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Photosynthetic Characteristics and a Sensitive Indicator for O_3-exposed *Platanus orientalis*

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오존에 노출된 버즘나무의 광합성 특성과 민감성 지표

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ABSTRACT

We investigated the effect of O_3 on the photosynthetic characteristics of oriental plane (*Platanus orientalis* L.) that is used as a side tree or ornamental tree in Korea. Two-year-old oriental plane seedlings were transplanted to pots and transferred into a closed O_3 chamber. Photosynthetic pigment content and photosynthetic characteristics of leaves were measured every three weeks during 100 ppb O_3 fumigation. There was no visible foliar injury by O_3 exposure and the content of photosynthetic pigments did not show significant differences between control and O_3-treated seedlings. But photosynthetic rate, stomatal conductance, and water use efficiency in leaves of O_3-treated seedlings were reduced after six weeks of ozone fumigation. In addition, reduction of carboxylation efficiency and photochemical efficiency was observed in leaves of O_3-treated seedlings after three weeks and six weeks. In accordance with our results, carboxylation efficiency, the most sensitive parameter to O_3 stress, was considered to be a suitable indicator of O_3 sensitivity.

***Key words*: Photosynthetic rate, Carboxylation efficiency, Photochemical efficiency, Water use efficiency**

I. INTRODUCTION

Due to industrialization and consumption of fossil fuel, air pollution problems have increased. Environmental problems result from primary pollutants and from photochemically generated secondary pollutants. O_3, a product of photochemical reaction, can harm not only plant but also human health. The phytotoxicity of O_3 inside the leaves is probably due to its ability to react with apoplast constituents, thus generating highly reactive oxygen species that are likely the direct cause of the negative effect of O_3 (Hippeli and Elstner, 1996). When plants uptake O_3, stomatal closure and mesophyll cell destruction will result, leading to a decrease of photosynthesis (Paakkonen *et al.*, 1996; Lee *et al.*, 2004). Also plant growth will be decreased by biochemical and physiological damage (Pye, 1988; Lee *et al.*, 2003).

O_3 effects on plant growth are usually related to an acceleration of leaf senescence involving chlorophyll degradation and reductions in CO_2 assimilation (Elvira *et al.*, 1998; Zheng *et al.*, 2002). During leaf aging and senescence, O_3 has been reported to accelerate the normal decline in chlorophyll content and photosynthesis

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(Reich, 1983). Photosynthesis is a core function in the physiology of plants, and its functional status has been considered an ideal physiological activity to monitor when the health and vitality of a plant is under scrutiny (Clark *et al.*, 2000). O_3 appears to alter photosynthetic activity through various mechanisms. A reduction in carboxylation efficiency has been considered to play a main role in the impairment of photosynthesis, and O_3 can alter the light reactions of photosynthesis, decreasing the electron transport rate between both photosystems (Calatayud *et al.*, 2002). O_3 reduces the amount of Rubisco independently of an effect on leaf conductance (Farage and Long, 1999; Nussbaum *et al.*, 2000).

The main objective of our study was to investigate the effect of O_3 on photosynthetic characteristics of oriental plane (*P. orientalis* L.) that is usually used as a side tree or ornamental tree in Korea.

II. MATERIALS AND METHODS

2.1. Plant material and growth condition

Oriental plane (*P. orientalis* L.) seeds were germinated in sand and transplanted into pots. Two-year-old seedlings were transplanted into large pots (30×34 cm) containing artificial soil, which consisted of 1:1:1 sand: peat moss: vermiculite (volume basis). Treatments were arranged in two blocks with 3 seedlings per treatment. Pots were transferred into the test chambers. The fumigation system was described in detail by Lee *et al.* (2003). Treatments were divided into two chambers, one for control (clean air) and the other for fumigation treatment at 100 ppb/hr of O_3. The fumigation duration was 8 hrs each day. O_3 concentration in chambers was registered at 5±1 ppb in control and 98 ±5 ppb in the treatment chamber during fumigation periods. Treatment began June 2, 2004 and continued for nine weeks.

