

Chapter 23

Growth responses of aspen clones to elevated carbon dioxide and ozone

J.G. Isebrands*

*Environmental Forestry Consultants, LLC, P.O. Box 54, E7323 Hwy 54,
New London, WI 54961, USA*

E.P. McDonald

USDA Forest Service, Forestry Sciences Laboratory, Rhinelander, WI 54501, USA

E. Kruger

*University of Wisconsin-Madison, Department of Forest Ecology, 1630 Linden Dr.,
Madison, WI 53706, USA*

G.R. Hendrey

Brookhaven National Laboratory, Upton, NY 11973, USA

K.E. Percy

Canadian Forest Service, Fredericton, NB E3B5P7, Canada

K.S. Pregitzer, J. Sober, D.F. Karnosky

*School of Forest Resources and Environmental Science, Michigan Technological University,
Houghton, MI 49931, USA*

Abstract

The International Panel of Climate Change (IPCC) has concluded that the greenhouse gases, carbon dioxide (CO₂) and tropospheric ozone (O₃), are increasing concomitantly globally. Little is known about the effect of these interacting gases on growth, survival, and productivity of forest ecosystems. In this study we assess the effects of three successive years of exposure to combinations of elevated CO₂ and O₃ on growth responses in a 5 trembling aspen (*Populus tremuloides*) clonal mixture in a regenerating stand. The experiment is located in Rhinelander, Wisconsin, USA (45° N 89° W) and employs free air carbon dioxide and ozone enrichment (FACE) technology. The aspen stand was exposed to a factorial combination of 4 treatments consisting of elevated CO₂ (560 ppm), elevated O₃ (episodic exposure—90 μl l⁻¹ h⁻¹), a combination of elevated CO₂ and O₃, and ambient control in 30 m treatment rings with 3 replications.

*Corresponding author.

Our overall results showed that our 3 growth parameters including height, diameter and volume were increased by elevated CO₂, decreased by elevated O₃, and were not significantly different from the ambient control under elevated CO₂ + O₃. However, there were significant clonal differences in the responses; all 5 clones exhibited increased growth with elevated CO₂, one clone showed an increase with elevated O₃, and two clones showed an increase over the control with elevated CO₂ + O₃, two clones showed a decrease, and one was not significantly different from the control. Notably, there was a significant increase in current terminal shoot dieback with elevated CO₂ during the 1999–2000 dormant seasons. Dieback was especially prominent in two of the five clones, and was attributed to those clones growing longer into the autumnal season where they were subject to frost. In summary, our results show that elevated O₃ negates expected positive growth effects of elevated CO₂ in *Populus tremuloides* in the field. Our results suggest that future climate model predictions should take into account the offsetting effects of elevated O₃ on CO₂ enrichment when estimating future growth of trembling aspen stands.

1. Introduction

There are increasing concerns about the effects of global climate change on the growth and sustainability of forest ecosystems. In their 2001 assessment report, the Intergovernmental Panel on Climate Change (IPCC) comprised of hundreds of scientists from many countries concluded that, “there is new and stronger evidence that human activities are affecting our climate.” Moreover, they cite the increase of the greenhouse gases carbon dioxide (CO₂) and tropospheric ozone (O₃) as important external factors affecting climate change (IPCC, 2001). This report also confirms the previous reports that CO₂ (Keeling et al., 1995) and O₃ (Marengo et al., 1994; Taylor et al., 1994; Stevenson et al., 1998; Fowler et al., 1999; Ryerson et al., 2001) are increasing steadily in the atmosphere.

Hundreds of research studies have been done on the single factor effects of elevated CO₂ and O₃ on plants, and there are numerous review articles available on these subjects. Ceulemans and Mousseau (1994), Ceulemans et al. (1999), and Saxe et al. (1998) reviewed the effects of elevated CO₂ on plants, and Jones and Curtis (1999) compiled an on-line bibliography of CO₂ effects on vegetation and ecosystems. In general, the effects of elevated CO₂ on most plants are increased growth through increased photosynthetic rates, individual leaf area, leaf area duration, and water use efficiency. The effects of O₃ on plants have been reviewed by Adams et al. (1989), and on forests by Taylor et al. (1994), Hogsett et al. (1997) and Bortier et al. (2000). In general, O₃ has profound negative effects on growth, development and productivity of many plants. The phytotoxic effects of O₃ on plants are generally due to decreased

photosynthetic rates, decreases in leaf surface area, premature leaf abscission, and weakened branch and root growth.

Little is known about interacting effects of elevated CO₂ and O₃ on plants, however (Allen, 1990; Isebrands et al., 2000). This lack of knowledge is problematic because both gases are concurrently increasing in the atmosphere, as mentioned above. This paucity of information is also related to the difficult logistical problems and expense of multiple factor experiments. The research on interacting effects of CO₂ and O₃ on plants has shown contradictory results. In some studies, elevated CO₂ offsets decreases in photosynthesis and growth caused by elevated O₃ (Barnes and Wellburn, 1998; Volin et al., 1998; Donnelly et al., 2000). In other studies, elevated CO₂ did not offset the effects of elevated O₃ on plants (Barnes et al., 1995; Barnes and Wellburn, 1998; Bortier et al., 2000). Most studies, however, did not evaluate the genetic responses to elevated CO₂ and O₃. We found the elevated CO₂ did not ameliorate the effects of elevated O₃ on photosynthesis parameters in trembling aspen clones (Kull et al., 1996). In hybrid poplar clones, the effect of elevated CO₂ on negating detrimental O₃ effects on growth varied by clone (Dickson et al., 1998).

Trembling aspen (*Populus tremuloides*), hereafter aspen, is an important ecological and economic tree species in the Great Lakes region of the USA and Canada. According to the International Poplar Commission, the aspen forest types make up more than 8.8 Mha in the US and 17.8 Mha in Canada. Moreover, other aspen species are significant in China, Russia and Scandinavia. It is known that aspen is sensitive to abiotic stressors (Kozłowski and Constantinidou, 1986). Berrang et al. (1989) collected aspen clones from across its natural range in the US to study the effects of O₃ on their growth and physiological processes. This collection led to a series of studies by D.F. Karnosky and coworkers on aspen clone vulnerability to O₃ and CO₂. In those studies, aspen clones were also shown to vary in their sensitivity to CO₂ (Kubiske et al., 1998; Zak et al., 2000), O₃ (Coleman et al., 1995a, 1995b; Karnosky et al., 1996), and to interacting effects of elevated CO₂ and O₃ (Karnosky et al., 1998; Kull et al., 1996; Isebrands et al., 2000). We also found that in certain aspen clones elevated CO₂ did not offset the detrimental effects of O₃ on growth and physiological processes (Isebrands et al., 2000).

Previous studies conducted in growth chambers and open-top chambers showed that the size and scale of the chamber studies were too small and there was a significant chamber effect that often confounded the results and conclusions of those experiments (Karnosky et al., 1999a). Therefore, we initiated a free air carbon dioxide and ozone enrichment experiment (Aspen FACE) near Rhinelander, Wisconsin, USA (Dickson et al., 2000) to study the effects of combinations of elevated CO₂ and O₃ and their interactive effects on regenerating northern hardwood stands under field conditions.

Other research groups are using FACE technology to expose forest ecosystems to elevated CO₂ (Hendrey et al., 1999). Results of those studies have shown that CO₂ increased growth in: (1) a loblolly pine plantation when nitrogen was not limiting (DeLucia et al., 1999), and (2) in sweetgum plantations (Norby et al., 1999), and a desert shrub ecosystem, when water was not limiting (Smith et al., 2000). Our FACE experiment is unique in that we are studying the combined effects of elevated CO₂ and O₃ predicted for the year 2100 on a regenerating trembling aspen stand beginning with establishment (Dickson et al., 2000).

Our objective was to determine the interacting effects of elevated CO₂ and O₃ alone and in combination, on trembling aspen growth, survival and productivity over time. Our results based upon 3 years of exposure in the field confirm our open-top chamber study findings that showed aspen clones differ in their sensitivity to elevated CO₂ and O₃, and that in some cases elevated CO₂ did not offset the detrimental effects of elevated O₃ on growth.

2. Materials and methods

2.1. Plant material

Five trembling aspen clones were selected from the Great Lakes Region for study based upon results of our previous open-top chamber research. Three of the clones (216, 259, 271) were selected for their differing sensitivity to O₃ (Karnosky et al., 1996) and two clones were selected for early (42E) and late (8L) leaf phenology and their differing response to elevated CO₂ (Kubiske et al., 1998). The clones were propagated from green wood cuttings, and grown in a greenhouse, then hardened in a shade frame before outplanting (Dickson et al., 2000).

