

# Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone

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Received August 16, 1993

**Summary** Rooted cuttings from three aspen (*Populus tremuloides* Michx.) clones (216, 271 and 259, classified as high, intermediate and low in O<sub>3</sub> tolerance, respectively) were exposed to either diurnal O<sub>3</sub> profiles simulating those of Michigan's Lower Peninsula (episodic treatments), or diurnal square-wave O<sub>3</sub> treatments in open-top chambers in northern Michigan, USA. Ozone was dispensed in chambers ventilated with charcoal-filtered (CF) air. In addition, seedlings were compared to rooted cuttings in their response to episodic O<sub>3</sub> treatments. Early in the season, O<sub>3</sub> caused decreased photosynthetic rates in mature leaves of all clones, whereas only the photosynthetic rates of recently mature leaves of the O<sub>3</sub>-sensitive Clone 259 decreased in response to O<sub>3</sub> exposure. During midseason, O<sub>3</sub> caused decreased photosynthetic rates of both recently mature and mature leaves of the O<sub>3</sub>-sensitive Clone 259, but it had no effect on the photosynthetic rate of recently mature leaves of the O<sub>3</sub>-tolerant Clone 216. Late in the season, however, photosynthetic rates of both recently mature and mature leaves of Clone 216 were lower than those of the control plants maintained in CF air. Ozone decreased the photosynthetic rate of mature leaves of Clone 271, but it increased or had no effect on the photosynthetic rate of recently mature leaves. Photosynthetic response patterns of seedlings to O<sub>3</sub> treatment were similar to those of the clones, but total magnitude of the response was less, perhaps reflecting the diverse genotypes of the seedling population. Early leaf abscission was observed in all clones exposed to O<sub>3</sub>; however, Clones 216 and 259 lost more leaf area than Clone 271. By late August, leaf area in the highest O<sub>3</sub> treatment had decreased relative to the controls by 26, 24 and 9% for Clones 216, 259 and 271, respectively. Ozone decreased whole-tree photosynthesis in all clones, and the decrease was consistently less in Clone 271 (23%) than in Clones 216 (56%) and 259 (56%), and was accompanied by declines in total biomass of 19, 28 and 47%, respectively. The relationship between biomass and whole-tree photosynthesis indicates that the negative impact of O<sub>3</sub> on biomass in the clones was determined largely by lower photosynthetic productivity of the foliage, rather than by potential changes in the carbon relations of other plant organs.

**Keywords:** biomass production, leaf abscission, leaf age classes, photosynthesis, photosynthetic light-response curves, *Populus tremuloides*, whole-tree photosynthesis.

## Introduction

Tropospheric ozone is a potent, widespread phytotoxin that causes significant damage to plants (Adams et al. 1989). But relatively little is known about O<sub>3</sub>-stress tolerance in forest trees (Sasek and Richardson 1989). Previous studies have shown that the negative effects of O<sub>3</sub> on productivity are correlated with decreasing stomatal conductance and photosynthesis, premature leaf abscission, and decreases in leaf size, all of which decrease total carbon accumulation (Reich 1987, Pye 1988, Matyssek et al. 1993). The decrease in photosynthesis in response to O<sub>3</sub> may result from oxidative damage to cell membranes, light harvesting processes or carbon fixation (Pell 1987, Landry and Pell 1993).

Genetic differences in the growth response of tree species to O<sub>3</sub> have been found (Wang et al. 1986), but little is known about the mechanisms underlying these differences. To investigate the physiological basis for genetic differences in O<sub>3</sub> sensitivity, we examined photosynthesis and biomass allocation responses to O<sub>3</sub> exposure in aspen (*Populus tremuloides* Michx.) clones and seedlings that differ in O<sub>3</sub> sensitivity.

## Methods

### Plant material

Aspen clones from the Great Lakes region ranging in sensitivity to O<sub>3</sub> (216, high O<sub>3</sub> tolerance; 271, intermediate O<sub>3</sub> tolerance; and 259, low O<sub>3</sub> tolerance) were selected based on susceptibility to foliar injury and decreases in biomass after O<sub>3</sub> exposure (Karnosky et al. 1992). Experimental plants were vegetatively propagated as softwood cuttings from vigorously growing shoots from potted stock material. Rooted cuttings were transplanted to 6-l pots containing perlite/peat moss/organic top soil (1/1/1 v/v) and 8 g of slow release (3- to 4-month release) fertilizer (17/6/12 N,P,K plus minor nutrients, Sierra

Chemical Corp., Milpitas, CA). Transplants were grown in a greenhouse (21 °C, 17-h photoperiod) until transferred to shaded field conditions on June 6, 1990. Plants were placed in open-top chambers located in northern Michigan on June 18, and ozone treatments were started June 20. In 1991, plants were transferred to shaded field conditions on June 4 and placed in open-top chambers on June 8; exposures started on June 9. In 1990, each plant had about 25 leaves before treatment, and in 1991, each plant had about 14 leaves.