2.2. Photosynthetic pigments

Leaves of control and O_3-treated oriental plane were excised and soaked in DMSO in a glass vial. The vial was tightly capped and incubated at 70°C for 2 h in the dark. The concentration of extracted pigments (total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid) was calculated based on absorbance values at 664, 645, and 470 nm, according to Lichtenthaler (1987).

2.3. Gas exchange and water use efficiency

Gas exchange of fully expanded leaves from the fourth or fifth whorl was measured using an infrared gas analyzer (Li-6400, LI-COR, USA). Environmental parameters were maintained stable during the measurements (mean temperature: 20.0±0.1°C relative humidity: 68.2±3.2%; leaf-to-air vapour pressure deficit (VPD): 1.2±0.2 kPa). All determinations were performed at 1200 μmol m^{-2} s^{-1} photon flux density (PFD). The gas exchange parameters determined at light saturation level were: photosynthetic rate (*A*, μmol CO_2 mm^{-2} s^{-1}), stomatal conductance to water vapor (*G*w, mol H_2O m^{-2} s^{-1}), and transpiration rate (E, mmol H_2O m^{-2} s^{-1}). Gas exchange determination was performed between 9 and 11 a.m.

Water use efficiency (WUE) was determined by dividing photosynthetic rate (A) by transpiration rate (E). To calculate carboxylation efficiency (CE), A/Ci-curve was measured (Farquhar *et al.*, 1980; Kim and Lee, 2001). The carboxylation efficiency was determined from a linear regression using the linear portion of the A/Ci-curve (0-150 ppm intercellular CO_2).

Apparent quantum yield (AQY) was used to calculate photochemical efficiency (PE), (Sharp *et al.*, 1984; Evans, 1987; Kim and Lee, 2001). Gas exchange was measured at 0, 20, 50, 100, 200, 500, 1000, 1500, and 2000 μmol m^{-2} s^{-1} PFD. The apparent quantum yield was determined from a linear regression using the linear portion of 0 to 100 μmol m^{-2} s^{-1} PFD.

2.4. Statistical analysis

Comparison of the effect on control and O_3 treatment was evaluated using the t-test (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).

III. RESULTS

3.1. Photosynthetic pigments

O_3-treated seedlings of *P. orientalis* did not show visible injury on leaves at the end of experiment. Chlorophyll a, b, and total chlorophyll contents of control plant decreased until six weeks and then stabilized, but chlorophyll contents of O_3-treated seedlings decreased to the end of the fumigation period (Fig. 1). Nevertheless, there was no significant difference in the content of photosynthetic pigments between control and O_3-treated seedlings. Also there was no significant difference in the ratio of chlorophyll a to b

