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Eco-physiological Responses of Two *Populus deltoides* Clones to Ozone

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ABSTRACT: One-year-old cottonwood (*Populus deltoides* Bartr.) clones, which were classified as sensitive or tolerant, were exposed to 150 nl/l ozone (O_3) over 8 days for 8 hours each day under glass chamber conditions with natural sunlight. The leaves of the sensitive clone had black stipple and bifacial necrosis after O_3 treatment. Photosynthesis and stomatal conductance were measured before, during, and after the O_3 treatment. The photosynthetic rates due to O_3 treatment were decreased 51 percent and 34 percent on the sensitive and tolerant clone, respectively. The stomatal conductance of the sensitive clone was more than 40 percent higher than that of the tolerant clone regardless of the O_3 treatment. As light intensity increased, the O_3 effect on photosynthesis was clear. Compared to the previous growth chamber studies, our natural light exposure system was able to maintain a stable photosynthetic responses of the control treatment throughout the fumigation period. In addition, changes in assimilation versus intercellular CO_2 concentration (A/C_i curves) showed that O_3 decreased the slope and asymptote of the curves for the sensitive clone. This indicates that O_3 decreases the biochemical capacity of photosynthesis on the sensitive clone. Chlorophyll contents and fluorescence of the two clones were analyzed to examine the O_3 effects on photosystem II, but O_3 did not impact these variables on either clone. Although the tolerant clone did not show any foliar injury, we could not find any ecophysiological defensive responses to O_3 treated. Stomatal conductance of the tolerant clone was originally much lower than that of the sensitive one. Thus, the mechanisms of the tolerant clone in this system are to narrowly open stomata and efficiently maintain photosynthesis with a more durable biochemical apparatus of photosynthesis under O_3 stress. The sensitive clone has higher photosynthetic capacity and more efficient light reaction activity than the tolerant one under charcoal filtered condition, but is not as resilient under stress.

Key Words: Chlorophyll, Fluorescence, Ozone, Photosynthesis, Poplar, Stomatal conductance

INTRODUCTION

Ambient ozone (O_3) causes a reduction of yield of plants even in the absence of visible injury (Krupa and Nosal 1989). Since photosynthesis is ultimately linked to plant yield, it is important in studies of air pollutant effects. Ozone effects within the photosynthetic apparatus are variously attributed to damage of stomatal conductance (Unsworth and Black 1981) or reduced capacity of mesophyll to fix CO_2 (Lehnherr *et al.* 1987). Reduction in photosynthesis due to O_3 depends on species, clones, and age of plants as well as pollutant concentration (Lee *et al.* 1988, Musselman *et al.* 1994). Photosynthetic rate is dependent upon stomatal control and there have been conflicting reports in the literature relating photosynthetic CO_2 uptake and stomatal conductance to injury (Reich 1987 Kull *et al.* 1996). Ozone, however, may also alter the structural and metabolic elements of photosynthesis (Pell *et al.* 1994).

Effects of O_3 on electron transport (Coulson and Heath 1974), PSI and/ or PSII (photosystem I / photosystem II) reactions (Schreiber *et al.* 1978), and on membrane permeability and structure (Matyssek *et al.* 1991) have been reported. In addition, changes in assimilation versus intercellular CO_2 concentration

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(A/Ci curves) due to O_3 stress, with decreased initial slopes and maximum plateaus, have been reported (Matyssek *et al.* 1993 Kull *et al.* 1996). Chlorophyll fluorescence emitted from the chloroplast thylakoid membrane is a complex but useful indicator of photosynthesis (Lichtenthaler 1988). Ozone treatment on bush bean affected the photosynthetic water-splitting enzyme systems, and then inhibited the electron transport between the two photosystems (Schreiber *et al.* 1978).

