The effects of ozone on growth and stomatal response in the F_2 generation of hybrid poplar (*Populus trichocarpa* × *Populus deltoides*)

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Abstract

Thirty-six F_2 hybrid poplar (*Populus trichocarpa* × *P. deltoides*) clones were fumigated with ozone to record its effects on growth, correlate them with stomatal response and screen for ozone sensitivity. Fumigation was applied for 6 to 9 h each day for approximately 3 months at ozone concentrations of 85 to 128 μg g^{-1} using open-top chambers. Height, diameter, number of leaves, stomatal conductance, transpiration rate, total biomass, biomass components and root/shoot ratios were reduced by ozone stress. Percent of leaf fall in ozone-treated plants was nearly three times higher than in control plants exposed to charcoal-filtered air. Leaf senescence, because of ozone exposure, did not appear to be associated with reduced biomass production. Some clones had a high percentage of leaf-fall with ozone exposure, but were able to maintain total biomass production near that of the control. Their response may be an example of an ability to adjust or compensate for ozone damage. There was no significant or consistent relationship between stomatal conductance and total biomass or the change in stomatal conductance as a result of ozone exposure and the change in total biomass. Taken together, these results suggest that effects of ozone on poplar growth cannot be solely correlated to changes in stomatal conductance, more physiological and biochemical parameters should be examined.

Additional key words: leaf senescence, open top chamber, relative growth rate, stomatal conductance, transpiration rate.

Introduction

Ozone is one of the most widespread air pollutants in the world, particularly in industrialized areas and countries. This pollutant affects physiological processes directly or indirectly; directly, by affecting the major enzyme of photosynthesis, Rubisco (Pell *et al*. 1992), and indirectly, by affecting stomatal aperture (Smeulders *et al*. 1995, Mansfield and Pearson 1996).

Several studies have demonstrated a relationship between stomatal responses to ozone and productivity (Taylor and Frost 1992, Matyssek *et al*. 1993). There are different patterns of stomatal behavior. Some plants with high stomatal conductances are more sensitive to ozone exposure (Reich 1987). In other words, for a given ozone exposure, plants with higher conductances receive a greater dose. In addition, high concentrations of O_3 usually cause stomatal closure, while low concentrations may cause either stomatal opening (Robinson *et al*. 1998, Darrall 1989) or closure (Reich and Lassoie 1984).

In this study, hybrid poplar was used because it is becoming important for the pulp and paper industry in short rotation plantations (Hinckley *et al*. 1989) and it is amenable to study because of its rapid growth, ease of clonal propagation, and well-characterized genetics (Stettler and Bradshaw 1994). In hybrid poplar, ozone generally reduces stomatal conductance (Reich and Lassoie 1984, Reich 1987). It is suggested that poplar clones that 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 stomatal closure occurs directly because of damage to guard cell membranes or indirectly because of damage to photosynthesis and therefore, normal stomatal responses

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Abbreviations: *ANOVA* - analysis of variance; LPI - leaf plastochron index; LSD - least significant difference; OTC - open top chamber; RGR - relative growth rate.
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to changes in internal CO_2 concentration (Farage *et al*. 1991). Many studies have shown that a high concentration of ozone does not always reduce stomatal conductance and total biomass (Dann and Pell 1989, Farage *et al*. 1991). These somewhat conflicting results indicate that an intensive study of poplar clones might help to elucidate the nature of these responses.

The first objective of this study was to characterize the relationship between stomatal responses and productivity of 36 different F_2 hybrid poplar clones (*Populus trichocarpa* × *P. deltoides*) when exposed to ozone. These F_2 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. The second objective was to rank the 36 clones according to their sensitivity as recorded by the different measured parameters.

Materials and methods

Plants: Thirty-six F_2 hybrid poplar (*Populus trichocarpa* × *P. deltoides*) clones from family 331 were collected as 20 cm cuttings from Washington State University Farm 5 in Puyallup (47° 12' N and 122° 20' E). Each cutting was dipped into 100 µg g^{-1} of indole-3-butyric acid, and then planted into in 7.6 dm^3 pots which contained peat, vermiculite and soil in a 1:1:1 ratio. Pots were watered daily and Peters NPK fertilizer (*Peat-Lite Special 15-16-17*) was used twice per week. The temperature in the greenhouse was between 23 and 25 °C, the relative humidity between 60 and 80 % at seedling height. The midday photosynthetic photon flux density (PPFD) ranged between 400 and 1300 µmol $m^{-2}s^{-1}$. Supplemental light was used because natural PPFD was fluctuated daily due to cloud cover and shade cast by greenhouse components (Table 1). This study was conducted in the Douglas Research Conservatory at the Center for Urban Horticulture of the University of Washington (47° 39´ N and 122° 18´ E).

Ozone fumigation: Three replicates of each clone were randomly assigned to each of open-top chambers (Heagle *et al*. 1973); ozone chambers and charcoal-filtered chambers as control. Open top chamber (OTC) has some disadvantages. Limited OTC space results in artificial shading of the plants due to the equipments frame, plastic material used to cover the chamber. This fact may induce the microclimatic differences inside OTC. But OTC has some advantages of being relative inexpensive, easy to replicated and accepted by other investigators. For seedlings and saplings, many researchers have used open top chamber to investigate air pollution effects on physiological process and growth (Pell *et al*. 1992, Reich 1984). Even though physiological differences exist between mature trees and seedlings, open-top chambers continue to be used in air pollution studies of trees (*e.g.*, seedlings and cuttings) because they offer many advantages (Heck *et al*. 1982). In addition, the pots inside chambers were reorganized every 2 weeks and maintained 20 cm distance between the seedlings.

