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# Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide

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**"Capsule"**: *Ozone decreased wood strength caused terminals and long shoots to droop and increased the branch angle of termination.*

## Abstract

To study the impact of ozone ($O_3$) and $O_3$ plus $CO_2$ on aspen growth, we planted two trembling aspen clones, differing in sensitivity to $O_3$, in the ground in open-top chambers and exposed them to different concentrations of $O_3$ and $O_3$ plus $CO_2$ for 98 days. Ozone exposure (58 to 97 $\mu l l^{-1}$-h, total exposure) decreased growth and modified crown architecture of both aspen clones. Ozone exposure decreased leaf, stem, branch, and root dry weight particularly in the $O_3$ sensitive clone (clone 259). The addition of $CO_2$ (150 $\mu l l^{-1}$ over ambient) to the $O_3$ exposure counteracted the negative impact of $O_3$ only in the $O_3$ tolerant clone (clone 216). Ozone had relatively little effect on allometric ratios such as, shoot/ root ratio, leaf weight ratio, or root weight ratio. In both clones, however, $O_3$ decreased the shoot dry weight/shoot length ratio and shoot diameter. This decrease in wood strength caused both current terminals and long shoots to droop and increased the branch angle of termination. These results show that aspen growth is highly sensitive to $O_3$ and that $O_3$ can also significantly affect crown architecture. Aspen plants with drooping terminals and lateral branches would be at a competitive disadvantage in dense stands with limited light. Published by Elsevier Science Ltd.

*Keywords: Populus tremuloides*; Ozone exposure; Elevated $CO_2$; Crown architecture; Genotypic response

## 1. Introduction

Tropospheric ozone ($O_3$) concentrations are increasing by about 1–2% per year (Marenco et al., 1994; Taylor et al., 1994) and may triple within the next 30 to 40 years in certain industrial regions (Chameides et al., 1994). Tropospheric ozone is a potent atmospheric pollutant that causes widespread damage to plants. Damage estimates based on current $O_3$ concentrations indicate billions of dollars in losses for agricultural crops annually (Adams et al., 1989) and significant impacts on forest tree productivity (Pye, 1988; Taylor et al., 1994). However, decreases in growth of forest trees from $O_3$ impacts are not well documented. To further complicate predictions of tree response to $O_3$, atmospheric carbon dioxide ($CO_2$) concentrations are increasing rapidly (Keeling et al., 1995). This increase has the potential to increase productivity in many agricultural crops (Wittwer, 1990) and forest trees (Ceulemans and Mousseau, 1994; Norby et al., 1999). In addition, increasing atmospheric $CO_2$ concentrations may offset the detrimental effects of increasing $O_3$ (Allen, 1990). Some studies have shown that $CO_2$ may counteract decreases in photosynthesis and growth caused by $O_3$ (Dickson et al., 1998; Volin et al., 1998), while others have shown that $CO_2$ did not protect against $O_3$ (Barnes et al., 1995; Kull et al., 1996).

Trembling aspen (*Populus tremuloides* Michx.) is very sensitive to $O_3$ exposure and responds with decreased photosynthetic rates (Coleman et al., 1995a), accelerated leaf senescence and abscission (Keller, 1988; Karnosky et al., 1996), and decreases in both shoot and root growth (Coleman et al., 1996; Karnosky et al., 1996). Ozone effects on the above physiological processes not only decrease total carbon fixation but may also

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influence carbon allocation within the plant and disrupt normal patterns of growth, functional equlibria between shoots and roots, and crown architecture. Root growth of aspen is particularly sensitive to ozone exposure with decreases in dry weight of 40–50% for ozone exposed plants compared to charcoal filtered control plants (Coleman et al., 1996; Karnosky et al., 1996). Root growth response to $O_3$ may induce other secondary effects such as decreases in cation uptake and other nutrient deficiencies (Matyssek et al., 1993a).

An adequate supply of carbohydrates is also necessary for maintaining normal crown architecture. Crown architecture of a plant is controlled by genetic and environmental interactions on meristem activity in space and time and the inherent degree of flexibility in expression of various morphological traits (Tremmel and Bazzaz, 1995). Crown and root deployment are important for resource acquisition e.g. light and nutrients, among neighboring plants and are important determinants of competitive ability. Trembling aspen is very shade intolerant (Perala, 1990). Given the importance of light interception, the display of branches for a given genotype could have substantial impact on the ability to survive in a competitive environment. There is evidence that ozone sensitive aspen genotypes may have already been eliminated from some regions of high ozone in the eastern United States (Berrang et al., 1991). Ozone has been shown to affect the crown architecture of European birch and aspen (Matyssek et al., 1992, 1993a). However, there are no reports of ozone effects on the crown architecture of trembling aspen. Therefore, we conducted this study to examine the effects of different ozone concentrations on growth and development of two aspen clones differing in sensitivity to ozone exposure and to determine if increased $CO_2$ concentrations would ameliorate $O_3$ effects on growth. Particular attention was given to changes in growth, allometric relations, and crown architecture. Deleterious changes in crown architecture could have significant effects on the competitive ability of different aspen genotypes.

## 2. Materials and methods

### 2.1. Plant material

The experiment was conducted at the Michigan Technological University, Ford Forestry Center, Alberta, Michigan. Two trembling aspen (*P. tremuloides* Michx.) clones differing in ozone ($O_3$) sensitivity (216 $O_3$ tolerant, 259 $O_3$ sensitive) were selected based on previous developmental characterization of a large number of aspen clones (Berrang et al., 1991; Karnosky et al., 1992) and from differential $O_3$ response in visual foliar symptoms, physiological parameters and growth parameters (Coleman et al., 1995a, 1995b; Karnosky et al., 1996). Softwood cuttings from greenhouse-grown stock plants were taken in March 1991, dipped in a mixture of the root-inducing compound "Hormodin 2" (K-IBA) and "Benelate 50DF" fungicide (1:3), placed in 40×20×8 cm plastic trays in a mixture of perlite and peat moss (3:1), and placed in a mist chamber for rooting. The greenhouse temperature was maintained at approximately 22 °C, the photoperiod was 16 h, and the average light intensity in the mist chamber was 80 $\mu$mol $m^{-2}$ $s^{-1}$.

Cuttings that developed roots within 4 weeks were transplanted into plastic 0.5 l containers (Stuewe & Sons, Corvallis, Oregon) with a growing medium containing topsoil, perlite and peat moss (1:1:1), supplemented with lime, superphosphate, and Peter's Hi-phosphate Special (15-30-15). After 2 months, plants were transplanted into 6-l plastic pots, in the same soil mixture described above plus a time release fertilizer (8 g of Sierra Osmocote (17-6-12) with micronutrients, 4-month formula Grace-Sierra Chemical Corp, Milpites, California). At the Alberta open-top chamber site, plants were placed outdoors under 50% shade cloth for 2 weeks and then grown outdoors in full sun for the rest of the summer. In these conditions, plants developed straight, branchless stems. In October 1991, 90 uniform plants per clone were transplanted from the pots into the ground within standard, 3-m-diameter, 2.3-m-tall open-top chambers (Heagle et al., 1973) with frustums added or within open, chamberless plots. During the 1992 field growing and exposure season, plants were maintained naturally with minimal as-needed watering, without additional fertilization, and with only occasional as-needed pesticide treatments to control fungi, insects, and mites.

### 2.2. Experimental design

The open-top chamber system, the ozone generating and monitoring equipment, and the episodic $O_3$ exposures were described in detail in Karnosky et al. (1996). Plants in the previous experiments were grown in pots and exposed to $O_3$ for one growing season (Karnosky et al., 1996). In the current experiment designed for long-term exposure, plants were placed in the ground (12 plants per chamber, 0.8 m apart around the circumference) so that plants in the final harvest would have three years of sequential $O_3$ exposure. In addition, an $O_3$ plus $CO_2$ treatment was added, and non-chambered open-air plots were established to test for chamber effects. The experiment was a randomized complete block design with three replicates of the following treatments: (1) charcoal-filtered air, (2) 1×$O_3$, (3) 2×$O_3$, (4) 2×$O_3$ plus 150 µl$l^{-1}$ $CO_2$ over 350 ambient, and (5) the open-air plots (OP). Each chamber or open-plot contained six plants per clone (6 plants×2 clones×3 chambers×5 treatments) for a total of 180 plants. In

1992, 90 plants (45 each clone, nine plants per treatment) were harvested and the remainder left in the ground for harvest in the next two years. The $O_3$ treatments were daily episodic cycles designed to simulate natural diurnal $O_3$ cycles. The episodic treatments were computer generated to match seasonal daily ambient $O_3$ concentrations obtained for Washtenaw County in southern Michigan, and then modified to produce the desired $O_3$ daily peak concentrations to better match the long-term $O_3$ profiles for the lower Great Lakes region (LeFohn and Pinkerton, 1988; Karnosky et al., 1996). Daily peak $O_3$ exposures were generated randomly with higher concentrations in late June as is common for the region. There were no $O_3$ treatments during rainy or foggy days or when the leaves were wet.