2.2. FACE experimental design

The Aspen FACE study is located within a fenced 32 ha field of sandy loam soil on the Harshaw Experimental Farm near Rhinelander, Wisconsin, USA, (45°N 89°W). The study consists of 12 individual treatment rings (30 m diameter), spaced 100 m apart to minimize drift of the CO₂ and O₃. The treatments are arranged in a full factorial design with 3 replications. Treatments include 3 control rings (ambient air), 3 elevated CO₂ (560 ppm), 3 elevated O₃ rings (episodic total seasonal exposure 90 µl l⁻¹ h⁻¹), and 3 rings of combination elevated CO₂ and O₃. Exposure was with a FACE delivery and control system designed by Brookhaven National Laboratory (Hendrey et al., 1999) consisting of a high volume blower, plenum pipe, vertical vent pipes for emitting CO₂

and O₃, and a computer control system linked to micrometeorology stations at the center of each ring. The gas delivery was controlled by valves that were opened in the upwind direction upon computer instruction. The FACE equipment was tested and calibrated in 1997, which was considered an establishment period and a full complement of treatments began on May 1, 1998. Details of exposures are outlined in Dickson et al. (2000).

The aspen clones were planted in June 1997, as randomized pairs of individual clones with a hand held 10 cm diameter auger in the east half of each treatment ring at 1-m spacing (Fig. 1). Each tree was given a unique study identification number and each ring was unique (Dickson et al., 2000, Fig. 8). The aspen clonal mix in each ring consisted of a 100-tree study core surrounded on all sides by six buffer rows. All growth measurements were made on the core trees only. See Dickson et al. (2000) for all details.

2.3. Plant growth measurements

Growth parameters were measured at the end of each growing season after leaf fall. A telescoping height pole was used for all tree height measurements, and a digital caliper was used to measure basal diameter at 3 cm above the soil surface. To minimize the potential for injury, a single basal diameter measurement was made along an N–S axis for consistency. By 1999 and 2000, the size of the trees allowed for caliper readings taken at N–S and E–W axes. The tree sizes at the end of the 1997 establishment period are considered the initial size of individuals for the subsequent growth analysis. The basal diameter² × height, or D^2H , is an exceptionally robust, non-destructive measure that is linearly related to aboveground tree biomass (Crow, 1988). Destructive biomass estimates are not yet available.

Each spring after bud-break, trees were visually inspected for damage or dieback on the current terminal. In May 2000, a high incidence of dieback was observed. We measured the length of dieback using the height pole, and the amount of dieback was expressed as a percentage of the total length of the 1999 current shoot length. Mortality at the end of 2000 ranged from 2–4% and was not significant among treatments.

2.4. Statistical analyses

The details of the Aspen-FACE design and statistical considerations are described thoroughly by Dickson et al. (2000). For analysis, the experiment was considered a randomized complete block design, with 3 replications of atmospheric treatments at the whole-plot level, and between clone effects and interactions of clone and treatments evaluated as sub-plot factors. The treatments represent ambient atmospheric conditions and a single elevated target

Ring 3.4

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
AD	271	271	259	42E												AD
AC	271	271	259	42E	42E	259	259									AC
AB	8L	42E	216	42E	271	271	216	42E	8L							AB
AA	8L	42E	216	42E	271	271	216	42E	8L	271						AA
A	259	259	259	42E	8L	259	42E	271	271	216	42E					A
B	259	259	259	216	8L	259	42E	271	271	216	42E	8L				B
C	259	259	42E	216	8L	216	8L	259	271	216	8L	8L				C
D	259	259	42E	216	8L	216	8L	259	271	8L	8L	216	271			D
E	216	8L	259	216	216	271	216	271	271	8L	42E	216	271	8L		E
F	216	8L	259	42E	216	271	216	271	271	8L	42E	271	42E	8L		F
G	216	271	8L	42E	42E	8L	271	259	259	8L	259	259	42E	8L		G
H	216	271	8L	42E	42E	8L	271	259	259	216	259	42E	8L	8L	8L	H
I	271	8L	259	42E	259	271	271	8L	216	216	8L	42E	8L	8L	216	I
J	271	8L	259	216	259	271	271	8L	216	259	8L	259	271	271	216	J
K	42E	42E	42E	216	216	8L	42E	42E	216	259	216	259	271	271	216	K
L	42E	42E	42E	42E	216	8L	42E	42E	216	216	216	42E	8L	8L	216	L
M	259	259	42E	42E	216	259	8L	216	42E	216	8L	42E	8L	8L		M
N	259	259	42E	8L	216	259	8L	216	42E	8L	8L	259	259	42E		N
O	259	216	8L	8L	216	271	271	259	8L	8L	216	259	259	42E		O
P	259	216	8L	259	216	271	271	259	8L	271	216	271	8L	271		P
Q	259	8L	271	259	259	259	271	42E	259	271	271	271	8L	271		Q
R	259	8L	271	259	259	259	271	42E	259	271	216	259	271			R
S	42E	8L	216	259	259	216	271	271	271	271	271	259	271			S
T	42E	8L	216	42E	259	216	271	271	271	42E	271	271				T
U	8L	42E	216	42E	42E	42E	42E	8L	259	42E	271					U
V	8L	42E	216	216	42E	42E	42E	8L	259	216						V
W	216	271	216	216	42E	8L	216	216	216							W
X	216	271	216	8L	42E	8L	216	216								X
Y	42E	271	42E	8L	259	259										Y
Z	42E	271	42E													Z
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	

Figure 1. Example treatment ring map showing paired arrangement of aspen clones and buffer rows. The dark line indicates the target exposure core. Ring 3.4 refers to replication 3, treatment 4 (elevated CO₂ + O₃) of the FACE array (Dickson et al., 2000).

level of the treatment gases, CO₂ and O₃. Because the aspen clones were chosen based on previous performance and we wished to continue long-term study of these genotypes, the treatment and clone effects were evaluated as fixed effects within analysis of variance. Replication effects and replication × treatment effects are considered random within this design. Thus, a mixed model analysis was required, and we used the PROC Mixed component of SAS software (SAS Institute Inc., 2000) for all analyses (Table 1). For the analysis of main effects of CO₂ and O₃, the replication × treatment error (a) terms can be pooled or partitioned, depending on the patterns of variation. To determine whether replication × CO₂ or replication × O₃ effects were significant partitioning was required, we tested the difference in Restricted Maximum Like-

Table 1. Analysis of variance table for growth measurements from 1998, 1999, and 2000 growing season

Source of variation	Degrees of freedom	Expected mean squares
Whole-plots		
Replications	2	$\sigma_{e(b)}^2 + nc\sigma_{e(a)}^2 + ntc\sigma_r$
Treatments	3	$\sigma_{e(b)}^2 + nc\sigma_{e(a)}^2 + nrcQ_t$
CO ₂	1	
O ₃	1	
CO ₂ + O ₃	1	
Error (a)	6	$\sigma_{e(b)}^2 + nc\sigma_{e(a)}^2$
Sub-plots		
Clones	4	$\sigma_{e(b)}^2 + nrtQ_c$
Clones × Treatments	12	$\sigma_{e(b)}^2 + nrQ_{tc}$
Clones × CO ₂	4	
Clones × O ₃	4	
Clones × CO ₂ + O ₃	4	
Error (b)	60(n - 1)	$\sigma_{e(b)}^2$
Total	60n - 1	

Analysis of variance (PROC MIXED SAS Institute Inc., 2000) for this split-plot design having *n* values per replication × treatment × clone combination, with the whole plot consisting of replications (*r*), treatments (*t*), and replications × treatments pooled for Error (a) (*n* varied from 100 to 110 for the treatments). Clones (*c*) and clones × treatments are the sub-plot factors, with Error (b) being a pooled term containing variation due to replication × subplot effect interactions and variation due to subsampling (individual trees). The specific errors associated with the Q terms are designated with subscripts. Replications, replications × treatments, Error (a) and Error (b) were assumed to be random; all other effects were assumed fixed. Variation due to CO₂, O₃, and CO₂ + O₃ effects was estimated by linear contrasts. *F*-ratios for subplot effects and Error (a) all used Error (b) as the denominator. Degrees of freedom for *F*-ratios are corrected for sampling imbalance using Satterthwaite approximations. *F*-ratios for whole-plot treatment combinations (single degree of freedom orthogonal linear contrasts) use the same denominator as the *F*-ratio for treatments.

likelihood indices between pooled and partitioned models as described by Littell et al. (1996). In the majority of analyses, pooled error terms were appropriate.