Ozone was generated by passing oxygen through a model *G-IL* generator (*PCI O_3 Corp.*, West Caldwell, NJ, USA, output capacity 0.45 kg d^{-1}). Ozone concentrations were monitored with a *Dasibi model 1008-AH* (Washington, USA) ozone monitor at seedling height (approximately 1 m above ground). Prior to the start of the entire experiment, both the monitor and generator were inspected and calibrated by an Environment Protection Agency-certified technician. Ozone concentrations varied from 85 to 128 µg g^{-1} for 89 and 65 d for the first and second screenings, respectively, 6 to 9 h in a day (Fig. 1). Ozone concentrations in the fumigated chamber remained below 9 µg g^{-1} after fumigation periods. Concentration in the charcoal-filtered chamber were always less than 9 µg g^{-1}. The monitor was re-calibrated daily before ozone fumigation. Because of the limited 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 periods. Twenty-four clones were exposed in the first period and 12 in the second. The plants for the first 24 clones were 32-d-old on the first day of the ozone treatment. The 12 clones used in the second period were 123-d-old when exposure started. Plants were allowed to acclimate to the chambers for one week before the ozone treatment was initiated.

Growth: Poplar cuttings were grown on a greenhouse bench for 4 weeks following shoot emergence before they were moved into ozone and charcoal-filtered chambers. Heights, diameters, number of leaves and percent of leaf fall were measured on each tree, every week. Diameters of new emergent shoot of poplars were measured. We marked the measuring point at the 3 cm above of the pots exactly. Percent of leaf fall was assessed using following formula: leaf fall [%] = (numbers of fallen leaves/total leaves of each plant) × 100. At the end of the experiment, plants were removed from soil, the roots were washed, separated into roots (excluding the cutting itself), shoots and leaves, then oven-dried to constant mass at 70 °C for 48 h and weighed (Reich 1984).

Relative growth rate was used to express the changes in biomass (total, stem, root and leaf) and growth (height and diameter) because of the two separate sequential exposure regimes (South 1991). Relative growth rate (RGR) was calculated using the following formula

(Tjoelker *et al*. 1993): RGR = (lg W_2 - lg W_1)/(T_2 - T_1), where W_2 is final dry mass (or height) at time T_2, W_1 is initial dry mass (or height) at time T_1, T_1 and T_2 are the first day of ozone fumigation and last day of harvest, respectively. Ten more cuttings per each clone were prepared and harvested at the first day of ozone fumigation to determine the initial dry mass. Comparisons were made between the average value found for the charcoal filtered clone versus the ozone treated clone and these values were ranked from 1 to 36, where 36 represented the smallest difference (most resistant) and 1 the greatest difference (most sensitive) between both chambers.

Stomatal conductance and transpiration rate: Every two weeks, stomatal conductance and transpiration ratewere measured on a single, recently mature leaf, LPI 11 (Leaf Plastochron Index; Larson and Isebrands 1971) on every tree in both chambers. They were measured with the broadleaf cuvette of the *Li-Cor 1600* (Lincoln, USA) porometer in both chambers. During the measurements, several environments inside chamber and greenhouse such as temperature, relative humidity and irradiance were manipulated by computer main system, greenhouse headquarter (Table 1).

Table 1. Average temperature, relative humidity and midday irradiation in open top chambers (OTC) and greenhouse during ozone exposure. First exposure was from March 31 to June 27 and second exposure from July 3 to September 8. Means ± SD.

		April	May	June	July	August	September
Temperature	greenhouse	23 ± 0.8	23 ± 1.1	23 ± 0.7	24 ± 0.8	25 ± 0.4	24 ± 0.9
[°C]	OTC	23 ± 2.7	24 ± 1.8	25 ± 1.4	25 ± 1.0	26 ± 1.2	25 ± 2.1
Relative	greenhouse	65 ± 3.4	70 ± 1.2	72 ± 1.8	73 ± 1.5	80 ± 2.8	78 ± 2.9
humidity [%]	OTC	64 ± 1.5	68 ± 1.1	70 ± 2.4	71 ± 1.8	78 ± 0.9	77 ± 1.3
PPFD	greenhouse	573 ± 28.4	408 ± 56.9	859 ± 89.4	1145 ± 548.0	1247 ± 59.7	1308 ± 49.8
[μmol $m^{-2} s^1$]	OTC	564 ± 50.3	405 ± 15.7	837 ± 45.8	1128 ± 248.0	1129 ± 46.3	1294 ± 48.7