Ozone was produced from bottled oxygen with an ozone generator (OREC model V10-$O_2$) and delivered with Teflon tubing into the charcoal filtered air stream of the treatment chambers. Ozone concentrations in the $O_3$ chambers were monitored with a time-share scanivalve system (Samplivalve S2565/IP-12T) and three $O_3$ analyzers (TECO model 49). Ozone concentrations were matched with the daily episodic profiles by mass-flow controllers regulated by computer. Carbon dioxide concentrations in the $O_3$ plus $CO_2$ treatment chambers were maintained at about 500 $\mu l l^{-1}$ 24 h per day with $CO_2$ gas delivered from a liquid $CO_2$ storage tank and monitored with an infrared $CO_2$ gas analyzer (Valtronics model 2008-SDL). Plant exposure to $O_3$ and $CO_2$ began on 10 June 1992, and continued until 14 September 1992 for a total of 98 days.

### 2.3. Plant harvest

At the end of the first treatment season (14 September 1992), three plants per clone from each chamber and the open plots (nine plants of each clone for each treatment) were randomly chosen for harvest (3 plants×3 chambers ×2 clones×5 treatments = 90 plants harvested, 90 plants were left in the ground). The shoots were cut off just above the ground and the root systems dug by hand with a potato fork. The plants were separated into shoots and roots, and the shoots were further divided into current terminal (height growth increment 2), 2-year-old stem (height growth increment 1), long shoots (branches longer than 16 cm), and short shoots. The plant material was dried (70 °C) and the dry weights of leaves, stems, and roots were determined. Total plant height, length of the current terminal, length of the long shoots, and basal diameters of the current terminals, long shoots, and main stem (growth increment 1) were determined. Total heights, branch lengths, and basal diameters were measured on all 180 plants (both harvested and left in the chambers). To further quantify crown architecture, branch angles were measured on all 90 plants left in the chambers. The angle of branch origin (Alpha) and the angle of branch termination (Beta) were measured for current terminals and all long shoots (Fig. 7).

### 2.4. Statistical analysis

Hypothesis tests of differences among treatments, clones, and treatment×clone interactions were conducted using analysis of variance (Proc GLM, SAS Institute, 1989–1996). The analysis model assumed a split plot design with treatments and chambers within treatments (error a; Table 1) in a completely random design at the whole plot level. Effects due to clones, treatment×clone interactions, clone × chambers within treatments and replicate clones within chambers (error b) were tested as subplot sources of variation. All effects were assumed fixed. Multiple comparison of means were calculated with Tukey's Studentized Range Test (HSD; Steel and Torrie, 1980).

## 3. Results

### 3.1. Ozone exposure

The episodic $O_3$ exposures were designed to mimic natural diurnal concentrations and were more realistic than the commonly used square wave exposure (Karnosky et al., 1996). Ambient diurnal concentrations can vary widely during the growing season depending on local meteorological conditions (Vukovich, 1994). Therefore, our exposures also varied widely from day to day. Because each of the $O_3$ exposures was generated independently by computer and monitored with different $O_3$ analyzers, it was difficult to exactly match the targeted seasonal exposures. Thus the $2\times O_3$ exposure was lower than the $2\times O_3+CO_2$ exposure (Table 2). Nevertheless, the treatments provided realistic $O_3$ exposures. By the end of August, the total $O_3$ exposure, (Sum-O, Table 2) ranged from 11 to 97 $\mu l l^{-1}$-h and average daily exposures (7 h mean) ranged from 10 to 72 $nl l^{-1}$ depending on treatment. Individual daily episodic peak exposures occasionally exceeded 100 $nl l^{-1}$ but rarely reached 150 $nl l^{-1}$. These relatively low $O_3$ exposures had important effects on plant growth and development.

### 3.2. Growth and dry weight responses

Chamber effects on growth were apparent even after only one season of growth. Height growth of the open-plot (OP) plants was considerably less than the $1\times O_3$ treated plants in the chambers (total height 20% less; current terminal length about 40% less; Fig. 1). Current terminal growth of the OP plants of clone 259 was even less than that found for the $2\times O_3$ treated plants (42 cm

Table 1
Analysis of variance for select morphological variables—probabilities

| Source | d.f. | Current terminal length | Total leaf weight | Total plant weight | Shoot/root ratio | Long shoot weight to length ratio |
|---|---|---|---|---|---|---|
| *Whole plot* | | | | | | |
| Treat | 4 | 0.0001 | 0.0297 | 0.0344 | 0.4130 | 0.0595 |
| Chamber/Treat (error a) | 10 | 0.0014 | 0.0740 | 0.0501 | 0.1398 | 0.2361 |
| *Split plot* | | | | | | |
| Clone | 1 | 0.0001 | 0.0013 | 0.0001 | 0.0110 | 0.1304 |
| Treat × Clone | 4 | 0.1354 | 0.2859 | 0.5445 | 0.0437 | 0.2020 |
| Clone × Chamber/Treat | 10 | 0.6826 | 0.8157 | 0.8269 | 0.9624 | 0.3081 |
| Error (error b) | 120 | | | | | |

Table 2
Summary of the ozone exposure for the 1992 growing season[a]

| Treatment | Total exposure Sum O $\mu$ll$^{-1}$-h | Highest 7 hour mean nll$^{-1}$ | Number of hours over 80 nll$^{-1}$ | Sum 80 $\mu$ll$^{-1}$-h |
|---|---|---|---|---|
| CF | 11 | 10 | 0 | |
| 1×$O_3$ | 58 | 47 | 72 | 6 |
| 2×$O_3$ | 71 | 61 | 202 | 16 |
| 2×$O_3$+$CO_2$ | 97 | 72 | 368 | 29 |
| OP (open plots) | 50 | 44 | 66 | 5 |

[a] CF, charcoal filtered treatment, Sum O, total seasonal exposure summed from zero. Sum O, seasonal ambient $O_3$ exposures at Alberta, Michigan may range from 40 to 65 $\mu$ll $^{1}$-h. Daily maximum exposures for the 2×$O_3$ treatments range up to 150 nll$^{-1}$ (Karnosky et al., 1996). Sum 80, total seasonal exposure over 80 nll$^{-1}$.

for OP plants vs. 61 cm for 2×$O_3$ plants or 31% less compared to the 2×$O_3$ plants). The chamber effects were greatest with the current terminal leaves. Current terminal leaf dry weights decreased by 50% in clone 216 (1×$O_3$ vs. OP) and 58% in clone 259 (Fig. 2). Dry weights of the other leaf categories for the OP plants did not differ from plants in the $O_3$ treatments.

Exposure to $O_3$ decreased height, diameter, and volume growth in both clones (Fig. 1). However, the relative growth response of the various parameters differed. Total height decreased by about 15%, current terminal length 33%, and stem volume ($D^2H$) 45% in the 2×$O_3$ plants compared to plants in the CF treatment (Table 3). The addition of $CO_2$ to the 2×$O_3$ exposure alleviated the $O_3$ impact for the $O_3$ tolerant clone 216 but had no effect on the $O_3$ sensitive clone 259. Changes in dry weights of the different plant parts were similar to that found for height and volume growth. Ozone exposure decreased dry weight production, and the addition of $CO_2$ to the 2×$O_3$ treatment alleviated $O_3$ impact only in clone 216. Ozone decreased total leaf dry weights for both clones, although the decrease was not always statistically significant at the 10% level (Fig. 2). Current terminal leaf dry weights were particularly responsive to treatment, decreasing by 49% in clone 216 (CF vs. 2×$O_3$) and 60% in clone 259 (Fig. 2). Total plant leaf dry weight shows not only plant response to treatment but also the importance of genetic or clonal differences in response. In the average response of the two clones, the 2×$O_3$+$CO_2$ treatment did not differ from the CF treatment (−12%). However, the individual clonal response was completely different. Leaf dry weight response of clone 216 was slightly positive (+7.6%), while the leaf dry weight of clone 259 decreased by 37% (Table 3). Response to treatment, however, was not consistent with all leaf categories; short shoot leaf weights increased in clone 216. The increase in short-shoot leaf weight with 2×$O_3$ in clone 216 was related to an increase in the number of short shoots for this clone in this treatment (average of 12 short shoots per plant in 216, compared to five per plant in the CF treatment and six short shoots per plant in 259 in the 2×$O_3$ treatment). Short shoots comprise about 5% of the total leaf dry weight for these 2-year-old aspen plants (Fig. 2), but increases to 30–40% in 3- and 4-year-old plants (Dickson unpublished).