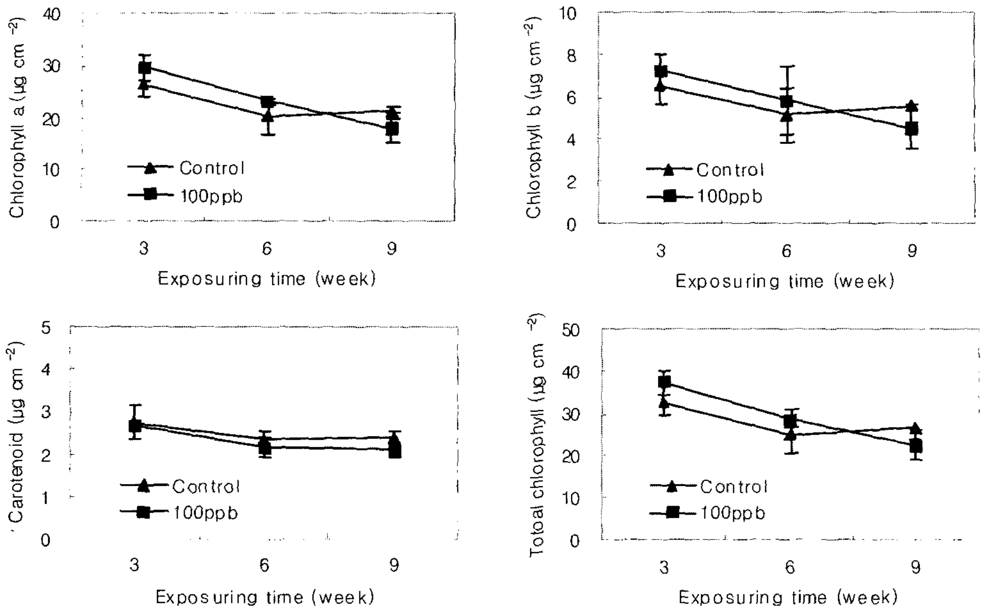

Fig. 1. Changes in content of photosynthetic pigments in the leaves of O_3-exposed *P. orientalis*. Each data point represents the mean of three replicates ± standard deviation.

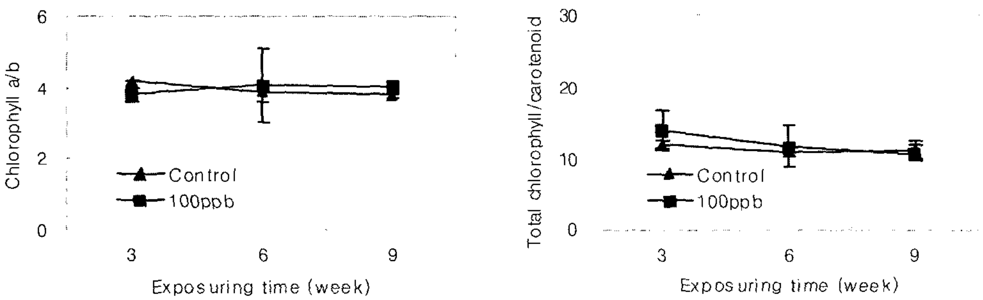

Fig. 2. Changes in relative ratios among photosynthetic pigments in the leaves of O_3-exposed *P. orientalis*. Each data point represents the mean of three replicates ± standard deviation.

or in the ratio of total chlorophyll to carotenoid (Fig. 2).

3.2. Gas exchange and water use efficiency

Photosynthetic rates did not show a significant difference between control and O_3-treated seedling at three weeks of O_3 fumigation, but there was a significant difference between treatments three weeks later (Fig. 3). Photosynthetic rate of O_3-treated seedling decreased to 72% of the control plant at six weeks; moreover it decreased to 42% of control at nine weeks. Reduction of photosynthetic rate in control plants continued for six weeks of the testing period, and then it stabilized. The rate for continuously O_3-treated seedlings decreased to the end of the O_3 fumigation. Stomatal conductance of control plants didn't change during the entire experimental period and that of the O_3-treated seedling didn't decreased until six weeks. However, it had decreased significantly at nine weeks (Fig. 3). Statistically, there was not a significant difference between control and O_3-treated seedling conductance from three weeks to six weeks. Stomatal conductance showed a significant difference between control and O_3-treated seedlings by nine weeks. The stomatal conductance was approximately 46% of the control seedlings.

WUE did not show a significant difference between control and O_3-treated seedlings during the first three weeks of the experiment, but it was significant at six

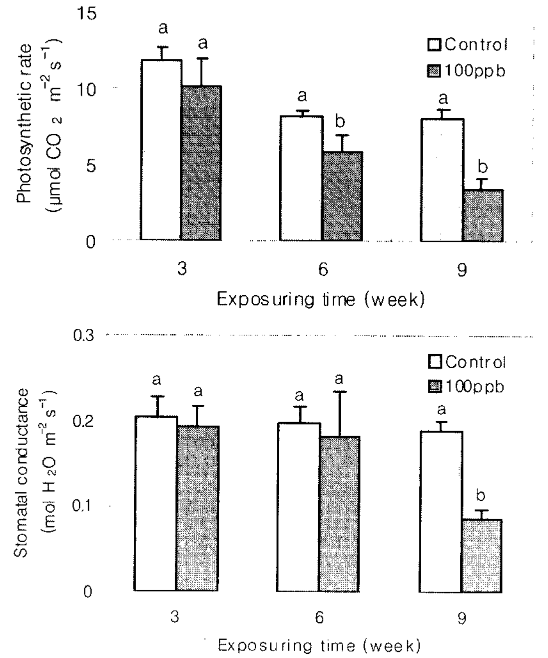

Fig. 3. Changes in photosynthetic rate (above) and stomatal conductance (below) at the leaves of O_3-exposed *P. orientalis*. Each bar represents the mean of six replicates ± standard deviation. Means with the same letter are not significantly different at the 5% probability level by the Duncan multiple range test.

