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Ozone-environmental Effects on Gas Exchange and Growth of Hybrid Poplar (*Populus trichocarpa* × *P. deltoides*) Seedlings

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오존 環境에 대한 雜種 포플러 苗木의 가스 交換과 生長에 관한 研究

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ABSTRACT

Hybrid poplar (*Populus trichocarpa* × *P. deltoides*) clones were fumigated with ozone. Fumigation was applied for 6 to 8 hours each day for approximately 3 months at ozone concentrations of 0.090 to 0.115 ppm using by open-top chambers.

Growth and biomass of hybrid poplar seedlings were reduced by ozone exposure. Mean percentage of falling leaves in ozone-treated plant was 6 times higher than that of charcoal-filtered plant. Among physiological responses, rate of photosynthesis, stomatal conductance, transpiration and initial Rubisco activity were significantly lower in seedlings grown in ozone environment compared seedlings grown in charcoal-filtered air. All these physiological results supported that biochemical process to be a key feature to understand reduction in photosynthesis.

Key words : Ozone environment, *Poplar*, Stomatal conductance, Photosynthesis, Rubisco.

INTRODUCTION

Ozone became one of the air pollutants in human environments (Treshow and Anderson 1991). Ambient ozone in many urban areas has negative impact on tree growth and vegetation (Reich 1987). Many experimental ozone studies and field surveys indicate that ozone alters physiological characteristics such as stomatal conductance, transpiration, photosynthesis, Rubisco activity and internal CO_2 concentration (Dann and Pell 1989, Volin *et al.* 1993, Hassan *et al.* 1994, Samuelson 1994).

Visible injuries on leaves exposed to ozone do not always indicate reductions in photosynthesis or tree growth. A decrease in photosynthesis or growth due to ozone can occur in the absence of visible symptoms on the leaves (Samuelson 1994). Various physiological parameters can be affected. Stomatal conductance, Rubisco activity and internal CO_2 concentration can be altered by ozone exposure. However, interactions among physiological responses to ozone have not yet been identified. For example, it is not clear whether reduction in photosynthesis with ozone exposure occurs indirectly because of altered Rubisco or directly because of closed stomata and therefore, stomatal responses to changes in internal CO_2 concentration (Lehnherr *et al.* 1987, Atkinson *et al.* 1988, Farage *et al.* 1991).

The objective of this study was to determine if elevated ozone induced changes in several important physiological characteristics mentioned previously and growth of hybrid poplar (*Populus trichocarpa* × *P. deltoides*). This study tested the hypothesis that elevated ozone influenced leaf gas exchange and growth.

MATERIAL AND METHODS

Plant culture

Cuttings of hybrid poplar clones (*Populus trichocarpa* × *P. deltoides*) were taken from a stool bed at Washington State University-Puyallup Farm 5 poplar plantation. For each cutting, root hormone (Indole 3 butyric acid) was applied to the distal end by dipping. One cutting was placed in a 7.3 liters pot which contained peat, vermiculite and soil as the 1:1:1 ratio. Peter's NPK fertilizer (Peter's professional water soluble fertilizer, M-77; Peat-Lite Special (15-16-17)) was used twice per week. Every pot was irrigated with tap water daily. Plants were grown on a greenhouse bench for four weeks following leaf emergence before being moved into the open-top chambers. Average temperature in the open-top chamber was maintained from 23 to 25 ℃ during the experimental period. Relative humidity was maintained from 70% to 85%. The midday photosynthetic photon flux density ranged between 400 and 1300 μ mol $m^{-2}s^{-1}$.

Ozone fumigation

Six replicates of each clone were randomly divided and placed into one of two open-top chambers, an ozone chamber and charcoal-filtered chamber (control). Each chamber was a two-tiered open-top chambers of the type described by Heagle *et al.* (1973). Plants were treated with charcoal-filtered air with or without ozone. Ozone was generated by passing oxygen through a model G-IL generator (PCI O_3 Corp., West Caldwell N. J., output capacity 0.5 kg d^{-1}), and was monitored with a Dashibi model 1008-AH ozone monitor. The accuracy of the monitor was EPA-certified and calibrated when the study began, and the calibration was checked daily before ozone fumigation. Following shoot emergence, cuttings were allowed to grow for one month before being placed in the open-top chambers. Cuttings were allowed to adjust to the chambers for one week before the ozone treatment was initiated. Ozone concentrations varied from 0.090 to 0.115 ppm for 126 days, 6 to 9 hours in a day (Table 1).

Measurements

1. Growth and biomass

At the end of the experiment, height, diameter, and percent of falling leaves were measured. Plants were harvested and removed from the soil in which they were grown, the roots were washed, and then the entire plant was separated into roots, shoots and leaves, oven-dried to constant weight in an oven [Dryer model: Hotpack] at 70 ℃, and weighed to the nearest 0.1g.