Dry weight response of stems and roots to treatment was similar to that found for leaves. Dry weight of the different plant components generally decreased with $O_3$ treatment, while the $CO_2$ addition counteracted the $O_3$ response in clone 216 (Fig. 3). Short-shoot stems showed the same increase in dry weight with the 2×$O_3$ treatment of clone 216 as the leaves. In contrast, long shoot dry weight decreased by 37% ($P=0.17$) in clone 216 and 43% in clone 259 (CF vs. 2×$O_3$). Similarly, current terminal dry weight decreased by 63 and 74%

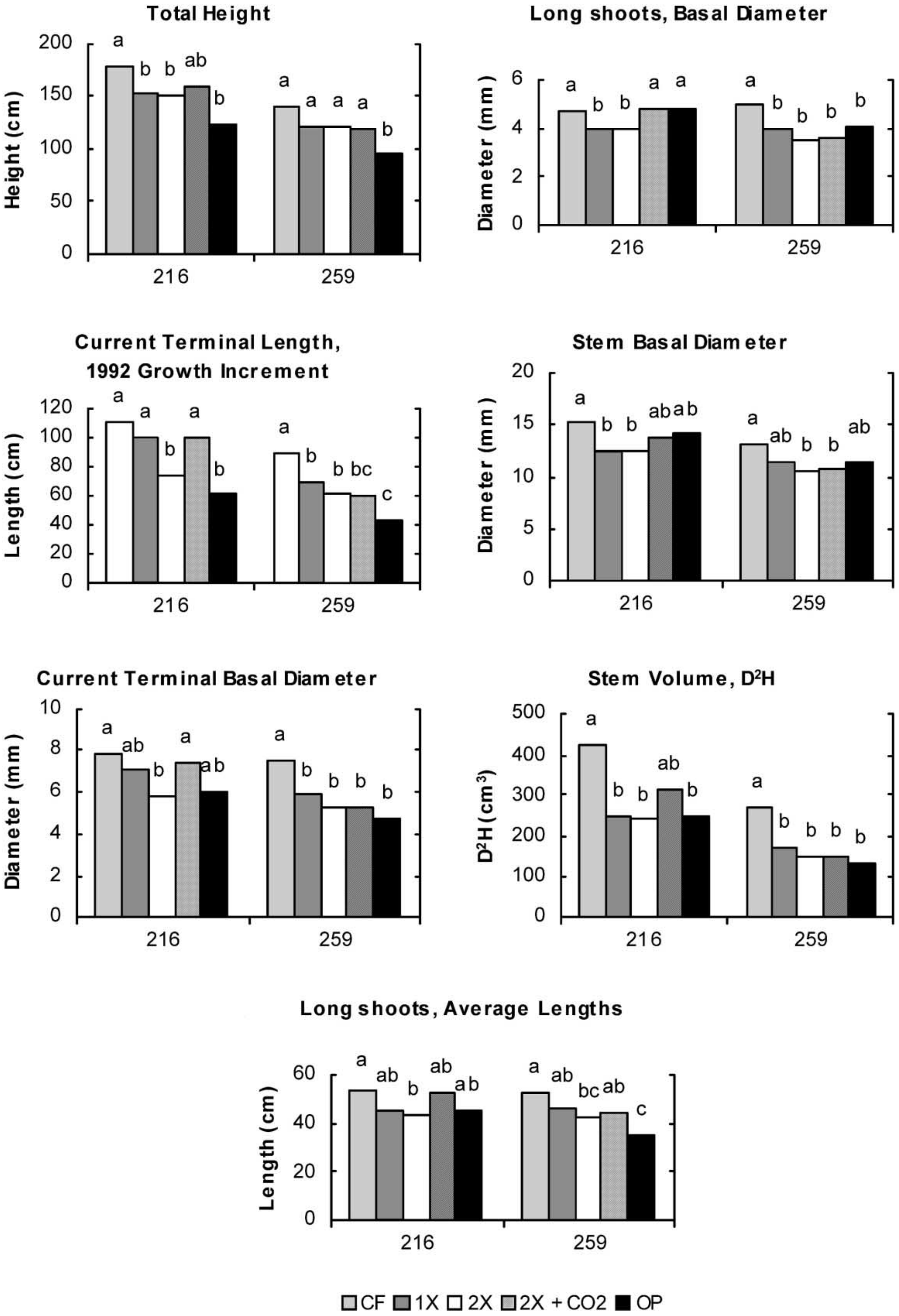

Fig. 1. Plant growth responses to elevated $O_3$ and $O_3$ plus $CO_2$. All measurements are from plants harvested in September 1992 (three plants per chamber, three chambers and nine plants per treatment for each clone). Means with the same letter are not statistically different at the 10% level. Treatments are: charcoal filtered air (CF), 1× ambient $O_3$, 2× ambient $O_3$. 2× ambient $O_3$ plus 150 ppm added $CO_2$, and non-chambered, open-air plots (OP). The clones are 216 ($O_3$ tolerant) and 259 ($O_3$ sensitive).

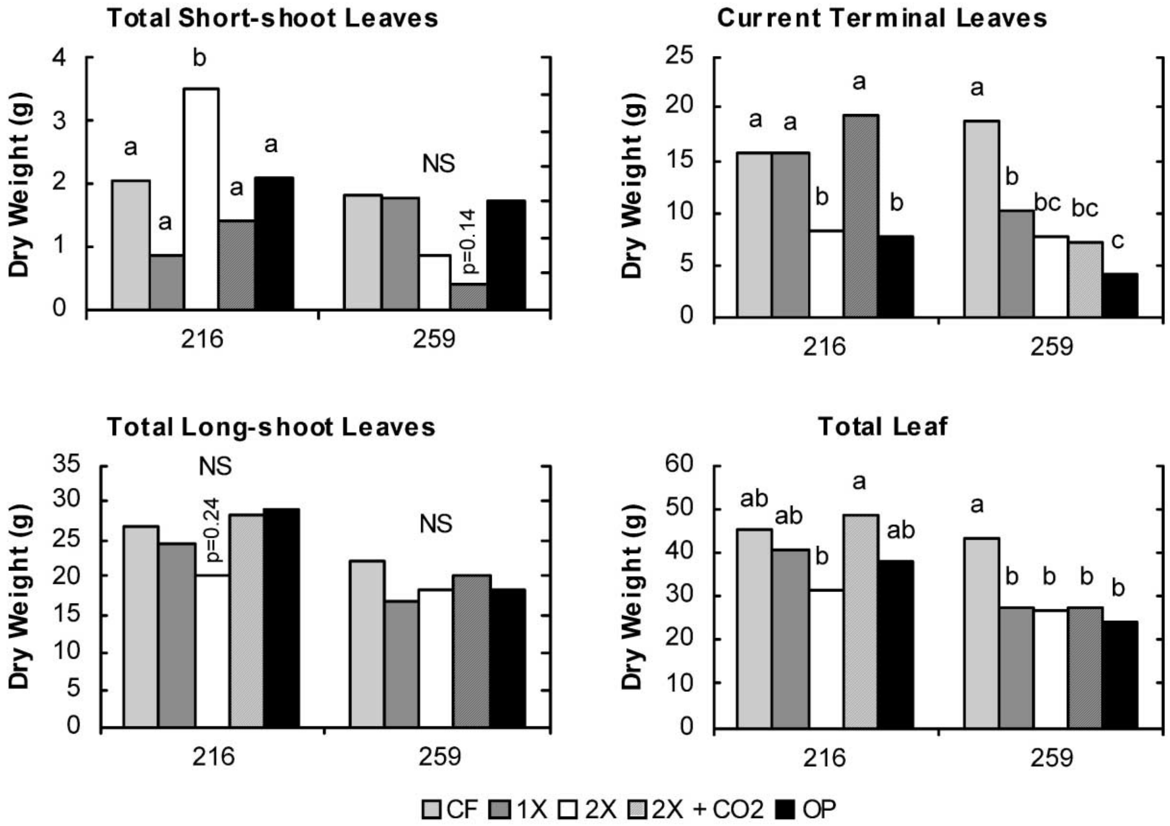

Fig. 2. Leaf dry weight responses to elevated $O_3$ and $O_3$ plus $CO_2$. Means with the same letter are not statistically different at the 10% level. Treatments as in Fig. 1. NS indicates no statistical difference at 10%. *P* values are from paired companions between the CF treatment and the treatment with the *P* value.

with the $2{\times}O_3$ treatment compared to the CF treatment in clone 216 and 259, respectively (Fig. 3). The negative impact of $O_3$ on stem weight, root weight, and total plant weight were consistently smaller in clone 216 compared to clone 259 (Fig. 3; Table 3), averaging −27% for 216 and -44% for −259 for these three fractions with the $2{\times}O_3$ treatment (Table 3).