In 1991, aspen seedlings, reared from seed collected from native northern Michigan populations, were also transplanted to the same potting mix described for the clonal plantlets. Seedlings were grown in a greenhouse for 4 weeks, transferred to shaded field conditions on June 4 and placed in open-top chambers on June 8. Seedlings (six leaves) were smaller than the clonal plants when placed in the open-top chambers. Plants subjected to episodic O<sub>3</sub> treatments in both 1990 and 1991 and plants subjected to square-wave treatments in 1991 were harvested in mid-September.

#### Ozone exposures

The episodic O<sub>3</sub> exposures of clones and seedlings designed to mimic natural O<sub>3</sub> exposures were based on diurnal ambient O<sub>3</sub> profiles constructed from O<sub>3</sub> data collected in Michigan's Lower Peninsula in cooperation with the US Environmental Agency (Karnosky, unpublished data). The simulated ambient profiles were, therefore, not related to or based on current ambient O<sub>3</sub> concentrations at the northern Michigan site. The simulated ambient profile (1×) provided hourly target concentrations achieved by means of automatic O<sub>3</sub> dispensing equipment. Target values were doubled for the twice ambient (2×) O<sub>3</sub> treatment. The O<sub>3</sub> treatments were compared to a charcoal-filtered (CF) air treatment (control). Ozone for the 1× and 2× treatments was generated from pure oxygen with an OREC Model V10-0 O<sub>3</sub> generator and dispensed into open-top chambers ventilated with CF air. There were three chambers (i.e., replications) per treatment. Square-wave O<sub>3</sub> exposures of clones, a procedure designed to deliver a constant amount of O<sub>3</sub>, were also applied in 1991. Two open-top chambers were ventilated with CF air (control) and two chambers were treated with 100 ppb O<sub>3</sub> for 6 h per day for four consecutive days per week. Target concentrations were maintained by manually adjusting the ozone generator output. Ozone concentrations were monitored in all chambers on a time-shared basis with a Scanivalve System and TECO model 49 O<sub>3</sub> analyzers and a Monitor Labs model 8500 O<sub>3</sub> calibrator. Mean hourly O<sub>3</sub> concentrations for each chamber were recorded and summed over the growing season. Values thus obtained were reflective of O<sub>3</sub> doses observed in the northeastern USA (Table 1) (cf. Lefohn and Pinkerton 1988).

#### Gas exchange measurement

Photosynthetic carbon exchange rate (CER) was measured under controlled environmental conditions and under ambient conditions in the open-top chambers with an LI-6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE). Environ-

Table 1. Cumulative O<sub>3</sub> doses for episodic treatments in 1990 and 1991 and the square-wave treatment in 1991. Dose values (ppm-h) are presented as cumulative hourly means of O<sub>3</sub> for three open-top chambers. Cumulative doses for the date given can be related to photosynthetic measurements (Figures 3–6). The last date represents the total seasonal exposure (charcoal-filtered air = CF, ambient O<sub>3</sub> = 1×, twice ambient O<sub>3</sub> = 2×).

Measurement period	Episodic treatment		
	CF	1×	2×
<i>1990</i>			
July 8	2	7	7
July 20	3	16	20
August 1	5	29	38
August 16	7	40	56
September 9	7	49	68
September 16	8	50	70
<i>1991</i>			
July 11	6	31	33
August 1	11	45	62
August 29	17	62	77
September 14	22	70	92
Square-wave treatment			
	CF	O <sub>3</sub>	
<i>1991</i>			
June 28	2	7	
July 26	7	22	
September 6	15	48	
September 14	16	53	

mental conditions were controlled by placing the LI-6200 1-liter cuvette in a small room cooled with a thermostatically controlled air conditioner. Air containing ambient CO<sub>2</sub> concentrations was pumped from outside at a height of 4 m into a precision temperature-controlled bubbler, to control vapor pressure, and then to the cuvette. During photosynthetic measurements, air flow was switched from the open-flow conditioned air source to the transient Li-Cor system for data collection. Photosynthetic light-response curves were developed for plants in each treatment. The CER of individual recently mature leaves was measured at different irradiances. Variable irradiances (0 to 1600 μmol m<sup>-2</sup> s<sup>-1</sup>) were produced by shading a high intensity OSRAM lamp (HQI-TS-250W/NOL, Berlin, Germany). Irradiances inside the cuvette were checked with a quantum sensor (LI-190S, Li-Cor Inc.).

To determine leaf positional and seasonal effects on gas exchange, plants were measured in the open-top chambers under ambient CO<sub>2</sub> concentrations and saturating light (> 600 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation, PAR). Photosynthesis and stomatal conductance were measured on a single, recently mature leaf and a single mature leaf, or on a series of leaves down the plant (Figure 1). Cloudy conditions often forced us to take measurements on several consecutive days. Respiration of plants in the episodic and square-wave O<sub>3</sub> exposure treatments was measured at least 1 h after dark.

