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Photosynthetic Inhibition in Leaves of *Ailanthus altissima* under O_3 Fumigation

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ABSTRACT: We investigated the effect of O_3 on the photosynthetic characteristics of tree of heaven (*Ailanthus altissima*) that is naturalized plant and used as restoration plant for contaminated area. Two-year-old seedlings were planted to pots and transferred into closed O_3 chamber. Photosynthetic pigments contents and photosynthetic characteristics were measured every three weeks under 100 ppb O_3 fumigation. There was no visible foliar injury by O_3 exposure and contents of photosynthetic pigments did not show significant differences between control and O_3-treated seedlings. Also there were no significant differences in stomatal conductance, and water use efficiency. But photosynthetic rate and apparent quantum yield (AQY) of O_3 treated seedlings were reduced after nine weeks of ozone fumigation. In addition, the reduction of carboxylation efficiency and photorespiration were observed in the leave of O_3 treated seedlings after six weeks. In accordance with our result, carbon fixation system of *A. altissima* was most sensitive to O_3 stress to evaluate physiological damage induced by O_3.

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

INTRODUCTION

Due to industrialization and consumption of fossil fuel, air pollution problem has been increased. It has been major environmental problem occurred by primary pollutants and secondary pollutants from photochemical reaction. Especially O_3 that is one of the products from 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 probably the real cause of the negative effect of O_3 (Hippeli and Elstner 1996). When plant uptakes O_3, stomatal closure and mesophyll cell destruction will be occurred, that leads to decrease of photosynthesis (Pääkkönen 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, 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 (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 plant is under scrutiny (Clark et al. 2000). In this sense, there is evidence that O_3 alters 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 photosynthetic inhibition induced by O_3 stress of tree of heaven (*Ailanthus altissima*) that is naturalized plant and used as restoration plant of contaminated area.

MATERIALS AND METHODS

Plant Material and Growth Condition

A. altissima 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 sand, peat moss and vermiculite (1:1:1 volume basis). Three seedlings per treatment arranged in two blocks. Each pot transferred into O_3 chamber, the fumigation system has been described in detail by Lee et al. (2003). Ozone treatment was divided into two chambers, one for control with clean air and the other for treatment with 100 ppb/hr of O_3 (short-term pollution standard) fumigation. Ozone fumigation time was 8 hrs a day. O_3 concentration in chamber was registered 5±1 ppb in control and 98±5 ppb in treatment chamber during fumigation period. The experiment was started on June 2,

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2004 and was conducted during nine weeks.

Photosynthetic Pigments

The leaves of control and O_3-treated *A. altissima* were excised and soaked in dimethyl sulfoxide (DMSO) in a glass vial. The vial was tightly capped and incubated at 70 ℃ for 2 hrs in the dark. The concentration of the extracted pigments (total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid) was calculated based on their absorbance values at 664, 645, and 470 nm, according to Lichtenthaler (1987).

Gas Exchange and Water Use Efficiency

Gas exchange of fully expanded leaves was measured between 9 and 11 a.m. using an infrared gas analyzer (Li-6400, LI-COR, USA). Environmental parameters were maintained during the measurements (mean temperature: 20.0 ± 0.1℃; 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 m^{-2} s^{-1}), stomatal conductance to water vapour (G_w, mol H_2O m^{-2} s^{-1}), and transpiration rate (E, mmol H_2O m^{-2} s^{-1}).

Water use efficiency (WUE) was determined by dividing photosynthetic rate (A) by transpiration rate (E). To calculate carboxylation efficiency (CE), A/C_i-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/C_i-curve (0～150 ppm intercellular CO_2), and photorespiration was estimated from a y-intercept of the linear regression (Ro et al. 2001).

Apparent quantum yield (AQY) was used to calculate photochemical efficiency (PE), (Sharp et al. 1984, Evans 1987, Kim and Lee 2001). Gas exchange were 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. Dark respiration and light compensation point was estimated from a y-intercept and x-intercept of the linear regression respectively.

Statistical Analysis

To compare the effect on control and O_3 treatment of each fumigation period, ANOVA was performed on experimental data (statistical significance, $P \leq 0.05$), 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).