Cottonwood (*Populus deltoides* Bartr.) or hybrid poplar included cottonwood has been widely studied because of its high sensitivity to O_3 and genetical uniformity (Tenga *et al.* 1993, Woodbury *et al.* 1994 Ainsworth *et al.* 1996). Gas exchange studies with cottonwood, exposed to 150 nl/l O_3 for 5 hours (Guidi *et al.* 1998) and 60 nl/l O_3, 5 hours per day, for 15 days (Soldatini *et al.* 1998), were conducted under artificial lamps and their comparisons were inter-poplar species. However, it is necessary to conduct O_3 exposure under natural light because O_3 effects on the photosynthetic apparatus may change due to the light source (Soldatini *et al.* 1998). The objectives of this study are to examine the ecophysiological responses under natural sunlight to O_3 stress from sensitive and tolerant clones of cottonwood based on their foliar responses to O_3 treatment. By measuring gas exchange before and after treatment, we determined O_3 altered the plant functions in the two-cottonwood clones.

MATERIALS AND METHODS

Ozone exposure

The two glass chambers with dimensions of 1.2 m x 1.5 m x 1.7 m (L x W x H, 3.06 m^3) were used in this study. Air was circulated through charcoal filters and O_3 was mixed into the airstream. Air temperature and relative humidity were controlled at 22 $\pm$ 2°C and 60-80% RH with a cooling, a heating, and a humidifying system. Natural sunlight for plant growth illuminated the inside of the chamber through single layer of 1.6 mm 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 1 m/sec. Ozone was generated by passing pure oxygen through a 110-V, single phase O_3 generator and was delivered to the chamber with a controlling system (Fig. 1). The O_3 concentration was continuously monitored during the exposure with an ultraviolet (UV) photometric O_3 analyzer (Model 1008-AH, Dashibi, Galendale, CA) installed with a zero/span calibrator (Model 5008, Dashibi, Galendale, CA). The non-O_3 treated chamber with charcoal filters maintained less than 10 nl/l of O_3. Plants were pre-adapted for 24 hours; conventional fumigations were then carried out at 150 $\pm$ 20 nl/l over 8 days for 8 h each day (9:00 -17:00 h) in August, 1999.

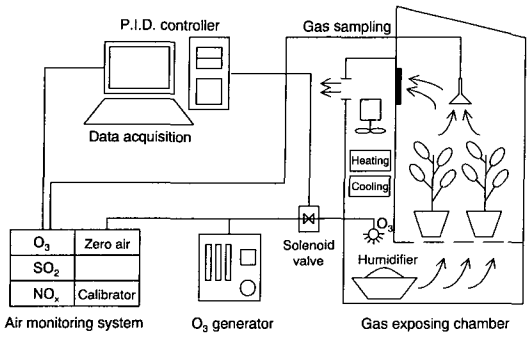

Fig. 1. Diagram of gas exposing system of the natural sunlight source.

Plant materials

The experiments were conducted on two cottonwood clones (*Populus deltoides*). Cottonwood cuttings were grown in 15-cm diameter pots containing top soil : peat : perlite (1:1:1, v/v/v) and maintained in a greenhouse at 18-22°C under ambient air condition. 5-months-old plants were used.

Net photosynthesis

The leaf net photosynthesis was measured three times (before, in the middle, and at the end of the fumigation) throughout the experiment with a photosynthesis measurement system (Li-Cor 6400, Lincoln, NE). The measured leaves were fully expanded and marked with string for repeated measurement. The leaf, still attached to the plant, was inserted into the 2 cm x 3 cm leaf chamber for broad leaves and allowed to acclimate for about 2 min. To precisely control gas exchange measurements, the leaf cuvette was maintained at 1000 μmol $m^{-2}s^{-1}$ with a light emitting diode (LED), 380 μl/l of CO_2 from the injector system (Li-Cor 6400-01, Li-Cor), and 25°C inside the chamber. The machine measured CO_2 change, water vapor change, light intensity, and leaf temperature.