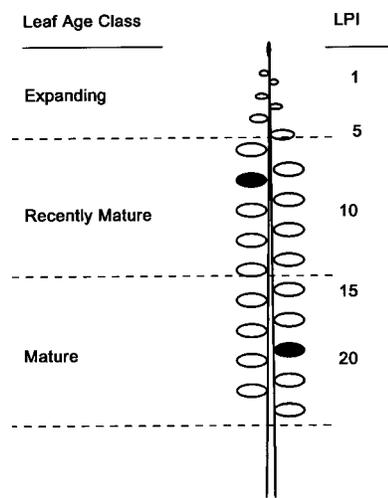


Figure 1. The relationship between leaf age class and leaf plastochron index (LPI, Larson and Isebrands 1971). The shading indicates the position of leaves in which photosynthesis was measured.

#### Leaf area

Leaf area was estimated from leaf length by leaf area regression equations developed for a set of companion plants. Leaf area was measured on detached leaves with an LI-3000A portable leaf area meter (Li-Cor Inc.). Data were collected and linear equations were developed for each clone  $\times$  treatment combination at each measurement period. Nondestructive leaf length measurements were then used to predict individual and whole-plant leaf areas.

#### Whole-tree photosynthesis

Whole-tree photosynthesis (WTP) was calculated as the product of leaf area and photosynthetic carbon exchange rate (CER) for each tree measured. In 1990, CER for the recently mature and mature leaf age classes was averaged and multiplied by whole-tree leaf area to calculate WTP. In 1991, two to five leaf age classes were defined by position on the stem. Photosynthesis for each leaf age class was estimated from the photosynthetic rate of one representative leaf and the age class leaf area. Photosynthetic values for each age class were summed to obtain WTP. Wolf et al. (1995) showed that measurement of any leaf in each of two stratified mature-leaf age classes is sufficient to define WTP of early season first-year trees. As plants grew larger, however, it was necessary to add additional leaf age classes in the mature leaf zone (i.e., one photosynthetic measurement leaf for an age class of 10 leaves). The area of leaves in the expanding leaf zone was included with the recently mature leaf age class because only the largest expanding leaves had enough area or CER to be of consequence.

#### Statistical analysis

Photosynthetic light-response curves were fitted to the model of Hanson et al. (1987) by means of quasi-Newton nonlinear iterative minimization methods (SYSTAT Inc., Evanston, IL

(Wilkinson 1990). The hypothesis that ozone treatment resulted in different photosynthesis versus irradiance relations was tested by comparing nonlinear regressions according to the  $f$ -test method of Ratkowsky (1983). Analysis of variance was used to test differences among model parameters. Photosynthetic measurements in the leaf position series and the relationship between WTP and biomass were analyzed by least squares linear regression analysis and 95% confidence intervals were calculated by means of a graphics and statistical analysis program (SigmaPlot, Jandel Scientific, San Rafael, CA). Mean and standard error for in-chamber measurements of photosynthesis, dark respiration, stomatal conductance and leaf area were calculated from two seedlings or two trees per clone in each episodic or square-wave exposure treatment chamber.

## Results

#### Photosynthetic light-response curves

The  $2\times O_3$  treatment decreased the light-saturated photosynthetic rate of recently mature leaves of the  $O_3$ -sensitive Clone 259, had little or no effect on Clone 216, and increased the photosynthetic rate of Clone 271 (Figure 2). A family of light-response curves developed from photosynthetic data collected under controlled environment conditions throughout the growing season showed more variability in response to the  $2\times O_3$  treatment than to the CF treatment (Table 2).

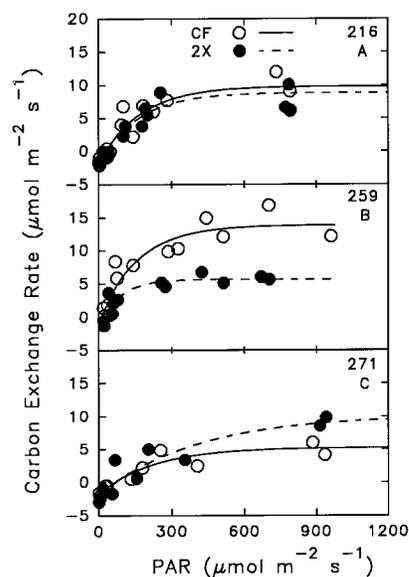


Figure 2. Photosynthetic light-response curves of aspen clones. Three aspen clones (216, 259, 271) were exposed to two episodic  $O_3$  treatments, charcoal-filtered air (CF) and twice ambient ( $2\times$ ). Recently mature leaves on two trees per clone in each treatment were measured to determine carbon exchange rates at several irradiances on July 19–20, 1990, for Clones 259 and 216 and on August 7–9, 1990, for Clone 271. Response curves were fitted according to the model of Hanson et al. (1987). Regressions differed between treatments for Clone 259 ( $P < 0.001$ ) and Clone 271 ( $P = 0.003$ ) but not for Clone 216 ( $P = 0.675$ ).