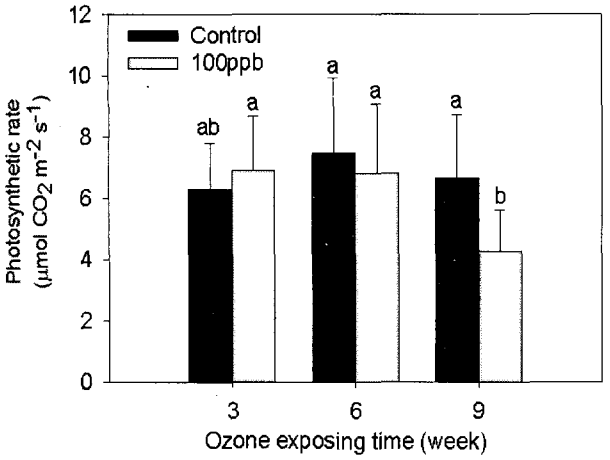
RESULTS

Photosynthetic Pigments

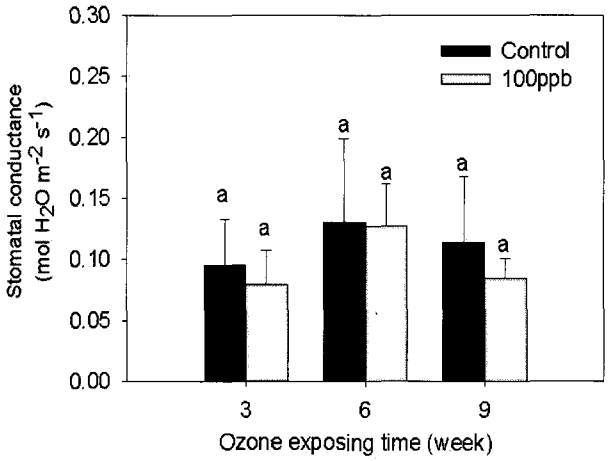
Ozone treated seedlings of *A. altissima* did not show visible injury on leaves at the end of experiment. Chlorophyll *a*, total chlorophyll, and carotenoid contents of control plant slightly decreased until six weeks and then stabilized, but chlorophyll contents of O_3-treated seedlings decreased to the end of fumigation period (Table 1). Especially after six weeks later chlorophyll *b* decreased up to 70 % of three weeks. Nevertheless there was no significantly different in contents of photosynthetic pigments between control and O_3-treated seedlings. Also there was no significantly different in the ratio of chlorophyll *a* and *b* and the ratio of total chlorophyll

Table 1. Changes in content of photosynthetic pigments in the leaves of O_3-exposed *A. altissima*. Each data represent mean of two replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05)

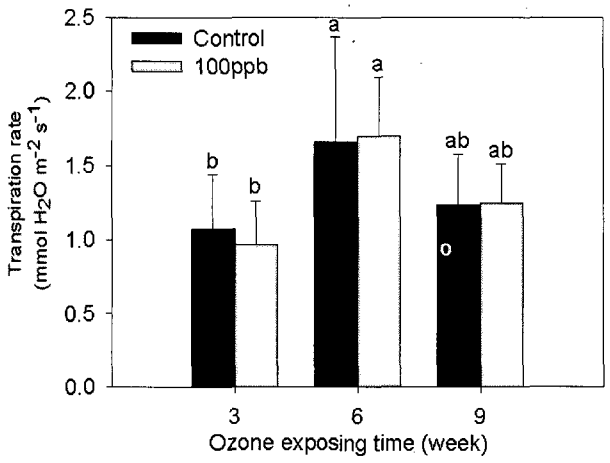
Exposing time (week)	O_3 treatment	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoid
		mg/g			
3	Control	26.4 ± 1.8^{a}	4.8 ± 0.8^{ab}	31.3 ± 1.4^{ab}	3.0 ± 0.1^{a}
	100 ppb	25.9 ± 3.8^{a}	5.8 ± 0.5^{a}	31.7 ± 4.1^{a}	2.9 ± 0.5^{a}
6	Control	24.0 ± 2.3^{a}	4.5 ± 0.5^{ab}	28.6 ± 1.8^{ab}	2.8 ± 0.2^{a}
	100 ppb	21.9 ± 2.5^{a}	4.3 ± 0.1^{ab}	26.2 ± 2.3^{ab}	2.6 ± 0.2^{a}
9	Control	23.5 ± 3.4^{a}	4.7 ± 0.9^{ab}	28.2 ± 4.4^{ab}	2.7 ± 0.3^{a}
	100 ppb	20.0 ± 2.0^{a}	4.0 ± 0.6^{b}	24.0 ± 1.4^{b}	2.4 ± 0.4^{a}