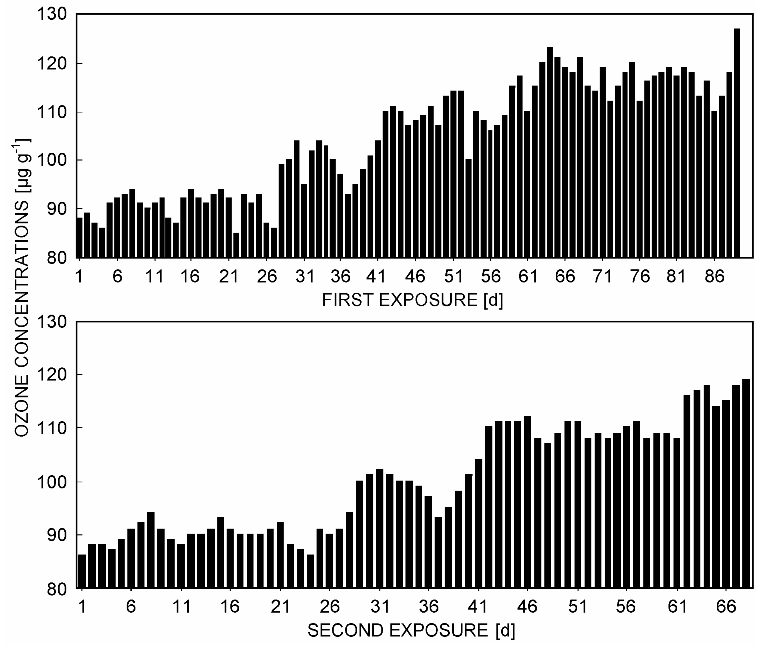

Fig. 1. Periods of ozone exposure for the two fumigation regimes during experiment. First exposure (89 d) was 31 March - 27 June and second exposure (65 d) was 3 July - 8 September. There were 24 clones in the first and 12 in the second. All clones were replicated 3 times in each chamber.

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*). Assumptions of the *ANOVA* were checked before proceeding. After each *ANOVA*, a Least Significant Difference (LSD) test ($P < 0.05$) was conducted on all variables. Height, diameter, number of leaves, percent of leaf fall and stomatal conductance responses to ozone were averaged for all measurements of every treatment replicate. Simple correlation coefficient was conducted on the every variable. Correlation coefficient calculated between changes in total biomass and studied variables such as leaf biomass, stem biomass, root biomass, leaf number, root/shoot ratio, diameter, height, stomatal conductance, and percent of leaf fall. All statistical analyses were conducted using the Statistical Package for the Social Sciences (*SPSS PC+, version 4.0*).

Results

Ozone impacts on growth: After the biomass and growth data had been converted to relative values using the relative growth rate formula, then the response of the 36 clones to ozone could be statistically compared. For all clones, leaf abscission and leaf numbers were the most sensitive study variables to ozone, and height growth was the least (Table 2). Growth responses, expressed in terms of relative growth rates, varied considerably among clones within the F_2 hybrid family 331 (Fig. 2, Table 3).

Table 2. Comparison between ozone-treated plants and charcoal-filtered plants for the mean of all clones. Correlation coefficients (r) between changes in total biomass and studied variables. All data except stomatal conductance and percent of leaf fall are in relative growth rate units. * - significantly different from ozone treatment at $P < 0.05$, $n = 3$.

Parameters	[% of control]	r
Total biomass	87.1	1.000
Leaf biomass	85.4	0.691*
Stem biomass	88.3	0.811*
Root biomass	81.7	0.708*
Leaf number	77.4	0.359
Root/shoot ratio	83.7	0.433*
Diameter	88.6	0.126
Height	90.4	0.101
Stomatal conductance	80.7	0.039
Percent of leaf fall	302.6	-0.230

The response of relative total biomass to ozone varied among clones within the study (Table 3). Clone 1698 showed the greatest reduction in relative total biomass (Fig. 2). Clones 1611, 1701, 1130, 1606, 1078, 1592, 1618, 1694, 1698, 1859 and 1917 also had significant reductions in relative total biomass as a result of ozone exposure. Clone 1955 did not show a significant reduction in relative total biomass (Table 3), but had a very large mean difference between the two treatments. For the remaining 23 clones, there were no significant differences. Six clones, 1939, 1751, 1064, 1634, 1608 and 1880, had insignificant and numerically small differences between relative growth rates in the charcoal filtered and ozone chambers (Table 3). Clone 1939 had the smallest difference in biomass (Fig. 2).

Reductions in leaf biomass were correlated significantly with reductions in total biomass ($r = 0.691$, Table 2). Generally, clones with large reductions in leaf biomass had greater decreases in total biomass than clones with only small decreases in leaf biomass. Clones 1078, 1095, 1130, 1611, 1618, 1698, 1701, 1772, 1859, and 1873 had statistically significant reductions in leaf biomass with ozone exposure (Table 3). Seven of these ten clones also had significant reductions in relative total biomass, whereas three did not (*e.g.*, clones 1095, 1772, and 1873). Clones 1064 and 1939 actually had numerically greater relative growth rates in the ozone chamber than in the charcoal filtered chamber. Clone 1701 showed the largest reduction in leaf biomass (Table 3).

Reductions in stem biomass due to ozone were also strongly correlated with reductions in relative total biomass among the 36 study clones ($r = 0.811$, Table 2).