Table 2. Mean ( $\pm$  standard errors) model parameters for light-response curves constructed for three aspen clones (216, 259, 271) exposed to two episodic O<sub>3</sub> treatments (charcoal-filtered air = CF, twice ambient O<sub>3</sub> = 2 $\times$ ,  $n$  = number of leaves measured and number of response curves). Probability values less than 0.05 are considered significant.

Clone	Treatment	Maximum CER $\mu\text{mol m}^{-2} \text{s}^{-1}$	Dark respiration $\mu\text{mol m}^{-2} \text{s}^{-1}$	Compensation irradiance $\mu\text{mol m}^{-2} \text{s}^{-1}$	Quantum efficiency $\mu\text{mol } \mu\text{mol}^{-1}$
216	CF, $n = 6$	9.2 $\pm$ 0.9	2.1 $\pm$ 0.3	48.9 $\pm$ 11.4	0.056 $\pm$ 0.009
	2 $\times$ , $n = 6$	9.2 $\pm$ 1.7	2.2 $\pm$ 0.2	88.3 $\pm$ 48.7	0.055 $\pm$ 0.013
259	CF, $n = 8$	9.3 $\pm$ 0.8	1.7 $\pm$ 0.2	40.5 $\pm$ 8.0	0.055 $\pm$ 0.009
	2 $\times$ , $n = 9$	5.8 $\pm$ 1.4	3.3 $\pm$ 0.6	49.6 $\pm$ 8.0	0.101 $\pm$ 0.037
271	CF, $n = 4$	5.8 $\pm$ 0.6	1.7 $\pm$ 0.1	60.1 $\pm$ 8.9	0.035 $\pm$ 0.005
	2 $\times$ , $n = 3$	9.8 $\pm$ 0.3	2.6 $\pm$ 0.3	75.7 $\pm$ 28.8	0.046 $\pm$ 0.011
Mean	CF, $n = 18$	8.5 $\pm$ 0.6	1.8 $\pm$ 0.1	47.7 $\pm$ 5.6	0.051 $\pm$ 0.005
	2 $\times$ , $n = 18$	7.6 $\pm$ 1.0	2.8 $\pm$ 0.3	67.8 $\pm$ 17.7	0.077 $\pm$ 0.020

Source	Analysis of variance probabilities			
Ozone	0.876	0.037*	0.292	0.392
Clone	0.371	0.638	0.470	0.355
O $\times$ C	0.041*	0.165	0.767	0.579

Model parameters calculated from the photosynthetic light-response curves followed the same pattern (Table 2). However, there was no effect of the 2 $\times$  O<sub>3</sub> exposure on photosynthetic rate averaged across all clones because of the contrasting responses of Clones 259 and 271. Ozone increased the dark respiration rates of Clones 259 and 271. In all clones, compensation points increased in response to O<sub>3</sub>, but the values did not differ significantly among clones. Quantum efficiency of all clones was similar in both the CF and 2 $\times$  O<sub>3</sub> treatments.

#### Photosynthetic carbon exchange

In all clones, photosynthetic rates of recently mature leaves

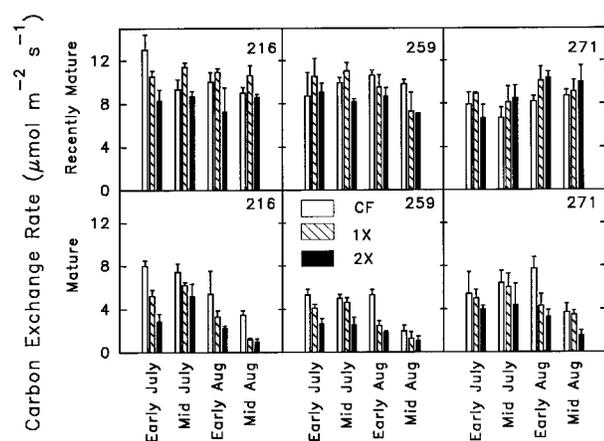


Figure 3. Photosynthetic carbon exchange rates (CER) for recently mature and mature leaves determined during the 1990 growing season. Three aspen clones (216, 259, 271) were exposed to three episodic O<sub>3</sub> treatments (CF, 1 $\times$  and 2 $\times$ ). Light-saturated photosynthetic CER was measured at approximately 2-week intervals throughout the 1990 growing season (average measurement dates: July 8 and 20, August 1 and 16). Each bar shows the mean and standard error of one leaf on each of six trees.

were greater than those of mature leaves (Figure 3). There was little seasonal effect on CER of recently mature leaves, but CER of mature leaves declined during the growing season. In recently mature leaves, the 1 $\times$  O<sub>3</sub> exposure often increased CER compared to the CF treatment. The 2 $\times$  O<sub>3</sub> exposure decreased CER in recently mature leaves of Clones 216 and 259, but increased the rate in recently mature leaves of Clone 271, particularly late in the growing season. Both ozone treatments decreased CER of mature leaves (Figure 3).