(a) Photosynthetic rate



(b) Stomatal conductance



(c) Transpiration rate

Fig. 1. Changes in photosynthetic characteristics at the leaves of O_3-exposed *A. altissima*. Each bar represents mean of six replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05).

Table 2. Changes in relative ratios among photosynthetic pigments in the leaves of O_3-exposed *A. altissima*. Each data represent mean of two replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05)

Exposing time (week)	O_3 treatment	Chlorophyll *a*/*b*	Total Chlorophyll/ carotenoid
3	Control	5.43 ± 0.83 [a]	10.40 ± 0.04 [a]
	100 ppb	4.42 ± 0.41 [a]	10.67 ± 0.73 [a]
6	Control	5.33 ± 1.16 [a]	10.00 ± 1.34 [a]
	100 ppb	5.01 ± 0.71 [a]	9.75 ± 0.04 [a]
9	Control	5.03 ± 0.31 [a]	10.59 ± 2.95 [a]
	100 ppb	5.06 ± 1.28 [a]	10.04 ± 1.43 [a]

and carotenoid (Table 2).

Gas Exchange and Water Use Efficiency

Photosynthetic rates did not show significant difference between control and O_3-treated seedling for six weeks of O_3 fumigation, but there was significantly difference between two treatments nine weeks later (Fig. 1). Photosynthetic rate of O_3-treated seedling decreased to 63 % of control plant nine weeks later. Stomatal conductance of control and O_3-treated seedling slightly increased after six weeks. And nine weeks later it was decreased up to 87 %

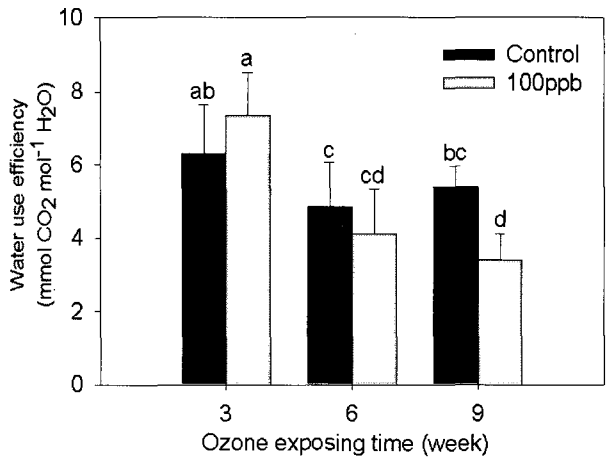
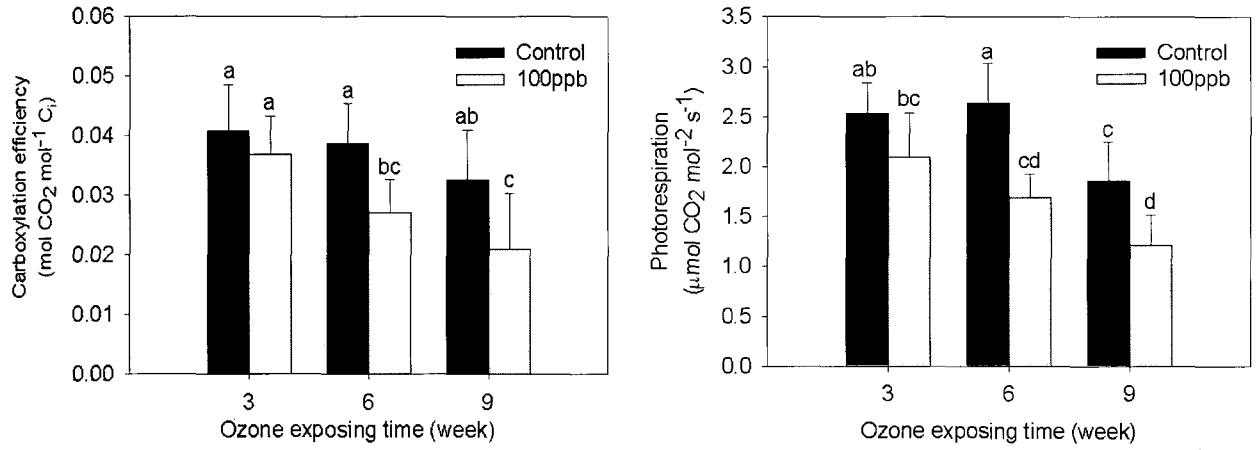


