes associated with general stress tolerance and increasing antioxidant concentration, which can avoid the effects of this pollutant (Conklin and Barth, 2004).

The need to alleviate the negative effects of O_3 on plants prompted the search for chemical compounds that are effective in counteracting O_3-induced phytotoxicity. A larger number of antioxidants have been evaluated for the suppression of injury caused by O_3 and/or other oxidants (Kendrick *et al.*, 1962; Manning *et al.*, 1974; Carnahan *et al.*, 1978; Ormrod and Beckerson, 1986; Pleijel *et al.*, 1999). The biochemical markers have been used to select the best tolerant species, cultivar and variety to O_3 or other pollutants (Han *et al.*, 2006a, b). However, there was not an exact answer about the differences of biochemical and physiological response among species, cultivar or varieties as well as among biochemical makers.

Maple trees that have been used as the ornamental or roadside tree are one of the most abundant tree species in Korean forest. In addition, they are known as a tolerance species to air pollutants, but there was only a few information on the biochemical and physiological responses and tolerant mechanisms of maple trees under environmental stress.

Therefore, this study was undertaken to investigate cellular responses to ozone as mechanism for oxidant impact on photosynthesis in seedlings of maple trees. Specifically, our objectives are to determine the effects of ozone exposure on photosynthetic pigments, photosynthesis, antioxidant enzyme activities and lipid peroxidation.

Materials and Methods

1. Plant material and growth condition

Seeds of five species of genus *Acer* (*Acer buergerianum*, *A. ginnala*, *A. mono*, *A. palmatum*, and *A. palmatum* var. *sanguineum*) were germinated in sand soil in spring, 2005. One-year-old seedlings were transplanted into plastic pots (Height 20 × Width 15 cm) containing artificial soil, which consisted of 1:1:1 sand: peat moss: vermiculite (volume basis). Five seedlings per treatment were transferred into O_3 chamber and were arranged in two blocks. O_3 treatment was divided two chambers: control chamber was circulated with the clean air and the other one was fumigated with 150 ppb O_3. O_3 fumigation time was 8 hrs a day. O_3 concentration in chamber was registered 5 ± 1 ppb in control and 150 ± 10 ppb in treatment chamber during fumigation period. The fumigation system has been described in detail by Lee *et al.* (2003). The experiment was started June 2, 2006 and it was conducted for four weeks.

2. Photosynthetic pigments

At the end of O_3 fumigation, the leaves of control and O_3-treated seedling of maple trees were excised and soaked in dimethyl sulfoxide (DMSO) in a glass vial. The vial was tightly capped and incubated at 70°C for 2 hrs in the dark. The concentration of the extracted pigments (total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid) was calculated on the basis of their absorbance values at 664, 645, and 470 nm according to Lichtenthaler (1987).

3. Photosynthesis

Net photosynthesis of fully expanded leaves was measured with an infrared gas analyzer (Li-6400, Li-COR, USA). Environmental parameters were maintained stably for measuring (mean temperature: 20.0 ± 0.1°C; relative humidity: 68.2 ± 3.2%; leaf-to-air vapour pressure deficit: 1.2 ± 0.2 kPa). All determinations were performed at 1100 μmol m^{-2} s^{-1} photon flux density (PFD). Net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}) was determined at light saturation level between 10 a.m. and 3 p.m.

To calculate carboxylation efficiency, *ACi*-curve was made (Farquhar *et al.*, 1980; Kim and Lee, 2001). The carboxylation efficiency was determined from the initial slope of a linear regression using the linear portion of

the *ACi*-curve (0-150 ppm intercellular CO_2).

4. Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by thiobarbituric acid reaction as described by Heath and Packer (1968). The crude extract was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3000×g for 10 min and the absorbance of the supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as μmol MDA g^{-1} FW.

5. Antioxidant enzyme activities

Fresh leaves (0.1g) were homogenized under ice-cold condition with 5 mL of 50 mM phosphate buffer (pH 7.0), 10 mM ascorbic acid (AsA) and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 20,000×g for 30 min, and the supernatant was collected for enzyme assays.

