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Growth responses of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone

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"Capsule": *Elevated ozone negates expected positive growth effects of elevated CO_2 in* Populus tremuloides *in the field.*

Abstract

The Intergovernmental Panel of Climate Change (IPCC) has concluded that the greenhouse gases carbon dioxide (CO_2) and tropospheric ozone (O_3) 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 CO_2 and O_3 on growth responses in a five 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 four treatments consisting of elevated CO_2 (560 ppm), elevated O_3 (episodic exposure-90 μ1 1^{-1} $hour^{-1}$), a combination of elevated CO_2 and O_3, and ambient control in 30 m treatment rings with three replications.

Our overall results showed that our three growth parameters including height, diameter and volume were increased by elevated CO_2, decreased by elevated O_3, and were not significantly different from the ambient control under elevated $CO_2 + O_3$. However, there were significant clonal differences in the responses; all five clones exhibited increased growth with elevated CO_2, one clone showed an increase with elevated O_3, and two clones showed an increase over the control with elevated $CO_2 + O_3$, 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 CO_2 during the 1999–2000 dormant season. 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. Our results show that elevated O_3 negates expected positive growth effects of elevated CO_2 in *Populus tremuloides* in the field, and suggest that future climate model predictions should take into account the offsetting effects of elevated O_3 on CO_2 enrichment when estimating future growth of trembling aspen stands.

Keywords: Global climate change; Aspen; Shoot dieback; FACE; CO_2; O_3

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 (CO_2) and tropospheric ozone (O_3) as important external factors affecting climate change (IPCC, 2001). This report also confirms the previous reports that CO_2 (Keeling et al., 1995) and O_3

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(Marenco 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 CO_2 and O_3 on plants, and, there are numerous review articles available on these subjects. Ceulemans and Mosseau (1994), Ceulemans et al. (1999), and Saxe et al. (1998) reviewed the effects of elevated CO_2 on plants, and Jones and Curtis (1999) compiled an on-line bibliography of CO_2 effects on vegetation and ecosystems. In general, the effects of elevated CO_2 on most plants are increased growth through increased photosynthetic rates, individual leaf area, leaf area duration, and water use efficiency. The effects of O_3 on plants has been reviewed by Adams et al. (1989), and on forests by Taylor et al. (1994), Hogsett et al. (1997) and Bortier et al. (1999). In general, O_3 has profound negative effects on growth, development and productivity of many plants. The phytotoxic effects of O_3 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 CO_2 and O_3 on plants, however (Allen et al., 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 CO_2 and O_3 on plants has shown contradictory results. In some studies elevated CO_2 offsets decreases in photosynthesis and growth caused by elevated O_3 (Barnes and Wellburn, 1998; Volin et al. ,1998; Donnelly et al., 2000). In other studies elevated CO_2 did not offset the effects of elevated O_3 on plants (Barnes et al., 1995; Bortier et al., 2000). Most studies, however, did not evaluate the genetic responses to elevated CO_2 and O_3. We found the elevated CO_2 did not ameliorate the effects of elevated O_3 on photosynthesis parameters in trembling aspen clones (Kull et al., 1996). In hybrid poplar clones the effect of elevated CO_2 on negating detrimental O_3 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 M ha in the USA and 17.8 M ha in Canada. Moreover, other aspen species are significant in China, Russia and Scandanavia. It is known that aspen is sensitive to abiotic stressors (Kozlowski and Constantinidou, 1986). Berrang et al. (1989) collected aspen clones from across its natural range in the USA to study the effects of O_3 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 O_3 and CO_2. In those studies, aspen clones were also shown to vary in their sensitivity to CO_2 (Kubiske et al., 1998; Zak et al., 1998), O_3 (Coleman et al., 1995a, 1995b; Karnosky et al., 1996), and to interacting effects of elevated CO_2 and O_3 (Karnosky et al., 1998; Kull et al., 1996; Isebrands et al., 2000). We also found that, in certain aspen clones, elevated CO_2 did not offset the detrimental effects of O_3 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 chambers studies were too small and there was a significant chamber effect that often confounded the results and conclusions of those experiments (Karnosky et al., 1999). 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 CO_2 and O_3 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 CO_2 (Hendrey et al. 1999). Results of those studies have shown that CO_2 increased growth in: (1) a loblolly pine plantation when nitrogen was not limiting (DeLucia et al., 2000), 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 CO_2 and O_3 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 CO_2 and O_3, 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 CO_2 and O_3, and that in some cases elevated CO_2 did not offset the detrimental effects of elevated O_3 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 O_3 (Karnosky et al., 1996) and two clones were selected for early (42E) and late (8L) leaf phenology and their differing response to elevated CO_2