Statistical analyses included only data from approximately 100 trees per FACE ring. To account for effects of initial size on growth, the height, diameter and D^2H values from 1997 were transformed to give linear relationships with their respective response variables in years 1998 through 2000. Once the linear relationships were established, the transformed initial size parameters were included as covariates in the mixed SAS models used in analyzing growth responses. The means and standard errors (based on $n = 3$) reported in tables and figures are for individual tree values adjusted for the covariate initial tree size by adding the residual from the expected value, based on initial size, to the overall grand mean for that parameter.

3. Results

3.1. Overall growth responses (1997–2000)

3.1.1. Height

During the study establishment year (1997), there were no elevated CO₂ or O₃ treatments administered during the season except for minor tests of equipment, so there were no observed treatment effects on growth. But there were clonal differences in height (cm) at the end of the first growing season related to establishment justifying the use of initial height as a covariate in the analyses. The height of the clones ranged from 35.6 cm for clone 8L to 68.0 cm for clone 271 (Table 2).

There were no overall treatment effects (i.e., CO₂, O₃, CO₂ + O₃) in subsequent year-end heights of the aspen stand (Table 3). In each of the three years, there was a highly significant effect of clone on year-end height. The CO₂ × clone interaction was not significant at the end of any of the years, but as expected from our previous research, the O₃ × clone interaction was significant each year. The CO₂ + O₃ × clone interaction was significant at the end of 1998 and 2000, but not in 1999 (Table 3).

3.1.2. Diameter

Aspen diameter growth in the elevated CO₂ treatment was not significant ($p < 0.05$) after the first treatment year (1998), but was near significant (0.06 level) at the end of the second and third treatment years (1999–2000). Similarly, the O₃ treatment did not affect diameter growth in 1998, but was near significant in 1999 and 2000. The CO₂ + O₃ treatment response for diameter

Table 2. Mean height (cm) ± SE of the 5 *Populus tremuloides* clones studied at the end of the establishment year (1997)

Clone	Mean height (cm)	SE
216	50.1	1.7
259	41.0	3.9
271	68.0	2.9
42E	48.0	2.5
8L	35.6	1.1

Table 3. Exact probability levels from SAS analyses of variance of the growth parameters—height (cm), diameter (cm), and diameter² × height (cm³) for three growing seasons (1998–2000)

Effect	Height (cm)			Diameter (cm)			D ² H (cm ³)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
CO ₂	0.519	0.491	0.115	0.326	0.056	0.064	0.962	0.015	0.040
O ₃	0.819	0.410	0.343	0.522	0.054	0.063	0.497	0.075	0.021
CO ₂ + O ₃	0.903	0.653	0.927	0.522	0.043	< 0.001	0.983	0.029	0.360
Clone	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CO ₂ × Clone	0.195	0.691	0.277	0.058	0.001	0.057	0.420	0.289	0.022
O ₃ × Clone	0.027	< 0.001	0.001	< 0.001	0.010	0.133	0.001	< 0.001	< 0.001
CO ₂ + O ₃ × Clone	0.036	0.161	0.003	0.065	0.002	< 0.001	0.337	0.031	0.078

growth was similar to the CO₂ effect, and the O₃ effect treatment for the first 2 years. However, at the end of the third treatment year it was highly significant (< 0.01). The clone effect for diameter at the end of each season was highly significant; and all treatment x clone interactions were significant or near significant for each year except at the end of 2000. The O₃ × clone interaction effect on diameter was highly significant at the end of 1998 and 1999, and the CO₂ + O₃ × clone interaction effect on diameter was highly significant at the end of the second and third treatment years (Table 3).

3.1.3. Volume estimate (D²H)

There was no significant effect of treatments on overall aspen stand volume estimates (i.e., D²H) after the first treatment year (Table 3). This finding was expected because it was the first year of exposure (1998) and volume estimate is a metric of height and diameter that had no treatment effects (above). However, volume growth was significantly affected by elevated CO₂ in the second and third treatment years (1999 and 2000). Elevated O₃ had a significant effect

on volume growth by the end of the second ($p = 0.08$) and third treatment year ($p = 0.02$). Elevated $\text{CO}_2 + \text{O}_3$ had a significant effect on the overall stand volume in the second year, but not the third.

At the end of each of the treatment years, the clone effect was significant as was the case with height and diameter data. There was no significant $\text{CO}_2 \times$ clone interactions after the first and second year, but there was after three years. Moreover, there was a highly significant effect of $\text{O}_3 \times$ clone interaction at the end of each year as expected from our previous research. The elevated $\text{CO}_2 + \text{O}_3 \times$ clone interaction was not significant at the end of the first treatment, but was significant at the end of the second ($p = 0.03$) and near significant at the end of the third year ($p = 0.08$).

3.2. Yearly growth responses of clones

The overall mean growth data and their standard errors for each treatment and clone for each of the first three treatment years 1998, 1999, and 2000 are given in Table 4.

1998

In the first year, there were few treatment effects on the mean height of the 5 aspen clones. Assessment of the effect of treatment on height was complicated by the initial height differences at the end of the establishment year (Table 2). The only significant treatment effect on height of a clone was elevated O_3 for clone 8L. The mean heights of the clones in the control at the end of 1998 ranged from 161 cm for clone 259 to 212 cm for clone 271 (Table 4).

Mean diameters for the aspen clones growing in the control treatment ranged from 1.92 cm for clone 259 and 2.35 cm for clone 271. Thus, a pattern of growth emerged in 1998 whereby clone 259 was the smallest clone and clone 271 the largest that continued throughout the three years. Mean diameter of individual clones was not significantly affected by elevated CO_2 in 1998, but was significantly decreased by elevated O_3 for clone 216 and 259, and increased for clone 8L (Table 4).

In 1998, the mean estimated volume increment (D^2H) of the clones in the control treatment ranged from 824 cm^3 for clone 259 to 1451 cm^3 for clone 271. Elevated CO_2 did not affect volume increment of individual clones in 1998; however, in all clones but one, diameter growth was decreased significantly by elevated O_3 . The exception was clone 8L that increased. In 1998, mean volume increment was significantly decreased for the elevated $\text{CO}_2 + \text{O}_3$ treatment for clones 216, 271, and 42E, and significantly increased for clone 8L, while clone 259 remained the same as the control (Table 4).

Table 4. Mean growth measurements (\pm SE) for each treatment and clone for each of the first three treatment years (1998–2000)

