Growth, Photosynthesis and Rubisco Activity of Resistant Hybrid Poplar(*Populus trichocarpa×P. deltoides*) to Ozone Exposure: A Link with Compensatory Strategy[1]

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오존에 露出시켰을 때 抵抗性을 갖는 雜種포플러의 生長, 光合成 그리고 Rubisco 活性에 關한 硏究: 樹木의 補償戰略과의 關係[1]

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ABSTRACT

The objective of this study was to investigate how resistant poplar hybrid makes compensation to ozone stress. Growth, net assimilation rate and initial Rubisco activity were investigated. This study elucidates the physiological mechanisms associated with ozone sensitivity and resistance in 3 selected F_2 hybrids, a family originating from a cross between *Populus trichocarpa*× *P. deltoides*. Open-top chambers were used. Ozone concentrations varied from 90 to 115 ppb for 126 days, 6 to 9 hours in a day. This study tested the hypothesis that resistant poplar hybrid maintains the biomass production to ozone exposure via increased net assimilation rate and Rubisco activity.

Growth, biomass, net assimilation rate and initial Rubisco activity were generally reduced by ozone treatment. In the tree parts, root under ozone stress was the most sensitive part. Reduced allocation of photosynthates to root growth might be due to increased respiratory demands for maintenance and repair of aboveground tissue damaged by ozone stress. Maintenance or increases remaining leaves in photosynthetic rates and Rubisco activity in resistant clone in response to ozone treatment were the results of biological compensation to ozone stress.

Key words : ozone, poplar, net assimilation rate, rubisco, compensation

要 約

雜種포플러가 어떻게 오존 stress에서 被害를 최소화하는가를 알아보기 위해서 生長, 光合成率, 그리고 初期 Rubisco의 活性을 調査하였다. 이 硏究에서는 *Populus trichocarpa× P. deltoides* 揷木苗를 材料로 使用하였다. 오존處理濃度는 90-115 ppb으로, 하루에 6-9시간씩, 126일 동안 처리하였다. 이 연구에서는, 오존에 抵抗性을 가지는 포플러는 光合成率과 Rubisco의 活性을 增加시켜서 그 物質 생산량을 維持하여 stress를 補償한다는 假說을 檢定하였다.

生長, 物質生産量, 光合成率, 그리고 初期 Rubisco 活性이 일반적으로 오존에 의해서 減少하였다. 뿌리의 생장량은 地上部의 被害를 보상하기 위한 炭素의 不均等한 分配때문에, 가장 피해가 심한 部位였다. 저항성을 가지는 포플러는 오존에 대해서 樹體內의 光合成率과 초기 Rubisco 활성을 높여 生産量을 維持하는 生物的인 補償을 하는 것으로 밝혀졌다.

INTRODUCTION

Ozone performs the important role by absorbing ultraviolet radiation in the stratosphere. In contrast, in the lower atmosphere near the ground, ozone is one of the air pollutants affecting plants and human(McLaughlin *et al.*, 1982). Among the air pollutants, ozone may have the greatest negative impacts on tree growth and physiology (Reich, 1987). Ozone concentrations differentially affect carbon fixation rates resulting in differences in sensitivity and resistance of tree species to ozone. Many physiological responses such as stomatal behavior, photosynthesis, Rubisco activity and respiration rate have been extensively documented to explain ozone sensitivity and resistance (Lehnherr *et al.*, 1989; Farage *et al.*, 1991; Pell *et al.*, 1992).

Compensations in trees have been taken place to overcome environmental stress. Resistant tree species to ozone exposure make compensation to maintain biomass production(Winner, 1994). Increased photosynthesis on leaf and stimulated apical growth were typical examples of compensation(Fredericksen *et al.*, 1996). However, less is known about how trees make compensation on Rubisco activity under ozone stress. Rubisco is most abundant enzyme in the leaves of plants and the enzyme responsible for the incorporation of CO_2 into the dark cycle of photosynthesis (Pell *et al.*, 1994).

The objective of this study was to determine how resistant poplar hybrid makes compensation to ozone stress. Therefore, growth, photosynthesis and initial Rubisco activity were investigated. This study elucidates the physiological mechanisms associated with ozone sensitivity and resis-

tance in 3 selected F_2 poplar hybrids. Two sensitive clones and a resistant clone, a family originating from a cross between *Populus trichocarpa* × *P. deltoides,* were used as plant materials. This study tested the hypothesis that resistant poplar hybrid maintains productivity to ozone exposure via increased photosynthesis and Rubisco activity.

MATERIALS AND METHODS

Plant culture

Cuttings of 3 selected poplar clones were taken from 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. 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). Ozone was generated by passing oxygen through a generator(PCI O$_3$ Corp., West Caldwell; model G-IL), and was monitored with a ozone monitor(Dashibi model 1008-AH). Ozone concentrations varied from 90 to 115 ppb for 126 days, 6 to 9 hours in a day (Table 1).