Carbon exchange rates decreased with leaf plastochron index (LPI, Larson and Isebrands 1971) in all clones when measured late in the season in both years of study under episodic and square-wave treatments (Figures 4 and 5). The relationship between CER and leaf position was linear; no significant improvement in prediction was found by adding a quadratic term to the regression model (Kilenbaum and Kupper 1978). Ozone exposure increased the decline in CER as leaves aged (Figures 4 and 5). Leaf aging patterns were similar for both the episodic and square-wave O<sub>3</sub>-treated plants, but O<sub>3</sub> effects were greater in plants in the square-wave treatment. Early in the growing season (June 28), CER of plants did not decline much with leaf position in the CF treatment, but the effect of O<sub>3</sub> was severe (Figure 5). Photosynthetic rates declined rapidly in older leaves. As the season progressed, CER curves of plants in the CF and 2 $\times$  O<sub>3</sub> treatments tended to converge. However, CER was always lower in O<sub>3</sub>-treated plants, particularly in Clone 259, than in plants in the CF treatment.

Stomatal conductance decreased during the growing season in both recently mature and mature leaves (Figure 6), and was generally greater in recently mature leaves than in mature leaves. In recently mature leaves, ozone effects on stomatal conductance varied among the clones and over the season. In mature leaves, O<sub>3</sub> decreased stomatal conductance, particularly early in the growing season.

Respiration rates were greater in recently mature than in

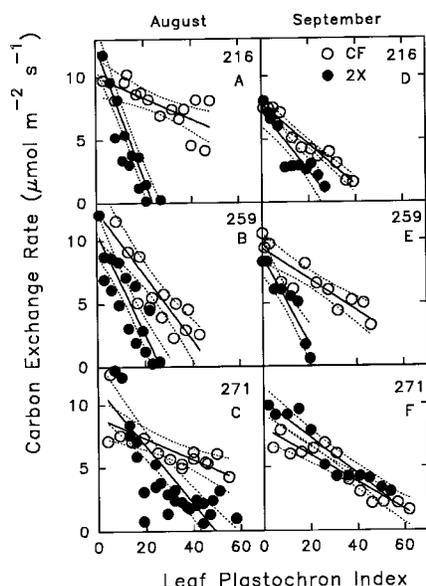


Figure 4. Changes in photosynthetic carbon exchange rates (CER) with leaf position in 1990. Three aspen clones (216, 259, 271) exposed to two episodic  $O_3$  treatments (CF and 2X) were measured on August 22 (A, B and C, two trees per treatment) and September 9, 1990 (D, E and F, one tree per treatment). Leaf position as determined by leaf plastochron index (LPI) increased from the top to the base of the plant (see Figure 1). Only fully expanded leaves were measured. Lines are plotted from linear regressions. Dotted lines represent the 95% confidence interval.

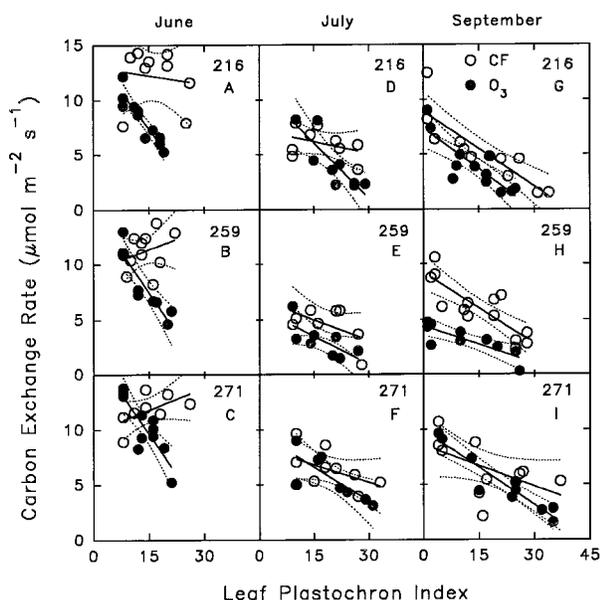


Figure 5. Changes in photosynthetic carbon exchange rates (CER) with leaf position in 1991. Three aspen clones (216, 259, 271) were exposed to two square-wave  $O_3$  treatments (CF and 100 ppb  $O_3$  for 6 h per day for 4 consecutive days per week). Leaf position was determined by leaf plastochron index (LPI). The CER was determined on June 28 (A, B, C), July 26 (D, E, F) and September 6, 1991 (G, H, I) on individual leaves from four trees per treatment. Only fully expanded leaves were measured. Lines are plotted from linear regressions. Dotted lines represent the 95% confidence interval.