2. Stomatal conductance, transpiration, photosynthesis and internal CO_2 concentration

Stomatal conductance, transpiration, photosynthesis and internal CO_2 concentration were measured on LPI (Leaf Plastachron Index) 12, in both chambers. These physiological characteristics were measured with a broad-leaf cuvette of the CIRAS-1 portable photosynthesis system (Chapman and Hall Corp., UK). In the cuvette, the leaf was sealed and the CO_2 concentration was allowed to be maintained at ambient

Table 1. Representation of ozone concentrations during exposure at ozone chamber for this study. There were three replications of each of 17 clones in both the control and ozone treatment (126 days of fumigation)

Date	O_3 Concentration	Exposure time
May 4 ~ May 27	0.090 ppm	9am − 3pm
May 28 ~ June 29	0.100 ppm	〃
June 30 ~ July 27	0.110 ppm	8am − 4pm
July 29 ~ Sep. 8	0.115 ppm	8am − 5pm

levels. Air flow through the analyzer was adjusted to maintain leaf cuvette relative humidity near ambient levels (ranged from 60 to 70%) during measurement. The average cuvette temperature was maintained at 25 ℃.

3. Rubisco activity

1) Extraction of leaves

Sample leaf pieces, 0.02 g (dry weight), were taken from a recently mature leaf, LPI 10, of every individual in both chambers. Each sample piece was immediately plunged into liquid nitrogen (< −80℃), and was ground in a mortar with a tissue homogenizer containing 30 mg insoluble PVPP and 2 ml CO_2-free extraction buffer to a fine powder. The extraction medium contained 100 nM BICINE (pH 8), 1mM EDTA, 5 mM $MgCl_2$, 5mM DTT, 0.02% BSA (w/v). The crude solution was transferred to a 1.5 ml micro-centrifuge tube, centrifuged for 30 seconds at 1,2000 × g (Model Marathon centrifuge 13 F/M; Fisher Scientific, 711 Forbes Ave., Pittsburgh PA), and supernatant retained on ice for the measurement of initial activity.

2) Initial Rubisco activity assay

Activity was measured at 25 ℃ by injecting 0.6 mM RuBP and 25 μL of soluble leaf extract into an assay mixture containing: 50 mM Bicine (pH 8.0), 1mM EDTA, 15 mM $MgCl_2$, 20mM NaCl, 9.2 mM DTT, 9.2 mM $NaHCO_3$, 0.4 mM NADH, 0.5 mM ATP, 4.6 mM phosphocreatine, 1.3 units per ml of phosphocreatine kinase, 47 units of phosphoglycerate kinase and glyceraldehyde 3-phosphate dehydrogenase. Rubisco activity was determined by the spectrophotometeric method at 340 nm (Lilley and Walker 1974). Spectrophotometeric assay (Bausch and Lomb Corp. : Model name; Spectronic 1001) for Rubisco activity used here is similar to those reported by other investigators (Sharkey *et al.* 1991).

Data analysis

A randomized block design was used to test for ozone treatment effects. Growth, biomass, stomatal conductance, transpiration, photosynthesis, internal CO_2 concentration and initial Rubisco activity responses to ozone were averaged for all measurements on every clone. The influence of ozone was determined by one way analysis of variance (ANOVA). All data were analyzed with the general linear model procedure. Assumptions (equality of variance and normality) in an ANOVA were checked before proceeding. After each ANOVA, tests for significance ($\alpha < 0.05$) between clonal treatment means for growth, biomass, stomatal conductance, transpiration, photosynthesis, internal CO_2 concentration and initial Rubisco activity were conducted using the Least Significant Difference (LSD) test. All statistical analyses were conducted using the procedures of Statistical Package for the Social Sciences (SPSS PC$^+$, Version 4.0).

RESULTS

Ozone environment generally affected the growth and biomass of hybrid poplar seedlings. Poplar seedlings grown in ozone for 126 days had smaller height, diameter, total dry weight and root-to-shoot ratio than seedlings grown in charcoal-filtered air. In contrast, mean percentage of falling leaves in ozone treated plant was 6 times higher than that of charcoal-filtered plant (Table 2).

Ozone environment generally reduced several physiological responses both 8 AM and 2 PM measurements (Table 3). At 8 AM measurement, pop-

Table 2. Growth of poplar hybrid in response to ozone exposure. Numbers show mean and standard error.

	Ozone	Control
Height(m)	1.7 ± 0.4*	2.2 ± 0.45
Diameter(cm)	2.0 ± 0.5*	2.7 ± 0.62
Total biomass(g)	57 ± 8*	94 ± 9
R/S ratio	0.58*	0.65
Percentage of falling leaves(%)	72*	12

*indicates a statistically significant difference between ozone treatment and control at the P≤0.05 level.