Clone	Height (cm)				Diameter (cm)				D^2H (cm ³)			
	Control	CO ₂	O ₃	CO ₂ + O ₃	Control	CO ₂	O ₃	CO ₂ + O ₃	Control	CO ₂	O ₃	CO ₂ + O ₃
1998												
216	205 \pm 7	192 \pm 24	194 \pm 5	202 \pm 1	2.26 \pm 0.09	2.26 \pm 0.10	1.95 \pm 0.06	2.07 \pm 0.06	1246 \pm 84	1246 \pm 284	984 \pm 67	1069 \pm 100
259	161 \pm 4	153 \pm 22	169 \pm 7	160 \pm 6	1.92 \pm 0.08	2.05 \pm 0.18	1.79 \pm 0.21	1.90 \pm 0.15	824 \pm 85	886 \pm 216	734 \pm 185	829 \pm 111
271	212 \pm 8	194 \pm 20	205 \pm 4	194 \pm 8	2.35 \pm 0.11	2.34 \pm 0.10	2.31 \pm 0.10	2.26 \pm 0.13	1451 \pm 219	1359 \pm 283	1236 \pm 149	1222 \pm 183
42E	175 \pm 7	165 \pm 16	162 \pm 8	164 \pm 8	2.09 \pm 0.06	2.17 \pm 0.12	2.00 \pm 0.09	2.06 \pm 0.14	998 \pm 30	977 \pm 128	837 \pm 67	833 \pm 123
8L	175 \pm 2	178 \pm 18	199 \pm 2	179 \pm 7	1.98 \pm 0.04	2.16 \pm 0.05	2.21 \pm 0.01	2.03 \pm 0.13	913 \pm 65	1089 \pm 84	1111 \pm 26	978 \pm 86
$\bar{X} \pm$ SE	194 \pm 4	180 \pm 20	184 \pm 3	180 \pm 5	2.18 \pm 0.08	2.23 \pm 0.10	2.03 \pm 0.10	2.08 \pm 0.12	1179 \pm 100	1150 \pm 211	963 \pm 99	987 \pm 109
1999												
216	302 \pm 12	318 \pm 33	294 \pm 7	309 \pm 11	3.58 \pm 0.11	3.86 \pm 0.08	3.09 \pm 0.11	3.33 \pm 0.11	4453 \pm 294	5691 \pm 822	3533 \pm 316	3966 \pm 204
259	248 \pm 10	260 \pm 28	252 \pm 10	250 \pm 8	2.83 \pm 0.09	3.25 \pm 0.11	2.46 \pm 0.25	2.69 \pm 0.19	2727 \pm 237	3526 \pm 459	2083 \pm 563	2669 \pm 457
271	346 \pm 11	356 \pm 23	321 \pm 6	328 \pm 11	3.76 \pm 0.09	3.98 \pm 0.04	3.68 \pm 0.15	3.45 \pm 0.17	5654 \pm 482	6833 \pm 364	4804 \pm 534	4533 \pm 545
42E	331 \pm 20	335 \pm 19	294 \pm 7	296 \pm 13	3.60 \pm 0.04	3.88 \pm 0.13	3.25 \pm 0.12	3.44 \pm 0.15	5040 \pm 318	5499 \pm 471	3749 \pm 173	4088 \pm 418
8L	291 \pm 14	342 \pm 20	319 \pm 6	308 \pm 14	3.30 \pm 0.10	3.76 \pm 0.09	3.61 \pm 0.02	3.14 \pm 0.21	3936 \pm 277	5172 \pm 230	4619 \pm 115	3972 \pm 370
$\bar{X} \pm$ SE	310 \pm 10	322 \pm 24	293 \pm 5	299 \pm 11	3.49 \pm 0.04	3.79 \pm 0.06	3.17 \pm 0.15	3.23 \pm 0.13	4584 \pm 199	5578 \pm 384	3644 \pm 337	3874 \pm 362
2000												
216	350 \pm 16	373 \pm 33	334 \pm 10	374 \pm 8	4.03 \pm 0.18	4.39 \pm 0.14	3.61 \pm 0.13	3.95 \pm 0.10	6542 \pm 734	8686 \pm 1359	5187 \pm 612	6750 \pm 189
259	287 \pm 9	307 \pm 28	294 \pm 14	296 \pm 11	3.07 \pm 0.12	3.57 \pm 0.10	2.68 \pm 0.27	2.89 \pm 0.18	3760 \pm 362	5105 \pm 509	2858 \pm 901	3760 \pm 778
271	411 \pm 3	419 \pm 25	373 \pm 16	415 \pm 15	4.39 \pm 0.07	4.81 \pm 0.02	4.21 \pm 0.21	4.15 \pm 0.15	8959 \pm 345	11706 \pm 940	7164 \pm 1202	8392 \pm 822
42E	433 \pm 30	455 \pm 18	377 \pm 12	411 \pm 26	4.38 \pm 0.15	4.66 \pm 0.15	3.86 \pm 0.15	4.14 \pm 0.25	9454 \pm 740	10637 \pm 989	6733 \pm 377	8098 \pm 1089
8L	364 \pm 15	436 \pm 12	397 \pm 5	393 \pm 16	3.98 \pm 0.10	4.61 \pm 0.06	4.35 \pm 0.04	3.76 \pm 0.28	7046 \pm 562	9275 \pm 505	8245 \pm 161	7112 \pm 883
$\bar{X} \pm$ SE	371 \pm 8	391 \pm 23	351 \pm 10	380 \pm 17	4.04 \pm 0.07	4.43 \pm 0.06	3.67 \pm 0.17	3.80 \pm 0.16	7291 \pm 199	9316 \pm 624	5793 \pm 668	6906 \pm 810

1999

At the end of the second treatment year (1999), the mean height of the individual clones ranged from 248 cm for clone 259 to 346 cm for clone 271 for the control treatment (Table 4). Elevated CO₂ did not significantly affect height of clones, except for clone 8L which increased. Elevated O₃ did not affect height for clones 216 and 259, but decreased it for clones 271 and 42E, and increased it for clone 8L (Table 4).

Diameter at the end of 1999 ranged from 2.83 cm for clone 259 to 3.76 cm for clone 271 in the control. In all clones, elevated CO₂ increased diameter growth (Table 4). However, elevated O₃ decreased diameter in clones 216, 259, and 42E, increased it in clone 8L, and was not significantly different in clone 271. Notably, at the end of 1999, the diameter of all clones was decreased by the combination treatment of elevated CO₂ and O₃, except 8L which was not significantly different.

Volume estimate in the control treatment at the end of 1999 ranged from 2727 cm³ for clone 259 to 5654 cm³ for clone 271. A clear separation in volume increment became evident at the end of two treatment years (i.e., 3 years old). All 5 clones showed increased volume increment in the elevated CO₂ treatment, although clone 42E had the least (Fig. 3). Moreover, the clones showed decreased volume increment with elevated O₃, except for clone 8L. This ozonephilic clone after two years of treatment continued to show a pattern of increased growth under elevated O₃ (Table 4). Volume increment at the end of 1999 was not significantly affected by elevated CO₂ and O₃ in clones 259 and 8L, but was decreased in clones 216, 271, and 42E.

3.3. Dormant season dieback 1999–2000

After the 1999–2000 dormant season, we observed significant current terminal (CT) shoot length dieback throughout the experiment (Table 5). Dieback symptoms were dead buds and discolored stems. An analysis of the dieback showed that the percent of aspen trees affected varied by treatment and clone. The elevated CO₂ treatment had an average frequency of 19% of the trees with dieback, while the other treatments averaged from 2–7%. Clones 271 and 216 accounted for most of the dieback observed with 20% and 10% of the trees experiencing dieback, respectively. The other three clones had minimal dieback in the range of 2–4%.

The severity of the dieback as evidenced by the percent of the current terminal shoot length that died back did not vary by treatment, but was significantly different among the clones (Table 5). About 28% of the shoot length died back in clones 216 and 271, 34% (although variable) in clone 259, 19% in clone 8L, and 10% of 42E. The dieback appeared to be associated with the prolonged

Table 5. Mean percent of aspen trees during the 1999–2000 dormant season with dieback by treatment and clone, and the percent of the current terminal (CT) shoot length that died back (\pm SE)

	% of trees with dieback	SE
Clone		
216	10	0.75
259	4	1.40
271	20	3.38
42E	2	0.00
8L	2	0.03
Treatment		
Control	7	1.26
CO ₂	19	4.65
O ₃	6	1.81
CO ₂ + O ₃	2	0.55
	% of CT length with dieback	SE
Clone		
216	28	6.67
259	34	11.57
271	28	0.30
42E	10	0.00
8L	19	0.29
Treatment		
Control	25	4.79
CO ₂	31	3.18
O ₃	32	10.62
CO ₂ + O ₃	23	7.03

growing season that the region experienced in the autumn of 1999. Clones 271 and 8L grew well into October and did not completely lose leaves until approximately November 1. The first freeze was in mid October, which was much later than normal in Rhinelander.

2000

At the end of the third treatment season (2000), the average height of the clones ranged from 287 cm for clone 259 to 433 cm for clone 42E for the control. Note that clone 42E surpassed clone 271 in height for the first time in the experiment. There was a significant effect of elevated CO₂ on height of clone 8L, but not the other clones. Elevated O₃ again significantly increased height of clone 8L, but decreased height in clones 271 and 42E. Elevated CO₂ + O₃

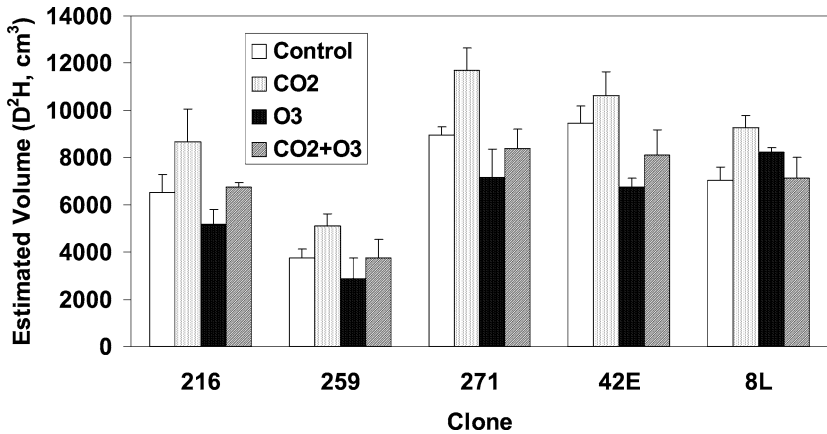


Figure 2. Summary of the effects of treatment for each clone on estimated volume increment (D^2H) at the end of the third treatment season (2000).

increased height in clones 216 and 8L, decreased height in 42E, and was not significantly different in clones 259 and 271 (Table 4).

Diameters at the end of 2000 ranged from 3.07 cm for clone 259 to 4.38 and 4.39 cm for clones 42E and 271, respectively, for the control treatments. In all clones elevated CO₂ increased diameter growth. Elevated O₃ decreased the diameter of all clones, except clone 8L, which increased. Elevated CO₂ + O₃ slightly decreased the diameter in clones 271 and 42E, and no significant effect on the other clones.