SOD was assayed based on the inhibition of reduction of nitro-blue tetrazolium in the presence of xanthine at 530 nm according to the method of Beauchamp and Fridovich (1971). APX activity was determined by the method of Nakano and Asada (1981). The assay was carried out in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM AsA, 0.1 mM EDTA, 0.1 mM H_2O_2, and 0.1 mL enzyme extract. The change in A_{290} was recorded for 1min after the addition of H_2O_2. Activity of GR was assayed as described in Carlberg and Mannervik (1985). The assay was carried out in a reaction mixture containing 50 mM phosphate buffer (pH 7.8), 0.1 mM NADPH, 0.5 mM GSSH and 0.1 mL enzyme extract. The change in A_{340} was recorded for 5 min after the addition of enzyme extract. CAT activity was determined by following a two-step procedure (Fossati *et al.*, 1980). The rate of dismutation of H_2O_2 to water and molecular oxygen is proportional to the concentration of catalase. Therefore, the sample containing catalase was incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute, the reaction was quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture was then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (HRP). The resulting quinoneimine dye was measured at 520 nm. All the activities of enzyme were measured using UV-120 (SHIMADZU, Japan).

6. Statistical analysis

To compare the effect on control and O_3 treatment, ANOVA was performed on experimental data (statistical significance, $P < 0.05$), and Duncan's multiple range tests were performed. Statistical analyses were performed using the statistical package SAS System for Windows, Version 8.01 (SAS Institute, USA).

Results

1. Photosynthetic pigments

After 28d of ozone exposure, the contents of photosynthetic pigments showed significant ($P < 0.05$) differ-

Table 1. Effects of ozone fumigation on photosynthetic pigments in the leaves of five species of genus *Acer*.

Species	Ozone	*chl* a	*chl* b	*chl* $_{a+b}$	car	*chl* a/b	*chl*/*car*
		mg g^{-1}					
A. buergerianum	Control	1.97 ± 0.24^{a}	1.65 ± 0.05^{a}	3.62 ± 0.28^{a}	0.55 ± 0.07^{a}	1.19 ± 0.12^{f}	6.58 ± 0.32^{a}
	150ppb	1.64 ± 0.41^{b}	1.51 ± 0.19^{b}	3.15 ± 0.27^{b}	0.56 ± 0.07^{a}	1.09 ± 0.06^{f}	5.69 ± 0.24^{b}
A. ginnala	Control	1.65 ± 0.24^{b}	0.76 ± 0.05^{c}	2.41 ± 0.28^{c}	0.47 ± 0.04^{b}	2.17 ± 0.21^{e}	5.11 ± 0.18^{c}
	150ppb	1.21 ± 0.14^{cd}	0.56 ± 0.10^{d}	1.77 ± 0.23^{de}	0.37 ± 0.03^{cd}	2.18 ± 0.22^{e}	4.76 ± 0.33^{d}
A. mono	Control	1.21 ± 0.28^{cd}	0.35 ± 0.06^{e}	1.56 ± 0.34^{def}	0.36 ± 0.06^{cd}	3.42 ± 0.29^{a}	4.37 ± 0.33^{e}
	150ppb	0.92 ± 0.07^{de}	0.29 ± 0.01^{e}	1.21 ± 0.07^{f}	0.27 ± 0.02^{e}	3.17 ± 0.18^{abc}	4.53 ± 0.12^{de}
A. palmatum	Control	1.18 ± 0.16^{cd}	0.35 ± 0.05^{e}	1.53 ± 0.21^{def}	0.34 ± 0.04^{cde}	3.37 ± 0.13^{ab}	4.53 ± 0.17^{de}
	150ppb	0.88 ± 0.15^{e}	0.34 ± 0.07^{e}	1.22 ± 0.21^{f}	0.27 ± 0.04^{e}	2.61 ± 0.25^{d}	4.50 ± 0.12^{de}
A. palmatum var. *sanguineum*	Control	1.40 ± 0.21^{bc}	0.46 ± 0.06^{d}	1.86 ± 0.25^{d}	0.39 ± 0.06^{c}	3.05 ± 0.47^{bc}	4.80 ± 0.16^{d}
	150ppb	1.07 ± 0.23^{de}	0.36 ± 0.06^{e}	1.43 ± 0.28^{ef}	0.30 ± 0.05^{de}	2.97 ± 0.36^{c}	4.71 ± 0.17^{d}
Species (S)		***	***	***	***	***	***
Ozone (O_3)		***	***	***	***	n.s.	***
S × O_3		n.s.	n.s.	n.s.	n.s.	n.s.	***

All the values are means of five replicates ± SD; Values in each column followed by the same letter indicate no significant differences ($P < 0.05$) according to Duncan's test; mixed effects linear model: ** $P < 0.01$, *** $P < 0.001$, and n.s.: not significant.