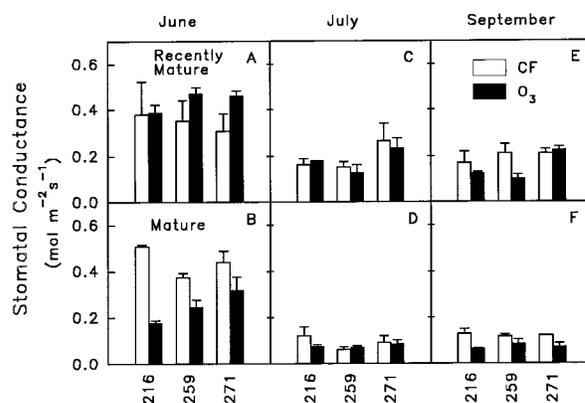


Figure 6. Stomatal conductance rates for recently mature (A, C, E) and mature (B, D, F) leaves determined in 1991. Three aspen clones (216, 259, 271) were exposed to two square-wave  $O_3$  treatments (CF and 100 ppb  $O_3$  for 6 h per day for 4 consecutive days per week). Stomatal conductance was determined during CER measurements on June 28 (A, B), July 26 (C, D) and September 6 (E, F). Each bar shows the mean  $\pm$  standard error derived from measuring one leaf on each of four trees per treatment.

older leaves and greater early in the season than later in the season. Ozone increased respiration rates in both leaf age-classes early in the growing season, but had little effect on mature leaves late in the season (Figure 7). In recently mature leaves, respiration rates also differed among the clones late in the growing season.

Seedling photosynthetic and respiration responses to episodic  $O_3$  exposures were less than in the clones, although the patterns were similar (Table 3). Ozone had little effect on CER of recently mature leaves throughout the growing season, but decreased CER of mature leaves. Respiration rates were greater in recently mature leaves than in mature leaves, but were not affected by the episodic  $O_3$  exposures.

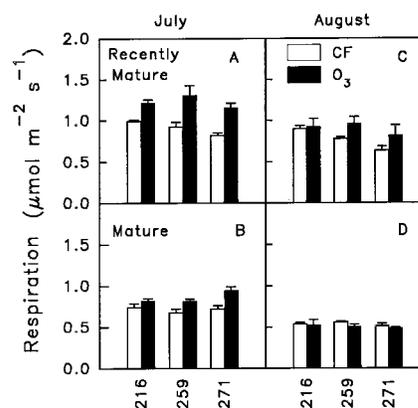


Figure 7. Respiration rates for recently mature (A, C) and mature (B, D) leaves determined in 1991. Three aspen clones (216, 259, 271) were exposed to two square-wave  $O_3$  treatments (CF and 100 ppb  $O_3$  for 6 h per day for 4 consecutive days per week). Respiration was measured 1 h after dark on July 5 (A, B) and August 23, 1991 (C, D). Each bar shows the mean  $\pm$  standard error derived from measuring one leaf on each of four trees per treatment.

Table 3. Photosynthesis and dark respiration of aspen seedlings. Data given are the means  $\pm$  standard error of one recently mature and one mature leaf on each of six plants grown under episodic  $O_3$  exposures in 1991 (see Table 1) (charcoal-filtered air = CF, ambient  $O_3 = 1\times$ , twice ambient  $O_3 = 2\times$ ).

	Photosynthesis $\mu\text{mol m}^{-2} \text{s}^{-1}$	Dark respiration $\mu\text{mol m}^{-2} \text{s}^{-1}$
<i>July 11—Recently mature leaf</i>		
CF	8.2 $\pm$ 0.27	1.2 $\pm$ 0.02
1 $\times$	8.8 $\pm$ 0.34	1.0 $\pm$ 0.05
2 $\times$	8.8 $\pm$ 0.95	1.1 $\pm$ 0.02
<i>July 11—Mature leaf</i>		
CF	6.2 $\pm$ 0.39	0.7 $\pm$ 0.05
1 $\times$	3.6 $\pm$ 0.41	0.7 $\pm$ 0.02
2 $\times$	2.4 $\pm$ 0.50	0.8 $\pm$ 0.04
<i>August 29—Recently mature leaf</i>		
CF	15.5 $\pm$ 0.82	1.3 $\pm$ 0.15
1 $\times$	14.7 $\pm$ 1.32	1.5 $\pm$ 0.13
2 $\times$	15.2 $\pm$ 0.99	1.6 $\pm$ 0.08
<i>August 29—Mature leaf</i>		
CF	5.8 $\pm$ 0.88	0.8 $\pm$ 0.08
1 $\times$	5.5 $\pm$ 0.70	1.0 $\pm$ 0.09
2 $\times$	3.5 $\pm$ 0.39	0.9 $\pm$ 0.09

### Whole-tree photosynthesis

Total leaf area increased during the growing season in all three clones in both the CF and 1 $\times$   $O_3$  treatments (Figure 8). Total leaf area in the 2 $\times$   $O_3$  treatment, however, remained constant during the season for Clones 216 and 259, but increased for Clone 271. Clone 271 retained more lower leaves in the 2 $\times$   $O_3$  treatment than the other clones. Whole-tree photosynthesis generally increased, then decreased during the growing season