The mean volume increment (D^2H) in the control treatment for 2000 ranged from 3760 cm³ for clone 259 to 9454 cm³ for clone 42E (Fig. 2). Again, that clone 42E surpassed clone 271 in size by the end of the third treatment season. In all clones, elevated CO₂ increased the volume estimate by the end of 2000. Moreover, elevated O₃ decreased volume increment in all clones, except the ozonephilic clone 8L. The effect of elevated CO₂ + O₃ was not significantly different than the control, except for clones 271 and 42E (Table 4).

A summary of the effect of treatments on estimated volume increment for 2000 is given in Fig. 2. Note that for the most part, all clones show the same pattern for treatment effects on volume estimate even though the absolute values of the clone volume estimates were quite different. Elevated CO₂ increases volume estimate for all clones, elevated O₃ decreases it, except for clone 8L, and elevated CO₂ + O₃ is not significantly different from the control, except for clone 42E which was lower.

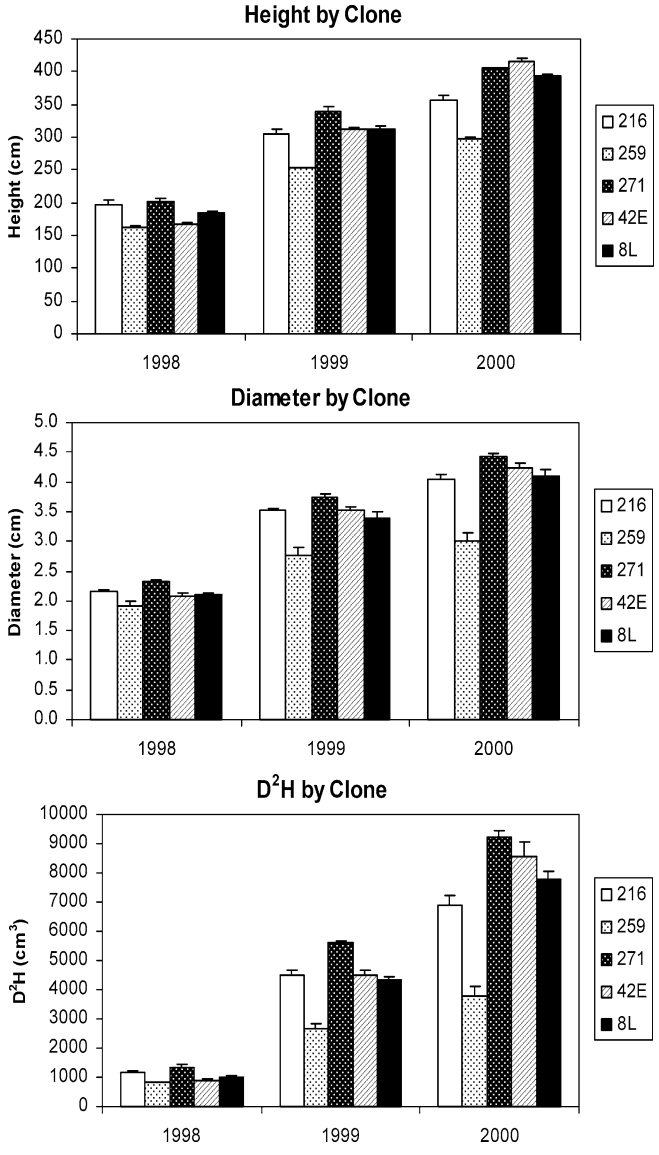


Figure 3. Overall growth response for each treatment and clone for three treatment years (1998–2000). (Continued on next page)

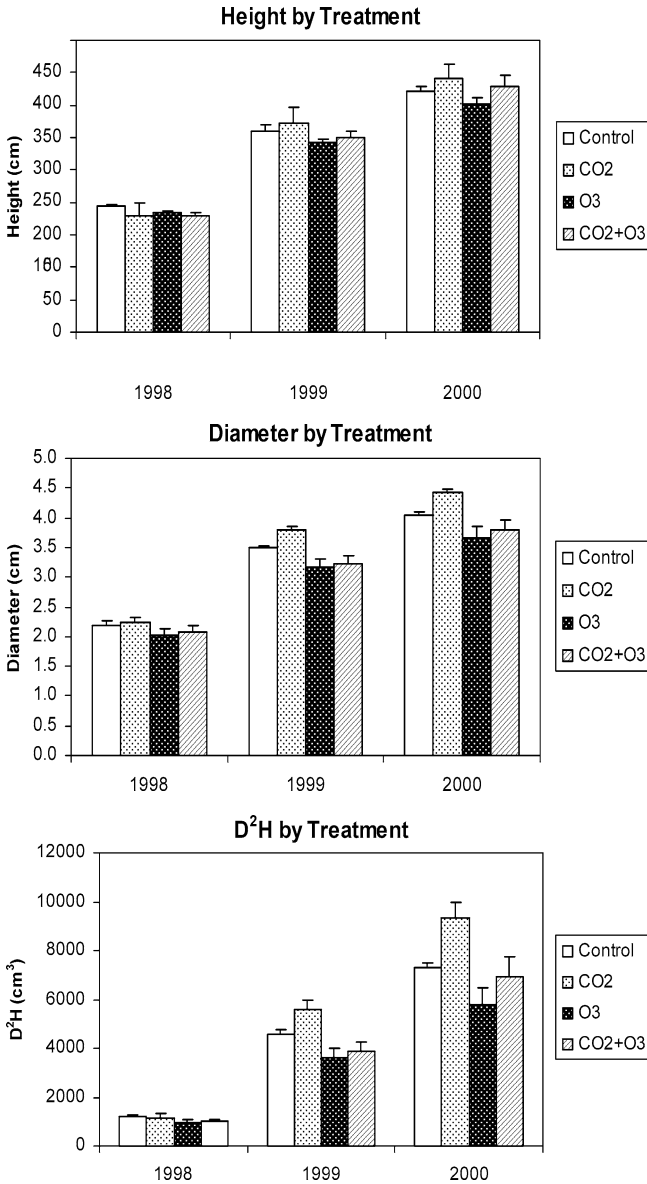


Figure 3. (Continued)

3.4. Overall growth response summary for three years

The overall growth response for the three treatment years is shown in Fig. 3. The three panels on the left summarize the overall experiment clone averages for height, diameter and volume increment (D^2H) for the three years. Note that clone 271 was the largest clone throughout the first two years, but that clone 42E equaled its size in the third year. Clone 259 was the slow grower throughout the three years, and the other 2 clones were intermediate.

The overall growth response of all clones in the aspen stand to treatment is shown on the right three panels of Fig. 3. Note that there was no difference in tree height of the stand with elevated CO_2 , and decreased height with elevated O_3 for the overall aspen stand at the end of 2000. Elevated CO_2 increased overall mean tree diameter for the stand, and elevated O_3 and elevated $\text{CO}_2 + \text{O}_3$ decreased mean tree diameter for the stand compared to the control.

The most significant treatment effects were an overall tree volume increment for the stand at the end of the second and third treatment year, compared to the control (Fig. 3). Elevated CO_2 alone increased tree volume by 22% by the end of 1999, and by 28% by the end of 2000. Elevated O_3 alone decreased tree volume by 26% by the end of 1999 and by 26% by the end of 2000. Elevated $\text{CO}_2 + \text{O}_3$ decreased tree volume compared to the control by 18% by the end of 1999, and by 6% by the end of 2000. The change in this decrease from 1999 to 2000 was due to the relative changes in individual clone responses to elevated $\text{CO}_2 + \text{O}_3$ (Fig. 4).

4. Discussion

What effect will atmospheric changes have on trembling aspen growth, survival and productivity by the year 2100? The Intergovernmental Panel on Climate Change (IPCC, 1998, 2001) predicts that atmospheric CO_2 will continue to rise by about 2% a year, so that the atmospheric CO_2 level in 2100 will approach 560 ppm. Moreover, tropospheric ozone (O_3) will concomitantly rise by 12–60% in certain regions, primarily due to rising atmospheric emission of several O_3 forming gases. Their report also predicts the freeze-free growing season will lengthen with increases in nighttime temperature of ca. 2 °C expected by 2100. The results of our experiment over the first three years of treatment, based upon a 2100 scenario, suggest that aspen growth, survival, and productivity will be greatly affected by the predicted 2001 climate. The extent of the effect will likely depend upon the genetic composition of the aspen stand, and the level of O_3 concentration in the region, as well as other interacting abiotic and biotic factors (Isebrands et al., 2000).