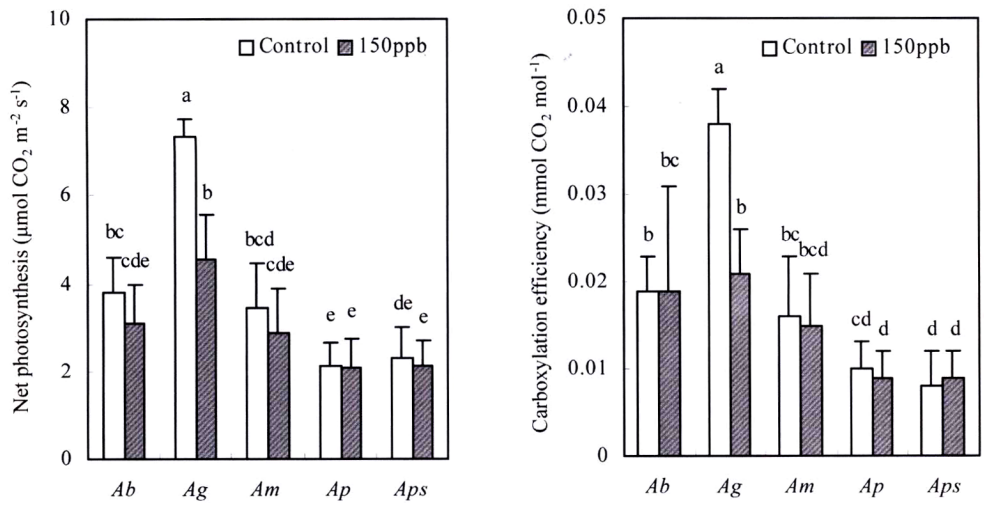

Figure 1. Effects of ozone fumigation on net photosynthesis and carboxylation efficiency in the leaves of five species of genus *Acer*. *Ab: Acer buergerianum, Ag: A. ginnala, Am: A. mono, Ap: A. palmatum, and Aps: A. palmatum var. sanguineum.* All the values are means of five replicates ± SD; The same letter indicates no significant differences ($P < 0.05$) according to Duncan's test; mixed effects linear model. Net photosynthesis: species P=0.0001*, ozone P=0.0011**; carboxylation efficiency: species P=0.0001***, ozone P=0.0279*; *, ** and *** significant at $P \leq 0.05$, 0.01 and 0.001.**

ences among species and between control and ozone treatment (Table 1). However, species × ozone treatment interaction wasn't observed for the content of photosynthetic pigment. Ozone treatment had a negative effect on the photosynthetic pigments in the leaves of five *Acer* species.

The content of chlorophyll (*chl*) a in the seedlings of five species was 16.8% (*buergerianum*) to 26.7% (*A. ginnala*) lower in ozone-exposed plant in comparison with the control. For the content of *chl* b, *A. ginnala* and *A. palmatum* var. *sanguineum* represented the high reduction rate of 26.3% and 23.6% respectively, whereas *A. palmatum* didn't reduced significantly under ozone exposure. In addition, the reduction rate of total *chl* content ranged from 20.3% (*A. palmatum*) to 26.6% (*A. ginnala*). Total carotenoid (*car*) content showed the highest reduction in *A. mono* (25.0%) under ozone fumigation. The *chl* a:b ratio was significantly different ($P < 0.05$) among species but not between control and ozone treatment. The highest reduction rate on the *chl* a:b ratio was observed in the leaves of *A. palmatum*. For the ratio of total *chl* to *car*, there were significant differences among five species and between control and ozone treatment, and species × ozone treatment interaction was observed. Especially the ratio in *A. buergerianum* decreased about 13.5% in comparison with control.

2. Net photosynthesis and carboxylation efficiency

The net photosynthesis showed significant ($P < 0.05$) differences among species and between control and ozone treatment at the end of ozone exposure (Figure 1). In addition, species × ozone treatment interaction was observed for net photosynthesis. The reduction rate of net photosynthesis in the seedlings of five species varied from 2.4% to 37.6%. Among five species, *A. ginnala* showed remarkable reduction rate (37.6%) for net photosynthesis in comparison with control. But net photosynthesis of *A. palmatum* didn't decrease significantly under ozone fumigation.

Carboxylation efficiency differed significantly ($P < 0.05$) among species and between control and ozone treatment at the end of ozone exposure (Figure 1). Species × ozone treatment interaction wasn't observed for carboxylation efficiency in the seedlings of five species. The reduction rate of carboxylation efficiency was the highest in the leaves of *A. ginnala* (44.7%), whereas carboxylation efficiency of *A. palmatum* var. *sanguineum* increased slightly but wasn't significant statistically.