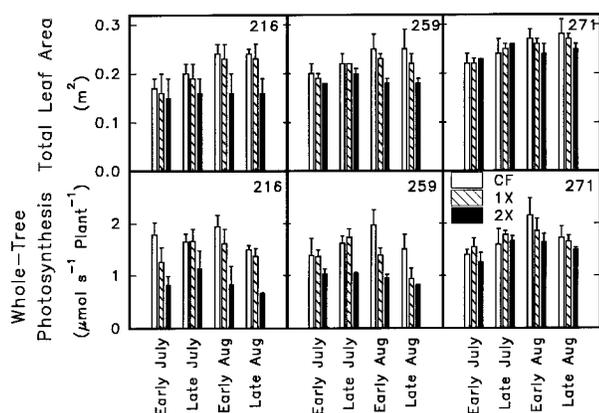


Figure 8. Total leaf area per plant and whole-tree photosynthesis (WTP) determined during the 1990 growing season. Three aspen clones (216, 259, 271) were exposed to three episodic  $O_3$  treatments (CF, 1 $\times$  and 2 $\times$ ). Total leaf area and CER (see Figure 3) were measured at approximately 2-week intervals throughout the 1990 growing season (average measurement dates: July 8 and 20, August 1 and 16). Each bar shows the mean  $\pm$  standard error of six trees per treatment. The WTP is calculated as the product of total leaf area and the average CER of recently mature and mature leaves on each tree.

for all three clones (Figure 8). Both the 1 $\times$  and 2 $\times$   $O_3$  treatments significantly inhibited whole-tree photosynthesis, particularly late in the growing season, as a result of decreased leaf area and CER. Whole-tree photosynthesis of Clone 259 was sensitive to the 1 $\times$   $O_3$  treatment, WTP of Clone 216 was sensitive to the 2 $\times$   $O_3$  treatment, and WTP of Clone 271 was not severely inhibited by either of the  $O_3$  treatments. Similar results were obtained in 1991 in both the episodic and square-wave  $O_3$  treatments.

### Discussion

#### Photosynthetic light-response curves

The analysis of photosynthetic response curves to increasing irradiance provides information about physical and biochemical factors that control photosynthetic rates such as quantum yield and other light energy reactions, and the regeneration of ribulose-1,5-bisphosphate (Stitt 1991). Our light-response curves, averaged over the growing season, suggest  $O_3$  exposure had little effect on quantum efficiency (Table 2). The upper asymptote or light-saturated CER was influenced by  $O_3$  and the effect differed among clones (Figure 2). Clone 259, the most productive clone when grown with high fertility (Coleman et al. 1993), was the most sensitive to  $O_3$  exposure. In general, the 2 $\times$   $O_3$  treatment caused a 50% reduction in maximum CER of recently mature leaves of Clone 259, had no effect on maximum CER of recently mature leaves of Clone 216, and increased CER of Clone 271. This pattern was consistent among clones, although the actual response differed with leaf age and season, indicating that  $O_3$  modified either the amount or activity of Rubisco, and this modification differed among the three clones.

#### Photosynthetic carbon exchange

Photosynthetic CER varied with leaf age, among clones and in response to  $O_3$ , and these patterns changed during the growing season (cf. Isebrands et al. 1988). In all clones in the CF treatment, photosynthetic rates increased as leaves expanded, reached a plateau with full expansion in recently mature leaves, and then decreased as leaves aged (cf. Dickmann et al. 1975, Mebrahtu and Hanover 1991, Horsley and Gottschalk 1993). Photosynthetic rates of older leaves declined more in response to  $O_3$  exposure than those of recently mature leaves as the growing season progressed (Figures 3 and 5), confirming that the  $O_3$  response is greater in old leaves than in young leaves (Reich 1983, Ballach et al. 1992, Matyssek et al. 1993).

The June measurements were made on leaves that were fully expanded before the plants were placed in the chambers. Measurements later in the season were made on leaves that had been exposed to  $O_3$  for some time or since their initiation. Thus, the finding that the photosynthetic response to  $O_3$  was greater early in the growing season than late in the season (Figure 5) suggests that the leaves became physiologically acclimated to  $O_3$  exposure. However, the capacity to acclimate differed among clones.

Photosynthesis in Clone 259 (an  $O_3$ -sensitive clone) decreased in response to 1 $\times$   $O_3$  exposure in both recently mature

and matures leaves throughout the season. Photosynthesis in recently mature leaves of Clone 216 (an O<sub>3</sub>-tolerant clone) increased in response to 1× O<sub>3</sub> exposure, but decreased in response to 2× O<sub>3</sub> exposure, whereas photosynthesis in recently mature leaves of Clone 271 (also O<sub>3</sub> tolerant) increased in response to both O<sub>3</sub> treatments (Figures 3–5). Photosynthetic rates declined with O<sub>3</sub> exposure in older leaves of all clones. Both the light-response curves and within chamber photosynthetic measurements for Clone 271 indicated increased photosynthetic activity in response to O<sub>3</sub> exposure (Figure 2, Table 2). This increase in the amount or activity of Rubisco compared with that in plants in the CF treatment indicates a compensatory response. Early senescence and loss of lower leaves in response to O<sub>3</sub> stress may initiate compensatory responses similar to those caused by defoliation (cf. Bassman and Dickmann 1982, Heichel and Turner 1983, Tschaplinski and Blake 1989). An increase in leaf production rate in response to O<sub>3</sub> was found in radish (*Raphanus sativus* L.) (Held et al. 1991), but we found no such response in these *Populus* clones. Compensatory responses are probably limited in these aspen clones because chronic O<sub>3</sub> exposure continuously damages leaves as they are produced and age. Loss of photosynthetic capacity, early senescence, and early leaf abscission severely limited carbon assimilation in these aspen clones, particularly in the O<sub>3</sub>-sensitive Clone 259.