### 3.3. Allometric responses

Allometric responses or changes in carbon allocation patterns within the plants differed with clone, with treatment, and with the particular response measured (Figs. 4 and 5). Allometric ratios, such as shoot/root ratio (leaf plus stem and branch dry weight/root dry weight; S/R), leaf weight ratio (total leaf dry weight/ total plant dry weight; LWR), shoot weight ratio (leaf plus stem and branch weight/total plant weight; SWR), and root weight ratio (root weight/total plant weight; RWR) changed relatively little with treatment (Fig. 4). The $O_3$ sensitive clone (259), while decreasing by 46% in volume and 43% in total plant weight in response to $2{\times}O_3$ (Table 3), maintained essentially the same within-plant distribution of carbon or relative weight of different plant modules. The $O_3$ tolerant clone (216), while also responding to the $2{\times}O_3$ treatment (volume decreased by 43% and total dry weight decreased by 28%), exhibited somewhat more flexible within-plant carbon allocation patterns (Fig. 4). The shoot/root ratio increased with the $2{\times}O_3+CO_2$ treatment with 216 indicating that more carbon was retained in the shoots in response to the increased $CO_2$ concentration. Greater leaf weight ratios and shoot weight ratios also indicate that more carbon was allocated to leaves and shoots in the $2{\times}O_3+CO_2$ treatment compared to the $2{\times}O_3$ treatment for clone 216.

Allometric relationships more closely related to crown architecture, such as leaf weight/branch weight ratios (g/g) and branch weight/branch length ratios (g/m), also responded to treatment. The leaf weight/branch weight ratios of both current terminals and long shoots increased with the $2{\times}O_3$ treatment compared to the CF treatment in both clones (56% and 41% in clone 216 and 259, respectively for the current terminals) (Fig. 5) the leaf weight/branch weight ratio of clone 216 increased only with the $2{\times}O_3$ treatment while this ratio increased in clone 259 with all of the $O_3$ treatments and the OP treatment compared to the CF treatment. An increase in the leaf weight/branch weight ratio indicates more leaves were required to produce the same unit weight of branches, or as was found here, while leaf weights decreased with the $O_3$ treatments branch weight

Table 3
Percentage change of different growth parameters in response to treatment compared to the charcoal filtered control plants[a]

| Treatment | Current terminal length | | | Volume, $D^2H$ | | |
|---|---|---|---|---|---|---|
| | $\bar{x}$216+259 | 216 | 259 | $\bar{x}$216+259 | 216 | 259 |
| 1×$O_3$ | −15.0[a] | −9.9 | −21.3 | −39.3 | −41.3 | −36.0 |
| 2×$O_3$ | −33.0 | −34.2 | −31.5 | −44.2 | −43.4 | −45.7 |
| 2×$O_3$+$CO_2$ | −20.0 | −9.9 | −32.6 | −32.9 | −25.2 | −45.4 |
| OP | −48.0 | −45.0 | −52.8 | −44.5 | −41.5 | −49.8 |
| | Total leaf weight | | | Total stem weight | | |
| | $\bar{x}$216+259 | 216 | 259 | $\bar{x}$216+259 | 216 | 259 |
| 1×$O_3$ | −22.5 | −8.9 | −36.6 | −24.4 | −11.3 | −40.8 |
| 2×$O_3$ | −33.6 | −29.2 | −38.0 | −37.4 | −30.5 | −45.9 |
| 2×$O_3$+$CO_2$ | −12.0 | +7.6 | −37.1 | −18.9 | −2.3 | −39.6 |
| OP | −28.6 | −14.7 | −43.2 | −24.4 | −9.0 | −43.7 |
| | Total root weight | | | Total plant weight | | |
| | $\bar{x}$216+259 | 216 | 259 | $\bar{x}$216+259 | 216 | 259 |
| 1×$O_3$ | −29.0 | −23.8 | −35.7 | −25.4 | −14.9 | −38.0 |
| 2×$O_3$ | −32.5 | −23.1 | −45.0 | −34.6 | −27.7 | −42.8 |
| 2×$O_3$+$CO_2$ | −25.5 | −13.8 | −40.8 | −19.8 | −3.6 | −39.3 |
| OP | −20.8 | −1.3 | −46.3 | −24.5 | −7.8 | −44.4 |

[a] All responses are negative compared to the CF plants except for total leaf weight, 2×$O_3$+$CO_2$, clone 216. For statistically significant differences, see Figs. 1–3.

decreased even more. In addition, while current terminal lengths decreased by about 32% with 2×$O_3$ (Table 3), branch weight/branch length ratios of current terminals decreased even more in both clones (−54% and −59% in 216 and 259, respectively) (Fig. 5), indicating greater $O_3$ impact on dry weight production of branches than on growth in length. The weight to length ratios of long-shoot branches of clone 259 also significantly decreased while the ratios of clone 216 were unaffected by $O_3$ treatment, indicating similar decreases in length and weight for 216.

Branch lengths relative to branch basal diameters also responded to treatment (Fig. 5). A smaller ratio compared to the CF treatment ratio indicates that the basal diameter was relatively larger for that treatment. Branch diameters of both current terminals and long shoots were relatively larger for the OP plants of both clones compared to the chamber treatments. In contrast, the larger ratios found for clone 259 in the 2×$O_3$ and 2×$O_3$+$CO_2$ treatments indicate a relatively smaller or thinner basal diameter compared to branch length (Fig. 5).

Treatment impacts on allometric relationships, such as branch weight/branch length ratios, result in changes in crown architecture. These changes in crown architecture are clearly visible and can be quantified with measurements of branch angles (Fig. 6). Branch angles of origin ($\alpha$) and angles of termination ($\beta$) were both affected by $O_3$ treatment of our aspen clones (Fig. 7). Current terminal angle of origin increased in the $O_3$ sensitive clone 259 with the 2×$O_3$+$CO_2$ treatments compared to the CF treatment (28° compared to 4.8°; Fig. 6), while this angle was not affected in the $O_3$ tolerant clone 216. In contrast, current terminal angle of termination increased in both clones with the 2×$O_3$ treatment. The addition of $CO_2$ did not alleviate the impact of $O_3$ but in fact made it greater in clone 259 (Figs. 6 and 7). Current terminals of 259 drooped or bent to greater than 90°. The angle of origin of long shoots was not consistent in respect to treatment with either clone; 2×$O_3$ and 2×$O_3$+$CO_2$ actually decreased this angle compared to the CF treatment in clone 259. The angle of termination for long shoots, however, did respond to treatment in clone 259, increasing from 87° for the CF treatment to 122° for the 2×$O_3$ treatment (Fig. 7).

## 4. Discussion

### 4.1. Ozone and carbon dioxide exposures

The ozone and carbon dioxide exposures in this experiment were chosen to simulate ambient concentrations expected within the next 50 years (Chameides et al., 1994; Marenco et al., 1994; Taylor et al., 1994; Keeling et al., 1995; Fowler et al., 1999). Although occasional hourly episodic $O_3$ exposures reached 150 nl $l^{-1}$ or greater, the total seasonal exposures of 58–97 μl $l^{-1}$-h accumulated over 98 days were not unusual for many areas in the eastern United States and Canada. Over this large area, summer daytime values of 50–70 nl $l^{-1}$ and seasonal ambient exposures of 60–100 μl $l^{-1}$-h for a 120-day growing season are common (Fuentes and Dann, 1994; Taylor et al., 1994; Vukovich, 1994; Hogsett et al., 1997). If tropospheric $O_3$ concentrations are increasing by 1–2% per year (Marenco et al., 1994) and $CO_2$ concentrations are increasing by 0.5% per year (Keeling et al., 1995), our highest experimental concentrations will equal ambient concentrations in less than 50 years. Currently regenerating aspen stands will experience these increasing concentrations throughout their rotation age of 30–50 years (Perala, 1990).