(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 CO_2 and O_3. The treatments are arranged in a full factorial design with three replications. Treatments include three control rings (ambient air), three elevated CO_2 (560 ppm), three elevated O_3 rings (episodic total seasonal exposure 90 μl l^{-1} $hour^{-1}$), and three rings of combination elevated CO_2 and O_3. 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 CO_2 and O_3, 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 1 May 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 a 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 diameter^2×height, or D^2H, is an exceptionally robust, non-destructive measure that is linearly related to above ground 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 to 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 three 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 level of the treatment gases, CO_2 and O_3. 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, 2000) for all analyses (Table 1). For the analysis of main effects of CO_2 and O_3, the replication×treatment error (1) terms can be pooled or partitioned, depending on the patterns of variation. To determine whether replication×CO_2 or replication×O_3 effects were significant and partitioning was required, we tested the difference in Restricted Maximum 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.

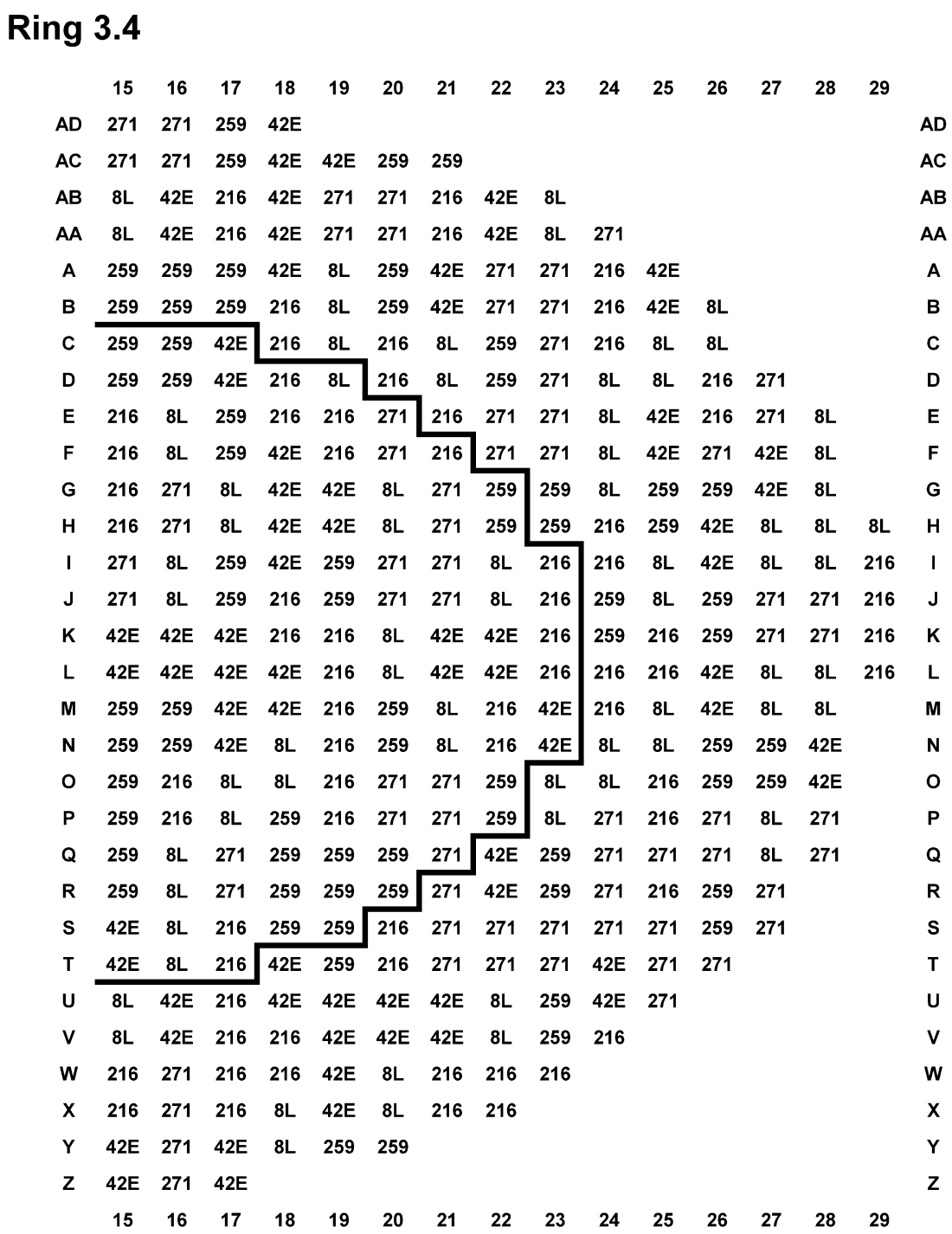

Fig. 1. Example treatment ring map showing paired arrangement of aspen clones and buffer rows. The dark line indicates the target exposure core. Ring 3.4 refers to replication 3, treatment 4 (elevated $CO_2 + O_3$) of the FACE array (Dickson et al., 2000).

3. Results

3.1. Overall growth responses (1997–2000)

3.1.1. Height

During the study establishment year (1997) there were no elevated CO_2, or O_3 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. CO_2, O_3, $CO_2 + O_3$) in subsequent year-end heights of the aspen

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

Source of variation	Degrees of freedom	Expected mean squares
Whole-plots		
Replications	2	$\sigma^2_{e(b)} + nc\sigma^2_{e(a)} + ntc\sigma_r$
Treatments	3	$\sigma^2_{e(b)} + nc\sigma^2_{e(a)} + nrcQ_t$
CO_2	I	
O_3	1	
$CO_2 + O_3$	1	
Error (a)	6	$\sigma^2_{e(b)} + nc\sigma_{e(a)}2$
Sub-plots		
Clones	4	$\sigma^2_{e(b)} + nrtQ_c$
Clones×treatments	12	$\sigma^2_{e(b)} + nrQ_{tc}$
Clone×CO_2	4	
Clone×O_3	4	
Clone×$CO_2 + O_3$	4	
Error (b)	60(n-1)	$\sigma^2_{e(b)}$
Total	60n-1	

[a] Analysis of variance (PROC MIXED SAS® Institute, 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 CO_2, O_3, and $CO_2 + O_3$ 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.

