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


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Received 1 October 2000; accepted 17 July 2001

“Capsule”: Elevated ozone negates expected positive growth effects of elevated CO₂ in *Populus tremuloides* in the field.

Abstract

The Intergovernmental 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 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 CO₂ (560 ppm), elevated O₃ (episodic exposure-90 μL 1⁻¹ hour⁻¹), a combination of elevated CO₂ and O₃, 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 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 five 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 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 O₃ negates expected positive growth effects of elevated CO₂ in *Populus tremuloides* in the field, and 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. © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Global climate change; Aspen; Shoot dieback; FACE; CO₂; O₃

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
Hundredsof research studies have been done on the single factor effects of elevated CO\textsubscript{2} and O\textsubscript{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\textsubscript{2} on plants, and Jones and Curtis (1999) compiled an on-line bibliography of CO\textsubscript{2} effects on vegetation and ecosystems. In general, the effects of elevated CO\textsubscript{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\textsubscript{3} on plants has been reviewed by Adams et al. (1989), and on forests by Taylor et al. (1994), Hoggsett et al. (1997) and Bortier et al. (1999). In general, O\textsubscript{3} has profound negative effects on growth, development and productivity of many plants. The phytotoxic effects of O\textsubscript{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\textsubscript{2} and O\textsubscript{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\textsubscript{2} and O\textsubscript{3} on plants has shown contradictory results. In some studies elevated CO\textsubscript{2} offsets decreases in photosynthesis and growth caused by elevated O\textsubscript{3} (Barnes and Wellburn, 1998; Volin et al., 1998; Donnelly et al., 2000). In other studies elevated CO\textsubscript{2} did not offset the effects of elevated O\textsubscript{3} on plants (Barnes et al., 1995; Bortier et al., 2000). Most studies, however, did not evaluate the genetic responses to elevated CO\textsubscript{2} and O\textsubscript{3}. We found the elevated CO\textsubscript{2} did not ameliorate the effects of elevated O\textsubscript{3} on photosynthesis parameters in trembling aspen clones (Kull et al., 1996). In hybrid poplar clones the effect of elevated CO\textsubscript{2} on negating detrimental O\textsubscript{3} effects on growth varied by clone (Dickson et al., 1998).

Trembling aspen, 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 Scandinavia. 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\textsubscript{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\textsubscript{3} and CO\textsubscript{2}. In those studies, aspen clones were also shown to vary in their sensitivity to CO\textsubscript{2} (Kubiske et al., 1998; Zak et al., 1998), O\textsubscript{3} (Coleman et al., 1995a, 1995b; Karnosky et al., 1996), and to interacting effects of elevated CO\textsubscript{2} and O\textsubscript{3} (Karnosky et al., 1998; Kull et al., 1996; Isebrands et al., 2000). We also found that, in certain aspen clones, elevated CO\textsubscript{2} did not offset the detrimental effects of O\textsubscript{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\textsubscript{2} and O\textsubscript{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\textsubscript{2} (Hendrey et al. 1999). Results of those studies have shown that CO\textsubscript{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\textsubscript{2} and O\textsubscript{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\textsubscript{2} and O\textsubscript{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\textsubscript{2} and O\textsubscript{3}, and that in some cases elevated CO\textsubscript{2} did not offset the detrimental effects of elevated O\textsubscript{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\textsubscript{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\textsubscript{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 CO₂ and O₃. The treatments are arranged in a full factorial design with three replications. Treatments include three control rings (ambient air), three elevated CO₂ (560 ppm), three elevated O₃ rings (episodic total seasonal exposure 90 μl 1⁻¹ hour⁻¹), and three 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 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 diameter²×height, or D³H, 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, 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, 2000) for all analyses (Table 1). For the analysis of main effects of CO₂ and O₃, the replication×treatment error (1) terms can be pooled or partitioned, depending on the patterns of variation. To determine whether replication×CO₂ or replication×O₃ 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 D³H 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


3.1.1. 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
Table 1
Analysis of variance table for growth measurements from 1998, 1999, and 2000 growing season

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>$\sigma_{o}(a)^2 + n \sigma_{e}(a)^2 + n \sigma_{r}$</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>$\sigma_{o}(b)^2 + n \sigma_{e}(b)^2 + n \sigma_{Qc}$</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>O$_3$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CO$_2$ + O$_3$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Error (a)</td>
<td>6</td>
<td>$\sigma_{e}(ba)^2 + n \sigma_{e}(a)^2$</td>
</tr>
<tr>
<td>Sub-plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clones</td>
<td>4</td>
<td>$\sigma_{o}(b)^2 + n \sigma_{Qc}$</td>
</tr>
<tr>
<td>Clones x treatments</td>
<td>12</td>
<td>$\sigma_{o}(b)^2 + n \sigma_{Qb}$</td>
</tr>
<tr>
<td>Clone x CO$_2$</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Clone x O$_3$</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Clone x CO$_2$ + O$_3$</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Error (b)</td>
<td>60(n-1)</td>
<td>$\sigma_{e}(b)^2$</td>
</tr>
<tr>
<td>Total</td>
<td>60(n-1)</td>
<td></td>
</tr>
</tbody>
</table>

* Analysis of variance (PROC MIXED SAS Institute, 2000) for this split-plot design having n values per replication x treatments x clone combination, with the whole plot consisting of replications (r), treatments (t), and replications x treatments pooled for Error (a) (m varied from 100 to 110 for the treatments). Clones (c) and clones x treatments are the sub-plot factors, with Error (b) being a pooled term containing variation due to replication x subplot effect interactions and variation due to subsampling (individual trees). The specific errors associated with the Q terms are designated with subscripts. Replications, replications x 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.

