韓國林學會誌 87(1) : 50 - 56. 1998
Jour. Korean For. Soc. 87(1) : 50 - 56. 1998

Effects of Ozone Environmental Stress on Growth and Stomatal Response in the F_2 Hybrid Poplar (*Populus trichocarpa* × *Populus deltoides*)[1]

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오존 環境이 雜種 포플러의 生長과 氣孔開閉에 미치는 影響[1]

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ABSTRACT

Thirty-six F_2 hybrid poplar (*Populus trichocarpa*×*P. deltoides*) clones were fumigated with ozone to select for ozone sensitive and resistant clones. Fumigation was applied for 6 to 8 hours each day for approximately 3 months at ozone concentrations of 90 to 115 ppb using by open-top chambers.

Height, diameter, number of leaves, total biomass, biomass components, root/shoot ratios, leaf drop and stomatal response were investigated. In summary, ozone generally reduced height, diameter, number of leaves, total biomass, and root/shoot ratios. Ozone stress induced leaf drop and foliar senescence in trees. This study showed very low relationship between total biomass and stomatal conductance. Increased plant resistant to ozone is not always correlated with stomatal behaviour. Probably, characterization of biochemical and other physiological responses to ozone exposure can provide a better understanding of tree response to ozone environment.

Key words : ozone, poplar, growth, stomata, physiological response

要 約

오존에 弱하거나 抵抗性을 가지는 포플러 個體를 選拔하기 위해서 36개의 雜種포플러 클론을 오존에 露出시켰다. Open-top chamber를 利用하여 하루에 6-8시간, 3개월 동안 90-115ppb의 濃度로 오존을 노출시켰다.

樹高, 直徑, 葉數, 物質生産量, root/shoot比率, 落葉率, 氣孔反應을 調査하였다. 수고, 직경, 엽수, 물질생산량, root/shoot비율, 기공 conductance는 오존 때문에 減少하였고, 낙엽률은 增加하였다. 이 연구에서는 기공 conductance와 물질생산량과의 關係는 아주 낮게 나타났다. 오존環境에 대해서 저항성을 갖는 것은 氣孔의 開閉와 恒常 關係가 있는 것은 아니라고 思料되며, 아마도 生化學的이고 生理的인 다른 要因에 對한 研究가 포플러 클론이 갖는 저항성을 理解하는데 도움이 될 것이다.

INTRODUCTION

Ozone is a major air pollutant that has been considered to be one of the most widespread air pollutants in the world (Reich, 1987). This pollutant affects physiological processes directly or indirectly; directly by affecting the major enzyme of photosynthesis, Rubisco (Dan and Pell, 1989) and indirectly by affecting stomatal aperture (Hassan et al., 1994).

The stomatal response of trees is a key process

[1] 接受 1997年 12月 8日 Received on December 8, 1997.
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regulating gas exchange. In hybrid poplar, ozone generally reduces stomatal conductance. It is suggested that poplar clones generally closed their stomata in response to elevated ozone would take up less ozone and thus be protected (Reich, 1987). However, it is not clear whether reduction in productivity with ozone exposure occurs indirectly because of closed stomata or directly because of damage to photosynthesis and therefore, normal stomatal responses to changes in internal CO_2 concentration (Farage et al., 1991; Samuelson, 1994). Many studies have shown that a high concentration of ozone does not always reduce stomatal conductance and biomass production (Farage et al., 1991; Clark et al., 1996). These studies supported that stomatal closure was not always the primary factor in limiting photosynthesis and reduction in total biomass, furthermore Clark et al. (1996) insisted that stomatal limitation to reduction in biomass increased only slightly, while biochemical limitation more than doubled. These somewhat conflicting reports indicate that an intensive study of poplar clones might help to elucidate the nature of these responses.

The objective of this study was to investigate growth and the relationship between stomatal responses and productivity of 36 poplar hybrid clones (*Populus trichocarpa* × *P. deltoides*) to ozone exposure. These poplar clones were chosen because they were part of an intensively studied pedigree (Stettler and Bradshaw, 1994) and they represented a wide range of different productivities and leaf phenologies.

MATERIALS AND METHODS

1. Plant culture

Thirty-six F_2 hybrid poplar(*Populus trichocarpa* × *P. deltoides*) clones from family 331 were harvested as 20cm stem cuttings. Each cutting was dipped into a root hormone (Indole 3 butyric acid), and then planted into in 2 gallon (7.6 liter) pots which contained peat, vermiculite and soil in a 1 : 1 : 1 ratio. The average temperature in the greenhouse was between 23℃ and 25℃ during the experimental period. The relative humidity was controlled between 60% and 80% at seedling height.