stand (Table 3). In each of the 3 years there was a highly significant effect of clone on year-end height. The CO_2 ×clone interaction was not significant at the end of any of the years, but as expected from our previous research the O_3 ×clone interaction was significant each year. The $CO_2 + O_3$ ×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 CO_2 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 O_3 treatment did not affect diameter growth in 1998, but was near significant in 1999 and 2000. The $CO_2 + O_3$ treatment response for diameter growth was similar to the CO_2 effect, and the O_3 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

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

Clone	Mean Height (cm)	S.E.
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

×clone interactions were significant or near significant for each year except at the end of 2000. The O_3×clone interaction effect on diameter was highly significant at the end of 1998 and 1999, and the $CO_2 + O_3$×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 (D^2H)

There was no significant effect of treatments on overall aspen stand volume estimate (i.e. D^2H) 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 CO_2 in the second and third treatment years (1999 and 2000). Elevated O_3 had a significant effect on volume growth by the end of the second ($P = 0.08$) and third treatment year ($P = 0.02$). Elevated $CO_2 + O_3$ 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 CO_2 ×clone interactions after the first and second year, but there was after 3 years. Moreover, there was a highly significant effect of O_3×clone interaction at the end of each year as expected from our previous research. The elevated $CO_2 + O_3$ ×clone interaction was not significant at the end of the first treatment year, 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.

3.2.1. 1998

In the first year there were few treatment effects on the mean height of the five aspen clones. Assessment of the effect of treatment on height was complicated by the

Table 3
Exact probability levels from SAS analyses of variance of the growth parameters—height (cm), diameter (cm), and diameter^2×height (cm^3) for three growing seasons (1998–2000)

Effect	Height (cm)			Diameter (cm)			D^2H (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×CIone	0.036	0.161	0.003	0.065	0.002	<0.001	0.337	0.031	0.078

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

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 3 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^2 H) 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 CO_2+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).

3.2.2. 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 CO_2 did not significantly affect height of clones, except for clone 8L which increased. Elevated O_3 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 CO_2 increased diameter growth (Table 4). However, elevated O_3 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 CO_2 and O_3 except 8L which was not significantly different.