Stomatal conductance decreased with O<sub>3</sub> exposure and with leaf age, but we found no consistently large clonal differences (Figure 6). Decreased stomatal conductance is a common response to O<sub>3</sub> exposure (Olszyk and Tingey 1986, Matyssek et al. 1991, 1993). Respiration rates increased in response to O<sub>3</sub> exposure, and this increase was greater early in the growing season (Figure 7). The seasonal shift in respiration response to O<sub>3</sub> may also indicate acclimation. The increase in respiration in response to O<sub>3</sub> was greater in the O<sub>3</sub>-sensitive Clone 259 than in the other clones. The gas exchange patterns were consistent for both treatment years (1990 and 1991), for both the episodic and square-wave O<sub>3</sub> treatments, and for both clonal plants and seedlings. However, seedling photosynthetic and respiration responses to O<sub>3</sub> were less than the clonal responses.

#### Whole-tree photosynthesis and biomass production

Plants exposed to O<sub>3</sub> initially showed decreases in photosynthetic rates. They also showed premature leaf senescence and abscission, particularly in older leaves (Reich and Lassoie 1985, Sasek and Richardson 1989, Matyssek et al. 1993). Decreases in leaf and whole-tree photosynthesis also lead to decreased leaf, stem and root biomass (McLaughlin 1985, Reich and Lassoie 1985). Studies on our aspen clones showed that stem and root biomass production was sensitive to O<sub>3</sub> exposure (Karnosky et al. 1992). Decreases in lower stem and root biomass were expected because the lower leaves on the stem are major contributors of fixed carbon downward to the root system (Dickson 1986).

In both years, total biomass production was linearly related to whole-tree photosynthesis (Figure 9) (cf. Isebrands et al. 1988). The decrease in biomass production in response to O<sub>3</sub>

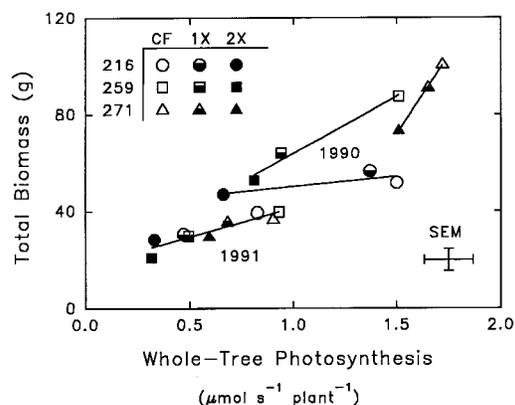


Figure 9. Total biomass and whole-tree photosynthesis (WTP) determined at the end of the growing seasons in 1990 and 1991. Three aspen clones (216, 259, 271) were exposed to three episodic O<sub>3</sub> treatments (CF, 1× and 2×). Whole-tree photosynthesis data are from the late August calculations (see Figure 8), and biomass data are from the final September harvest. Each point is the average of six trees per treatment. The average standard error (SEM) is also given.

exposure was greater for the O<sub>3</sub>-sensitive Clone 259 than for the O<sub>3</sub>-tolerant Clones 216 and 271. Whole-tree photosynthesis of Clone 271 was least sensitive to O<sub>3</sub> because it was able to retain lower leaves (Figure 8) and increase photosynthetic rate of recently mature leaves (Figure 3). In all clones, O<sub>3</sub> decreased whole-tree photosynthesis as a result of decreasing individual leaf photosynthetic rates, particularly in older leaves, and increasing leaf senescence and abscission.

We conclude that chronic exposure to low concentrations of tropospheric O<sub>3</sub> commonly found within the natural range of trembling aspen can severely depress photosynthesis and decrease productivity (about 35–40% in the O<sub>3</sub>-sensitive Clone 259) during the first year of growth in both O<sub>3</sub>-sensitive and O<sub>3</sub>-tolerant genotypes of aspen. Differences in O<sub>3</sub> sensitivity among genotypes indicate that aspen may be undergoing natural selection for O<sub>3</sub> tolerance in many locations across the USA leading to decreases in productivity and losses in biodiversity (Berrang et al. 1989, 1991).

#### Acknowledgments

We gratefully acknowledge the technical assistance given by Zophie Gagnon and Fei Li for the plant culture, ozone treatments, and leaf and photosynthetic measurements. Funding was provided by the USDA Forest Service, Northern Global Change Program, USDA-CSRS Grant No. 89-34171-4384 and by US EPA Grant No. DW12934109-0. This paper was presented at the international workshop entitled "Ecophysiology and Genetics of Trees and Forests in a Changing Environment" in Viterbo, Italy, in May 1993.

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