Table 1. Periods of ozone exposure for the two exposure treatments. There were three replicates of each 36 clones in both the control and ozone chamber.

First exposure (24 clones, 89 days)

Date	O_3 (ppb)	Time
Mar. 31-April 27	90	0900-1500
April 28-May 10	100	〃
May 11-May 27	110	0800-1700
May 28-June 27	115	〃

Second exposure (12 clones, 67 days)

Date	O_3 (ppb)	Time
July 3-Aug. 1	90	0900-1500
Aug. 2-Aug. 15	100	〃
Aug. 16-Sep. 2	110	0800-1600
Sep. 3-Sep. 8	115	0800-1700

ppb; part per billion

2. Ozone fumigation

Six replications of each clone were randomly divided into two open-top chambers (Heagle et al., 1973). Plants were treated with charcoal filtered air with and without supplemental ozone. Ozone was generated by passing oxygen through a model G-IL generator. Ozone concentrations were monitored with a Dasibi model 1008-AH ozone monitor. Because of the size of the chambers, only 24 clones could be studied at one time. Therefore, the exposure of study material to ozone required two separate, consecutive exposure regimes. Twenty-four clones were exposed in the first and 12 in the second. Ozone concentrations varied from 90 ppb (part per billion) to 115 ppb for 89 and 65 days for the first and second screenings, respectively, 6 to 9 hours in a day (Table 1).

MEASUREMENTS

1. Growth

Height, diameter, number of leaves and percent of abscised leaves were measured on every individual every week. At the end of the experiment, plants were harvested and removed from soil, the roots were washed, separated into roots (excluded cuttings), shoots and leaves, then oven-dried to constant weight in an oven at 80℃, and weighed.

2. Relative growth rate (RGR)

Relative growth rate was used to express the changes in total biomass because of the two separate sequential exposure regimes. Relative growth rate (RGR) was calculated basis using the following formula (Tjoelker et al., 1993) :

$$RGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

Where W_2 is final dry mass at time T_2, W_1 is initial dry mass at the time, T_1 and T_2 are the first day of ozone fumigation and last day of harvest, respectively.

3. Stomatal conductance and transpiration

Every two weeks, stomatal conductance and transpiration were measured on a single leaf, LPI (Leaf Plastachron Index) 11 on every individual in both chambers. Stomatal conductance was measured with the broadleaf cuvette of the Li-Cor 1600 porometer (Li-Cor. Inc., Steady State Porometer).

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, Least Significant Difference (LSD) test ($\alpha < 0.05$) was conducted on all measured growth, biomass variables and stomatal conductance responses to ozone. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS PC+, version 4.0).

RESULTS

1. Growth

The response of growth-parameters to ozone exposure varied at the final harvest. All of growth-parameters in poplar hybrid, except leaf abscession, were decreased by ozone treatments (Fig. 1). In all clones, leaf abscission of ozone exposed clones was three times higher than that of charcoal-filtered clones. Root biomass in a plant was the most sensitive growth-parameter to ozone exposure. Reductions in root biomass compared with

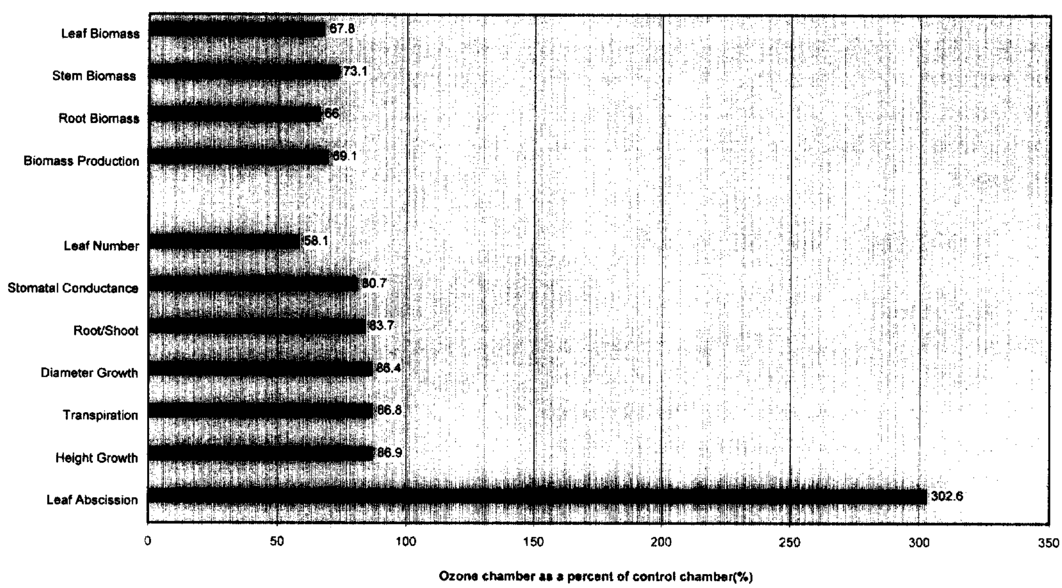

Fig. 1. Growth comparisons between ozone chamber and charcoal filter chamber for the mean of all clones.

