Growth, Physiological Responses and Ozone Uptake of Five *Betula* Species Exposed to Ozone

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ABSTRACT : The objectives of this study were to examine the physiological responses to ozone and to measure ozone uptake rates of *Betula* species exposed to relatively high concentration of pollutants. At the end of the growing season, photosynthesis, pigments contents, antioxidants (SOD and GR) and ozone uptake rates were measured or estimated at the leaves of five *Betula* species (*Betula costata, B. davurica, B. platyphylla* var. *japonica, B. schmidtii* and *B. ermanii*) exposed to 100ppb ozone concentration. On the termination of the experiment, growth effects were determined by measuring leaf area and dry weights of leaf, stem and root. Ozone treatment showed the significant reduction the leaf area and dry weight of four *Betula* species, except for *B. ermanii*. Shoot / root (SR) ratio of five species represented two different types. SR ratio of *B. costata* and *B. davurica* were lower than control, in contrast, SR ratio of *B. platyphylla* var. *japonica, B. schmidtii* and *B. ermani* were higher than that of control. The photosynthetic responses of five species were different in responses to ozone exposure. Four species, except for *B. ermanii*, maintained or increased the stomatal conductance, but *B. ermanii* decreased both stomatal conductance and photosynthesis. SOD activities of five species decreased by the ozone exposure, especially *B. ermanii* showed the largest reduction, GR activities of *B. platyphylla* var. *japonica* and *B. schmidtii* increased, *B. costata* and *B. ermanii* decreased. Instantaneous ozone uptake rate was the highest at the leaves of *B. ermanii* and *B. costata*, ozone uptake per seedling was the highest at the leaf of *B. schmidtii* and *B. ermanii*. It was concluded that *B. costata*, *B. davurica* and *B. platyphylla* var. *japonica*, appeared the growth reduction and visible ozone injury, were sensitive species to ozone, and *B. schmidtii* with the increased antioxidant activity and *B. ermanii* without the growth reduction were relatively resistant species to high ozone concentration at the early growing stage.

Key words : Antioxidants, *Betula*, GR, Growth responses, Ozone exposure, Ozone uptake rate, Photosynthesis, SOD

INTRODUCTION

Air pollution is an important stress factor affecting vegetation (Reich 1987). Of the air pollutants, the biological impact of ozone is complex and still to be deciphered in detail. Ozone, absorbed through leaf stomata, disturbs the physiological and biochemical functions of plants producing a range of effects such as visible injury, growth and yield reduction (Guderian *et al.* 1985). Ozone absorbed in the leaves through stomata, combines with biological molecules on the cell membranes, generates reactive oxygen species (superoxide, hydroxyl radical, hydrogen peroxide, singlet oxygen), the reactive oxygen species occur the leakage of cell membranes by the lipid peroxidation (Lidon and Henriques 1993). The physiological disturbances by ozone result in the growth reduction by low photosynthetic efficiency, leaf senescence, chlorosis and necrosis.

However, despite the toxicity of pollutants, many plants are able to tolerate polluted environments and continue functioning. The tolerance mechanisms of plants are resistance and avoidance mechanism. Avoidance mechanism is to suppress ozone flux into leaf through the stomatal closure in response to higher ozone concentrations, resistance mechanism is to develop a mechanism for the neutralization of the damage caused by ozone for living cells. This mechanism involves the resynthesis of damaged enzyme molecules or membrane fractions, and it causes increased maintenance/respiration rates (Heath 1980). Plants with the tolerance mechanisms might be reduced the concentration of ozone in the ambient air. But a few experiments have shown large differences in ozone sensitivity between species (Mortensen and Skre 1990; Chappelka and Chevone 1992). Therefore to use the woody plants as a shade trees, we have to select and

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to plant the trees species suitable to the required function in considerable with kinds or concentration of pollutants.

Recently, *Betula* species naturally growing at the high altitude mountains in South Korea, have been used for mine reclamation, and have been used on a trial basis as shade or ornamental trees in the city. Despite these diverse uses for natural *Betula* species, little is known about their physiological responses and tolerance mechanisms for ozone and other pollutants.