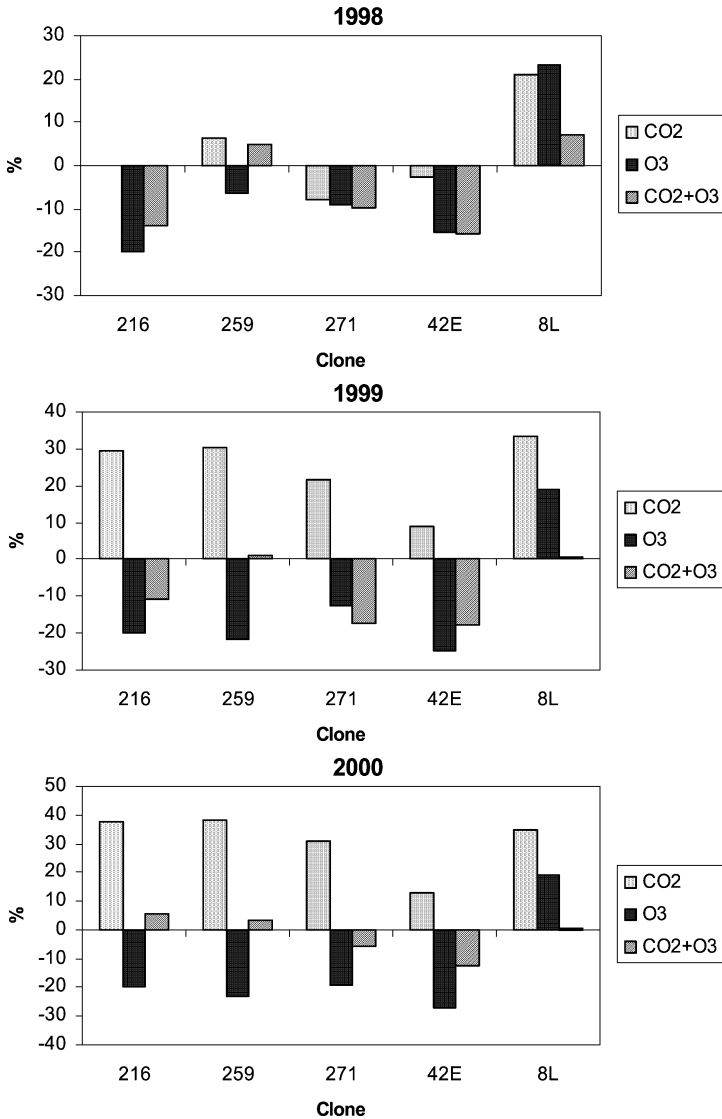


Figure 4. Volume increment (D^2H) for treatments, expressed as percent of control for five aspen clones over three treatment years (1998–2000).

If elevated CO_2 were to increase independently, as many researchers have suggested, our results suggest aspen volume growth would likely increase significantly (28%, Table 4), if all other factors such as soil fertility, water avail-

ability, temperature, and pests and diseases were equal. This result agrees with other research findings in open-top chambers with aspen (Curtis et al., 2000; Zak et al., 2000) and hybrid poplars (Curtis et al., 1995; Dickson et al., 1998; Wang et al., 2000), and is in agreement with the results of other field experiments conducted on loblolly pine (DeLucia et al., 1999) and desert ecosystems (Smith et al., 2000) using FACE technology. Height growth, on the other hand, would not be significantly affected as others have shown (Kubiske et al., 1998); however, diameter growth would increase dramatically with elevated CO₂, thereby contributing to increased volume growth.

If tropospheric ozone doses were to increase in aspen forests to the current level in many areas surrounding of our mid-western US cities, aspen volume growth would decrease significantly (26%, Table 4), if all related abiotic and biotic factors including CO₂ concentrations remained the same (an unlikely scenario). These results agree with many growth chamber and open-top chamber studies on the effects of elevated O₃ on aspen (Coleman et al., 1995a; Karnosky et al., 1996, 1998) and hybrid poplar (Dickson et al., 1998), and are in general agreement with reported elevated O₃ effects on woody plants (Bortier et al., 2000). Again, height growth was not affected by elevated O₃, while diameter growth decreased markedly as we found in our previous open-top chamber studies with aspen and hybrid poplar (Karnosky et al., 1996; Dickson et al., 1998).

If the predicted increases of atmospheric CO₂ and O₃ for the year 2100 occur concomitantly, our results suggest that elevated CO₂ will not ameliorate the negative effects of elevated O₃ on growth of aspen. In fact overall, the volume growth would be about 6% less than growth of the control. Again, these results assume that all interacting related abiotic and biotic factors would remain the same, which is unlikely. Our findings are counterintuitive with respect to results of most growth chamber and open-top chamber experiments with other plants (Olszyk and Wise, 1997; Barnes and Wellburn, 1998; Grams and Matyssek, 1999; Donnelly et al., 2000), and suggests that there is some doubt as to whether open-top experiments with aspen showing an amelioration effect (Volin and Reich, 1996; Volin et al., 1998) are valid under field conditions. No significant effect of elevated CO₂ + O₃ would be expected on height and/or diameter in aspen.

In our experiment, the growth response dynamics of aspen under elevated CO₂ and O₃ varied significantly by clone (Fig. 4). Moreover, the elevated CO₂ and O₃ effects in our FACE experiment developed over time. But, unlike our previous studies that showed clone 259 as an O₃ sensitive clone and clone 216 tolerant (Karnosky et al., 1996), we found that the five clones responded quite similarly to long-term field exposure to elevated CO₂ and O₃. It is notable that the faster-growing clones 271 and 42E responded negatively to the combination treatment of elevated CO₂ + O₃ over time. In addition,

one of the clones, namely 8L, was ozonephilic throughout the three treatment years, growing greater than the control in all three years. Also, clone 8L exhibited a unique response to elevated $\text{CO}_2 + \text{O}_3$, in that it exhibited decreased volume growth negating the positive effect of either the elevated CO_2 and elevated O_3 treatment alone (1999 and 2000, Fig. 4). These dynamic and counterintuitive results illustrate the importance of long-term field experiments of climate change variables as opposed to short-term chamber experiments.

Another important prediction of the IPCC (2001) report was the extension of the freeze-free period in the autumn. In 1999, the growing season was extended by over 50 days. During that period, clones 271 and 216 grew late in the growing season in the elevated CO_2 treatments. They did not grow later in nearby ambient experimental plots (Karnosky et al., 1999a). The observed dormant season dieback that occurred in these clones under elevated CO_2 illustrates the potential negative effect that elevated CO_2 can have on plant growth. Apparently, certain aspen clones will be more vulnerable to freezing under an elevated CO_2 scenario, because over-wintering buds on the current terminal shoots do not adequately harden. This negative effect of elevated CO_2 prolonging the growing season has been observed by others (Lutze et al. 1998) and is yet another important ramification of global climate change.

There are many complex interacting factors in our experiment. One of the most important considerations in interpretation of the field results is soil nitrogen (N) availability. Many studies have shown the importance of soil N to understanding elevated CO_2 experiments (Oren et al., 2001). Kubiske et al. (1998) and Zak et al. (2000) showed that N levels greatly influence the results of elevated CO_2 with aspen and poplars. In their studies, lower N availability exhibited a lesser response to elevated CO_2 . These findings have been found to be generally true of all plants (Jones and Curtis, 1999). Our experimental site had a history of agriculture prior to the establishment of our FACE study. As a result, the site remains high in soil N even after four growing seasons (Dickson et al., 2000). Moreover, Sober et al. (2003) have shown the importance of N nutrition to interpretation of our FACE study results. Leaf N content is highly correlated with photosynthetic rate responses under elevated CO_2 and O_3 . In their studies, they found that leaf N is declining over the course of the three treatment years in our experiment. If soil N is quite high at our site, but is declining, and if soil N is often limiting in northern forests, one must ask the questions: (1) "How would aspen trees respond to elevated CO_2 when soil N is limiting?", and (2) "Is elevated CO_2 likely to draw down available soil N needed for sustainable growth of aspen in future climates?"

Another important consideration raised in our field study is "what effect will the differential growth response of clones to elevated CO_2 and O_3 have with stand development?" Clone 259 is rapidly being over-topped over time

by the competing faster-growing clones. Moreover, clones 271 and 8L are, after three treatment years, assuming a more dominant role in the aspen clone mixture. Clonal mixtures are a common feature of aspen stands (Berrang et al., 1989) and our results suggest that certain aspen clones will not compete well with neighbors under future climate scenarios (McDonald et al., 2002). Such a response to elevated CO₂ and O₃ could lead to significant mortality in aspen stands in the future. In fact, we have observed substantial mortality in clone 259 in aspen clone mixtures growing in an elevated O₃ ambient environment near Kenosha, WI at present (Karnosky et al., 1999b).