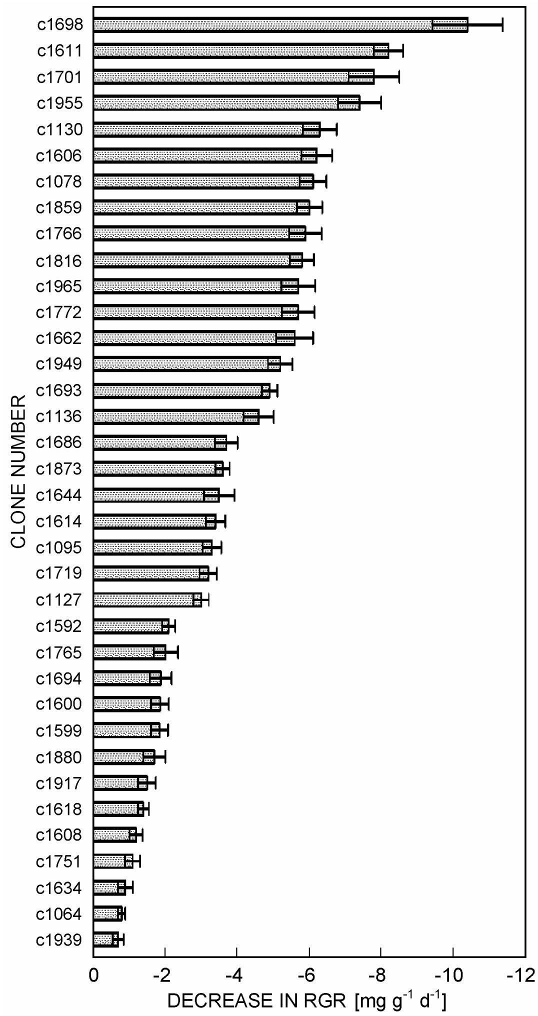

Fig. 2. The effects of ozone exposure on RGR (expressed as decrease from the control) for 36 F_2 study clones is presented in descending order. Means ± SD of 3 replicates.

Table 3. Rank for each of 36 different F_2 hybrid poplar clones for ten different study variables. The changes of biomass (height, diameter, root mass, leaf mass and stem mass) were calculated using the relative growth rate formula and then values were compared. The numbers in parentheses are the absolute differences between control and ozone chamber. Absolute values were calculated as value in control - value in ozone chamber. g_s - stomatal conductance, E - transpiration rate, R/S - root/shoot ratio, NR - no rank (could not make rank due to lack of data), * - significant at $P < 0.05$.

Clone	Leaf fall [%]	g_s	E	Height	Number of leaves	Diameter	R/S	Biomass root	leaf	stem	total
1064	34(0.20)	4(299)*	28(-0.05)	6(0.950)	30	1(1.8100)*	36(-0.15)	35(0.00)	36(-0.02)	22(2.53)	35
1078	13(19.3)*	19(65)	7(2.19)*	8(0.660)	2*	6(0.6700)*	3(0.24)	7(9.89)*	16(3.23)*	5(7.09)*	7*
1095	31(0.80)	20(52)	9(2.00)*	22(0.140)	22	21(0.0400)	34(-0.04)	25(2.51)	17(3.03)*	15(4.88)	20
1127	16(15.8)*	25(33)	4(3.17)*	14(0.290)	10*	8(0.3980)	17(0.14)	26(2.47)	22(1.80)	28(1.28)	23
1130	26(4.80)	29(2)	1(8.97)*	11(0.370)	27	32(0.0025)	11(0.17)	10(9.23)*	7(3.98)*	4(8.65)*	5*
1136	25(5.20)	3(402)*	34(-1.65)	35(-0.002)	11*	34(0.0023)	16(0.15)	13(8.24)	18(2.64)	20(3.04)	16
1592	22(8.10)	34(-21)	20(0.91)	21(0.150)	18	22(0.0180)	31(0.01)	28(2.32)*	19(2.52)	25(2.05)*	24*
1599	12(19.4)	31(-2)	11(1.81)	34(-0.001)	31	30(0.0029)	27(0.05)	29(2.31)	24(1.26)	29(1.22)	29
1600	6(27.7)*	26(32)	16(0.97)	31(0.001)	20	16(0.1500)	18(0.13)	21(3.25)*	27(0.88)	27(1.31)	28
1606	21(8.70)	5(251)*	30(-0.17)	29(0.002)	24	29(0.0032)	20(0.10)	11(8.98)*	6(4.40)	3(9.23)*	6*
1608	4(33.2)*	24(41)	29(-0.08)	13(0.300)	24	17(0.1300)	26(0.05)	31(1.97)	30(0.49)	30(0.99)	32
1611	23(8.00)	11(104)	21(0.68)	30(0.003)	13*	3(1.1500)*	1(0.35)	2(13.83)*	5(4.85)*	26(1.77)*	2*
1614	3(34.5)*	35(-22)	6(2.24)*	24(0.120)	28	25(0.0070)	8(0.19)	14(7.77)	20(2.14)	19(3.06)	21
1618	7(25.6)*	33(-3)	19(0.92)	26(0.080)	29	13(0.1700)	29(0.04)	24(2.69)	26(0.88)*	24(2.25)*	26*
1634	2(35.1)*	28(20)	22(0.61)	28(0.002)	1*	11(0.2500)	15(0.15)	27(2.41)	34(0.00)	35(0.00)	34
1644	17(14.6)	15(80)	17(0.97)	9(0.610)	8*	33(0.0024)	21(0.09)	1(18.59)*	20(0.14)	17(3.83)	19
1662	30(2.30)	9(122)	26(0.17)	16(0.260)	36	35(-0.001)	7(0.19)	5(10.57)	14(3.45)	9(5.89)	10
1686	36(0.00)	27(30)	25(0.27)	32(0.0004)	17	31(0.0027)	4(0.23)	15(6.75)	33(0.00)	32(0.67)	17
1693	14(18.2)	2(403)*	35(-1.82)	2(1.460)*	9*	2(1.1800)*	22(0.07)	16(6.64)	13(3.53)	8(6.12)	15
1694	18(13.5)	6(222)*	33(-1.46)	20(0.170)	5*	10(0.2800)	12(0.17)	20(3.26)	31(0.48)	21(2.91)*	27*
1698	23(8.00)	21(51)	10(1.90)	7(0.720)	4*	18(0.1200)	28(0.03)	6(10.48)*	3(8.15)*	1(10.82)*	1*
1701	15(17.2)*	18(70)	15(1.46)	17(0.230)	15	14(0.1600)	9(0.18)	3(12.89)*	1(19.58)*	6(7.02)*	3*
1719	1(39.3)*	15(99)*	32(0.91)	12(0.350)	32	26(0.0056)	10(0.18)	17(6.55)*	28(0.67)	23(2.41)*	22*
1751	28(4.40)	33(-3)	3(4.00)*	1(1.610)*	34	27(0.0042)	24(0.07)	23(3.00)	32(0.25)	31(0.79)	33
1765	33(0.80)	1(421)*	36(3.91)	10(0.450)	21	12(0.2000)	23(0.07)	19(3.32)	29(0.56)	16(4.38)	25
1766	20(8.80)	13(100)	12(1.56)	25(0.100)	3*	15(0.1600)	13(0.16)	18(5.13)	8(3.83)	7(6.57)	9
1772	34(0.20)	21(51)	14(1.47)	19(0.197)	14	4(1.0700)*	32(0.01)	33(0.001)	2(10.60)*	14(5.10)	12
1816	19(10.0)	17(70)	8(2.10)*	36(0.0004)	6*	36(-0.023)	5(0.23)	8(9.67)	9(3.77)	10(5.88)	11
1859	26(4.80)	36(-50)	2(6.32)*	3(1.380)*	12	5(0.7100)*	6(0.22)	4(11.84)*	11(3.62)*	11(5.82)*	8*
1873	8(23.9)*	12(100)	18(0.95)	15(0.290)	35	9(0.3400)*	35(0.08)	32(0.30)	15(3.39)*	13(5.27)*	18
1880	10(20.2)	23(49)	24(0.42)	33(0.000)	23	19(0.1100)	19(0.11)	22(3.18)	25(1.15)	33(0.44)	30
1917	9(22.2)*	10(119)*	27(0.04)	27(0.009)	33	20(0.0970)	30(0.03)	30(2.09)	23(1.50)	34(0.42)	31*
1939	29(2.60)	30(0)	5(3.12)*	5(1.030)*	16	24(0.0090)	33(0.02)	34(0.001)	35(-0.01)	36(-0.01)	36
1949	5(32.4)*	7(205)*	31(-0.57)	4(1.060)*	26	7(0.6300)	2(0.28)	NR	12(3.54)	18(3.40)	14
1955	11(19.6)	15(80)	13(1.53)	18(0.200)	6*	23(0.0860)	25(0.06)	9(9.27)	4(4.91)	2(10.42)*	4
1965	31(0.80)	8(201)*	23(0.55)	23(0.140)	19	28(0.0040)	14(0.16)	12(8.73)	10(3.71)	12(5.33)	13