Stomatal conductance

The stomatal conductance was measured with a porometer (model 1600, Li-Cor). The stomatal ratio of the poplar leaf between adaxial and abaxial sides was checked before the O_3 treatment by measuring both sides of all leaves. Measurements were conducted four times during the O_3 exposures: before exposure, 3 days, 6 days, and 8 days after (final day) O_3 fumigation began. Stomatal conductance was measured on the same leaves used for photosynthesis measurement. A total of four leaves per clone were measured, i. e. two leaves per treatment.

Light response curve

The light response curves of the leaf photosynthetic potential were obtained by controlling the light intensity inside the Li-Cor chamber. Irradiance was decreased in 7 steps: 2000, 1500,

Table 1. Changes in net photosynthesis (A: μmol CO_2 m^{-2} s^{-1}) in the sensitive and the tolerant clones of *Populus deltoids* on before, 4 days after, and the last day of fumigation with 150 nl/l O_3 between 9:00 and 17:00 h. Net photosyntheses were determined at 1000 μmol m^{-2} s^{-1} light, 380 μmol CO_2, 25°C in the controlled measurement chamber. Numbers in parentheses indicate the standard deviation of the mean of two replicate samples

Days after ozone fumigation begin	Sensitive		Tolerant	
	Control	Ozone	Control	Ozone
0	21.75 (1.48)	20.50 (0.85)	14.35 (0.35)	18.45 (3.04)
4	18.80 (0.14)	9.56 (1.47)	15.65 (0.35)	15.60 (0.00)
8	19.40 (0.00)	9.53 (3.36)	7.30 (0.28)	11.50 (1.13)

1000, 700, 500, 300, 150, 50, 0 μmol m-2s-1 of photosynthetic active radiation (PAR) on the adaxial side of the leaf. Once a leaf was inserted into the chamber, gas exchange measurements were automatically conducted via a programmed setting of irradiation for the light curve. Four light response curves were obtained with leaves from two treated measurement alternately. The chamber environment was controlled at 380 μl/l CO_2, 25°C, and 50-60% RH during the measurements.

A/Ci curve

The programed levels of CO_2 concentration inside the measurement chambers were controlled with the CO_2 injector system (Li-Cor 6400-01, Li-Cor) and liquid CO_2. This system can deliver precisely controlled CO_2 concentrations with rapid detection and compensation of CO_2 change in the chamber (C_a). Autoprograms within the Li-Cor 6400 system can automatically change setpoints to 1000, 700, 400, 200, 100, 50, 0 μl/l C_a and log data for each setpoint. Internal CO_2 concentration (C_i) was calculated based on the gas exchange theory (Farquhar and Sharkey 1982). Four leaves were sequentially measured on the two clones and two treatments. The measured chamber was controlled at 1000 μmol m^{-2} s^{-1} PAR, 25°C, and 50-60% RH.

Chlorophyll contents

The leaves measured for gas exchange were sampled. 0.1 g of the fresh weight without veins was placed in 50 ml vials. The samples soaked with 10 ml of dimethyl sulfoxide (DMSO) were in a 65°C water bath for 6 hours (Hiscox and Israelstam 1979). The amount of total chlorophyll, chlorophyll a, and chlorophyll b extracted from these leaf pieces was determined from optical density readings at 645, 652, and 663 nm (MacKinney 1941). Total chlorophyll content was determined by adding chlorophyll a and chlorophyll b per unit leaf fresh weight (mg chlorophyll/ fresh weight).

Fluorescent measurement

Chlorophyll fluorescence was measured using a chlorophyll fluorescence measurement system (Model CF-1000, Morgan Scientific, Inc., Andover, MA). The pulsed measuring light and the actinic light were 1-3 and 1000 μmol m^{-2} s^{-1}, respectively. The measurements were carried out under chamber condition on the adaxial surface of the same leaves measured for the gas exchange determinations, but adapted in the dark for 1 minute. The fast kinetics phase of the induction curve was used to characterize the PSII activity using the value Fv (variable fluorescence in dark-adapted leaves)/Fm (maximal flourescence) (Bjorkman and Demming 1987), which was taken to be the maximum relative efficiency of photochemistry in the dark-adapted state.