Volume estimate in the control treatment at the end of 1999 ranged from 2727 cm^3 for clone 259, to 5654 cm^3 for clone 271. A clear separation in volume increment became evident at the end of two treatment years (i.e. 3 years old). All five clones showed increased volume increment in the elevated CO_2 treatment, although clone 42E had the least (Fig. 4). Moreover, the clones showed decreased volume increment with elevated O_3, except for clone 8L. This ozonephilic clone after 2 years of treatment continued to show a pattern of increased growth under elevated O_3 (Table 4). Volume increment at the end of 1999 was not significantly affected by elevated CO_2 and O_3 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 CO_2 treatment had an average frequency of 19% of the trees with dieback, while the other treatments averaged from 2 to 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 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.

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 (±S.E.)

	% of Trees with dieback	S.E.
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	S.E.
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

3.3.1. 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 CO_2 on height or clone of 8L, but not the other clones. Elevated O_3 again significantly increased height of clone 8L, but decreased height in clones 271 and 42E. Elevated $CO_2 + O_3$ 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 CO_2 increased diameter growth. Elevated O_3 decreased the diameter of all clones, except clone 8L, which increased. Elevated $CO_2 + O_3$ 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 cm^3 for clone 259 to 9454 cm^3 for clone 42E (Fig. 2). Again, that clone 42E surpassed clone 271 in size by the end of the third

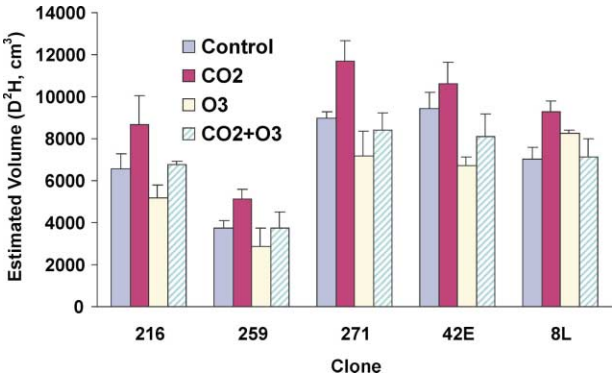

Fig. 2. Summary of the effects of treatment for each clone on estimated volume increment (D^2H) at the end of the third treatment season (2000).

treatment season. In all clones, elevated CO_2 increased the volume estimate by the end of 2000. Moreover, elevated O_3 decreased volume increment in all clones, except the ozonephilic clone 8L. The effect of elevated $CO_2 + O_3$ 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 CO_2 increases volume estimate for all clones, elevated O_3 decreases it, except for clone 8L, and elevated $CO_2 + O_3$ is not significantly different from the control, except for clone 42E which was lower.

3.4. Overall growth response summary for 3 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 2 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 two 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 $CO_2 + O_3$ decreased mean tree diameter for the stand compared to the control.

The most significant treatment effects were on 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 $CO_2 + O_3$ 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 $CO_2 + O_3$ (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 ca. 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 to 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 night-time temperature of ca. 2 °C expected by 2100. The results of our experiment over the first 3 years of treatment, based upon a 2100 scenario, suggest that aspen growth, survival, and productivity will be greatly affected by the predicted 2100 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).