Fig. 2. Changes in water use efficiency of O_3-exposed *A. altissima*. Each bar represents mean of six replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05).

in control and 66 % in O_3-treated seedling (Fig. 1). However statistically there was not significantly difference between control and O_3-treated seedling during whole O_3-fumigation period.

WUE did not show significant difference between control and O_3-treated seedling until six weeks of experiment, but it showed significant difference between each other after nine weeks (Fig. 2). WUE of control plant decreased till six weeks and then stabilized but WUE of O_3-treated seedlings decreased to the end of fumigation period.

CE and photorespiration showed significant difference between control and O_3-treated seedling from six weeks to nine weeks (Fig. 3). CE of O_3-treated seedling was reduced 69 % and 64 % of control plant at six, and nine weeks respectively. Photorespiration of O_3-treated seedling was reduced 64 and 65 % of control plant at six, and nine weeks, respectively. AQY was decreased conti- nuously but there was no significant difference between control and O_3-treated seedling until six weeks, and after nine weeks AQY of O_3-treated seedling was reduced 76 % of control plant (Table 3). Dark respiration and light compensation point showed significant difference between control and O_3-treated seedling until six weeks, but it did not show significant difference between each other after nine weeks (Table 3). Dark respiration of O_3-treated seedling was increased 69 and 57 % of control plant at three and six weeks, respectively. And light compensation point of O_3-treated seedling was increased 103 and 125 % of control plant at three and six weeks, respectively.



(a) Carboxylation efficiency (b) Photorespiration

Fig. 3. Changes in carboxylation efficiency and photorespiration of O_3-exposed *A. altissima*. Each bar represents mean of six replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05).

Table 3. Changes in apparent quantum yield, dark respiration, and light compensation point in the leaves of O_3-exposed *A. altissima*. Each data represent mean of six replicates ± standard deviation. Means with the same letter are not significantly different by the ANOVA (α=0.05)

Exposing time (week)	O_3 treatment	Apparent quantum yield (mmol CO_2 mol^{-1})	Dark respiration rate (μmol CO_2 m^{-2} s^{-1})	Light compensation point (μmol m^{-2} s^{-1})
3	Control	0.0463 ± 0.0037^{a}	0.5730 ± 0.1499^{b}	10.6512 ± 2.6974^{c}
	100 ppb	0.0497 ± 0.0016^{a}	0.9734 ± 0.2749^{a}	21.6271 ± 4.5143^{a}
6	Control	0.0377 ± 0.0069^{b}	0.3808 ± 0.1781^{bc}	8.5970 ± 3.0247^{c}
	100 ppb	0.0327 ± 0.0083^{bc}	0.6007 ± 0.1906^{b}	19.3866 ± 8.0045^{ab}
9	Control	0.0328 ± 0.0034^{bc}	0.3046 ± 0.1578^{c}	12.3318 ± 4.8556^{bc}
	100 ppb	0.0252 ± 0.0091^{c}	0.2749 ± 0.1140^{c}	11.6452 ± 1.3617^{c}