3. Lipid peroxidation and antioxidant activity

There were significant differences in leaf MDA content among five species and between control and ozone treatment (Figure 2). Especially, among five species, *A. palmatum* var. *sanguineum* showed the highest increase rate (41.7%) for MDA content, whereas the increase rate for MDA in *A. ginnala* was the lowest (5.0%) under ozone fumigation.

The responses of antioxidative enzymes varied each other under ozone fumigation. For SOD, there was significant ($P < 0.05$) difference among five species. However, there wasn't significant difference between control and ozone treatment (Table 2), and species × ozone treatment interaction wasn't also observed for SOD activity. *A. palmatum* showed the highest increase rate of SOD activity (26.1%) and *A. mono* didn't show significantly the increase of SOD activity.

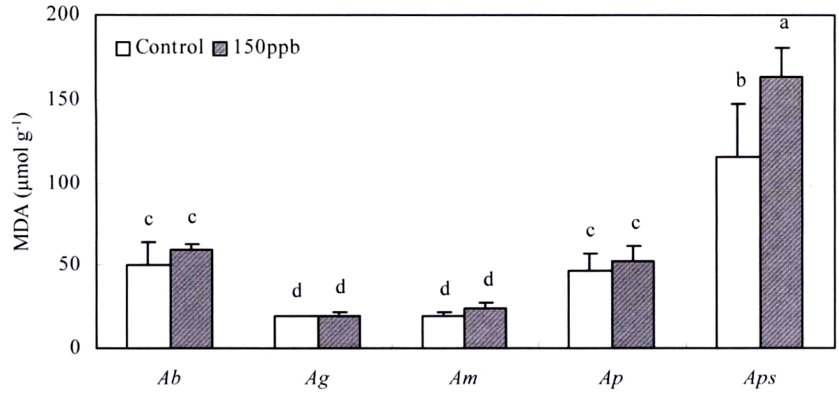

Figure 2. Effect of ozone fumigation on MDA content in the leaves of five species of genus *Acer*. All the values are means of five replicates ± SD; The same letter indicates no significant differences ($P < 0.05$) according to Duncan's test; mixed effects linear model. Species P=0.0001*, ozone P=0.0001***; *** significant at $P \leq 0.001$.**

Table 2. Effects of ozone fumigation on antioxidative enzyme activities in the leaves of five species of genus *Acer*.

Species	Ozone	SOD (unit g^{-1})	APX (μmol g^{-1})	GR (nmol g^{-1})	CAT (unit g^{-1})
A. buergerianum	Control	2366 ± 317^{c}	1767 ± 64^{b}	579 ± 197^{cd}	761 ± 29^{c}
	150ppb	2623 ± 284^{b}	2340 ± 411^{a}	824 ± 237^{bc}	696 ± 33^{c}
A. ginnala	Control	4441 ± 292^{a}	346 ± 52^{cd}	448 ± 168^{cd}	655 ± 24^{c}
	150ppb	4829 ± 608^{a}	403 ± 84^{c}	247 ± 101^{d}	923 ± 175^{b}
A. mono	Control	1391 ± 103^{d}	195 ± 40^{d}	249 ± 64^{d}	1743 ± 84^{a}
	150ppb	1400 ± 227^{d}	259 ± 56^{cd}	1187 ± 129^{ab}	642 ± 99^{c}
A. palmatum	Control	967 ± 192^{d}	345 ± 44^{cd}	1243 ± 537^{ab}	652 ± 58^{c}
	150ppb	1219 ± 200^{d}	422 ± 57^{c}	832 ± 306^{bc}	1756 ± 121^{a}
A. palmatum var. *sanguineum*	Control	1023 ± 181^{d}	228 ± 60^{cd}	1387 ± 618^{a}	725 ± 49^{c}
	150ppb	1125 ± 83^{d}	340 ± 75^{cd}	612 ± 239^{cd}	943 ± 68^{b}
Species (S)		***	***	***	***
Ozone (O_3)		n.s.	***	n.s.	**
S × O_3		n.s.	***	***	***

All the values are means of five replicates ± SD; Values in each column followed by the same letter indicate no significant differences ($P < 0.05$) according to Duncan's test; mixed effects linear model: ** $P < 0.01$, *** $P < 0.001$, and n.s.: not significant.