Seven clones (1078, 1130, 1606, 1698, 1701, 1859 and 1955) showed significant and large differences between the two treatments. In contrast, clones 1634, 1880, 1917 and 1939 had very small reductions in stem biomass over the control as a result of ozone exposure.

Decreases in the relative growth rates of root biomass were also significantly associated with decreases in the relative growth rates of total biomass among clones ($r = 0.708$, Table 2). For most clones large reductions in root biomass had corresponding large reductions in total biomass. Clones 1078, 1130, 1611, 1644, 1698, 1701 1719 and 1859 had large and significant reductions in root biomass (Table 3). All of these clones, except 1644, had large and significant reductions in total biomass. Although clone 1644 had a very large reduction in root biomass (Table 3), it did not have a very large reduction in total biomass (Fig. 2), probably because leaf and stem biomasses (Table 3) were only slightly affected by ozone. In contrast, clones 1064, 1772, 1873 and 1939 did not have significant decreases in root biomass with ozone exposure (Table 3).

The relationship between changes in relative total biomass and number of remaining leaves was not significant ($r = 0.359$, Table 2). Some clones including 1078, 1634, 1694, 1698 and 1766 had large reductions in their leaf numbers under ozone treatment (Table 3).

Except for clones 1634 and 1766, four of the six clones also showed significant reductions in total biomass. In contrast to this group of clones, clones 1064, 1599, 1614, 1662, 1751 and 1873 demonstrated very little difference in the number of leaves between the two treatments.

Percent of leaf fall is one of the important factors used in evaluating the effect of ozone on plants. Leaf fall, either due to normal or premature leaf abscission, will affect the total number of leaves present on a plant. In addition, the number of leaves and their leaf area in poplar are important factors determining total biomass production (Ridge *et al*. 1986). A wide variation of responses to ozone was found among the clones. Generally, clones with a large percent of leaf fall had only a small difference in total biomass between the two treatments (r = -0.230, Table 2). For example, 12 out of 36 clones, including clones 1608, 1614, 1634 and 1949, had significantly higher rates of leaf fall in the ozone chamber than in the control chamber (Table 3) while showing only a small change in total biomass. The biggest difference in percent of leaf fall between the ozone and control chamber was 39.3 % (Table 3) in clone 1719, whereas the reduction in total biomass was only 3.2 mg g^{-1} d^{-1} (Fig. 2). In contrast, clones 1130, 1611, 1698 and 1859 had no significant differences between the two treatments in terms of percent of leaf fall, but were very sensitive in total biomass (Table 3). Because there was a significant, positive correlation between the reduction in leaf biomass and relative total biomass (Fig. 2), changes in leaf abscission as the result of ozone did not appear to translate directly into changes in total leaf biomass.