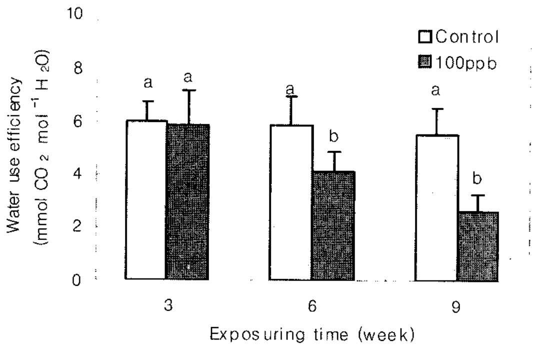

Fig. 4. Changes in water use efficiency of O_3-exposed *P. orientalis*. Each bar represents the mean of six replicates ± standard deviation. Means with the same letter are not significantly different at the 5% probability level by the Duncan multiple range test.

and nine weeks (Fig. 4). In addition, WUE exhibited a similar pattern with photosynthetic rate. WUE of the control plants did not change during the test period, but that of O_3-treated seedlings decreased significantly. WUE of O_3-treated seedlings was reduced to 70% and

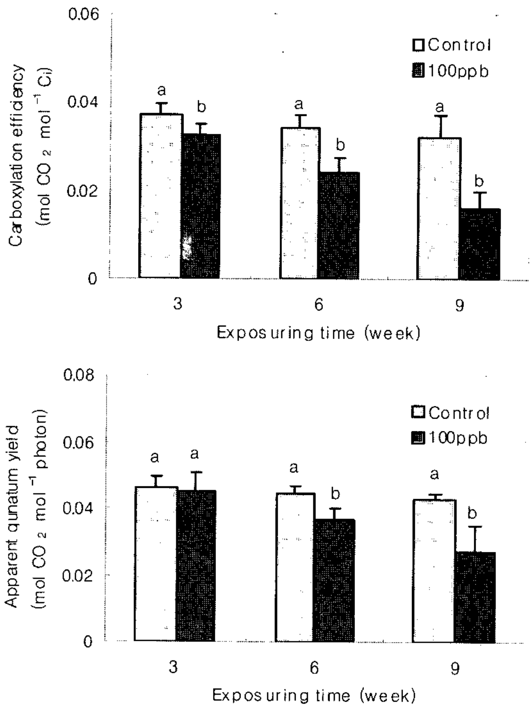

Fig. 5. Changes in carboxylation efficiency (above) and apparent quantum yield (below) of O_3-exposed *P. orientalis*. Each bar represents the mean of six replicates ± standard deviation. Means with the same letter are not significantly different at the 5% probability level by the Duncan multiple range test.

46% of control plants at six and at nine weeks of O_3 fumigation, respectively.

CE exhibited significant differences between control and O_3-treated seedling from three weeks to nine weeks (Fig. 5). CE of control plants did not decrease but CE of O_3-treated seedlings decreased continuously. CE of O_3-treated seedling was reduced 86%, 70% and 50% of the control plants at three, six, and nine weeks, respectively. AQY was significantly different between control and O_3-treated seedlings at six weeks and at the ninth week of O_3 fumigation (Fig. 5). AQY in O_3-treated seedlings at six and nine weeks was marked at 81% and 62% of the control seedlings, respectively.