The analysis of variance for growth measurements from 1998, 1999, and 2000 growing season was highly significant; and all treatment effects and CO$_2$ + O$_3$ effects were estimated by linear contrasts. All other effects were assumed fixed. Variation due to CO$_2$, O$_3$, and the second and third treatment years were significant or near significant for each year except at the end of 2000. The O$_3$ x clone interaction effect on diameter was highly significant at the end of 1998 and 1999, and the CO$_2$ + O$_3$ x clone interaction effect on diameter was highly significant at the end of the second and third treatment years (Table 3).

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

<table>
<thead>
<tr>
<th>Clone</th>
<th>Mean Height (cm)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>216</td>
<td>50.1</td>
<td>1.7</td>
</tr>
<tr>
<td>259</td>
<td>41.0</td>
<td>3.9</td>
</tr>
<tr>
<td>271</td>
<td>68.0</td>
<td>2.9</td>
</tr>
<tr>
<td>42E</td>
<td>48.0</td>
<td>2.5</td>
</tr>
<tr>
<td>8L</td>
<td>35.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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$_2$ x clone interactions after the first and second year, but there was after 3 years. Moreover, there was a highly significant effect of O$_3$ x clone interaction at the end of each year as expected from our previous research. The elevated CO$_2$ + O$_3$ x 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 stand (Table 3). In each of the 3 years there was a highly significant effect of clone on year-end height. The CO$_2$ x clone interaction was not significant at the end of any of the years, but as expected from our previous research the O$_3$ x clone interaction was significant each year. The CO$_2$ + O$_3$ x 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 ($P<0.01$). 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 O$_3$ x clone interaction effect on diameter was highly significant at the end of 1998 and 1999, and the CO$_2$ + O$_3$ x clone interaction effect on diameter was highly significant at the end of the second and third treatment years (Table 3).
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 ($D^2H$) 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 $CO_2 + O_3$ 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. 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.

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\(^2\)H) 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 year.
treatment season. In all clones, elevated CO\textsubscript{2} increased the volume estimate by the end of 2000. Moreover, elevated O\textsubscript{3} decreased volume increment in all clones, except the ozonephilic clone 8L. The effect of elevated CO\textsubscript{2} + O\textsubscript{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\textsubscript{2} increases volume estimate for all clones, elevated O\textsubscript{3} decreases it, except for clone 8L, and elevated CO\textsubscript{2} + O\textsubscript{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\textsuperscript{2}H) 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\textsubscript{2}, and decreased height with elevated O\textsubscript{3} for the overall aspen stand at the end of 2000. Elevated CO\textsubscript{2} increased overall mean tree diameter for the stand, and elevated O\textsubscript{3} and elevated CO\textsubscript{2} + O\textsubscript{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\textsubscript{2} alone increased tree volume by 22% by the end of 1999, and by 28% by the end of 2000. Elevated O\textsubscript{3} alone decreased tree volume by 26% by the end of 1999 and by 26% by the end of 2000. Elevated CO\textsubscript{2} + O\textsubscript{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\textsubscript{2} + O\textsubscript{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\textsubscript{2} will continue to rise by ca. 2% a year, so that the atmospheric CO\textsubscript{2} level in 2100 will approach 560 ppm. Moreover, tropospheric ozone (O\textsubscript{3}) will concomitantly rise by 12 to 60% in certain regions primarily due to rising atmospheric emission of several O\textsubscript{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\textsubscript{3} concentration in the region, as well as other interacting abiotic and biotic factors (Isebrands et al., 2000).

If elevated CO\textsubscript{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.
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 our mid-western US cities, aspen volume growth would decrease significantly (ca. 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; Karnosky et al., 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 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).
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 (Kar nosky 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 CO2 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 CO2 illustrates the potential negative effect that elevated CO2 can have on plant growth. Apparently, certain aspen clones will be more vulnerable to freezing under an elevated CO2 scenario, because overwintering buds on the current terminal shoots do not adequately harden. This negative effect of elevated CO2 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 CO2 experiments (Oren et al., 2001). Kubiske et al. (1998) and Zak et al. (2000) showed that N levels greatly influence the results of elevated CO2 with aspen and poplars. In their studies, lower N availability exhibited a lesser response to elevated CO2. 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 CO2 and O3. 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 CO2 when soil N is limiting?”, and (2) “Is elevated CO2 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 CO2 and O3 have with stand development?” Clone 259 is rapidly being overtopped developed over time by the competing fast-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 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., 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 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 was highly significant (Karnosky et al., in press). 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, cartenoids, 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 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 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 dieback that 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 (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.

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