There was no significant relationship between reductions in height growth and reductions in relative total biomass (r = 0.101, Table 2). Clones 1693, 1751, 1939 and 1949 had very large and significant changes in height growth, but were not significantly different in total biomass (Table 3). In contrast, clone 1859 had very large changes in both height and total biomass (Table 3). Clones 1136, 1599, 1816 and 1880 had very small changes in height with ozone treatment. Furthermore, clones 1136, 1599 and 1816 were actually slightly taller in ozone treated plants.

Reductions in relative diameter growth rate were insignificantly associated with reductions in relative growth rate in total biomass (r = 0.126, Table 2). Large reductions in diameter did not appear to be observed with large reductions in total biomass. For example, clones 1064, 1693 and 1772 had large reductions in diameter, but were not significantly different in total biomass. Clones 1136, 1644, 1662 and 1816 had very small changes in diameter growth under ozone treatment (Table 3).

Ozone impacts on stomatal conductance: The relationships between stomatal conductance, growth and ozone were investigated from two perspectives: *1*) Was there a positive relationship between stomatal conductance and production? and *2*) Was there a relationship between the change in stomatal conductance as a result of ozone exposure and the change in biomass? There were no significant relationships between stomatal conductance and relative total biomass. Initially, data were pooled (*e.g*., control, ozone-treated, and exposure regime 1 and 2 were all combined). Then data were analyzed separately (*e.g.*, stomatal conductance control plants from exposure regime 1 *vs*. relative biomass growth control plants, exposure regime 1). As a result, some r values improved but were still less than 0.30; F values were still insignificant. Fast-growing plants did not necessarily have more open stomata. There was no pattern between the changes in relative growth rate of total biomass and stomatal conductance as a result of ozone (r = 0.039, Fig. 3, Table 2). Clones 1599 and 1751 approximately maintained stomatal conductances the same as found in the charcoal filtered chamber. In contrast, clones 1064, 1136 and 1765 had very large reductions in stomatal conductances and with only small reductions in biomass. Another group of three clones demonstrated the exact opposite responses. Clones 1078, 1130 and 1606 all had significant and large reductions in relative total biomass between the two chambers. In this situation, only clone 1606 had a statistically significant reduction in stomatal conductance.

A few clones had more open stomata in the ozone chamber than in the control. For example, clones 1592, 1599, 1614, 1618, 1751 and 1859 had greater stomatal conductances in the ozone than in the charcoal-filtered chamber (Fig. 2).

Ranking of clones for ozone sensitivity: The 36 study clones showed a wide range of responses in all measured variables (Table 3). Clones 1064 (rank # 35), 1608 (32), 1634 (34), 1751 (33), 1880 (30), 1917 (31) and 1939 (36) had very small changes in relative total biomass between the two treatments, but had very different responses in the other studied variables. For example, clone 1064 had ranks of 1 (diameter), 4 (stomatal conductance), and 6 (height). In contrast, clone 1608 had very large changes in percent of leaf fall (rank #4), but very small changes in stomatal conductance (rank #24). These clones, however, have relatively consistent and small changes in root/shoot ratio, stem, leaf and root biomasses between the two treatments.

Selection of ozone resistant and sensitive clones: Selection of ozone-sensitive and -resistant clones was based on relative biomass ranking (Fig. 2, Table 3). Total biomass was given a greater consideration than other study variables for two reasons. First, it is regarded as a reliable predictor and as an excellent and sensitive integrator of plant function under stress (Dueck *et al*. 1987). Second, biomass production is one of the main goals of short rotation intensive culture. Seven resistant clones were selected and they are: 1064, 1599, 1634, 1751, 1765, 1880, and 1939. Six sensitive clones were selected: 1078, 1130, 1606, 1611, 1698 and 1701. These 13 clones were then used in a subsequent study where the physiological mechanisms associated with ozone sensitivity and resistance in hybrid poplar would be further elucidated. Although clone 1955 showed large, absolute differences in relative total biomass between the two chambers, it was excluded from the final selection because of the lack of a statistically significant difference.

Based upon the rank of each of three variables (relative total biomass, stomatal conductance and number of leaves), properties associated with each of the resistant and sensitive clones are illustrated in Fig. 4. Generally, there was consistency of rank within a clone; however, stomatal conductance's rank in clones 1064 and 1765, and the rank of leaf number in clone 1634 did not correspond to total biomass rank in these resistant clones.