### 4.2. Growth responses

Trembling aspen and *Populus* taxa in general have rapid growth rates and a competitive growth strategy designed to take advantage of favorable environmental conditions. Because of these characteristics (high photosynthetic rates and stomatal conductance, rapid leaf production, and rapid height growth), taxa in the genus

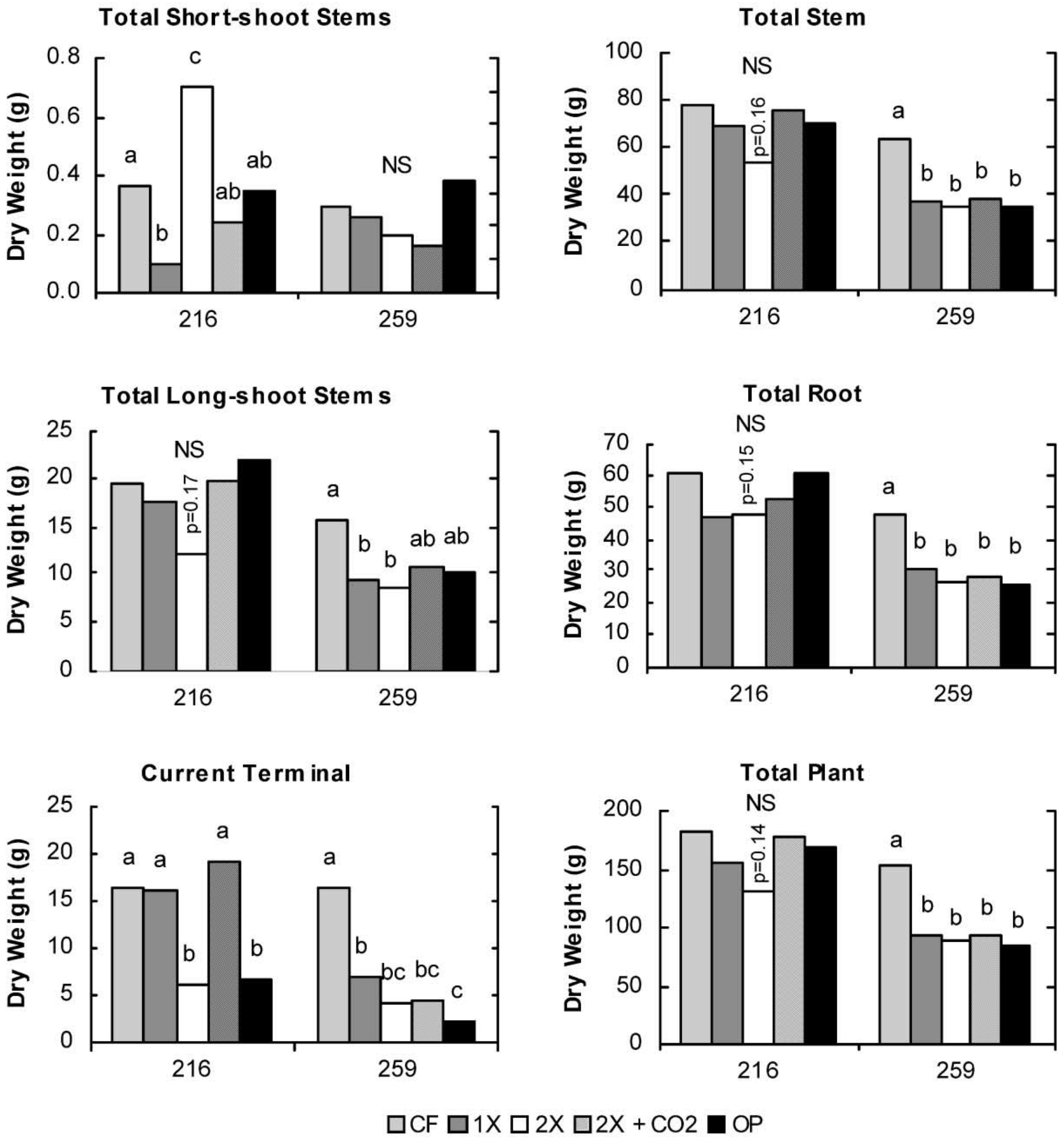

Fig. 3. Stem and root dry weight responses to elevated $O_3$ and $O_3$ plus $CO_2$. Means with the same letter are not statistically different at the 10% level. Treatments as in Fig. 1.

*Populus* may be quite sensitive to environmental stresses, such as $O_3$ exposures (Reich, 1987; Laurence et al., 1994; Karnosky et al., 1996; Dickson et al., 1998). The aspen clones in this experiment were no exception. Growth of all parameters measured (i.e. shoot length; shoot diameter; stem volume; and leaf, stem, and root dry weight) decreased with $O_3$ exposure, particularly with the $O_3$ sensitive clone 259 (Figs. 1–3; Table 3). The addition of $CO_2$ to the $2\times O_3$ treatment alleviated the impact of $O_3$ on growth only with the $O_3$ tolerant clone 216.

Leaf production, height growth, and branch elongation usually respond less to $O_3$ exposure than other growth parameters such as weight of plant parts (Karnosky et al., 1996). In this current study, the length of current terminals and long shoots of the sensitive clone 259 decreased by 32 and 19%, respectively with the $2\times O_3$ treatment compared to the CF treatment (Fig. 1; Table 3). In contrast, dry weight of the current terminals and long-shoots decreased 74 and 43%, respectively (Fig. 3). Such differences in response may be expected because young expanding leaves and internodes are strong sinks and receive most of the photosynthate required for growth from the subtending, recently mature leaves (Dickson, 1989, 1991). This leaf age class is less responsive to $O_3$ exposure compared to

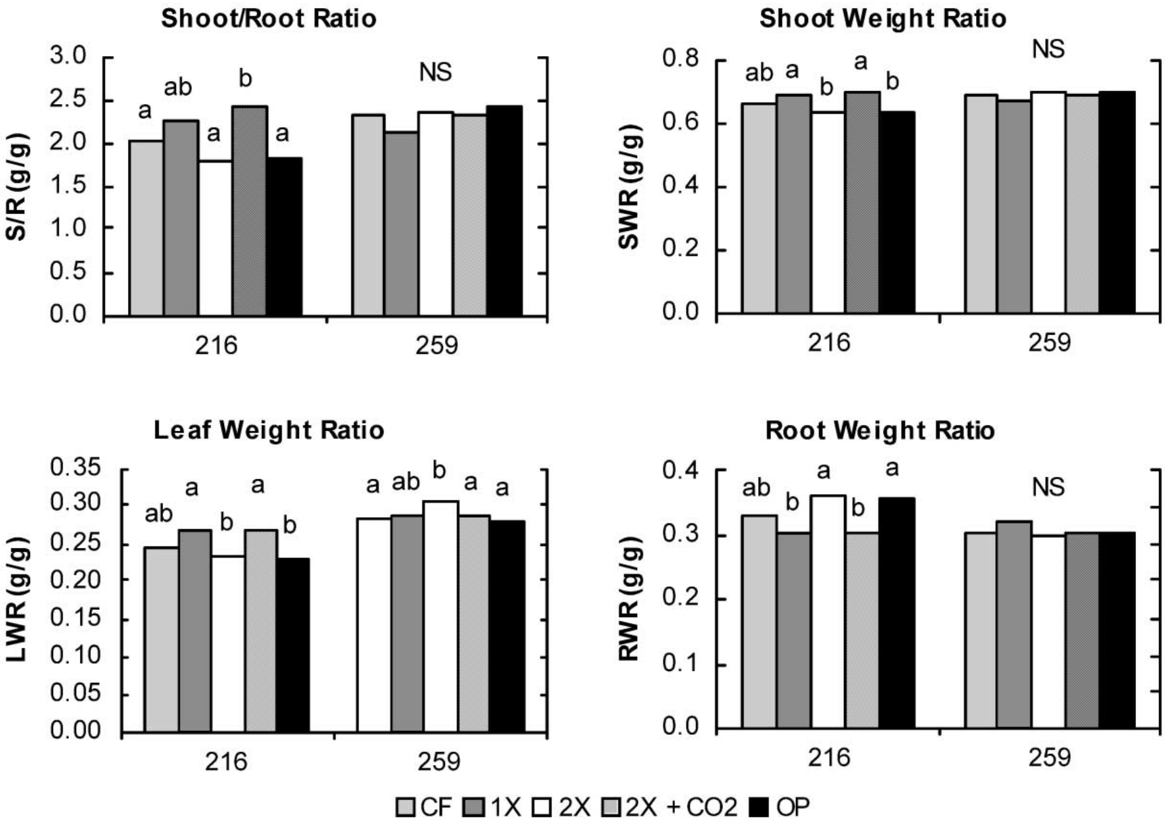

Fig. 4. Allometric responses of leaves, stems, and roots to elevated $O_3$ and $O_3$ plus $CO_2$. Means with the same letter are not statistically different at the 10% level. Treatments as in Fig. 1. Leaf weight ratio (LWR) is the total leaf weight/total plant dry weight in g/g. Shoot weight ratio (SWR) and root weight ratio (RWR) are calculated like the LWR.

older mature leaves (Coleman et al., 1995a). In addition, ozone exposure may have little impact on the expanding leaves during development. Frost et al. (1991) found that $O_3$ exposure had no effect on any parameter involved in leaf expansion or in final leaf size of hybrid poplar (*Populus*×*euramericana* Dode. Guinier). However, such results are not universal. In previous studies with our aspen clones, leaf initiation rate, internode length, leaf size, leaf weight, and height growth were not generally affected by $O_3$ exposures (Li et al., 1991; Karnosky et al., 1996). Later studies showed, however, that internode length and leaf area decreased with $O_3$ exposure while leaf initiation rate increased, particularly in clone 259 (G.A. Pieters, 1996 unpublished). Similarly, other studies with aspen (Matyssek et al., 1993a; Greitner et al., 1994) and hybrid poplar (Woodbury et al., 1994) showed that leaf size, internode length, and branch length decreased with $O_3$ exposure. In contrast, studies with birch (*Betula pendula* and *B. pubescens*) found that leaf production and height growth increased with exposure to low concentrations of $O_3$ (1.5× ambient) while essentially all other factors that were measured decreased (Pääkkonen et al., 1996, 1997).