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	90 ppb	9am - 3pm
May 28 - June 29	100 ppb	〃
June 30 - July 27	110 ppb	8am - 4pm
July 29 - Sep. 8	115 ppb	8am - 5pm

Measurements

1. Growth and biomass

At the end of the experiment, height and diameter 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°C, and weighed to the nearest 0.1g.

2. Photosynthesis

Photosynthesis was measured on every leaf from top of the shoot, every individual in both chambers. Photosynthesis was measured with a broad-leaf cuvette of the portable photosynthesis system(Chapman and Hall Corp., UK; CIRAS-1). 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°C.

3. Rubisco activity

A) Extraction of leaves for Rubisco activity

Sample leaf pieces, 0.02g(dry weight), were taken from a recently mature leaf of every individual in both chambers. Each sample piece was immediately plunged into liquid nitrogen($<$ -80°C), and was ground in a mortar with a tissue homogenizer containing 30mg insoluble PVPP and 2ml CO_2-free extraction buffer to a fine powder. The extraction medium contained 100nM BICINE(pH 8), 1mM EDTA, 5mM $MgCl_2$, 5mM DTT, 0.02% BSA(w/v). The crude solution was transferred to a 1.5ml micro-centrifuge tube, centrifuged for 30 seconds at $12,000 \times$ g(Fisher Scientific; model name; Marathon centrifuge 13

F/M), and supernatant retained on ice for the measurement of initial activity.

B) Initial Rubisco activity assay

Activity was measured at 25°C by injecting 0.6mM RuBP and $25\mu\text{L}$ of soluble leaf extract into an assay mixture containing : 50mM Bicine (pH 8.0), 1mM EDTA, 15mM $MgCl_2$, 20mM NaCl, 9.2mM DTT, 9.2mM $NaHCO_3$, 0.4mM NADH, 0.5mM ATP, 4.6mM 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 340nm. 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. The influence of ozone was determined by one way analysis of variance(ANOVA). After each ANOVA, tests for significance($\alpha < 0.05$) between clonal treatment means for growth, biomass, photosynthesis 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

Growth and biomass

As illustrated in Table 2, growth parameters

Table 2. Growth of hybrid poplar in responce to ozone

Growth-parameter	Clone number and treatment					
	1606		1130		1634	
	Control	Ozone	Control	Ozone	Control	Ozone
Height (m)	$2.8^+0.2^*$	1.9 ± 0.4	$2.9^+0.3$	1.8 ± 0.3	3.1 ± 0.3	2.6 ± 0.2
Diameter (cm)	2.4 ± 0.5	1.8 ± 1.2	2.5 ± 0.9	1.4 ± 0.4	2.9 ± 1.1	2.0 ± 0.8
Leaf (g)	$19^+3.4$	12 ± 3.7	18 ± 2.3	9 ± 2.1	$20^+3.3$	18 ± 3.3
Stem (g)	$33^+4.2$	20 ± 6.7	38 ± 6.2	18 ± 3.6	35 ± 5.7	32 ± 3.6
Root (g)	$36^+5.8$	18 ± 5.8	$35^+5.8$	9 ± 3.2	35 ± 2.7	27 ± 2.9
Total (g)	$88^+13.4$	50 ± 16.2	$90^+14.3$	36 ± 8.9	90 ± 11.7	77 ± 9.8

* : standard error

Table 3. Net assimilation (μmol CO_2 m^{-2} s^{-1}) of three clones for leaves of different LPI in the both control and ozone chamber at 1 PM. Clones 1130 and 1606 are classified as sensitive and 1634 as resistant.

Clone number Treatment LPI	1606		1130		1634	
	Control	Ozone	Control	Ozone	Control	Ozone
1	4.63±0.90*	5.33±0.59	4.63±0.88	4.77±0.33	1.40±0.16	3.13±0.64
2	5.57±0.67	8.00±0.16	10.33±0.33	6.10±0.40	3.07±0.30	3.87±1.37
3	7.13±1.27	4.40±0.25	7.03±1.24	7.50±0.1	3.33±0.41	3.43±0.92
4	9.63±1.23	6.03±0.20	11.57±0.12	6.03±0.20	2.97±0.56	5.10±1.03
5	12.47±0.87	5.77±0.67	11.90±0.85	5.90±0.65	4.17±0.43	6.23±0.97
6	11.27±0.99	5.17±0.32	12.43±0.88	3.77±0.44	4.03±0.32	9.77±1.27
7	10.73±0.81	7.83±0.24	14.83±0.97	2.93±0.15	5.10±0.46	8.60±1.27
8	12.73±0.55	8.70±0.75	14.60±0.40	3.70±0.32	4.37±0.23	8.60±1.27
9	11.40±0.76	6.00±0.85	14.60±0.23	6.63±0.78	3.63±0.35	7.73±0.52
10	11.47±0.53	6.40±0.85	13.50±0.47	#	4.87±0.41	5.80±0.35
11	14.00±0.95	5.03±1.48	15.90±0.75	#	5.23±0.64	4.40±0.17
12	11.63±0.56	5.73±0.43	7.60±0.46	#	5.13±0.29	#
13	13.30±0.59	#	#	#	9.00±0.60	#