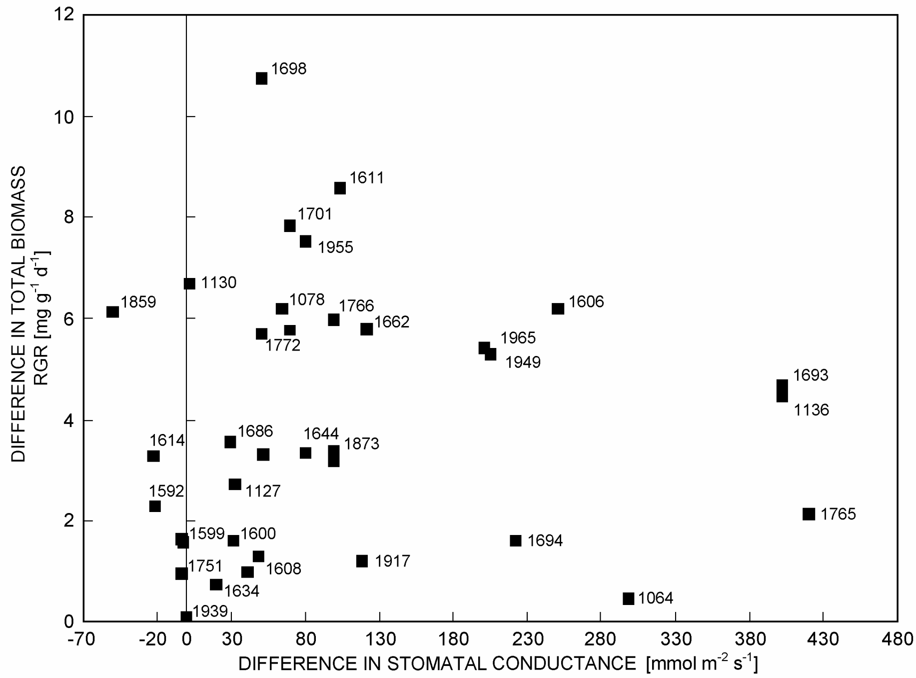

Fig. 3. Relationship between the changes in relative growth rates of total biomass and the changes in stomatal conductance.

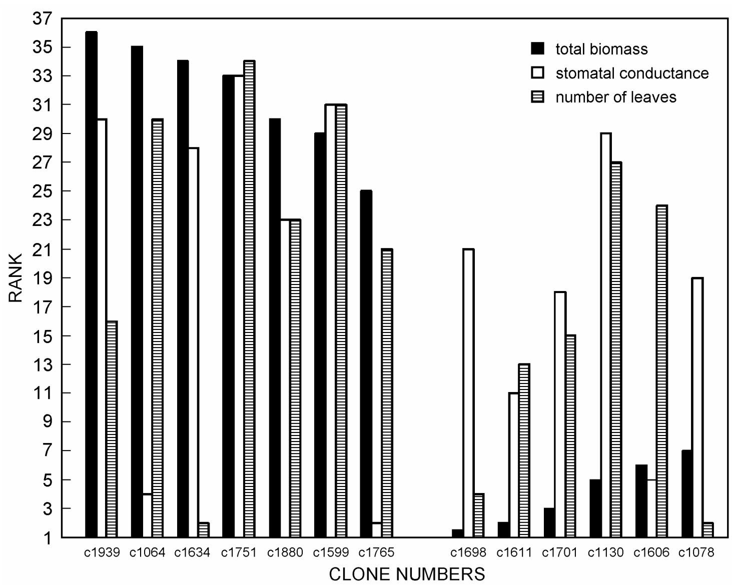

Fig. 4. The ranked response of relative growth rate in total biomass to ozone for the six sensitive and seven resistant clones is presented by going from the most sensitive (rank #1) to the least sensitive or most resistant (rank #36). In addition, the clone specific ranking in terms of the decrease in stomatal conductance or change in number of leaves on the plant is also shown.

The six sensitive clones all demonstrated large changes in total biomass (rank #s < 7); however, except for clone 1606, ranks in stomatal conductance are much higher.

Discussion

Growth: In this study, ozone stress affected the growth and physiology of 36 F_2 hybrid clones of *Populus*. Growth of all clones treated with ozone was reduced; however, there was considerable variation among clones in which physiological and growth variables responded to ozone treatment. Variables associated with foliage development, foliage number and leaf drop, were the most sensitive whereas height growth was the least sensitive. Other investigators have also noted reductions in growth parameters in poplar species with ozone stress (Taylor and Frost 1992, Tenga *et al*. 1993). All of these studies found that reductions in height and diameter growth in response to ozone treatment were accompanied by reductions in biomass production.

In this study, ozone-treated F_2 hybrids generally demonstrated reduced biomass production in total and some of its component parts (Tables 2, 3). Root, stem and leaf biomass were all sensitive to ozone exposure. Reduction of root biomass in plants is a response typically associated with aboveground stresses such as air pollution (Mooney and Winner 1991, Barnes *et al*. 1995). In this study, 31 out of 36 clones in the control chamber had higher root/shoot ratios than those in the ozone chamber (Woo, 1996). A reduction in the root/shoot ratio is one of the response patterns associated with a physiological/morphological adjustment to ozone stress (Winner 1994, Woodbury *et al*. 1994). Such responses are often referred to as examples of compensation. In the case of ozone stress, the impact of ozone on foliage function and foliage loss is compensated for by an increased carbon investment in the foliage and an associated decrease in the root system. Ozone likely inhibits photosynthesis (Pell *et al*. 1992), thus reducing carbon assimilation. Not only is carbon production reduced, but carbon allocation is altered with increasing carbon being allocated to foliage repair and replacement and decreasing carbon to the roots. The reduced translocation of assimilates to the root system appears to be due to the disruption of carbon partitioning after exposure to ozone (Smeulders *et al*. 1995). In this study, leaf fall was a very sensitive parameter to ozone. Premature senescence and abscission of leaves may be due to both accelerated leaf aging by ozone and also by reduced root production, thus altering nutrient and water uptake from the soil (Taylor and Frost 1992). It could be also attributed to increased ethylene production due to ozone stress (Kargiolaki *et al*. 1991).