The reason(s) for these different results are not known but probably involve differences in experimental procedures, environments, and genotypes. The aspen clones in our current study had rather large negative growth responses of branch lengths and weights to $O_3$ exposure, current terminal lengths decreased by 33% and terminal dry weights decreased by 68% ($2\times O_3$ compared to the CF treatment). Additional stress such as nitrogen deficiency or water stress may limit the ability of aspen plants to compensate for $O_3$ exposures (Pell et al., 1994). Studies with birch (*B. pendula* Roth) showed that low N levels increased the response to $O_3$ exposure (Pääkkonen and Holopainen, 1995). Another study showed that negative growth responses of aspen to $O_3$ or $O_3$ plus N stress and $O_3$ plus water stress were additive (Greitner et al., 1994). The soil at the Alberta, Michigan open-top site was relatively infertile (leaf N content for current terminal leaves was 2.4% for the CF and $2\times O_3$ treatment plants and 1.6% for the $2\times O_{3+}CO_2$ plants). The low N availability may have increased the $O_3$ response.

Young developing leaves and expanding stem internodes are the strongest sinks in rapidly growing *Populus* plants, and up to 70% of the photosynthate from recently mature leaves may be allocated to their development (Coleman et al., 1995b). In contrast, over 90% of the photosynthate from mature leaves is allocated to lower stem and roots. Because $O_3$ impacts are greatest

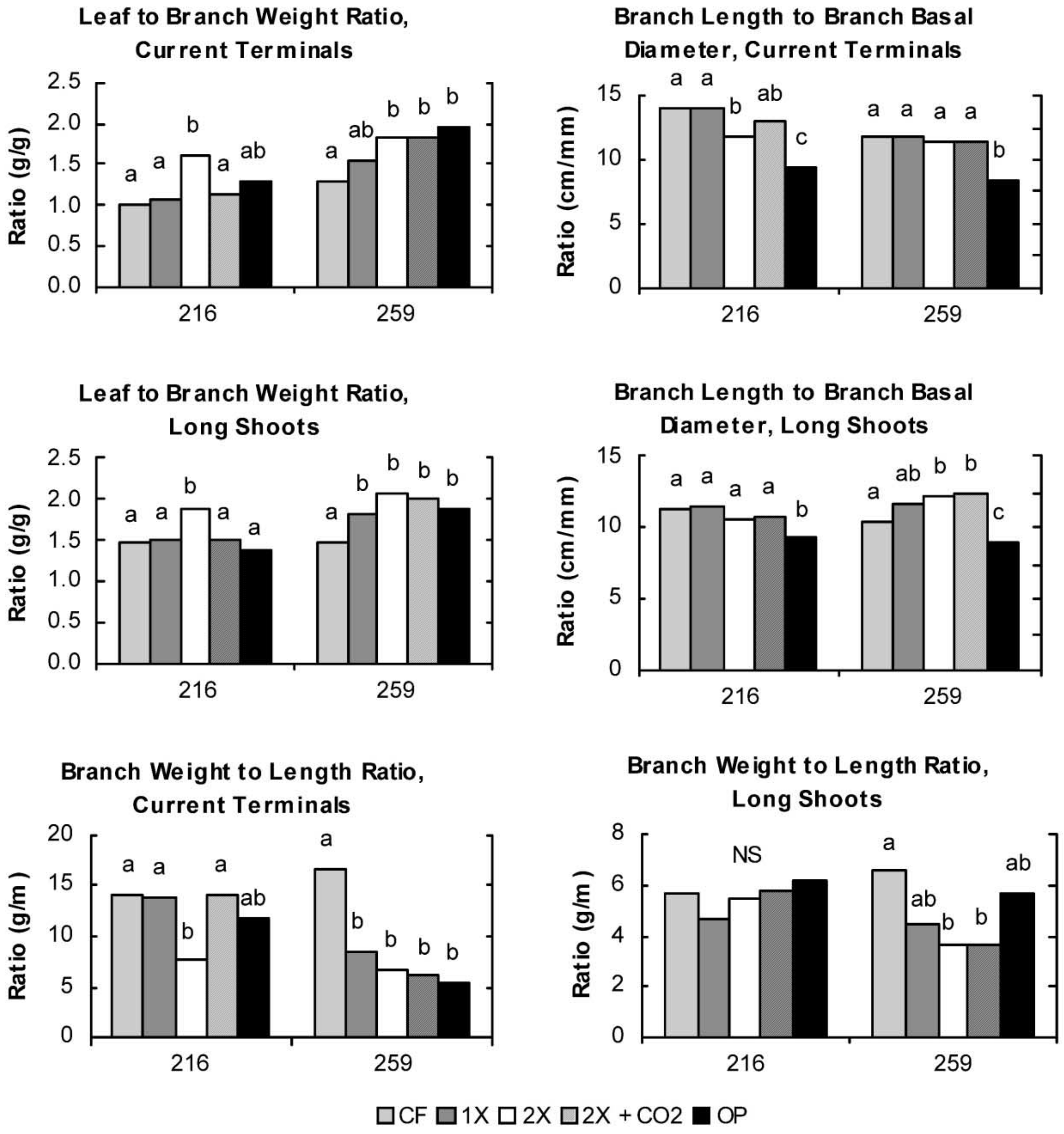

Fig. 5. Allometric responses of current year leaves and branches to elevated $O_3$ and $O_3$ plus $CO_2$. Means with the same letter are not statistically different at the 10% level. Treatments as in Fig. 1.

in the lower, mature leaf age class, the response to $O_3$ exposure should be greatest in lower stem and roots. The average decrease in stem and root dry weight for the two aspen clones with the 2×$O_3$ treatment in this experiment was 37 and 32%, respectively (Table 3). These $O_3$ impacts on growth are similar to our previous findings with aspen where stem and root growth decreased by 20 and 36%, respectively (Karnosky et al., 1996), but they are less than we found for hybrid poplar clones (stem and root dry weights decreased on average for the five clones by 51% and 56%, respectively; Dickson et al., 1998).

The response to $O_3$ exposure was always greater in the $O_3$ sensitive clone 259. For example, with the 2×$O_3$ treatment, total root dry weight decreased by 23% for clone 216 and 45% for clone 259, and total plant dry weight decreased by 28% for 216 and 43% for 259. The addition of $CO_2$ to the 2×$O_3$ treatment largely alleviated $O_3$ impacts in clone 216 but did not improve growth of clone 259 (Table 3), pointing out the importance of genotype in the response obtained. In a recent study with hybrid poplar, the addition of $CO_2$ to the $O_3$ exposure alleviated the detrimental response to $O_3$ for all five hybrid clones. However, $O_3$ exposure negated