Table 3. Net assimilation rate (Ps), stomatal conductance (gs), transpiration (Tr), internal CO_2 concentration (C*i*) and initial Rubisco activity of 8 AM and 2 PM measurements in responses to ozone exposure

	8 AM		2 PM	
	Ozone	Control	Ozone	Control
Ps (μmol CO_2 m^{-2} sec^{-2})	3.4^b	3.5^b	5.6^b	11.7^a
gl (μmol m^{-2} sec^{-2})	345^b	568^a	458^b	682^a
Tr (μmol m^{-2} sec^{-2})	2.7^c	3.4^b	2.9^b	4.2^a
C*i* (μ l^{-1})	287^a	304^a	298^a	311^a
Rubisco activity (μmol CO_2 min^{-1} dry $weight^{-1}$)	3^c	5^c	9^b	18^a

Different small letters(a, b and c) indicate differences among means when $P \leq 0.05$.

lar seedlings grown in ozone environment showed significantly lower stomatal conductance, transpiration and internal CO_2 concentration, but similar rate of photosynthesis and initial Rubisco activity compared to seedlings grown in charcoal-filtered air. When gas exchange was measured at 2 PM, internal CO_2 concentration was only slightly lower in ozone-treated plants, but rate of photosynthesis, stomatal conductance, transpiration and initial Rubisco activity were significantly lower in seedlings grown in ozone environment compared seedlings grown in charcoal-filtered air.

DISCUSSION

Many researchers reported that ozone reduced growth and biomass on many other species (Reich 1983, Taylor and Frost 1992, Tjoelker *et al.* 1993, Samuelson 1994). Increased percentage of leaf fall and reduced root-to-shoot ratio were previously reported for poplar species in response to ozone environment (Mooney and Winner 1991). Especially, decreased root-to-shoot ratio in response to ozone suggests that normal carbon allocation patterns were disrupted. Reduction in root biomass may resulted from respiratory demand for above ground tissue (Edwards *et al.* 1994). At the foliar level, ozone reduces photosynthesis and causes internal damage, thus increasing dark respiration and the demand for the already reduced carbon (Barnes *et al.* 1995). Furthermore, broadleaf plants such as poplar generally have higher respiration rates than conifers (Reich 1987).

Internal CO_2 concentration is important to understand how ozone inhibits photosynthesis. In this study, internal CO_2 concentration was only slightly decreased with increasing ozone concentration. This is in agreement with findings found by Reich and Amundson (1985) and Matyssek *et al.* (1992). The results suggest that ozone environment reduced photosynthesis not only through reductions in stomatal conductance, but also through reduction in biochemical processes (Rubisco activity) with the leaf mesophyll. Farage *et al.* (1991) reported that the internal CO_2 concentration rose gradually to ozone exposure, suggested that the closure of stomata did not cause the decrease in photosynthesis directly.

Effect of ozone on physiological responses could be demonstrated in many other tree species (Farage *et al.* 1991, Pell *et al.* 1992). All these studies showed# reduction in photosynthesis, stomatal conductance transpiration and Rubisco activity to ozone exposure. All these authors reported that biochemical process to be a key feature to understand reduction in photosynthesis. Furthermore, Dann and Pell (1989) showed that a reduction of Rubisco content could result in symptoms of early senescence in *Solanum tuberosum*.

CONCLUSION

Growth and biomass of hybrid poplar seedlings were reduced by ozone exposure. Mean percentage of falling leaves in ozone treated plants was 6 times higher than that of charcoal-filtered plant. Among physiological responses, rate of photosynthesis, stomatal conductance, transpiration and initial Rubisco activity were significantly lower in seedlings grown in ozone environment compared with seedlings grown in charcoal-filtered air. All these physiological results supported that biochemical process to be a key feature to understand reduction in photosynthesis.

摘 要

오존 環境에 대한 가스 交換을 觀察하기 위해서 雜種 포플러 苗木을 오존에 露出시켰다. Open-top chamber를 利用하여 하루에 6～8시간, 3개월 동안 0.090～0.115 ppm의 濃度로 오존을 노출시켰다.

잡종 포플러 묘목의 生長, 物質生產이 오존에 의해서 줄어 들었고, 平均 落葉率은 오존에 노출된 苗木이 6배 높은 結果를 보여 주었다. 이는 오존에 대해서 필요없는 組織을 떨어뜨려서 被害를 최소화하려는 포플러의 生存 戰略으로 보인다. 生理的인 反應가운데 光合成速度, 氣孔전도도, 蒸散率 그리고 Rubisco의 活性은 오존으로 인해서 유의적으로 줄어 들었다. 위의 사실은 生化學的인 過程도 光合成을 妨害하는데 관여하는 重要한 요인이라는 事實을 뒷받침해 준다고 사료된다.

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