The objectives of this study were to examine the physiological responses to ozone and to measure ozone uptake rates in order to be convinced of the capability of the use for *Betula* species as a shade tree in the city.

MATERIALS AND METHODS

Plant materials

In late spring, One-year-old seedlings of five *Betula* species (*Betula costata, B. davurica, B. platyphylla* var. *japonica, B. schmidtii* and *B. ermanii*) were transplanted to 0.5-liter plastic pots containing a soil: peat: perlite (1:1:1, v/v) mixture and the potted plants were grown in a greenhouse for 2 months. During this period, temperature ranged between 15 and 26℃ and relative humidity (RH) ranged between 55 and 85%. Ten uniform plants of each species were selected when leaves were fully expanded. Fifty plants were acclimated at light and humidity conditions in exposure chamber for a week.

Exposure chambers and fumigation techniques

Ten plants of each species were treated for 5 weeks in two exposure chambers. One chamber was used as a control, the other chamber was fumigated with O_3 during 8h day^{-1} at a concentration of 100 ppb. Ozone exposures were performed during the summer of 2001 in a Plexiglas fumigation apparatus. Two-exposure chamber with dimensions of 3m(L)×3m(W)×1.8m(H) were used in this study. Air was circulated through charcoal filters and O_3 was mixed into the air stream. Air temperature and relative humidity were controlled at 22± 2℃ and 60~80% RH with a cooling, a heating and a humidifying system. Light for plant growth illuminated the inside of the chamber through single layer of 1.6mm thick glass. The mixed humid and ozonated air entered the bottom of each chamber and exited the chamber top via two exhaust filters. Airflow was maintained at about 1m/sec. Temperature was maintained at 25± 1℃, RH at 60±5%; a photosynthetic photon flux at plant height of about 550μmol m^{-2} s^{-1} was provided for a 12-h photoperiod. Plants within the chamber spread out randomly to minimize the edge effects, and changed the locations of individual plants throughout the study.

Ozone was generated by Corona Discharge System of ozone generator (Model H450, Harim engineering, Inc., Korea) with oxygen of ambient air through a charcoal filter, was mixed with the air filtered by Zero Air System (Model 701, API, Inc., USA) with charcoal and Furafil, and was delivered to each chamber with Gas Exposing System (Model H1800, Harim Engineering, Inc., Korea). The O_3 concentrations at plant height were continuously monitored with a photometric O_3 analyzer (Model 400, API, Inc., USA), automatically controlled with PWM(Pulse Width Modulated) system, and calibrated once a week. All measuring values stored every three-second in HARE 600 data logger (Harim Engineering, Inc., Korea). The control plants were exposed to charcoal-filtered air only.

For the experimental period, average ozone concentration in two chambers were 5±1ppb(control chamber) and 98±5ppb(fumigation), respectively. Physiological responses and ozone uptake were measured at the end the 35-day fumigation period.

Growth analyses and chlorophyll contents

On termination of the experiment, Effects on growth were evaluated by measuring leaf area and dry weight of leaf, stem and root. Leaf area was determined with a Li-Cor leaf area meter. Pigment contents were measured at the five leaves per seedling with portable chlorophyll meter (SPAD-502, Minolta), their mean values were used to data analysis.

Net photosynthesis and stomatal conductance

Both net photosynthesis and stomatal conductance were measured with a portable photosynthesis system (LI-6400, Li-Cor Inc., USA). Three fully expanded stem-attached leaves (leaf position from fourth to sixth from the top) per plant were measured at light saturation (1200μmol m^{-2} s^{-1}) provided by an LED light module after determining the light-response curve (between 0 and 2000μmol m^{-2} s^{-1}) and steady-state rate of photosynthesis. The CO_2 concentration during measurements was maintained between 340 and 360 μmol CO_2 mol^{-1} air, leaf temperature was 24.0±0.2℃ and RH was 60± 5%. Replicate data were averaged for each plant. After determining A-Ci curve according to the internal CO_2 concentration (between 0 and 350μmol CO_2 mol^{-1} air), carboxylation efficiency was estimated based on the method of Farquhar *et al.*(1980).