* : Standard error,   # : No leaf available to measure

were impacted by ozone exposure. Ozone treatment generally reduced height, diameter and biomass. Clones 1606 and 1130(sensitive clones to ozone treatment) had significant reductions in growth and biomass as a results of ozone exposure. In contrast, clone 1634(resistant clone to ozone treatment) did not show a significant reduction in growth and biomass(Table 2).

Net assimilation rate in different leaves

Sensitive clones, 1606 and 1130, illustrated a large difference in the net assimilation rate at the same location between control and ozone chamber. In contrast, clone 1634 did not show a significant reduction in net assimilation rate (Table 3). Actually, most of leaves had higher net assimilation rate in ozone chamber than in the control.

Diurnal changes in net assimilation rate and initial Rubisco activity

Net assimilation rate and initial Rubisco activity appeared to be related to each other at the both 8 AM and 2 PM measurements(Fig. 1-3). Net assimilation rate and initial Rubisco activity in plants exposed to charcoal-filtered air were generally greater than for those plants exposed to ozone. Most plants with less initial Rubisco activities illustrated less net assimilation

rate in both chambers.

Due to ozone treatment, the two selected sensitive clones(1606 and 1130) demonstrated large reductions in the both net assimilation rate and initial Rubisco activity during a day. In contrast, the selected resistant clone(1634) illustrated no or small changes in both net assimilation rate and initial Rubisco activity between two treatments. Net assimilation rate at the both noon and 5 PM actually had higher in ozone chamber than in the control.
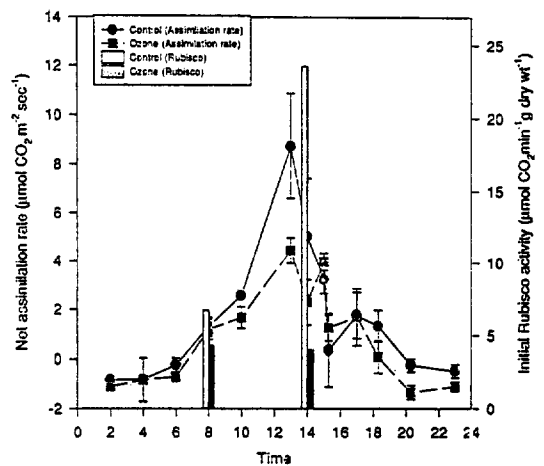

Fig. 1. Diurnal changes in net assimilation rate and initial Rubisco activity(Sensitive clone 1606). Fumigation began at 8 AM and ended at 5 PM.
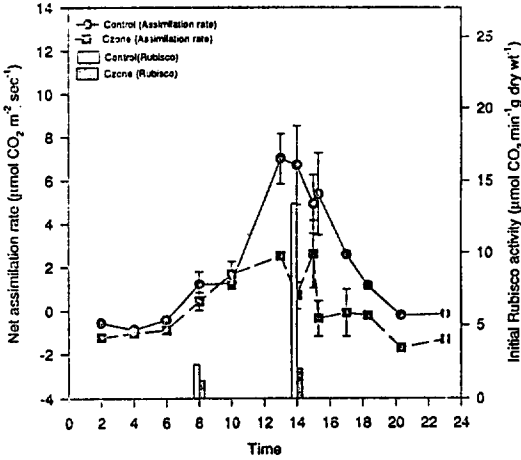
Fig. 2. Diurnal changes in net assimilation rate and initial Rubisco activity(Sensitive clone 1130). Fumigation began at 8 AM and ended at 5 PM.
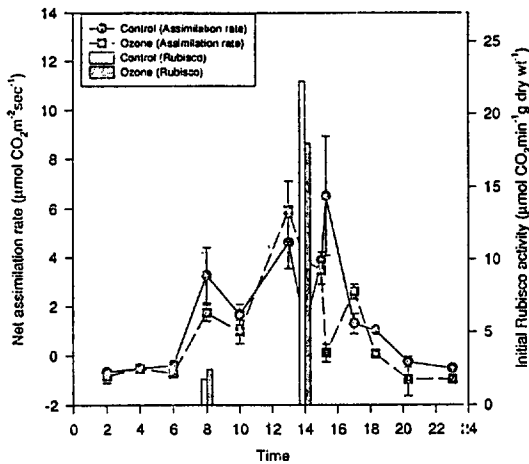

Fig. 3. Diurnal changes in net assimilation rate and initial Rubisco activity(Resistant clone 1634). Fumigation began at 8 AM and ended at 5 PM.