IV. DISCUSSION

4.1. Photosynthetic pigments

O_3 is a highly oxidative pollutant, so it can damage any part of plants. Chlorophylls are in an oxidative condition during photosynthetic processes, so they are easily damaged by O_3. Many studies have reported the effects of O_3 to photosynthetic pigments. According to tolerance against O_3 toxicity, plants show different responses and injuries (Bortier *et al.*, 2000a). This study, found no visible injury on leaves and no significant difference in chlorophyll content between control and O_3-treated seedlings (Fig. 1). Meanwhile, chlorophyll a of the control seedlings decreased from the beginning to six weeks and then stabilized. Growth chamber differences from natural conditions for plant growth appeared to require a plant adaptation period to the environmental changes. In addition, the ratio of chlorophyll a and b and the ratio of total chlorophyll and carotenoid did not show significant differences between treatments (Fig. 2). Therefore we considered that photosynthetic pigments of two-year-old oriental plane were not affected by 100 ppb O_3 fumigation. Longer O_3 exposure may produce a different result.

4.2. Gas exchange and water use efficiency

Many experiments have demonstrated the relationships between O_3 exposure and reductions in physiological gas exchange and growth (Bortier *et al.*, 2000b; Schaub *et al.*, 2003). In this study, short-term O_3 fumigation (three weeks) did not affect photosynthetic rate (Fig. 3), but O_3-treatment for six weeks resulted in reduction of the photosynthetic rate. Reduction increased with the duration of O_3 fumigation. After 9 weeks, photosynthetic rate of O_3-treated seedling was reduced to about 40% of control; that is, seedlings were seriously influenced by O_3.

Stomata on the leaf can limit carbon uptake, a crucial process for plant growth. Closing of stomata generally prevents further O_3 uptake. It has also been suggested that O_3 may directly inhibit stomatal opening, leading to the decrease of carbon assimilation (Torsethaugen *et al.*, 1999). Therefore, it is important to understand the relationship between net photosynthesis and stomatal conductance to assess sensitivity to O_3 exposure among plant species (Fredericksen *et al.*, 1996). Until six weeks of exposure stomatal, conductance did not show a significant difference between control and O_3-treated seedlings, but that of the O_3-treated seedlings decreased to 45% of control by nine weeks (Fig. 3). This indicated that short-term O_3 fumigation does not affect the photosynthetic apparatus of oriental plane.

WUE represents carbon fixation rate to unit transpiration rate. There are many reports of WUE response to O_3 and O_3-induced increase or decrease in WUE for some herbaceous plants (Greitner and Winner, 1988; Miller *et al.*, 1994). For example, Shan *et al.* (1996) reported that WUE of *Pinus armandi* was reduced by O_3 exposure. In our study, WUE was reduced to 70% and 46% of control as a result of O_3 exposure after six and nine weeks of O_3 fumigation, respectively (Fig. 4). Meanwhile WUE showed a similar pattern with photosynthetic rate because transpiration rate did not change during the O_3 fumigation period. In general, plants expend more water to fix the same amount of carbon with increasing O_3 exposure time. Therefore, if plants are exposed to O_3 fumigation for a long time, they may easily suffer from drought stress.

Pell *et al.* (1992) reported that the decline of net photosynthesis in O_3-treated hybrid poplar was correlated with decreased activity and quantity of Rubisco. In our study, CE of two-year-old oriental plane reduced after short-term O_3 exposure. Therefore, it may imply that Rubisco activity or quantity is sensitive to O_3 exposure. Mehta *et al.* (1992) showed that Rubisco protein is highly sensitive to oxidative stress *in vivo*, which affects its translocation and degradation as well as cross-linking of the large subunit. In addition, the early decline in Rubisco mRNA immediately after O_3 exposure indicates that O_3 may be capable of directly affecting synthesis of Rubisco (Reddy *et al.*, 1993).

O_3 and other environmental stresses can limit the capacity of plants to use light energy (Pell *et al.*, 1992). Thus, in the absence of any mechanism to avoid the potentially damaging accumulation of excitation energy in the photochemical apparatus, the decrease in CO_2 fixation could result in large reductions of the number of active reaction centers, leading to photo-inhibition (Castagna *et al.*, 2001; Ort, 2001). In our study, AQY of O_3-treated seedling was not reduced at the initial stage of O_3 fumigation. However, several studies have shown that the carboxylation process is the first to be inhibited; this is followed by decreased stomatal conductance as a means of maintaining the internal CO_2 concentration (Bortier *et al.*, 2000b; Clark *et al.*, 2000).