APX activity differed significantly ($P < 0.05$) between control and ozone treatment as well as among five species, and species × ozone treatment interaction was also observed for APX activity. The increase rate of APX activity ranged from 16.5% (*A. ginnala*) to 49.1% (*A. palmatum* var. *sanguineum*).

For GR activity, the differences among species were obvious ($P < 0.05$), and species × ozone treatment interaction was also observed for GR activity. In special, *A. mono* showed the highest increase rate (376.6%) of GR activity under ozone fumigation, and A. *buergerianum* also represented high increase rate (42.3%) of GR activity. On the contrary, *A. ginnala, A. palmatun* and *A. palmatum* var. *sanguineum* showed the lower GR activity than control plants.

CAT activity differed significantly ($P < 0.05$) between control and ozone treatment as well as among five species, and species × ozone treatment interaction was also observed for CAT activity. CAT activity increased in the leaves of *A. ginnala, A. palmatun* and *A. palmatum* var. *sanguineum* under ozone exposure, whereas *A. buergerianum and A. mono* decreased in comparison with control plants.

Discussion

Ozone (O_3) alters characteristics or functions of physiological and biochemical parameters in plant cell and the sensitivity to O_3 varies among these parameters. In addition, the sensitivity/resistance to O_3 is obviously different from species to species and varieties even among cultivars of a single species. The analysis of modifications in plant metabolism through the study of physiological and biochemical markers could represent more reliable tool to characterize the differential responses of plants to O_3 (Schraudner *et al.*, 1997).

Chlorophyll and carotenoid were one of the most sensitive parameters to O_3. In our results, the contents of chlorophyll and carotenoid significantly decreased in the leaves of five species treated with O_3, and *chl* a was more sensitive than *chl* b under ozone fumigation (Table 1). Reduction in the *chl* content of leaves, following exposure of plants to ozone, has been reported in many species such as *Picea abies* (Robinson and Wellburn, 1991), and *Pinus ponderosa* (Anderson *et al.*, 2003). Knudson *et al.* (1977) and Sakaki *et al.* (1983) found higher reduction in *chl* a in ozone-exposed plants of *Phaseolus vulgaris* L., and *Spinacia oleracea* L. like our results (Table 1). Robinson and Wellburn (1991) also observed reduction in the *chl* a:b ratio in *Picea abies* L. plants, due to summer ozone exposures. It supports our results that *chl* a:b ratio decreased in the leaves of the ozonated *A. mono*, *A. palmatum* and *A. palmatum* var. *sanguineum* (Table 1). The decreased *chl* a:b ratio in the leaves of the ozonated plants suggests a greater reduction of *chl* a than in *chl* b in these leaves. Moreover, Dhindsa *et al.* (1981) and Mikkelsen *et al.* (1995) found greater reduction in *chl* a with increasing age of leaves as a result of the physiological senescence in non-ozonated plants. Therefore, these findings support the suggestions of several investigations (Pleijel *et al.*, 1994; Welfare *et al.*, 1996) that ozone advances the senescence process in leaves. The change in *chl* a:b ratio, that implies a preferential susceptibility to ozone of one of the *chl* forms, was initially thought that it would be considered additionally informative in revealing the ozone effects on plants (Saitanis *et al.*, 2001).

Exposure to ozone results in lower net photosynthesis (Reich and Amundson, 1985; Reich, 1987), frequently concomitant with increased foliar dark respiration (Reich, 1983; Skrby *et al.*, 1987), attributed to increased maintenance respiration (Amthor, 1988). In our results, the analysis of photosynthetic light response curves showed ozone-induced decreases in net photosynthesis in the seedlings of maple trees (Figure 1). This is consistent with other reports of O_3-induced reductions in photosynthesis (Reich, 1987; Pye, 1988; Dizengremel, 2001; Matyssek and Sandermann, 2003). It has been suggested that the decrease in photosynthetic capacity may be a result of accelerated physiological aging, increased respiration, and/or other biochemical effects (Ormrod *et al.*, 1981; Pell and Pearson, 1983; Lehnherr *et al.*, 1987; Sasek and Richardson, 1989). In addition, the ozone-induced reduction of net photosynthesis is closely associated with the decrease of chlorophyll content. In Table 1 and Figure 1, *A. ginnala* showed the highest reduction of chlorophyll content and net photosynthesis, whereas *A. palmatum* and *A. palmatum* var. *sanguineum* didn't show the reduction of net photosynthesis in spite of low reduction rate of chlorophyll content. Especially *chl* b content in the leaves of *A. palmatum* didn't decrease when compared with non-ozonated plant. Meanwhile, Reich and Amundson (1985) and Reich (1987) found that interspecific differences in ozone response were strongly related to inherent differences in stomatal conductance; species with characteristically high values of conductance showed a greater proportional reduction in light-saturated photosynthetic rate than those with lower conductances.