Table 2. Stomatal conductance(Gs : $mmol/m^2/s$) and relative growth rate(RGR) differences in total biomass(mg/g/day) between ozone and charcoal-filter chamber grown hybrid poplar.

Clone No.	Gs difference (Control-ozone)	RGR differences (Control-ozone)
1064	299*	0.462
1078	65	6.177***
1095	52	3.312
1127	33	2.710
1130	2	6.678*
1136	402**	4.439
1592	-21	2.288**
1599	-2	1.562
1600	32	1.610
1606	251*	6.195*
1608	41	0.987
1611	104	8.574**
1614	-22	3.277
1618	-3	1.653*
1634	20	0.752
1644	80	3.341
1662	122	5.780
1686	30	3.569
1693	403*	4.665
1694	222*	1.621**
1698	51	10.717*
1701	70	7.820*
1719	99*	3.177*
1751	-3	0.958
1765	421**	2.123
1766	100	5.964
1772	51	5.678
1816	70	5.767
1859	-50	6.130**
1873	100	3.384
1880	49	1.291
1917	119*	1.208*
1939	0	0.083
1949	205*	5.300
1955	80	7.600
1965	201*	5.679

* : Significant at 0.05% significant level
** : Significant at 0.01% significant level
*** : Significant at 0.001% significant level

above-ground biomass resulted in decreases in root to shoot ratios with ozone treatment

Relative growth rate reduction in total biomass to ozone exposure varied among clones (Table 2). Total biomass of clone 1698 to ozone exposure was the most sensitive and that of clone 1939 was the least. The relationship between percent of leaf fall and relative growth rate in total biomass showed a wide array of variation to ozone exposure (Fig. 2). Clone 1719 had largest difference in percent of leaf fall between the ozone and control chamber (39%). In contrast, clones 1772, 1686 and 1064 had no significant differences between the two treatments. In general, the charcoal-filtered plants had more surviving leaves at final harvest than ozone-treated plants.

2. Stomatal response

Thirty-six hybrid poplar clones demonstrated a diversity of stomatal responses as a result of ozone exposure. In general, the reduction in stomatal conductance associated with ozone exposure is not a satisfactory explanation for the observed decreases in total biomass (Table 2). Clones 1064, 1765 and 1694 had very large changes in stomatal conductance between both treatments, but clones 1939, 1634 and 1608 had very small changes in stomatal conductance reductions between two treatments, though these six clones have similar relative growth rate in total biomass. More open stomata to ozone exposure have been observed. Clones 1592, 1599, 1614 and 1618 had higher stomatal conductances in the ozone chamber.

DISCUSSION

1. Reduced biomass

Many investigators have shown that ozone stress reduces plants growth and changes carbon partitioning to root (Samuelson, 1994; Clark et al., 1996). In this study, ozone generally reduces the amount of root dry matter in the whole plants. Increased leaf drop and ozone damage on older leaves may contribute to reduced root biomass and root to shoot ratio.

Old leaves, mature lower leaves, which act as the main source of photosynthates for root growth, were the most damaged, offering an explanation for decreases in partitioning to roots (Cooley and Manning, 1987). A similar effect on older leaves and roots has been reported in agricultural species