Unfortunately, not all biotic stressors will remain constant with respect to any future climate scenario. We found in open-top chamber experiments that elevated CO₂ and O₃ and their combination affects chemical composition of aspen foliage, which in turn influences the incidence and severity of insect attack and disease occurrence (Isebrands et al., 2000). Herms et al. (1996) showed that insect feeding and larvae growth were affected by elevated CO₂ and O₃ with aspen. With elevated CO₂, the leaf feeding insects consumed more material because of the lower N content per unit area of leaves. Preliminary results at the Aspen FACE experiment suggest that both leaf feeding and wood boring insect populations, as well as their larval growth, will be affected by elevated CO₂ and O₃ (Mattson et al., unpublished).

Moreover, we have found in our Aspen FACE experiment that disease incidence and severity of the pathogens *Melampsora* and *Venturia* vary by clone and treatment. For example, the percent of trees infected by *Venturia* in 2000 was significantly different among clones and treatments, and the treatment x clone interactions were highly significant (Karnosky et al., 2002). These findings suggest that in an elevated CO₂ and O₃ world in 2100, pests and diseases may become very important interacting stressors for aspen stands. These stressors will likely have an impact on growth, survival and productivity of aspens in the future, and they should be taken into account by any models aimed at predictions of future climate effects on aspen forests.

The mechanisms of the counterintuitive effects of elevated CO₂ and O₃ on aspen clones that we observed are not yet clearly understood. However, we have some mounting anatomical, biochemical, and physiological evidence that may help explain our observed growth responses in aspen. Elevated O₃ and CO₂ + O₃ have direct effects on leaf surface chemical composition. For example, the epicuticular wax chemical composition varied by clone and treatment and may be implicated in the above-mentioned host pathogen/insect interactions (Percy et al., 2000). In addition, we find that elevated CO₂ did not ameliorate the negative effect of O₃ on biochemical and molecular responses (Karnosky et al., 1998). For example, significant decreases in chlorophyll, carotenoids, starch and Rubisco concentrations were observed in aspen under elevated CO₂ and O₃ in our experiment (Oksanen et al., unpublished). These

results may explain why we found counterintuitive decreases in photosynthesis in aspen clones grown under elevated CO₂ and O₃ in open-top chambers (Kull et al., 1996) and in the field at Aspen FACE (Noormets et al., 2001).

In summary, the results of our research team at the Aspen FACE experiment suggest that for trembling aspen the favorable reports and model predictions of enhanced growth and productivity of seedlings grown in chambers under elevated CO₂ (Saxe et al., 1998) probably overestimate field performance (Norby et al., 1999). Many questions remain concerning the effects of interacting abiotic and biotic stressors on aspen growth. We found in our experiments that relatively low concentrations of tropospheric ozone (i.e., currently present in regions with aspen) dramatically decrease growth and productivity of an aspen stand. Moreover, the fact that soil N will probably decrease over time, will likely further decrease growth of aspen under elevated CO₂ and O₃. Add to that decrease the enhanced probability that dieback will occur in certain aspen clones with lengthened freeze-free periods by the year 2100, and the differential competition effects of elevated CO₂ and O₃ will have on growth of certain clones. In addition, there are higher probabilities that aspen damaging insects and diseases will increase under future climate scenarios. All in all based on these aforementioned considerations, the complex effects of elevated CO₂ and O₃, and climate change along with associated abiotic and biotic stressors are not likely, in our view, to enhance aspen growth, survival, and productivity in the future.

Acknowledgements

We gratefully acknowledge the support for this research by the US Department of Energy, Office of Biological and Environmental Research (BER) (DE-FG02-95ER62125), the USDA Forest Service Northern Global Change Program, the Canadian Forest Service, the USDA Forest Service, North Central Research Station and Michigan Technological University. We also thank Audra Kolbe for her valuable technical assistance and data analysis, and Penny Kluetz and Janet Pikkarainen for typing the manuscript.

References

- Adams, R.M., Glycer, J.D., Johnsen, S.L., McCarl, B.A., 1989. A reassessment of the economic effects of ozone on US agriculture. *J. Air Pollut. Control Assoc.* 39, 960–968.
- Allen Jr., L.H., 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. *J. Environ. Quality* 19, 15–34.
- Barnes, J.D., Pfirman, T., Steiner, K., Lutz, C., Busch, U., Kuchenhoff, H., Payer, H.D., 1995. Effects of elevated CO₂, elevated O₃, and potassium deficiency on Norway spruce. *Plant Cell Environ.* 18, 1345–1357.

- Barnes, J.D., Wellburn, A.R., 1998. Air pollutant combinations. In: De Kok, L.J., Stulen, I. (Eds.), Responses of Plant Metabolism to Air Pollution and Global Change. Backhuys Publishers, Leiden, The Netherlands, pp. 147–164.
- Berrang, P.C., Karnosky, D.F., Bennett, J.P., 1989. Natural selection for ozone tolerance in *Populus tremuloides* II. Field verification. *Can. J. Forest Res.* 19, 519–522.
- Bortier, K., Ceulemans, R., Temmerman, L., 2000. Effects of tropospheric ozone on woody plants. In: Agrawal, S.B., Agrawal, M. (Eds.), Environmental Pollution and Plant Responses. CRC Press, pp. 153–174.
- Ceulemans, R., Mousseau, M., 1994. Effects of elevated atmospheric CO₂ on woody plants. *New Phytol.* 127, 425–446.
- Ceulemans, R., Janssens, I.A., Jach, M.E., 1999. Effects of CO₂ enrichment on trees and forests: Lessons to be learned in view of future ecosystem studies. *Ann. Bot.* 84, 577–590.
- Coleman, M.D., Dickson, R.E., Isebrands, J.G., Karnosky, D.F., 1995a. Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiol.* 15, 593–604.
- Coleman, M.D., Isebrands, J.G., Dickson, R.E., Karnosky, D.F., 1995b. Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiol.* 15, 585–592.
- Curtis, P.S., Vogel, C.S., Pregitzer, K.S., Zak, D.R., Teeri, J.A., 1995. Interacting effects of soil fertility and atmospheric CO₂ on leaf area growth and carbon gain physiology in *Populus x euramericana* (Dode) Guinier. *New Phytol.* 129, 253–263.
- Curtis, P.S., Vogel, C.S., Wang, X., Pregitzer, K.S., Zak, D.R., Lussenhop, J., Kubiske, M., Teeri, J.A., 2000. Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO₂-enriched atmosphere. *Ecol. Applic.* 10, 3–17.
- Crow, T.R., 1988. A guide to using regression equations for estimating tree biomass. *North. J. Appl. For.* 5, 15–22.
- DeLucia, E.H., Hamilton, J.G., Naidu, S.L., Thomas, R.B., Andrews, J.A., Finzi, A., Lavine, M., Matamala, R., Mohan, J.E., Hendrey, G.R., Schlesinger, W.H., 1999. Net primary production of a forest ecosystem with experimental CO₂ enrichment. *Science* 294, 1177–1179.
- Dickson, R.E., Coleman, M.D., Riemenschneider, D.E., Isebrands, J.G., Hogan, G.D., Karnosky, D.F., 1998. Growth of five hybrid poplar genotypes exposed to interacting elevated CO₂ and O₃. *Can. J. Forest Res.* 28, 1706–1716.
- Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Riemenschneider, D.E., Sober, J., Host, G.E., Hendrey, G.R., Pregitzer, K.S., Karnosky, D.F., 2000. Forest atmosphere carbon transfer storage (FACTS II)—The aspen free-air CO₂ and O₃ enrichment (FACE) project in an overview. USDA Forest Service, North Central Experiment Station, General Technical Report, NC-214.
- Donnelly, A., Jones, M.B., Burke, J.I., Schnieders, B., 2000. Elevated CO₂ provides protection from O₃ induced photosynthetic damage and chlorophyll loss in flag leaves of spring wheat (*Triticum aestivum* L., cv ‘Minaret’). *Agric. Ecosyst. Environ.* 80, 159–168.
- Fowler, D., Cape, J.N., Coyle, M., Flechard, C., Kuylenstierna, J., Hicks, K., Derwent, D., Johnson, C., Stevenson, D., 1999. The global exposure of forests to air pollutants. *Water Air Soil Pollut.* 116, 5–32.
- Grams, T.E.E., Matyssek, R., 1999. Elevated CO₂ counteracts the limitation by chronic ozone exposure on photosynthesis in *Fagus sylvatica* L.: Comparison between chlorophyll fluorescence and leaf gas exchange. *Phyton (Austria)* 39, 31–40.
- Hendrey, G.R., Ellsworth, D.S., Lewin, K.F., Nagy, J., 1999. A free air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biol.* 5, 293–309.
- Hermes, D.A., Mattson, W.J., Karowe, D.N., Coleman, M.D., Trier, T.M., Birr, B.A., Isebrands, J.G., 1996. Variable performance of outbreak defoliators on aspen clones exposed to elevated CO₂ and O₃. In: Hom, J., Birdsey, R., O’Brian, K. (Eds.), Proceedings, 1995 Meeting of the