There was no visible foliar injury such as chlorosis or necrosis after O_3 fumigation during nine weeks. Our results are in accordance with previous results that physiological and metabolic damage precedes visible injuries (Bray *et al.*, 2000). CE showed the most sensitive response to O_3; therefore, this photosynthetic parameter is suitable for use as an indicator of O_3 stress.

적 요

오존 노출이 버즘나무의 광합성 특성에 미치는 영향을 조사하기 위하여, 2년생 버즘나무 유묘에 하루에 8시간씩 100 ppb의 오존을 처리하였다. 오존 처리를 진행하는 동안, 3주마다 버즘나무의 엽내 엽록소 함량과 광합성 특성을 측정하였다. 오존에 의한 잎의 가시적 피해는 나타나지 않았으며, 또한 엽내 엽록소 함량은 대조구와 뚜렷한 차이를 보이지 않았다. 그러나 광합성량, 기공전도도, 수분이용효율은 오존 처리 6주 후에 대조구보다 감소하였다. 한편 탄소고정효율과 광화학효율은 오존 처리 3주와 6주 후에서 감소한 것으로 나타났다. 위의 결과를 토대로 볼때, 탄소고정효율은 오존 스트레스에 가장 민감한 파라미터로 나타났으며, 이것은 오존 민감성을 평가하기 위한 매우 적당한 지표로 생각되었다.

REFERENCES

Bortier, K., L. De Temmerman, and R. Ceulemans, 2000a: Effects of ozone exposure in open-top chambers on poplar (*Populus nigra*) and beech (*Fagus sylvatica*): a comparison. *Environmental Pollution* **109**, 509-516.

Bortier, K., L. De Temmerman, and R. Ceulemans, 2000b: Effects of ozone exposure on growth and photosynthesis of beech seedlings (*Fagus sylvatica*). *New Phytologist* **146**, 271-280.

Bray, E. A., J. Bailey-Serres, and E. Weretilnyk, 2000: Responses to abiotic stresses. *Biochemistry & Molecular Biology of Plants*, In B. B. Buchanan, W. Gruissen, R. L. Jones (eds), American Society for Plant Physiology, New York, pp 1158-1203.

Calatayud, A., J. W. Ramirez, D. J. Iglesias, and E. Barreno, 2002: Effects of ozone on photosynthetic CO_2 exchange, chlorophyll a fluorescence and antioxidant systems in lettuce leaves. *Physiologia Plantarum* **116**, 308-316.

Castagna, A., C. Nali, S. Ciompi, G. Lorenzini, F. E. Soldatini, and A. Ranieri, 2001: Ozone exposure affects photosynthesis of pumpkin (*Cucurbita pepo*) plants. *New Phytologist* **152**, 223-229.

Clark, A. J., W. Landolt, J. B. Bucher, and R. J. Strasser, 2000: Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environmental Pollution* **109**, 501-507.

Elvira, S., R. Alonso, F. Castillo, and B. S. Gimeno, 1998: On the response of pigments and antioxidants of *Pinus halepensis* seedlings to Mediterranean climatic factors and long-term ozone exposure. *New Phytologist* **138**, 419-432.

Evans, J. R., 1987: The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal of Plant Physiology* **14**, 69-79.

Farage, P. K. and S. P. Long, 1999: The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: an in vivo analysis. *Photosynthesis Research* **59**, 1-7.

Farquhar, G. D., S. von Caemmerer, and J. A. Berry, 1980: A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **149**, 78-90.

Fredericksen, T. S., K. C. Steiner, J. M. Skelly, B. J. Joyce, T. E. Kolb, K. Kouterick, and J. A. Ferdinand, 1996: Daily and seasonal patterns of leaf gas exchange and xylem water potentials of different-sized *Prunus serotina* Ehrh. Trees. *Forest Science* **42**, 359-369.

Greitner, C. S. and W. E. Winner, 1988: Increases in ^{13}C values of radish and soybean plants caused by ozone. *New Phytologist* **27**, 397-423.

Hippeli, S. and E. Elstner, 1996: Mechanisms of oxygen activation during plant stress: biochemical effects of air pollutants. *Journal of Plant Physiology* **148**, 249-257.