Antioxidants

The superoxide dismutase (SOD) activity assay was carried out according to the NBT (nitro blue tetrazolium)-xanthine oxidase method (Beauchamp and Fridovich 1971). 0.1g of homogenized fresh leaf was added to 1.5ml of cold buffer, which was prepared by dissolving 3.72mg of EDTA and 1g of PVP in 100ml of 50mM KH_2PO_4 at pH 7.8. after centrifugation at 20,000×g for 15min,

0.3ml of supernatant was obtained and added to the reaction mixture, which consisted of 0.6ml of 200μM NBT and 1.8ml of 53 μM xanthine. The reaction was inhibited by adding 0.3ml of 60μ g/ml xanthine oxidase to the reaction mixture. Reduction rates over 2min were determined as an increase of absorbance of 530nm according to Asada *et al.* (1974), SOD activity was calculated by the equation (V/v-1), where V and v represent the reduction rates of the reaction when the buffer or the supernatant was added, respectively.

Glutathione reductase (GR) activity was measured by following the decrease in absorbance at 340nm due to NADPH oxidation (Carlberg and Mannervik 1985). The reaction mixture contained 0.2M potassium phosphate buffer, pH 7.0, 2mM EDTA, 2mM NADPH, 20mM GSSG. The reaction was initiated by the addition of the sample extract, and was monitored for 5min.

Calculations of ozone uptake rate

To estimate foliar O_3 uptake for each tree species we used the stomatal conductance (g_{gw}) values and average hourly O_3 concentrations. The g_{gw} measurements were done at saturating PAR (1200 μmol m^{-2} s^{-1}), and we assumed the values were representative for a period centered around each measurement day. The instantaneous uptake rates were calculated as the product of the 8-h average O_3 concentration and g_{gw}, adjusted for conductance to O_3 by dividing by 1.68, as follows; $Q=Za\times g_{gw}/1.68$ (Laisk *et al.* 1989), where Za is the ambient ozone concentration, g_{gw} the gas phase conductance for water vapor and 1.68 the theoretical ratio of diffusion coefficients for water vapor and ozone in air.

Data analyses

The statistical analysis of the data was performed with SAS System for Windows Version 6.12 (SAS Institute Inc. USA). Mean values per treatment were compared by ANOVA. When ANOVAs showed significant differences ($P\leq 0.05$), Duncan's multiple range tests were performed.

RESULTS AND DISCUSSION

Growth responses

Growth, measured as leaf area, dry weight and shoot/root ratio, was affected by the O_3 treatments at the early stage of growing season (Table 1). Ozone treatment decreased the leaf area and dry weight of five *Betula* species. The reduction of leaf area was significantly different among five species, showed the range from 38.6 to 90.6%. Leaf area of *B. costata* decreased up to 61.4% of control, but leaf areas of *B. schmidtii* and *B. ermanii* were not significantly different with control.

The leaf area reduction in the ozone treatment was caused by the early stage senescence and leaf chlorosis, especially *B. costata* was observed a lot of leaf senescence at the lower leaves of the main stem after 10 days of ozone exposure (data not shown), and *B. davurica* and *B. platyphylla* var. *japonica* were observed the visible injury (chlorosis) on the surface of leaves. But *B. schmidtii* and *B. ermanii* were not visible ozone injury on the leaves.

Dry weights of leaf, stem and root were significantly reduced relative to control (Table 1), especially total dry weights of *B. costata*, *B. davurica* and *B. platyphylla* var. *japonica* showed 64.0, 56.6, 62.1% reduction of control, respectively, and they represented larger reduction at the leaves than at the stems and roots by the effects of leaf senescence or chlorosis. Leaf dry weights of *B. costata*, *B. davurica* and *B. platyphylla* var. *japonica* indicated 77.5, 66.2 and 61.5% reduction relative to control, respectively. The reduction of leave dry weights resulted in the growth reduction of stem and root.

Shoot / root ratio of five species showed two different types (Table 1). Shoot/root ratios of *B. costata* and *B. davurica* were lower than control, but shoot / root ratios of *B. platyphylla* var. *japonica*, *B. schmidtii* and *B. ermanii* were higher than control. The lower shoot/root ratio of two species of the former resulted from the larger reduction at the above-ground including leaf and stem than at the below-ground, on the contrary, the higher shoot/root ratio of three species of the later resulted from the lower reduction at the above- ground than at the below-ground.