- Northern Global Change Program, March 14–16, Pittsburgh, PA, Radnor PA. US Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, General Technical Report NE-214, pp. 43–55.
- Hogsett, W.E., Weber, J.E., Tingey, D., Herstrom, A., Lee, E.H., Laurence, J.A., 1997. Environmental auditing: an approach for characterizing tropospheric ozone risk to forests. *Environ. Manag.* 21, 105–120.
- Intergovernmental Panel on Climate Change (IPCC), 1998. The regional impacts of climate change: An assessment of vulnerability. In: Watson, R.T., Zinyowera, M.C., Moss, R.H., Dokken, D.J. (Eds.), *Special Report of IPCC Working Group II*. Cambridge Univ. Press, New York, NY.
- IPCC 2001. Third Assessment Report. Summary for policy makers. <http://www.ipcc.ch/>.
- Isebrands, J.G., Dickson, R.E., Rebbeck, J., Karnosky, D.F., 2000. Interacting effects of multiple stresses on growth and physiological processes in northern forests. In: Mickler, R.E., Birdsey, R.A., Hom, J. (Eds.), *Responses of Northern US Forests to Environmental Change*. In: *Ecological Studies*, Vol. 139. Springer-Verlag, pp. 149–180.
- Jones, M.H., Curtis, P.S., 1999. Bibliography on CO₂ Effects on Vegetation and Ecosystems: 1990–1999, The Ohio State University (OSU) and produced in cooperation with the Institute for Scientific Information. <http://www.biosci.ohio-state.edu/~pcurtis/>.
- Karnosky, D.F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Can. J. Forest Res.* 26, 23–37.
- Karnosky, D.F., Podila, G.K., Gagnon, Z., Pechter, P., Akkapeddi, A., Coleman, M., Dickson, R.E., Isebrands, J.G., 1998. Genetic control of responses to interacting O₃ and CO₂ in *Populus tremuloides*. *Chemosphere* 36, 807–812.
- Karnosky, D.F., Mankovska, B., Percy, K., Dickson, R.E., Podila, G.K., Sober, J., Noormets, A., Hendrey, G., Coleman, M.D., Kubiske, M., Pregitzer, K.S., Isebrands, J.G., 1999a. Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: Results from an O₃-gradient and a FACE experiment. *J. Water Air Soil Pollut.* 116, 311–322.
- Karnosky, D.F., Percy, K.E., Mankovska, B., Dickson, R.E., Isebrands, J.G., Podila, G.K., 1999b. Genetic implications for forest trees of increasing levels of greenhouse gases and UV-B radiation. In: Matyas, C. (Ed.), *Forest Genetics and Sustainability*. In: *Forest Gen. Sust.*, Vol. 63, pp. 111–124.
- Karnosky, D.F., Percy, K.E., Xiang, B., Callan, B., Hopkin, A., Dickson, R., Isebrands, J.G., 2002. Interacting elevated CO₂ and tropospheric ozone disposes aspen to infection by rust (*Melampsora* spp.). *Global Change Biol.* 8, 329–338.
- Keeling, C.D., Whort, T.P., Wahlen, M., van der Plicht, J., 1995. Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* 375, 666–670.
- Kozłowski, T.T., Constantinidou, H.A., 1986. Responses of woody plants to environmental pollution. *Forestry Abstracts* 47, 5–51.
- Kubiske, M.E., Pregitzer, K.S., Zak, D.R., Mikan, C.J., 1998. Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO₂ and soil N availability. *New Phytol.* 140, 251–260.
- Kull, O., Sober, A., Coleman, M.D., Dickson, R.E., Isebrands, J.G., Gagnon, Z., Karnosky, D.F., 1996. Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO₂. *Can. J. Forest Res.* 26, 639–648.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS[®] System for Mixed Models. SAS Institute Inc, Cary, NC.
- Marenco, A., Gouget, H., Nedelec, P., Pages, J.P., 1994. Evidence of a long term increase in tropospheric ozone from Pic du Midi series: consequences: Positive radiative forcing. *J. Geophys. Res.* 99 (D8), 16617–16632.

- McDonald, E.P., Kruger, E.L., Riemenschneider, D.E., Isebrands, J.G., 2002. Competitive status inferences tree-growth responses to elevated CO₂ and O₃ in aggrading aspen stands. *Functional Ecology* 16, 792–801.
- Noormets, A., McDonald, E.P., Kruger, E.L., Söber, A., Isebrands, J.G., Dickson, R.E., Karnosky, D.F., 2001. The effect of elevated carbon dioxide and ozone on leaf-and branch level photosynthesis and potential plant level carbon gain in aspen. *Trees* 15, 262–270.
- Norby, R.J., Wullschleger, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R., 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* 22, 683–714.
- Olszyk, D.M., Wise, C., 1997. Interactive effects of elevated CO₂ and O₃ on rice and *flacca* tomato. *Agricult. Ecosyst. Environ.* 66, 1–10.
- Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schäfer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature* 411, 469–472.
- Percy, K.E., Karnosky, D.F., Mankovska, B., Sober, J., Isebrands, J.G., Hendrey, G., Pregitzer, K., 2000. Interactive O₃ and CO₂ effects on leaf surface physicochemical characteristics in paper birch (*Betula papyrifera* Marsh) and three aspen (*Populus tremuloides* Michx.) clones: results from the aspen FACE project (FACTS II). In: Proc. Air Pollution, Global Change and Forests in the New Millennium, The 19th International Meeting for Specialists in Air Pollution Effects on Forest Ecosystems, Houghton, Michigan USA, p. 67, Abstract.
- Ryerson, T.B., Trainer, M., Holloway, J.S., Parrish, D.D., Huey, L.G., Sueper, D.T., Frost, G.J., Donnelly, S.G., Schauffler, S., Atlas, E.L., Kuster, W.C., Goldan, P.D., Hubler, G., Meagher, J.F., Fehsenfeld, F.C., 2001. Observations of ozone formation in power plant plumes and implications for ozone control strategies. *Science* 292, 719–723.
- SAS Institute Inc., 2000. Computer Software, SAS[®] System for Windows: Release 8.1. SAS Institute, Cary, NC.
- Saxe, H., Ellsworth, D.S., Heath, J., 1998. Tree and forest functioning in an enriched CO₂ atmosphere Tansley Review No. 98. *New Phytol.* 139, 395–436.
- Smith, S.D., Huxman, T.E., Zitzer, S.F., Charlet, T.N., Housman, D.C., Coleman, J.S., Fenstermaker, L.K., Seemann, J.R., Nowak, R.S., 2000. Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature* 408, 79–82.
- Sober, A., Noormets, A., Isebrands, J.G., Dickson, R.E., Sober, J., Karnosky, D.F., 2003. Photosynthetic parameters in aspen grown with interacting elevated CO₂ and tropospheric ozone concentrations as affected by leaf nitrogen. *Tree Physiol.* (in press).
- Stevenson, D.S., Johnson, C.E., Collins, W.J., Derwent, R.G., Shine, K.P., Edwards, J.M., 1998. Evolution of tropospheric ozone radiative forcing. *Geophys. Res. Lett.* 25, 3819–3822.
- Taylor Jr., G.E., Johnson, D.W., Andersen, C.P., 1994. Air pollution and forest ecosystems: a regional to global perspective. *Ecol. Applic.* 4, 662–689.
- Volin, J.C., Reich, P.B., 1996. Interaction of elevated CO₂ and O₃ on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiol. Plant.* 97, 674–684.
- Volin, J.C., Reich, P.B., Givnish, T.J., 1998. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant function group. *New Phytol.* 138, 315–325.
- Wang, X., Curtis, P.S., Pregitzer, K.S., Zak, D.R., 2000. Genotype variation in physiological and growth responses of *Populus tremuloides* to elevated atmospheric CO₂ concentration. *Tree Physiol.* 20, 1019–1028.
- Zak, D.R., Pregitzer, K.S., Curtis, P.S., Vogel, C.S., Holmes, W.E., Lussenhop, J., 2000. Atmospheric CO₂ soil-N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. *Ecol. Applic.* 10, 34–46.