RESULTS

Ozone injury

Typical O_3 injury occurred only on the leaves of the sensitive clone. The symptoms were black stipple with some severely injured part showing bifacial necrosis. The tolerant clone did not show any injury. The damaged area was about 50 - 60 percent of the leaf. The symptoms appeared on 2nd day after O_3 fumigation commenced. The damage mostly occurred on fully expanded leaves in the middle of the tree. The bottom leaves of the fumigated plants had mild symptom but did not show any early senescence.

Net photosynthesis

Net photosynthesis of the two clones and two treatments were not different at the first measurement before the treatment began, but variability was high. The decrease of photosynthesis on O_3-treated sensitive clone started from 4th day after the fumigation began. There was a decrease in photosynthesis in the tolerant clone continuously throughout the measurement. After O_3 fumigation, the average of both clones showed 42 percent of photosynthesis compared to the control; the sensitive clone decreased 51 percent, whereas the tolerant clone decreased 34 percent. Although the photosynthetic decrease due to O_3 was less severe on the tolerant clone, the absolute amounts of photosynthesis on both clones with O_3 treatment were the same (Table 1). The photosynthetic rate of the sensitive clone was a little greater than that of tolerant clone throughout all three measurements.

Table 2. Changes in stomatal conductance (g_s: mmol H_2O m^{-2} s^{-1}) in the sensitive and the tolerant clones of *Populus deltoides* on before, 3 days after, 6 days after, and the last day of fumigation with 150 nl/l O_3 between 9.00 and 17.00 h. Stomatal conductances were measured at natural sunlight (600-900 μmol m^{-2} s^{-1}) under ambient air conditions in summer. Numbers in parentheses indicate the standard deviation of the mean of two replicate samples

Days after ozone fumigation begin	Sensitive		Tolerant	
	Control	Ozone	Control	Ozone
0	0.92 (0.24)	1.33 (0.30)	0.81 (0.08)	1.04 (3.04)
3	1.22 (0.01)	1.42 (0.20)	0.08 (0.02)	0.53 (0.09)
6	1.13 (0.10)	1.19 (0.14)	1.00 (0.03)	0.93 (0.08)
8	1.05 (0.02)	1.20 (0.04)	0.60 (0.09)	0.66 (0.04)

Stomatal conductance

The clonal difference of stomatal conductance was distinct throughout all four measurements (Table 2). The stomata of the sensitive clone were always open wider than that of the tolerant clone regardless of the O_3 treatment. The average of the sensitive clone on both treatments throughout all measurements was 1.182 H_2O mmol m^{-2} s^{-1}, whereas that of the tolerant clone was 0.796 H_2O mmol m^{-2} s^{-1}. The average stomatal conductance of O_3 treated plants was 1.037 H_2O mmol m^{-2} s^{-1} and that of controls 0.941 H_2O mmol m^{-2} s^{-1}. These two poplar clones did not close their stomata to O_3 stress.

Light response curve

The photosynthetic decrease due to O_3 started at 150 μmol m^{-2} s^{-1} of light and the O_3 effect became more distinct as light levels increased (Fig. 2). The O_3 effect on the sensitive clone under 1000 mmol m^{-2} s^{-1} or greater light was approximately 67 percent, whereas, that of the tolerant clone under the same conditions was 34 percent. Even at 2000 μmol m^{-2} s^{-1} of light, the poplar leaves were not light saturated since the potential of photosynthesis seemed to be increased as the light level increased. Dark respiration, the release of carbon dioxide without light, was measured on both clones and the two treatments. It was hard to detect an O_3 effect because of low rates of respiration. Under 50 μmol m^{-2} s^{-1} of light, the photosynthetic average of the tolerant clone was 1.3 CO_2 μmol m^{-2} s^{-1}, whereas that of the sensitive one was 0.3 CO_2 μmol m^{-2} s^{-1}. Thus, the light compensation point of the tolerant clone would be much lower than that of the sensitive one.