Accelerated leaf senescence during ozone stress in deciduous trees has been well documented in earlier studies (Wang *et al.* 1986; Pye 1988), and negative effects of ozone on growth have been reported in several tree species (Darrall 1989; Chappelka and Chevone 1992). However, the mechanisms responsible for these growth reductions are not completely understood.

Mortensen and Skre (1990) reported that increasing the concentration significantly enhanced leaf senescence and visible ozone injury was observed at 53nl l^{-1} and above. However early leaf abscission may have been enhanced in seedlings because of their rapid growth rates and indeterminate shoot growth habit and may be indicative of a greater ability to compensate for foliar injury compared to larger trees (Fredericksen *et al.* 1996). Woodbury *et al.* (1994) found that hybrid popular (*Populus* species) leaves exposed to ozone compensated for early leaf abscission by an increase in new leaf production.

Mortemsen and Skre (1990) found that shoot / root and leaf / stem dry weight ratios were unaffected by ozone concentration. while leaf senescence was significantly enhanced by increasing the concentration in the *Betula* species.

In this study we could not observe that *Betula* species increased

Table 1. Dry weight of leaf, stem and root, and shoot/root(SR) ratio of five *Betula* species exposed to 100ppb O_3. Mean for the same species and in the same column with the same letter are not significantly different at $p \leq 0.05$(Duncan's multiple test). The number in the parenthesis indicates standard deviation

Species	Treatment	Leaf area (cm2)	Dry weight(g)				SR ratio
			Leaf	Stem	Root	Total	
B. costata	Control	326.4[a] (72.6)	1.42[a] (0.40)	0.98[a] (0.38)	0.69[a] (0.22)	3.11[a] (0.65)	3.47[a] (1.16)
	100ppb	126.2[b] (48.0)	0.32[b] (0.16)	0.47[b] (0.12)	0.32[b] (0.10)	1.12[b] (0.37)	2.47[b] (0.84)
	%	61.4	77.5	52.0	53.6	64.0	28.8
B. davurica	Control	390.0[a] (60.9)	2.01[a] (0.31)	1.10[a] (0.35)	0.66[a] (0.08)	3.78[a] (0.42)	4.71[a] (0.84)
	100ppb	251.7[b] (94.6)	0.68[b] (0.28)	0.56[b] (0.16)	0.40[b] (0.12)	1.64[b] (0.49)	3.1[b] (0.81)
	%	35.5	66.2	49.1	39.4	56.6	34.19
B. platyphylla var. *japonica*	Control	398.2[a] (107.7)	1.35[a] (0.39)	0.80[a] (0.31)	0.74[a] (0.28)	2.90[a] (0.94)	2.9[a] (1.19)
	100ppb	224.6[b] (76.7)	0.52[b] (0.18)	0.35[b] (0.09)	0.22[b] (0.05)	1.10[b] (0.25)	3.95[a] (1.26)
	%	43.6	61.5	56.3	70.2	62.1	-36.21
B. schmidtii	Control	269.4[a] (47.3)	1.11[a] (0.27)	0.60[a] (0.17)	0.49[a] (0.11)	2.21[a] (0.55)	3.49[b] (0.28)
	100ppb	244.0[a] (41.2)	0.65[b] (0.13)	0.55[a] (0.13)	0.27[b] (0.06)	1.48[b] (0.32)	4.44[a] (0.76)
	%	9.4	41.4	8.3	44.9	33.0	-27.22
B. ermanii	Control	240.0[a] (36.4)	0.92[a] (0.06)	0.70[a] (0.31)	0.58[a] (0.12)	2.21[a] (0.42)	2.79[a] (0.66)
	100ppb	220.6[a] (58.2)	0.88[a] (0.17)	0.50[a] (0.18)	0.47[a] (0.17)	1.87[a] (0.52)	2.93[a] (0.54)
	%	8.1	4.3	28.6	19.0	15.4	- 5.02

the new leaf production to compensate the early leaf abscission, and shoot and root dry weight of five *Betula* species decreased, and shoot /root dry weight ratio was also affected by ozone, because it was exposed to the higher ozone concentration (100ppb) than the above other studies. Oksanen *et al.* (2001) reported that ozone caused a shift in resource allocation toward stem height growth, thereby altering the shoot to root balance, in the free-air O_3 enrichment experiment, low O_3 concentrations tended to stimulate growth of most clones, whereas 100 and 150ppb O_3 in the chamber experiment impaired growth of most clones.