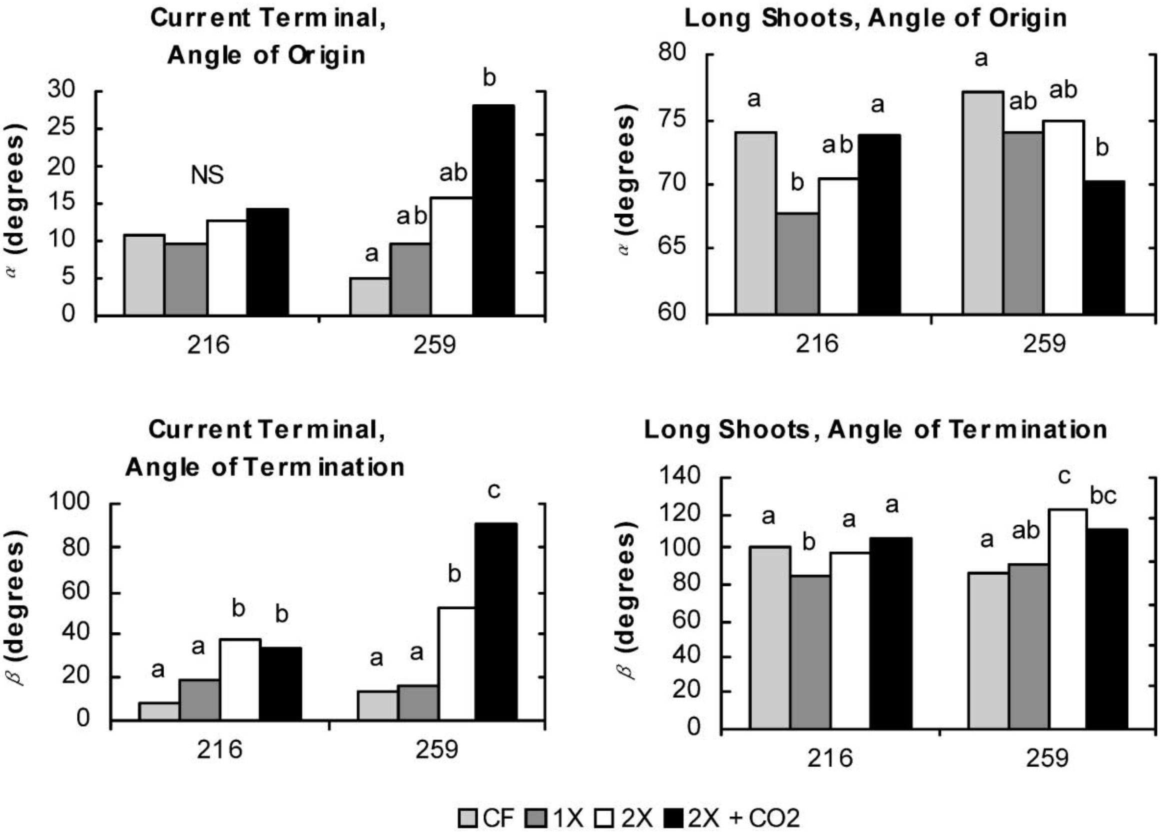

Fig. 6. Crown architectural responses of branches to elevated $O_3$ and $O_3$ plus $CO_2$. The angle of origin (α) and angle of termination (β)(degrees of displacement from vertical or 0°) were measured on all plants not harvested and left in the ground in the open-top chambers (nine plants per treatment for each clone). Means with the same letter are not statistically different at the 10% level. Treatments as in Fig. 1. Branch angles were not measured on the open-plot (OP) plants. Long-shoot angles are the average of all long shoots on the nine plants per treatment for each clone.

increased growth from the addition of $CO_2$ (Dickson et al., 1998).

### 4.3. Allometric responses

Plants respond to stress not only with changes in photosynthetic rates and growth rates but also with changes in carbon allocation within the plant. Carbon allocation within a plant depends on inherent growth strategy and response to different environments (Chapin, 1991; Laurence et al., 1994). Rapidly growing plants such as poplars store relatively little carbon during the growing season (Nelson and Dickson, 1981) and use most of the carbon fixed in photosynthesis for growth. Such plants respond to stress with changes in photosynthetic rates and shifts in carbon allocation within the plant (Pell et al., 1994; Dickson et al., 1998). The sensitivity to a particular stress in aspen has a strong genetic component (Karnosky et al., 1996, 1999). The clones in this current study were chosen for their relative sensitivity to $O_3$ exposure; clone 216 is fairly tolerant while clone 259 is quite sensitive. This relative sensitivity results from differing impacts of $O_3$ on carbon fixation rates and the ability to compensate for $O_3$ stress in different ways (Coleman et al., 1995a, 1995b; Kull et al., 1996; Sheng et al., 1997; Karnosky et al., 1999).

Changes in allometric ratios such as shoot/root ratio (S/R), leaf weight ratio (LWR), or root weight ratio (RWR) in response to treatment indicate major changes in carbon allocation within the plant. Shoot/root ratios are probably the most common allometric parameter measured in studies of plant response to stress. Shoot/root ratios usually increase in plants exposed to $O_3$ (Cooley and Manning, 1987), and this increase is particularly common in rapidly growing plants such as hybrid poplars where recently fixed carbon is preferentially allocated to new leaf and shoot growth (Matyssek et al., 1993b; Woodbury et al., 1994; Dickson et al., 1998). No change in S/R may indicate that carbon allocation to shoots and roots declines equally with $O_3$ exposure (Cooley and Manning, 1987), or it may indicate a shift of carbon from recently mature leaves to root growth instead of new leaf development. Similarly, a decrease in S/R may indicate an increase in carbon allocation to roots to compensate for $O_3$ stress. The ability to increase photosynthetic rates in recently mature leaves and reallocate carbon to lower stem and roots after $O_3$ damage to lower mature leaves is an

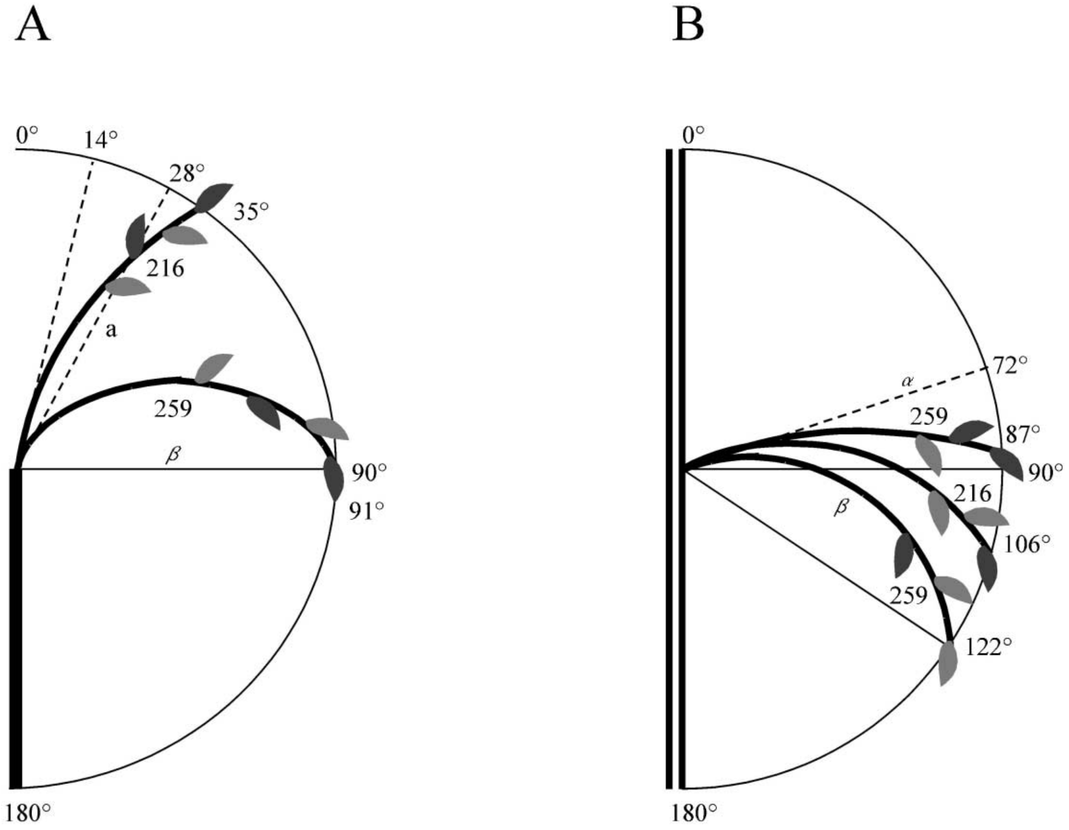

Fig. 7. Representative maximum crown architectural responses of current terminal and lateral branches to elevated $O_3$ and $O_3$ plus $CO_2$. The angle of branch origin (α, dotted line) and the angle of branch termination (β, solid line) were measured as degrees from vertical. (A) Current terminal. Angle α for the $2{\times}O_3 + CO_2$ treatment was 14° and 28°, and angle β was 35° and 91° for clone 216 and 259, respectively. (B) Lateral branches. Angle α was 72° for both 216 and 259. Angle β for clone 216 was 100° and 106° for CF and $2{\times}O_3 + CO_2$ treatment, respectively. Angle β for clone 259 was 87° and 122° for the CF and $2{\times}O_3$ treatments, respectively. Number of plants measured and significant differences for all treatments are given in Fig. 6. Numbers given above are average values for all current terminals and lateral branches measured.