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

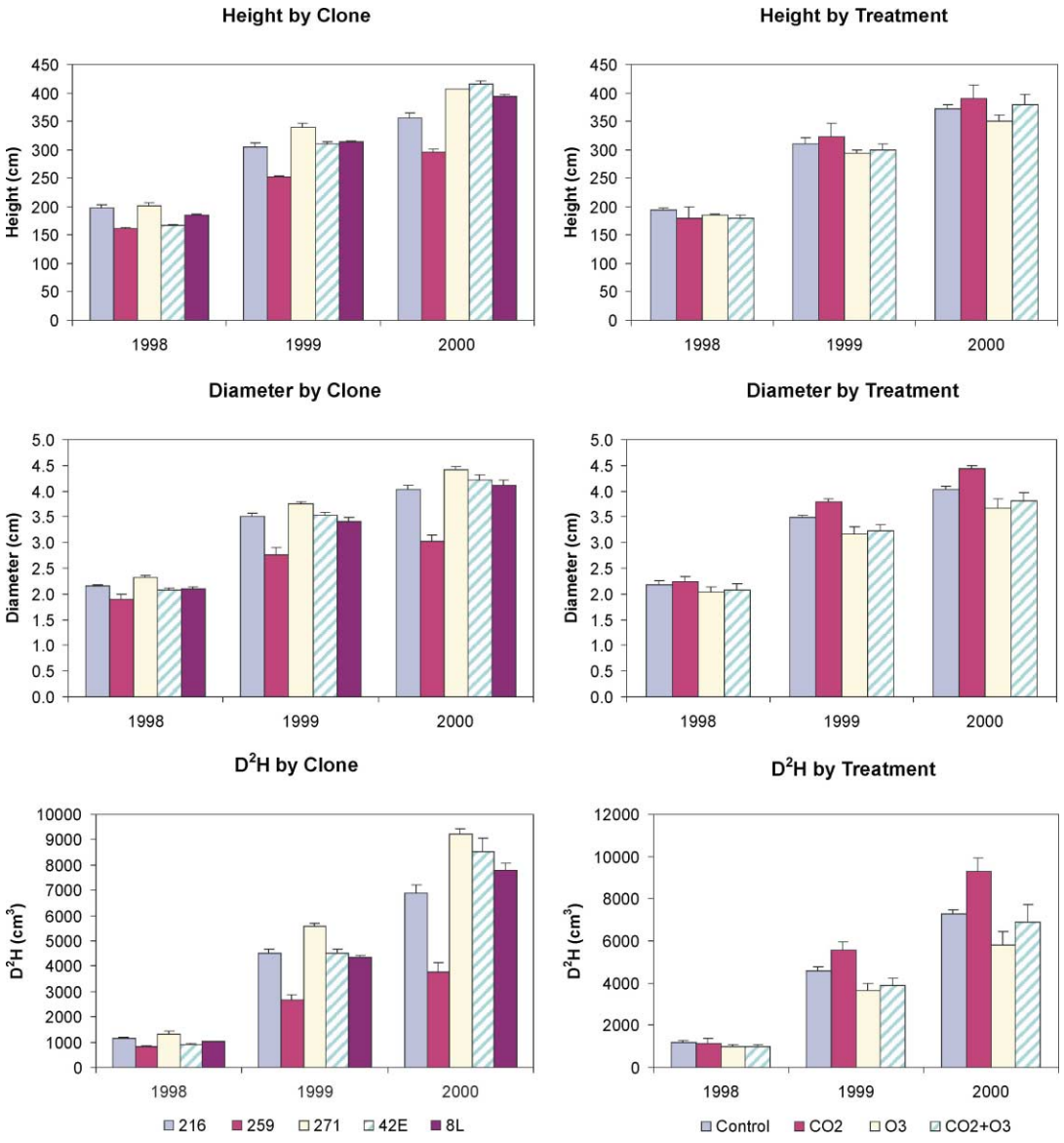

Fig. 3. Overall growth response for each treatment and clone for three treatment years (1998–2000).

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 CO_2, thereby contributing to increased volume growth.

If tropospheric ozone doses were to increase in aspen forests to the current level in many areas surrounding our mid-western US cities, aspen volume growth would decrease significantly (ca. 26%; Table 4) if all related abiotic and biotic factors including CO_2 concentrations remained the same (an unlikely scenario). These results agree with many growth chamber and open-top chamber studies on the effects of elevated O_3 on aspen (Coleman et al., 1995a; Karnosky et al., 1996; Karnosky et al., 1998) and hybrid poplar (Dickson et al., 1998), and are in general agreement with reported elevated O_3 effects on woody plants (Bortier et al., 2000). Again, height growth was not affected by elevated O_3, 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 CO_2 and O_3 for the year 2100 occur concomitantly, our results suggest that elevated CO_2 will not ameliorate the negative effects of elevated O_3 on growth of aspen. In fact, overall the volume growth would be ca. 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, 1998; Donnelly et al., 2000),