Net photosynthesis and chlorophyll contents

The results for photosynthesis, stomatal conductance, carboxylation efficiency and SPAD values are shown in Table 2. Photo-

Table 2. Net photosynthesis(A_{sat}) and stomatal conductance(g_{gw}) of five *Betula* species exposed to 100ppb O_3. Mean for the same species and in the same column with the same letter are not significantly different at $p \leq 0.05$(Duncan's multiple test). The number in the parenthesis indicates standard deviation

Species	Treatment	A_{sat} ($\mu molCO_2/m^2/s$)	g_{gw} ($molH_2O/m^2/s$)	Carboxylation efficiency	SPAD values
B. costata	Control	8.21[a] (1.28)	0.199[b] (0.009)	42.1[a] (8.0)	36.6[a] (2.7)
	100ppb	8.92[a] (1.28)	0.320[a] (0.042)	36.6[a] (3.4)	31.8[b] (1.4)
	%	-8.7	- 60.8	13.1	13.1
B. davurica	Control	5.52[a] (1.83)	0.184[a] (0.019)	28.7[a] (6.8)	34.0[a] (2.7)
	100ppb	5.81[a] (0.96)	0.202[a] (0.014)	26.6[a] (3.9)	29.3[b] (2.8)
	%	-5.2	- 9.8	7.3	13.8
B. platyphylla var. *japonica*	Control	7.06[a] (0.87)	0.199[a] (0.026)	31.5[a] (7.2)	33.9[a] (2.8)
	100ppb	5.22[b] (1.97)	0.234[a] (0.039)	23.4[a] (7.9)	25.6[b] (5.0)
	%	26.1	- 17.6	25.7	24.5
B. schmidtii	Control	5.87[a] (1.17)	0.137[b] (0.033)	30.6[a] (5.9)	35.5[a] (1.4)
	100ppb	6.95[a] (0.97)	0.314[a] (0.038)	25.2[a] (4.8)	31.8[b] (1.7)
	%	-18.4	-129.2	17.6	10.4
B. ermanii	Control	12.6[a] (1.6)	0.392[a] (0.046)	61.9[a] (9.6)	37.6[a] (2.0)
	100ppb	9.0[b] (1.6)	0.338[b] (0.038)	35.7[b] (9.1)	37.2[a] (1.8)
	%	28.6	13.8	42.3	1.1

synthesis at saturating light intensity was significantly different with species. The photosynthetic capacity of *B. costata*, *B. davurica* and *B. schmidtii* were not reduced in the 100ppb, but those of *B. platyphylla* var. *japonica* and *B. ermanii* were significantly reduced in the 100ppb. The reduction of *B. platyphylla* var. *japonica* and *B. ermanii* were 26.1% and 28.6%, respectively.

Except for *B. platyphylla* var. *japonica*, stomatal conductances of four species were higher or equaled to control. Especially stomatal conductance of *B. schmidtii* showed the largest increase of five *Betula* species (129.2% of control). Carboxylation efficiency decreased in the leaves of the all species exposed to ozone, and *B. ermanii* showed the largest reduction of 42.3% of control. Chlorophyll content indicated with SPAD values, decreased in the leaves of four *Betula* species except for *B. ermanii*.

Five species were different in response to ozone exposure. *B. costata*, *B. davurica* and *B. schmidtii*, appeared the visible ozone injury or lower SPAD values, increased or withstood the stomatal conductance in order to maintain the photosynthetic capacity. Although *B. platyphylla* var. *japonica* increased the stomatal conductance, the photosynthetic capacity significantly reduced at the ozone treatment.

B. ermanii, not indicated a visible injury and the reduction of SPAD values, significantly decreased the stomatal conductance, photosynthesis capacity and carboxylation efficiency. However the growth reduction was not larger compared to other tree species.