DISCUSSION

Growth and biomass

Ozone treatment reduced growth and biomass overall compared control. Many researchers have reported these observations on many other plants species. All of these studies illustrated that reductions in growth and biomass in response to ozone stress were accompanied by reductions in net assimilation rate and initial Rubisco activity

(Pell et al., 1992; Dizengremel et al., 1994; Vozzo et al., 1995).

Resistant clone(1634) maintained higher root biomass in ozone treatment. Ozone treatment in sensitive clones(1606 and 1130) reduced root biomass overall by 62% compared to control, and the reduction was larger than did resistant clone (27%; Table 2). In the tree parts, root under ozone stress has been reported to be the most sensitive part(Karnosky et al., 1996). Ozone generally altered carbon portioning to root. The reduction in root biomass may negatively affect nutrient and water uptake. Ozone reduces carbon production and inhibits allocation of carbon to the root system, which may lead to reduced or altered water and nutrient uptake. Reduced allocation of carbon to roots may to result from increases in respiratory demands for maintenance and repair of aboveground tissue damaged by ozone stress(Winner, 1994).

Net assimilation rate and initial Rubisco activity

Ozone stress decreased the net assimilation rates and initial Rubisco activity in many other plants species(Pell et al., 1992; Dizengremel et al., 1994). A declining net assimilation rate in leaves may lead to lower productivity(Winner, 1994). As mentioned previously, resistant clone (1634) were able to make physiological adjustment to ozone stress. Increasing net assimilation rate in the remaining leaves is one of the compensatory strategies that maintains high productivity under ozone stress(Reich, 1984).

Resistance to ozone occurs because clones are able to maintain high net assimilation rate and Rubisco activity. Resistant clone produces a large number of new leaves(rapid leaf turnover strategy). There are two ways to achieve high total carbon production. First, maintain or protect photosynthetic tissues in leaves. Second, increase photosynthetic capacity in the remaining leaves (Winner, 1994; Fredericksen, et al., 1996).

Plants frequently make physiological adjustments to an environmental stress. Reducing root-to-shoot ratio and accelerated rates of leaf maturation are examples of these adjustments(Mooney et

al., 1988). Many changes in a plant physiology and growth, such as those caused by ozone, are the results of biological compensatory responses to an environmental stress(Kelley et al., 1993). The main strategy of compensation for stress in plants is to minimize damage from stress. Therefore, ozone can reduce biomass but trees may increase their photosynthetic capacity for different leaves in order to maintain productivity. According to growth data in this study, clone number 1634 (resistant clone) had maintained biomass production and showed little difference in growth between the charcoal-filtered and ozone-treated ramets (Table 2). In contrast, clone numbers 1130 and 1606(Sensitive clones) in ozone chamber had lower biomass production than controls and showed large differences in growth rates between the two treatments(Table 2). The ability of clone 1634 to maintain biomass production may be related to its ability to increase photosynthetic rates and Rubisco activity in the remaining leaves(Table 3).

It is well known that net assimilation rate is closely related to Rubisco activity. Lehnherr(1989) even suggested that the response of photosynthesis to elevated ozone was the result of a limitation in the amount of Rubisco present in the leaves and not of changes in CO_2 conductance. Stomatal behaviour is one of the important physiological responses to ozone exposure(Reich, 1987). However, it is not clear whether the stomatal closure occurred directly as a result of damage to the guard cell membrane or indirectly because of ozone impacts on the photosynthetic apparatus.

In the future, several detailed studies are needed. Antioxidant systems are known to be very important in determining a plant's reaction to ozone and yet these systems were not studied in poplar. SOD(Superoxide dismutase), Glutathione, a-tocoperol and Ascorbic acid are important enzymes involving not only compensation but also antioxidant system(Kangasjarvi et al., 1994).

CONCLUSIONS

Growth, biomass, net assimilation rate and ini-

tial Rubisco activity were generally reduced by ozone treatment. Reduced allocation of photosynthates to root growth might be due to increased respiratory demands for maintenance and repair of aboveground tissue damaged by ozone stress. Maintenance or increases in photosynthetic rates in resistant clone in response to ozone treatment were the results of biological compensation to ozone stress. Ozone can reduce foliar biomass, but poplar maintained their photosynthetic rates in remaining leaves, thereby minimizing the loss in productivity.

ACKNOWLEDGEMENT

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