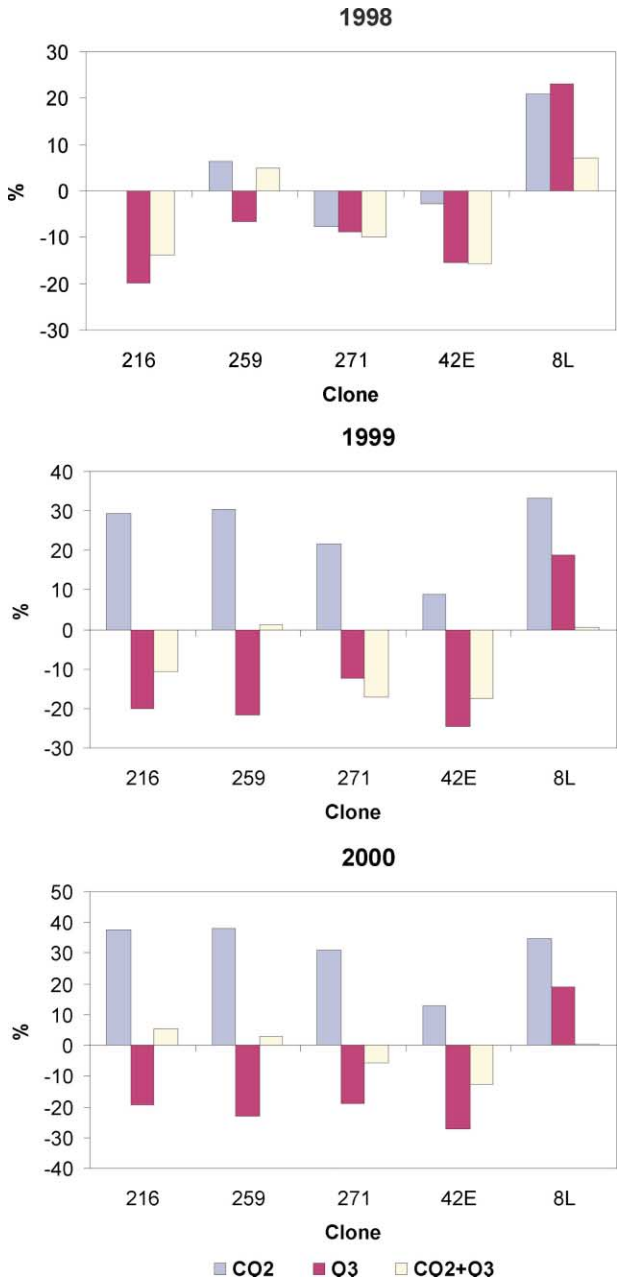

Fig. 4. Volume increment (D^2H) for treatments, expressed as percent of control for five aspen clones over three treatment years (1998–2000).

and suggests that there is some doubt as to whether open-top experiments with aspen showing an amelioration effect (Volin et al., 1996, 1998) are valid under field conditions. No significant effect of elevated $CO_2 + O_3$ would be expected on height and/or diameter in aspen.

In our experiment, the growth response dynamics of aspen under elevated CO_2 and O_3 varied significantly by clone (Fig. 4). Moreover, the elevated CO_2 and O_3 effects in our FACE experiment developed over time. But, unlike our previous studies that showed clone 259 as an O_3 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 CO_2 and O_3. It is notable that the faster-growing clones 271 and 42E responded negatively to the combination treatment of elevated $CO_2 + O_3$ over time. In addition, one of the clones, namely 8L, was ozonephilic throughout the three treatment years, growing greater than the control in all 3 years. Also, clone 8L exhibited a unique response to elevated $CO_2 + O_3$, 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., 1999). 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 overwintering 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. (2001) 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 overtopped developed 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., 2000). Such a response to elevated CO_2 and O_3 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 O_3 ambient environment near Kenosha, WI at present (Karnosky et al., 1999).

Unfortunately, not all biotic stressors will remain constant with respect to any future climate scenario. We found in open-top chamber experiments that elevated CO_2 and O_3 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 CO_2 and O_3 with aspen. With elevated CO_2 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 CO_2 and O_3 (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×clone interactions was highly significant (Karnosky et al., in press). These findings suggest that in an elevated CO_2 and O_3 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 CO_2 and O_3 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 O_3 and CO_2+O_3 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 CO_2 did not ameliorate the negative effect of O_3 on biochemical and molecular responses (Karnosky et al., 1998). For example, significant decreases in chlorophyll, cartenoids, starch and Rubisco concentrations were observed in aspen under elevated CO_2 and O_3 in our experiment (Oksanen et al., unpublished). These results may explain why we found counterintuitive decreases in photosynthesis in aspen clones grown under elevated CO_2 and O_3 in open-top chambers (Kull et al., 1996) and in the field at Aspen FACE (Noormets et al., 2001).

In summary, the study results of our research team at the Aspen FACE experiment suggest that the favorable reports and model predictions of enhanced growth and productivity of trembling aspen seedlings grown in chambers under elevated CO_2 (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 CO_2 and O_3. Add to that decrease the enhanced probability dieback that will occur in certain aspen clones with lengthened freeze-free periods by the year 2100, and the differential competition effects of elevated CO_2 and O_3 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 CO_2 and O_3, 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.

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