In general, ozone has been reported to accelerate the normal decline in chlorophyll content and photosynthesis (Thomas and Stoddardt 1980; Reich 1983) and in the activity and quantity of rubisco (Farage and Long 1999; Shavnin *et al.* 1999).

In this study the decline in chlorophyll content and photosynthesis were different among species. Four species, except for *B. ermanii*, increased stomatal conductance to compensate for the loss of photosynthesis by ozone injury at the early stage (Oksanen *et al.* 2001). The differences in ozone responses among five species might be related to the maintenance of high photosynthetic rate in recently mature leaves (Coleman *et al.* 1995). Also despite the increased stomatal conductance, the growth reduction of all species that tested in this study might be resulted from the disturbances of physiological and biochemical systems and the deformations of anatomical structures by the relatively high ozone concentration (Minnocci *et al.* 1999).

Antioxidants

SOD and GR, as a antioxidant were analyzed in the leaves at the end of treatment (Table 3). SOD activities at the 100ppb ozone treatment, except for *B. schmidtii*, were slightly lower than that of control, but the SOD activity of *B. ermanii* showed considerable reduction by the ozone exposure.

In general, when plants have a certain oxidative stress, SOD activities increase to scavenge the free radical generating from the complex with cell membranes. But when stress is excessive concentration or lasted at the long-term exposure, SOD activity decreases by the over-expression of defense mechanism. We could also find out the same results. Five *Betula* species, especially *B. ermanii*, showed lower activities than control.

GR activity, similar to SOD, indicates the increasing tendency under the condition of ozone exposure. In this study three species (*B. davurica*, *B. platyphylla* var. *japonica* and *B. schmidtii*) were higher than control or maintained GR activity at the level equal with control. Especially *B. schmidtii* showed the highest activity of five species. However the GR activities of *B. costata* and *B. ermanii* were significantly lower than that of control, The increased activity resulted in the loss of energy, eventually the growth of tree would be reduced, like *B. schmidtii*. On the contrary, lower activity, the lack of induction of antioxidant defense processes causes O_3-induced cell death and lesion formation under ozone stress (Koch *et al.* 2000). These results might be appeared in the visible injury, like at the leaves of *B. costata* exposed to 100ppb ozone. Although *B. ermanii* showed the considerably lower activity, was not observed the visible injury on the foliar surface during the current growing season. It probably means that *B. ermanii* has relatively high tolerance to the ozone toxicity.

Table 3. SOD and GR of five *Betula* species exposed to 100ppb O_3. Mean for the same species and in the same column with the same letter are not significantly different at $p \leq 0.05$(Duncan's multiple test). The number in the parenthesis indicates standard deviation

Species	Treatment	SOD	GR
B. costata	Control	98.6[a] (7.1)	3.273[a] (0.261)
	100ppb	87.6[a] (7.8)	2.153[b] (0.499)
	%	11.1	34.2
B. davurica	Control	76.6[a] (10.9)	3.629[a] (0.149)
	100ppb	63.2[a] (10.1)	3.707[a] (0.282)
	%	17.5	−2.1
B. platyphylla var. *japonica*	Control	90.6[a] (8.7)	3.021[b] (0.309)
	100ppb	86.0[a] (10.9)	3.499[a] (0.236)
	%	5.1	−15.8
B. schmidtii	Control	79.0[a] (7.6)	2.118[b] (0.298)
	100ppb	88.4[a] (11.8)	2.752[a] (0.349)
	%	−11.9	−29.9
B. ermanii	Control	108.2[a] (10.0)	4.019[a] (0.349)
	100ppb	59.0[b] (13.0)	1.814[b] (0.166)
	%	45.5	54.9