A/Ci curves

Ozone effects on A/C_i curves occurred only on the sensitive clone, except for one datam point at about 500 μl/l C_i on the tolerant clone (Fig. 3). Ozone decreased the slope and the asymptote of the curves. The clonal difference on A/C_i curves between the two control curves seemed to be the same on the graphs, but Ci was significantly different between the two clones. The internal CO_2 concentrations between the two clones and the two treatments were similar until 100 μl/l CO_2, but the C_i of the sensi-

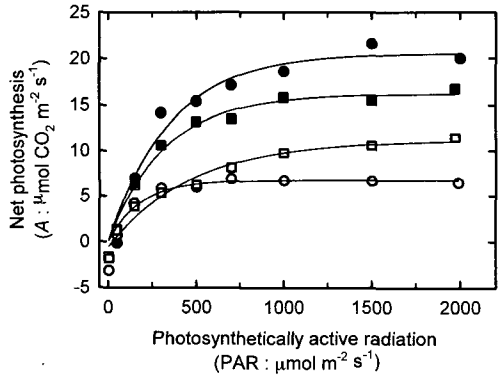

Fig. 2. Effect of ozone on the response of photosynthesis to variation in photosynthetically active radiation (PAR) of the two poplar clones. Solid circle, sensitive clone and control; Open circle, sensitive clone and O_3 treated; Solid square, tolerant clone and control; Open square, tolerant clone and O_3 treated. Measurement conditions were; chamber temperature 25°C, 380 μl/l CO_2, 25°C, and 30-60% RH.

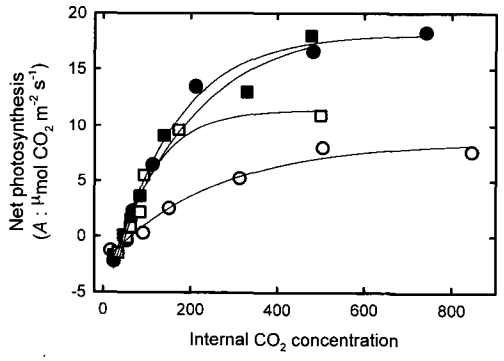

Fig. 3. Effect of ozone on the response of photosynthesis to variation in intercelluar CO_2 concentration of the two poplar clones. The curves represent a biochemical model fitted to the data. Solid circle, sensitive clone and control; Open circle, sensitive clone and O_3 treated; Solid square, tolerant clone and control; Open square, tolerant clone and O_3 treated. Measurement conditions were; chamber temperature 25°C, photon flux density 1000 μmol $m^{-2}s^{-1}$.

Table 3. Internal CO_2 concentrations (Ci) of the two *Populus deltoides* clones exposed to O_3 (150 nl/l for 8 h day^{-1}) for 8 days. The controlled plants were kept in charcoal-filtered air. The gas exchage parameters were determined at 380 ml/l PAR, 25°C and 50-60% RH

Supplied CO_2[1]	Internal CO_2 concentration[2]			
	Sensitive		Tolerant	
	Control	Ozone	Control	Ozone
1000	741.0	845.0	478.0	500.0
700	483.0	504.0	330.0	173.1
400	212.0	312.0	139.0	92.7
200	112.0	151.0	84.6	84.6
100	67.5	91.5	63.2	62.5
50	49.8	53.8	46.8	54.2
0	24.0	17.0	24.1	34.1

[1] The concentrations of the supplied CO_2 inside the measured chamber was precisely controlled with the CO_2 injector system (Li-Cor 6400-01).

[2] The calculated Ci was according to Farquar and Sharkey (1982).