important component of compensation in aspen (Coleman et al., 1995 a, b). Previous studies with our different aspen clones have shown that the clones differ in ability to reallocate carbon to roots (271 > 216 > 259; Coleman et al., 1995b). In this current study, $O_3$ exposure of clone 259 decreased total plant weight by 43% (Table 3), but there were no significant changes in S/R, SWR, and RWR (Fig. 4). Leaf weight ratio increased somewhat with the $2{\times}O_3$ treatment, indicating either that more carbon was allocated to leaves or that more leaves were required to produce the same total plant dry weight. In other words, when leaf photosynthetic rates decreased with the $O_3$ exposure, the total carbon budget was smaller, and less carbon was available for stem growth. In contrast, $O_3$ exposure of clone 216 decreased S/R, LWR, and SWR and increased RWR, indicating a potential shift in carbon allocation from shoots to roots. These results were similar to those found previously for these clones (Coleman et al., 1995b). Although both clones increased carbon allocation from recently mature leaves to roots with $O_3$ exposure compared to the CF treatment, clone 216 allocated significantly more total carbon to roots than clone 259 because $O_3$ exposure of clone 259 decreased photosynthetic rates and increased loss of lower leaves compared to clone 216 (Coleman et al., 1995a). The addition of $CO_2$ to the $O_3$ treatment increased carbon allocation to shoots compared to the $2{\times}O_3$ treatment in clone 216 but had no effect on clone 259. The decrease in S/R with $O_3$ exposure in clone 216 is probably a real change in carbon allocation and not the result of different plant size (Gebauer et al., 1996) because total plant dry weight decreased less in clone 216 than in clone 259 for the $2{\times}O_3$ treatment (Fig. 3; Table 3).

Allometric changes occur not only within the whole plant such as changes in S/R or LWR but also within branches and within the crown. These allometric changes within the crown determine crown architecture and may have significant impacts on the ability to compete for light. Ozone exposure initially impacts leaf mesophyll cells and decreases photosynthetic rates and carbon fixation (Landry and Pell, 1993; Coleman et al., 1995a). Decreased photosynthetic rates mean less carbon is available for metabolic processes within the leaf and for transport to other parts of the plant. Premature leaf senescence and abscission also decrease total carbon availability. Mature leaves are largely autonomous with respect to assimilation and use of carbon (Dickson, 1991). Mature source leaves fix carbon, use some for respiration and tissue maintenance, and export the remainder. Less carbon available for transport decreases branch growth and wood formation within the branch and changes branch dimensions. Leaf weight/branch weight ratios increased for both clones with the $2\times O_3$ treatment (Fig. 5). An increase in this ratio indicates more leaves were required to produce the same unit weight of branches. In actuality, both the effective leaf area and leaf weight decrease with $O_3$, but the relative weight of branches decreases even more.

The decrease in branch wood formation is clearly shown with the branch weight/branch length (g/m) ratios. The ratios for current terminals decreased in both clones (54 and 59% in 216 and 259, respectively) with the $2\times O_3$ treatment compared to the CF treatment. Similar decreases in photosynthetic efficiency, leaf area, and branch weights and lengths were found with 100 $nl l^{-1}$ square wave $O_3$ treatments of European birch (*B. pendula* Roth; Matyssek et al., 1992), aspen (*P. tremula* L.; Matyssak et al., 1993a), and hybrid poplar (*Populus × euramericana*; Matyssek et al., 1993b). The decrease in branch weight/length ratio was the result of thinner secondary cell walls in the xylam in response to $O_3$ (Matyssak et al., 1993a). Secondary cell wall thickness is an important component of wood formation.

These changes in wood formation of branches have a direct effect on crown architecture. The internally weak and thin branches droop conspicuously as shown by the large angle of termination for both clones (Fig. 7). These structurally weak and drooping branches are not efficient in light capture compared to a fastigiate crown with upright, acute branch angles (Nelson et al., 1981; Dickmann and Keathley, 1996). In contrast to other factors measured, the addition of $CO_2$ to the $2\times O_3$ treatment did not alleviate branch droop in clone 216 and made it greater in clone 259. Additional changes in crown architecture in response to $O_3$ exposure were noted that may also influence productivity. More short shoots were produced on clone 216 (Figs. 2 and 3) and more short shoots elongated into long shoots on clone 259, suggesting a change in hormonal relations with $O_3$ exposure (data not shown). In addition, there was significantly more branch abscission for both clones with $O_3$ exposure. Short shoots and smaller long shoots with large amounts of leaf abscission were particularly vulnerable to premature abscission. Short shoots may contain a major proportion of leaf area in young aspen (Pollard, 1970) and birch (Kauppi et al., 1988) and contribute significantly to total carbon fixation and tree or stand productivity because they provide a lot of leaf area with little branch mass to support.

### 4.4. *Interactions of ozone and carbon dioxide*

Because elevated $CO_2$ exposure usually increases carbon fixation, decreases stomatal conductance, and increases resistance to other environmental stresses, it is generally believed that increasing atmospheric $CO_2$ concentrations may alleviate the negative effects of $O_3$ on plant growth (Allen, 1990). However, recent studies on the interacting effects of $CO_2$ and $O_3$ are contradictory. Some studies have shown that elevated $CO_2$ may counteract negative $O_3$ responses (Mortensen, 1995; Mulholland et al., 1997; Volin et al., 1998), while others have shown that $CO_2$ did not protect against $O_3$ (Balaguer et al., 1995; Barnes et al., 1995). In a study of the interacting effects of $CO_2$ and $O_3$ with hybrid poplars, $CO_2$ alleviated the negative growth responses of $O_3$ in all five hybrid clones. However, the $O_3$ exposure negated the increase in growth from $CO_2$ (Dickson et al., 1998). In another study with the aspen clones 216 and 259, $CO_2$ did not counteract the negative effects of $O_3$ on photosynthesis. In fact, photosynthesis of the $O_3$ tolerant clone 216 decreased more with $O_3$ exposure in the $CO_2$ plus $O_3$ treatment compared to $O_3$ alone (Kull et al., 1996). The reasons for these differing responses between studies to interacting $CO_2$ and $O_3$ are not known but probably result from differences in procedures and environmental stresses. All open-top studies involve environmental stresses other than those imposed by the exposure gases (McLeod and Long, 1999). Such multiple environmental stresses may increase or decrease the response to $CO_2$ and $O_3$, and the actual response obtained will be modified by the genotypic response of the different clones.

Open-top chambers are widely used to study the response of plants to air pollutants, but chamber effects are always present and complicate the interpretation of responses (Olszyk et al., 1986; McLeod and Long, 1999). Chamber effects were particularly large in this study with aspen. Plants grown in the open plots (OP) were smaller in essentially all growth parameters measured compared to the CF treatment (Figs. 1–3). Clone 259 was the most severely impacted by the open-growth conditions. In most cases, growth of clone 259 in the open was much less than that found in the CF treatment and was similar to that found in the $1\times O_3$ and $2\times O_3$ exposures. Ambient seasonal $O_3$ exposures at the Alberta, Michigan site were similar to the experimental $1\times O_3$ treatment (Table 2). The large decrease in growth of clone 259 in the open-air plots may indicate that this $O_3$ sensitive clone is responding to the relatively low ambient $O_3$ seasonal exposure (50 plus $\mu l l^{-1}$-h) along with other open-grown stresses at the Alberta, Michigan location. Clone 259 was also the most responsive of all clones tested to ambient $O_3$ concentrations in an $O_3$ gradient study (Karnosky et al., 1999).

## 5. Conclusions

Increasing atmospheric $CO_2$ concentrations and tropospheric $O_3$ concentrations will have widespread impacts on tree growth in the near future. Trembling aspen, a widespread and economically important tree species in the northern United States and Canada, is very sensitive to $O_3$ exposure. Decreases in shoot and root production of 20 to 30% after one growing season of $O_3$ exposure have been reported (Karnosky et al., 1996). Similar responses were found in this current study and these impacts are expected to compound with $O_3$ exposure during subsequent growing seasons. In addition to decreases in dry weight, crown architecture is also affected. Decreases in carbon fixation that result in less carbon available for wood formation produce structurally weak and drooping branches that are inefficient in light capture. All of these growth responses have a strong genetic component. Aspen clones sensitive to $O_3$ and other atmospheric pollutants show the greatest response and will be at a competitive disadvantage in dense stands.

Elevated $CO_2$ concentrations are expected to alleviate the detrimental effects of $O_3$ and other environmental stresses. The addition of $CO_2$ to the highest $O_3$ exposure in this study counteracted the $O_3$ response only in the $O_3$ tolerant clone (216), but had no effect on the $O_3$ sensitive clone (259). Genetic variation in response to atmospheric pollutants must be considered when interpreting current studies and when predicting future responses.

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