Among ozone responses in poplar, increase in the rate of leaf senescence is a typical response (Keller 1988, Woodbury *et al*. 1994, Gravano *et al*. 2003). In their studies, increased senescence and abscission are associated with declines in growth. However, in this study, total biomass productivity was not always strongly related to the number of remaining leaves on individual clones. For instance, clone number 1634 (one of the resistant clones) had a very high rate of leaf senescence and had the fewest remaining leaves within the ozone chamber; however, its relative growth rate in total biomass remained relatively unchanged (Table 3). In contrast, clones 1130 and 1606 (both classified as sensitive clones) have very low rates of leaf senescence and relatively large numbers of retained leaves; however, these clones showed large changes in total biomass with ozone exposure. Leaf retention or loss does not appear to translate directly into changes in productivity. Generally, seedlings have a greater ability to compensate than saplings or mature trees because seedlings can more easily translocate and mobilize nutrients from old to newly expanding leaves (Laurence *et al*. 1994, Pell *et al*. 1994). Leaf yellowing prior to abscission in this study suggested that nutrients were being re-translocated from these older leaves. Such a pattern of rapid leaf expansion coupled with the re-translocation of nutrients may result in the maintenance of relatively high biomass productivity under ozone stress (Taylor and Frost 1992). In addition, some clones may make a physiological adjustment to ozone stress. These clones may increase the photosynthetic ability of the remaining leaves in order to maximize their growth. Previously mentioned clone 1634 had a very high level of leaf abscission and had the fewest remaining leaves within the ozone chamber. However this clone did not show a decrease in total biomass probably because of the increase in photo-synthesis in the remaining leaves as an adjustment to ozone stress (Woo 1996).

Stomatal conductance and productivity: Changes in stomatal aperture are the most important means by which a plant leaf can regulate gas exchange with the atmosphere (Kozlowski *et al*. 1991). Generally, those species with greater stomatal conductances are likely to be more sensitive to air pollutants (Heath 1994, Wang *et al*. 1995, Fredericksen *et al*. 1996). Plants with greater stomatal conductances, and thus higher potential for pollutant uptake, exhibited greater growth losses when exposed to ozone than plants with lower stomatal conductances (Reich and Amundson 1985). Some authors (Tingey and Taylor 1982, Reich and Amundson 1985) have suggested that one can largely explain a plant's relative sensitivity or tolerance to ozone solely by knowing that plant's maximum stomatal conductance.

In this study, a wide variety of stomatal responses were observed. In general, stomatal conductances in ozone-treated plants were less than those in control plants. However, there was neither a significant relationship between stomatal conductance and relative growth rate nor the change in stomatal conductance as a result of ozone treatment and the reduction in biomass (r = 0.039, Fig. 3, Table 2). If one examines nine clones which demonstrated similar small reductions in total biomass as a result of ozone treatment, six of the clones (1599, 1608, 1634, 1751, 1880, and 1939) had only small changes in stomatal conductances whereas three (1064, 1694, and 1765) had very large changes (Fig. 3). A similar observation results when an additional eight clones, all of which had a very large change in biomass, are examined. Five (1078, 1130, 1698, 1701, and 1955) had small changes in stomatal conductances and three had large changes (1606, 1693 and 1949). Specifically, clone 1606 was the fifth and sixth most sensitive clone in terms of changes in stomatal conductance and total biomass with ozone exposure, respectively. Only in this case, there appeared to be a clear association between the two variables. Clone 1130 was the fifth most sensitive in terms of biomass and yet was the eighth least sensitive in terms of stomatal conductance. An examination of the data from all 36 clones suggests that sensitivity or tolerance to ozone in poplar is determined by more than stomatal conductance and the response of stomatal conductance to ozone.

A number of other studies have also demonstrated no relationship between total biomass and stomatal conductance (Tjoelker *et al*. 1993, Volin *et al*. 1993, Fredericksen *et al*. 1995). Tjoelker *et al*. (1993) and Volin *et al*. (1993) have reported greater damage in hybrid poplar leaves with lower stomatal conductances. These results support evidence that other factors, such as net photosynthesis or Rubisco activity, are the physiological processes affected by ozone. The exposure of some plants to ozone can lead to partial closure of stomata (Sauer *et al*. 1991), or with other plants, photosynthetic ability is directly affected with little or no stomatal closure (Pell and Pearson 1983). Pell and Pearson (1983) found up to an 80 % reduction in the quantity of Rubisco in alfalfa foliage exposed to ozone. In another study, Omielan and Pell (1988) used mesophyll cell suspension of soybean to study the direct effects of O_3 without the possible indirect effects of stomata activity being present. They demonstrated that the effects of O_3 on photosynthesis were direct rather than through changes in cell viability. Other studies, in which stomatal closure has been observed, have determined that closure was the result of photosynthetic induced increases in internal CO_2 levels rather than the direct effects of ozone on stomata (Robinson *et al*. 1998, Farage *et al*. 1991).

Among highly-related F_2 hybrid clones of *Populus*, ozone exposure resulted in a highly variable response. Both reductions in total biomass and growth and physiological variables affected varied considerably. Other than measuring biomass directly, no single indicator variable was found. In contrast to previous studies, neither stomatal conductance nor leaf senescence proved to be a useful variable in predicting biomass changes with ozone exposure. Although many studies appear to demonstrate a relationship between ozone induced stomatal closure, reduced CO_2 uptake and decreased productivity (Reich 1984, Reich and Amundson 1985), an equal number of studies fail to demonstrate such a linkage. These later studies have often shown that altered photosynthesis is accompanied by a reduction in the quantity and activity of Rubisco (Pell and Pearson 1983, Dan and Pell 1989, Heath 1994). The data from this study show no evidence of a direct relationship between stomatal conductance and productivity as a response to O_3 treatment. A more detailed physiological and biochemical analysis is needed in order to explain O_3 effects on productivity.

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