Table 4. The maximum quantum yield (Fv/Fm) and chlorophyll contents of a (Chl a), b (Chl b), and total (total Chl) of mature leaves of cottonwood (*Populus deltoides*) clones exposed to O_3 (150 nl/l for 8 h per day) for 8 days. The controlled plants were kept in charcoal-filtered air. The chlorophyll fluorescence parameters were determined in leaves dark-adapted for 1 min. Chlorophyll contents were determined by spectrophotometric absorbance of dimethyl sulfoxide extracts from 0.1 g fresh weigh leaf samples without foliar injury at the end of fumigation. The significance of the F-value following the two way ANOVA is shown. *, $p<0.05$; **, $p<0.01$

		Flourescence (Fv/Fm)	Chl a	Chl b	Total Chl
Treatment	Control	0.6927	212.0	83.0	294.9
	Ozone	0.6913	203.6	87.3	290.8
Clone	Sensitive	0.7517	195.1	74.74	269.8
	Tolerant	0.6323	220.5	95.48	315.9
F-values	Treat effect	1.0000	0.6517	0.5052	0.8646
	Clone effect	0.0110	0.2129	0.0245**	0.1083
	O_3 x Clone Interaction	0.7021	0.0464**	0.0750*	0.0482**

tive clone was much higher than that of the tolerant one (Table 3).

Fluorescent responses and chlorophyll contents

Ozone did not change any fluorescence response (Table 4). The major difference in fluorescent response was due to clonal difference. That is, Fv/Fm of the sensitive clone was 19 percent higher than that of the tolerant clone and it means the photosynthetic ability on light reaction of the sensitive clone was much higher than that of the tolerant one.

The chlorophyll content was not affected by O_3 treatment or clonal difference (Table 4). The clonal difference was much greater than the treatment difference. Chlorophyll a was about 70 percent of total chlorophyll in both clones. Significant interactions between treatment and clone were found on Chlorophyll a and total chlorophyll. Although obvious O_3 damage occurred on the sensitive clone, chlorophyll contents on the sensitive clone did not significantly decrease due to O_3 treatment.

DISCUSSION

Natural light maintained A and g_s on both clones throughout the measurement, compared to the decreasing of gas exchange parameters under artificial light sources (Reich 1983, Yun and Laurence 1999). In addition, our light curve results showed that light intensity is an important factor to discriminate O_3 effects on photosynthetic responses clearly. Our results are consistent with previous studies that cottonwood is highly responsive to O_3 (Tenga *et al.* 1993, Woodbury *et al.* 1994 Woo 1997,). Ozone causes leaf injury, early leaf senescence, and abscission of older cottonwood leaves (Coleman *et al.* 1988, Sen *et al.* 1991, Soldatini *et al.* 1998). It also causes a reduction of net photosynthetic capacity (Reich 1983, Jensen and Nobel 1984, Guidi *et al.*

1998, Soldatini *et al.* 1998) in both the sensitive and the tolerant clones of cottonwood.

The previous studies (Guidi *et al.* 1998, Soldatini *et al.* 1998) reported that the mechanism of a decrease is stomatal closure and inhibition of mesophyll conductance. Moreover, PSII was affected by O_3 according to the significant Fv/Fm decrease. However, the significant changes of gas exchange were due to the clonal differences rather than O_3 treatment, in stomatal conductance, A/Ci curves, Fv/Fm, as well as in the visible symptom on the leaves caused by O_3. If O_3 exposure is not so severe, the sensitive clone has greater photosynthetic activity than the tolerant one due to greater light reaction efficiency (fluorescence) and stomatal conductance. However, the tolerant clone under high O_3 condition did efficiently photosynthesize while closing stomata, and thus has unchanged Rubisco activity. Thus, the mechanism of the O_3-tolerance can be a biochemically efficient photosynthesis with low stomatal opening and a durable Rubisco activity under O_3 stress. Although O_3 could not change the chlorophyll contents and the maximal quantum yield, we found severe damage on the biochemical appartus.