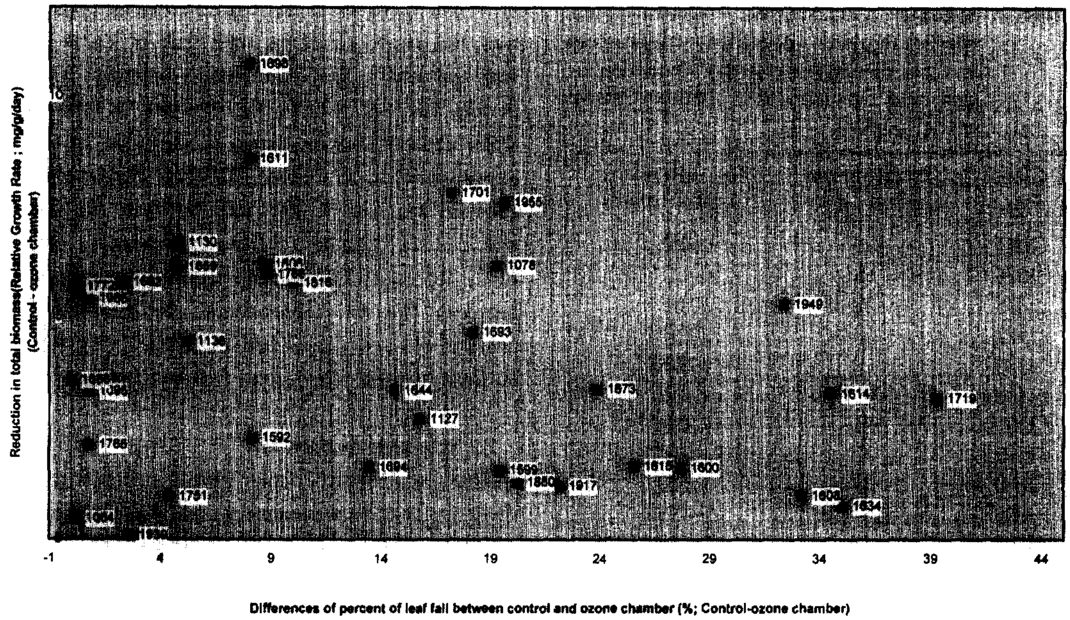

Fig. 2. Percent of leaf fall and total biomass RGR between two chambers.

(Heagle et al., 1993). These studies have reported that older leaves which supplied most of the photosynthate partitioned to roots, and assimilation rate of older leaves was reduced more than that of young leaves.

Increased respiratory demand may also be a contributor to reduced root biomass. Ozone induced large amount of carbon to repair damage in the leaf and an altered balance between carbon source and allocation caused by reduced photosynthetic carbon fixation and greater demand for carbon. Growth and maintenance respiration may be increased by ozone exposure (Winner, 1994).

2. Accelerated leaf drop

Accelerated leaf drop is a another typical responce to ozone exposure (Woodbury et al., 1994). Ozone stress induces leaf drop or foliar senescence in trees. Leaf drop may induce the time a tree has to photosynthesize, thus reducing productivity in trees (Cooley and Manning, 1987). Leaf fall affected the total number of leaves remaining on a plant. The number of leaves is important factor determining total biomass production. In this study, many clones with high leaf drop showed large reduction in total biomass. Accelerated rates of leaf maturation is an example of biological adjustment to ozone stress (Winner, 1994). To minimize ozone damage on trees, poplar clones removed old leaf. Ozone treated-poplars had stippling and necrosis on old leaves. Ozone stress damaged to old leaves which need lots of respiration rate to recover. Therefore, poplar drop the old leaves or damaged tissiues to minimize respiratory demand.

3. Stomatal behaviour and productivity

Stomatal behaviour is one of the most important physiological function to understand ozone effects on tree development because stomata control gas exchanges between leaf and atmosphere. Stomatal closure under ozone stress has been observed in most species(Tjoelker et al., 1993; Clark et al., 1996). But, many other investigators also have reported the stomatal open to ozone exposure(Keller and Häsler, 1987; Hassan et al., 1994). In this study, poplar have different responses of stomata to ozone stress. Clone 1599 and 1951 maintained stomatal conductances approximately the same as reported in the control. In contrast, clones showed very large reductions in stomatal conductance.

In general, those species with lower stomatal conductances are to be more tolerant to ozone exposure (Reich, 1987). Stomatal closure can reduce ozone damage by reducing ozone uptake. For example, water stressed-plants are more tolerant of ozone exposure than well-watered plants. Drought may lead to stomatal closure, therefore decrease in ozone uptake (Vozzo et al., 1995). In this study, stomatal conductance in charcoal-filtered plants were higher than those in ozone-treated plants. However, no association between reduction in stomatal conductance and relative growth rate was found. As previously mentioned, clones 1064, 1765 and 1694 had very large changes in stomatal conductance between both treatments, but clones 1939, 1634 and 1608 had very small changes in stomatal conductance reductions between two treatments, though these six clones have similar relative growth rate in total biomass.

Several studies have reported no relationship between total biomass and stomatal conductance (Tjoelker et al., 1993; Frederickson et al., 1996). Increased plant resistant to ozone is not always correlated with stomatal behaviour(Dan and Pell, 1989). Probably, characterization of biochemical (Rubisco activity and quantity) and physiological responses (net assimilation rate) to ozone exposure can provide a better understanding of tree responce to ozone environment (Sasek and Richardson, 1989).

CONCLUSION

In this study, ozone generally reduced height, diameter, number of leaves, total biomass, and root/shoot ratios. Ozone stress induced leaf drop or foliar senescence in trees. In addition, this study indicates that ozone reduced biomass production not only through alteration in stomatal conductance, but also through unknown altered physiological processes. Further physiological and bichemical study will be needed to identify this pathway.

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