The exact mechanisms of O_3 effects on photosynthesis are still unclear, but it has been suggested that carboxylation processes (related to Rubisco activity) are more sensitive than electron transport processes (Fiscus *et al.*, 2005). In practice, decreases in Rubisco activity are often inferred from decreases in the maximum rate of carboxylation, as indicated by a decrease in the initial slope of an A/Ci curve. However, unlike some previous studies we did not observe a significant effect of ozone treatment on carboxylation efficiency, except for *A. ginnala* (Figure 1), which might have been functionally related to lower nitrogen content per unit area in O_3-treated trees (Warren *et al.*, 2007). Unfortunately, we couldn't show the same results in our study.

The activity of PSII is also closely associated with lipid peroxidation (Xu and Zhou, 2005). Prior to the onset of any visible injury, there is an increase in membrane damage in the leaves exposed to O_3. Concentration of MDA, that estimates the state and integrity of membrane through the degree of lipid peroxidation, has been shown to correlate with the level of ozone exposure. In addition, the O_3-treated plants showed an increase in MDA content, which indicates the state of membrane lipid peroxidation and has been shown to be correlated with the degree of O_3 exposure to plants (Price *et al.*, 1990; Yoshida *et al.*, 1994; Ranieri *et al.*, 1996).

As reported in *Pinus densiflora* (Lee *et al.*, 2006) and *Citrus clementina* (Iglesias *et al.*, 2006), it was found that lipid peroxidation increases after short or long exposures to O_3, by disorganizing the membrane structure, and altering membrane permeability finally (Weber *et al.*, 2004). In our studies, however, the increase of lipid peroxidation wasn't obvious in O_3-treated plants (Figure 2). Exceptionally MDA content of *A. palmatum* var. *sanguineum* increased by 41.7% of control plants. In addition, the reductions of chlorophyll content and net photosynthesis were not consistent with the increase of MDA content, which might show that the changes of physiological parameters were not affected by lipid peroxidation directly.

Active oxygen radicals have been proposed as lipid peroxidation initiators (Elstner *et al.*, 1988; Winston, 1990). Higher activities of scavenger antioxidant enzymes

may help in protecting plants from oxidative stress (Lee *et al.*, 1984; Bowler *et al.*, 1992; Brunschon-Harti *et al.*, 1995; Asada, 1997). However, in the present study there were non-significant differences in the activities of SOD and GR between treatments. On the contrary, APX and CAT were significant differences between treatments. For SOD and APX, in O_3-treated A. *buergerianum* the activities of SOD and APX were higher than that of control plants (Table 2). O_3 has been reported to degrade into superoxide, hydrogen peroxide and hydroxyl radical and also singlet oxygen from reaction with biological molecular (Foyer *et al.*, 1994). This H_2O_2 formation apparently caused the inactivation of endogenous SOD, together with a reversible inhibition of Calvin cycle enzymes (Tanaka *et al.*, 1982). Sometimes, the low SOD activity in O_3-treated plants could be due a higher H_2O_2 concentration that inhibited the SOD activity. The induction of SOD activity in O_3-treated plants, as compared to control, may be explained in terms of ozone concentration, an increase in SOD activity can alleviate the oxidative stress. There are, however, conflicting reports of the effects of O_3 on antioxidant enzyme activity (Bowler *et al.*, 1992; Lyons *et al.*, 1999). Several cultivars of spinach with differing ozone sensitivities were examined, but no clear correlation was found between resistance and levels of SOD, catalase, ascorbate peroxidase, or glutathione reductase (Tanaka *et al.*, 1985).

In conclusion, physiological markers such as chlorophyll content and photosynthesis that responded sensitively to O_3 in maple trees were considered as the very important indicators in order to evaluate the tolerance against O_3 stress, and parameters were closely related with each other. MDA content wasn't feasible as a indicator on the physiological damage due to the irregular responses. Among antioxidative enzymes, SOD and APX might be contributed to alleviate to O_3 toxicity through the increase of activity in all maple trees. Therefore, these compounds can be used as a biochemical maker to assess the stress tolerance to O_3. However, GR and CAT weren't suitable as a biochemical indicator due to the contradictory results among species.

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