Kim, P. G. and E. J. Lee, 2001: Ecophysiology of photosynthesis 1: Effects of light intensity and intercellular CO_2 pressure on photosynthesis. *Korean Journal of Agricultural and Forest Meteorology* **3**, 126-133.

Lee, J. C., S. H. Han, P. G., Kim, S. S. Jang, and S. Y. Woo, 2003: Growth, physiological responses and ozone uptake of five *Betula* species exposed to ozone. *Korean Journal of Ecology* **26**, 165-172.

Lee, J. C., C. S. Kim, S. H. Han, and P. G. Kim, 2004: Stomatal and photosynthetic responses of *Betula* species exposed to ozone. *Korean Journal of Agricultural and Forest Meteorology* **6**, 11-17.

Lichtenthaler, H. K., 1987: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350-382.

Mehta, R. A., T. W. Fawcette, D. Porath, and A. K. Mattoo, 1992: Oxidative stress causes rapid membrane translocation and *in vivo* degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Journal of Biological Chemistry* **267**, 2810-2816.

Miller, J. E., F. L. Booker, E. L. Fiscus, A. S. Heagle, W. A. Pursley, S. F. Vozzo, and W. W. Heck, 1994: Ultraviolet-B and ozone effects on growth, yield, and photosynthesis of soybean. *Journal of Environmental Quality* **23**, 83-91.

Nussbaum, S., M. Geissmann, M. Saurer, R. Siegwolf, and J. Fuhrer, 2000: Ozone and low concentrations of nitric oxide have similar effects on carbon isotope discrimination and gas exchange in leaves of wheat (*Triticum aestivum* L.). *Journal of Plant Physiology* **156**, 741-745.

Ort, D. R., 2001: When there is too much light. *Plant*

Physiology **125**, 29-32.

Paakkonen, E., J. Vahala, T. Holopainen, R. Karjalainen, and L. Karenlampi, 1996: Growth responses and related biochemical and ultrastructural changes of the photosynthetic apparatus in birch (*Betula pendula*) saplings exposed to low concentrations of ozone. *Tree Physiology* **16**, 597-605.

Pell, E. J., N. A. Eckardt, and A. J. Enyedi, 1992: Timing of ozone stress and resulting status of ribulose bisphosphate carboxylase/oxygenase and associated net photosynthesis. *New Phytologist* **120**, 397-405.

Pye, J. M., 1998: Impact of ozone on the growth and yield of trees: A review. *Journal of Environmental Quality* **17**, 347-360.

Reddy, G. N., R. N. Arteca, Y. R. Dai, H. E. Flores, F. B. Nerm, and E. J. Pell, 1993: Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose bisphosphate carboxylase/ oxygenase in one-stressed potato foliage. *Plant Cell Environment* **16**, 819-826.

Reich, P. B., 1983: Effects of low concentration of O_3 on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. *Plant Physiology* **73**, 291-296.

Schaub, M., J. M. Skelly, K. C. Steiner, D. D. Davis, S. P. Pennypacker, J. Zhang, J. A. Ferdinand, J. E. Savage, and R. E. Stevenson, 2003: Physiological and symptom responses of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* seedlings to varying soil moisture and ozone. *Environmental Pollution* **124**, 307-320.

Shan, Y. F., Z. W. Feng, and T. Izuta, 1996: The individual and combined effects of ozone and simulated acid rain on growth, gas exchange rate and water-use efficiency of *Pinus armandi* Franch. *Environmental Pollution* **91**, 355-361.

Sharp, R. E., M. A. Mattews, and J. S. Boyer, 1984: Kok effect and the quantum yield of photosynthesis. *Plant Physiology* **75**, 95-101.

Torsethaugen, G., E. J. Pell, and S. M. Assmann, 1999: Ozone inhibits guard cell K^+ channels implicated in stomatal opening. *Plant Biology* **96**, 13577-13582.

Zheng, Y., H. Shimizu, and J. D. Barnes, 2002: Limitations to CO_2 assimilation in ozone-exposed leaves of Plantago major. *New Phytologist* **155**, 67-78.