The O_3-induced different A/C_i curves on the sensitive clone (Fig. 3) mean that O_3 is associated with lower ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco) enzyme activity, limitation of ribulose-1,5-bisphosphate (RuBP) regeneration, and limitation of inorganic phosphate supply (Ball *et al* 1987, Flanagan and Jefferies 1989). The saturation level of A/C_i means that either the limitation is caused by the regeneration of RuBP or the limited supply of inorganic phosphate on ATP regeneration (Wullschleger 1993). The O_3 in this study can explain the damage on the supply of inorganic phosphate (Pi) and RuBP in the dark reaction. Our results indicate that Rubisco in the O_3-treated leaves on the sensitive clone was damaged because there was a slower increase in carbon fixation as CO_2 increased. Thus, the severe O_3 damage on the sensitive clone can explain the damage to carbon metabolism after CO_2 entered through stomata into intercellular air spaces (Ball *et al.* 1987). However, our A/C_i results with the tolerant clone mean that O_3 affects the regeneration of biochemical responses for photosynthesis, but did not affect the RUBP enzyme activity on the tolerant clone.

The O_3 effect on gas exchange parameters, such as photosynthesis and stomatal conductance of the sensitive clone was that greatest on the third day after the fumigation began. However, the stomata of the sensitive clone did not respond to O_3 treatment. Because the quantum yield and the stomatal sensitivity of the sensitive clones were much lower than those of the tolerant clone, the stomata should be open regardless of the O_3 stress from the outside to maintain photosynthesis. On the other hand, O_3 greatly reduced stomatal conductance in the tolerant clone and decreased photosynthetic rates; stomatal closure was the mechanism in this clone.

The analysis of photosynthetic curves with increasing irradiance provides information on quantum yield and other light energy reactions (Stitt 1991). Our results showed that O_3 had little effect on quantum efficiency, the slope of the curve. Rather, the upper asymptote (near light-saturated photosynthesis) was influenced by O_3 and the effect differed between the clones (Fig. 2). Decreased photosynthesis on the upper asymptote due to O_3 treatment differed with clones, indicating that O_3 modified either the amount or activity of Rubisco, depending on the clones. The sensitive clone was the most productive when grown under high light. These clonal and O_3 effects on the light response curves were reported on *Populus tremuloides* (Coleman *et al.* 1995).

Fluorescence response can indicate direct photosynthetic disruption (Lichtenthaler 1988); O_3 changed the chlorophyll fluorescence kinetics on *Vicia faba* (Guidi *et al.* 1993), *Plantago major* (Reiling and Davison 1994), and barley (Rowland-Bamford *et al.* 1989). These previous studies found that the changes in fluorescence induction caused by O_3 started at leaf before any visible sign of leaf necrosis and that these changes correlated well with the alteration in fluorescence and the extent of visible injury. However, our results did not show any O_3 effects on fluorescent response, although 150 nl l^{-1} O_3 is quite severe compared to other studies. Because Fv/Fm of the sensitive clone was significantly higher, the photosystem of the sensitive clone is more efficient in the photochemcal functioning of the thylakoids than that of the tolerant one.

Chlorophyll contents of the treated leaves did not significantly reduced. The remained healthy parts of the leaves were not damaged by O_3. Because photosynthesis was reduced due to O_3 treatment without changes in fluorescence or chlorophyll contents, O_3 in this study did not significantly affect light reactions of the two clones of cottonwood.

Since O_3 fumigation occurred for relatively short periods of time during the tree growth, we did not measure biomass of the tree. However, biomass is important to relate whole-tree photosynthesis and carbon assimilation throughout the growing season. Photosynthetic decrease due to O_3 can be easily measured with gas exchange, but long-term fumigations should be conducted to investigate the O_3 effect on biomass. Our fumigation level of 150 nl/l for 8 days is unlikely to occur in the ambient air in Korea (Yun *et al.* 1999), however, this level can be reached in our circumstances and the O_3 damage shown in the results can be used as a warning of increasing O_3 levels.

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