DISCUSSION

Photosynthetic Pigments

Ozone is highly oxidative pollutant, so it can damage any part of organism of plant. Especially chlorophylls are oxidative condition during photosynthetic procedure, so they are easily damaged by O_3. Many previous studies have reported for the effects of O_3 to photosynthetic pigments, and according to tolerant ability against O_3 toxicity plants show different response and injury (Bortier et al. 2000a). In this study, we could not find out any visible injury on leaves as well as significant difference in chlorophyll contents between control and O_3-treated seedling (Table 1). Meanwhile chlorophyll a of control seedling decreased from beginning to six weeks of experiment and then was stabilized. It is because growth chamber is different from natural condition for plant growth, and seedlings seemed to be required adaptation period for environmental changes. In addition to, the ratio of chlorophyll *a* and *b* and the ratio of total chlorophyll and carotenoid did not show significant difference between treatments (Table 2). Therefore we considered that photosynthetic pigments of two-year-old *A. altissima* were not affected by 100 ppb O_3 fumigation. But the longer O_3 exposure may give us a different result.

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, until mid-term O_3 fumigation (three weeks) did not affected photosynthetic rate (Fig. 1). But O_3-treated seedling for nine weeks were affected and resulted in reduction of photosynthetic rate. After 9 weeks photosynthetic rate of O_3-treated seedling was reduced to about 63 % of control, that is, seedlings were seriously influenced by O_3.

Stomata on the leaf can control carbon uptake as a crucial process for plant growth. Generally plants close their stomata in order to avoid 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). In this study, there was no significant difference between control and O_3 treatment to the end of O_3 fumigation period (Fig. 1), so we may conclude photosynthetic apparatus especially stomatal control system of *A. altissima* was not affected by 100 ppb O_3 fumigation.

WUE represents carbon fixation rate to unit of transpiration rate. There are a lot of different reports of WUE responses to O_3 and O_3-induced increase or decrease in WUE of some herbaceous plant was also reported (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 showed continuously decreasing pattern in O_3-treated seedling and after nine weeks it decreased up to 63 % of control (Fig. 2). In general plants need more water to fix same amount of carbon with increasing of O_3 exposure time. Therefore if plants leave under O_3 fumigation for a long time, they may be suffered from drought stress easily.

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 2-year-old *A. altissima* reduced after mid-term O_3 exposure. Therefore it may represent that Rubisco activity or quantity is sensitive to O_3 exposure. Rubisco is a key enzyme of photorespiration (Douce and Neuburger 1999). In our study, photorespiration was reduced after six weeks O_3 exposure as CE. Therefore photorespiration was reduced by decreasing of activity or quantity of Rubisco. 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).

Ozone 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 *A. altissima* did not show significant difference between each other until six weeks O_3 fumigation, so it seems AQY was not affected by O_3 fumigation until six weeks. But after nine weeks O_3 fumigation AQY was decreased, therefore photochemical pathway affected by O_3 fumigation.

It is widely admitted that the respiratory processes are increased in response to ozone (Darrall 1989). In our study dark respiration of O_3 fumigated plant showed higher than control after three and six weeks, but after nine weeks it did not show significant difference between each other. This increasing of respiration was considered as adaptation to stress by energy consumption for detoxification of O_3 and the repairing of damaged membranes and proteins (Amthor 1988). Therefore the result of our study showed that *A. altissima* increased respiration to resist the stress induced by O_3 fumigation. But the substrate was not infinite, and duration of stress caused cell damage, so it may lead continuously decreasing of dark respiration. This increase of dark respiration was expressed

in light compensation point. So light compensation point followed dark respiration as same pattern.

In photosynthetic system, several studies have shown that the carboxylation process is the first to be inhibited; this is followed by decreased stomatal conductance as a mean of maintaining the internal CO_2 concentration (Bortier et al. 2000b, Clark et al. 2000). There was not 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). Especially CE showed the most sensitive response to O_3, therefore this photosynthetic parameter would be able to use as suitable indicator to O_3 stress. Besides *A. altissima* showed that it was not affected by O_3 stress in many photosynthetic parameter, so *A. altissima* may have high tolerance ability to ozone.

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