Ozone uptake rate

In Fig. 1 the instantaneous ozone uptake rate and ozone uptake

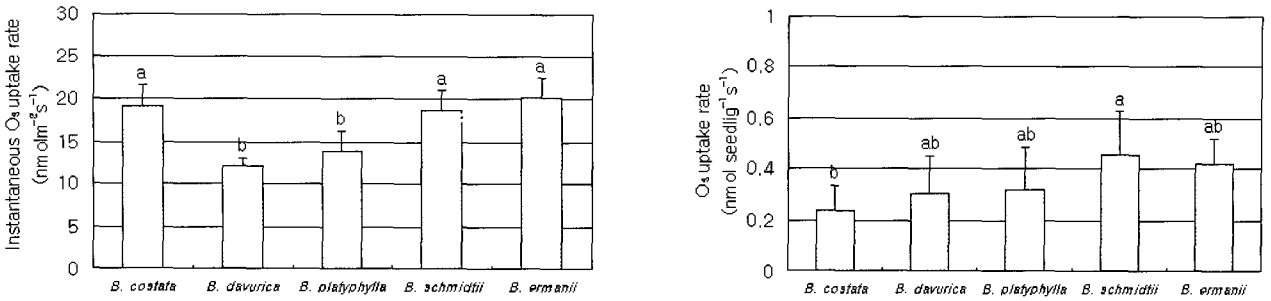

Fig. 1. Instantaneous ozone uptake rate and ozone uptake per seedling of five *Betula* species exposed to 100ppb O_3. Significant differences are indicated by different letters at $p \leq 0.05$(Duncan's multiple test).

rate per seedlings were significantly different with tree species exposed to 100ppb ozone. The instantaneous O_3 uptake rate, based on stomatal conductance and O_3 concentration, showed the highest value in the leaf of *B. costata* (19.1nmol m^{-2} s^{-1}) and *B. ermanii* (20.1nmol m^{-2} s^{-1}). Ozone uptake rate per seedling was the highest in the leaf of *B. ermanii* (0.418nmol $seedling^{-1}$ s^{-1}) and *B. schmidtii* (0.458nmol $seedling^{-1}$ s^{-1}) and the lowest in the leaves of *B. costata* (0.237nmol $seedling^{-1}$ s^{-1}).

Many researchers have been studied for ozone uptake rates at the various species (Wang *et al.* 1995; Fredricksen *et al.* 1996; Bortier *et al.* 2001). Heath (1980) and Guderian *et al.* (1985) reported that stomatal conductance was the principal regulator of ozone uptake. Ozone uptake is thus largely related to stomatal conductance because stomatal guard cells control diffusion of ozone into the leaf.

Most researchers reported that tree species exposed to ozone had lower stomatal conductance due to the stomatal closure (Bortier *et al.* 2001), but in the present study except for *B. ermanii*, four species represented higher stomatal conductance at the 100ppb ozone exposure. The higher stomatal conductance was considered as a response in order to compensate for the growth reduction by the ozone exposure. It also means that the responses for the ozone are different with the physiological characteristics of tree species.

Because of this important regulatory role in uptake, it has been proposed that differences in ozone sensitivity within a species could be explained to a large degree by differences in stomatal conductance (Reich and Amundson 1985; Runeckless 1992).

In this study *B. costata* had higher ozone uptake rates, and apparently greater foliar ozone injury, because of their higher stomatal conductance. However, Fredericksen *et al.* (1995) found greater injury in lower compared to upper crown leaves of black cherry trees despite greater uptake for upper crown leaves. These results, as well as those from other studies (Tjoelker *et al.* 1993; Volin *et al.* 1993) indicate that other factors, besides stomatal conductance and ozone uptake, may be important in determining ozone injury.

Tjoelker *et al.* (1993) proposed that the ratio of stomatal conductance, or uptake, to net photosynthesis could possibly be used as an index of sensitivity to ozone because more photosynthate is necessary with increasing ozone uptake for anti-oxidant defense and repair of damaged tissue. However, Fredricksen *et al.* (1996) reported that such an index was not useful in predicting ozone sensitivity. Taylor and Hanson (1992) suggested that the pathway of ozone from the leaf surface to target sites within the cell is less than a millimeter, but processes occurring at this level may be important in determining the effects of ozone on the plant.

We concluded that *B. costata, B. davurica* and *B. platyphylla* var *japonica* were sensitive species to ozone, and *B. schmidtii* with the increased antioxidant activity and *B. ermanii* without the growth reduction, were relatively resistant species to high ozone concentration at the early growth stage.

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