MODEL

EFFECTS OF TROPOSPHERIC O_3 ON TREMBLING ASPEN AND INTERACTION WITH CO_2: RESULTS FROM AN O_3-GRADIENT AND A FACE EXPERIMENT

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Abstract. Over the years, a series of trembling aspen (*Populus tremuloides* Michx.) clones differing in O_3 sensitivity have been identified from OTC studies. Three clones (216 and 271[(O_3 tolerant] and 259 [O_3 sensitive]) have been characterized for O_3 sensitivity by growth and biomass responses, foliar symptoms, gas exchange, chlorophyll content, epicuticular wax characteristics, and antioxidant production. In this study we compared the responses of these same clones exposed to O_3 under field conditions along a natural O_3 gradient and in a Free-Air CO_2 and O_3 Enrichment (FACE) facility. In addition, we examined how elevated CO_2 affected O_3 symptom development. Visible O_3 symptoms were consistently seen (5 out of 6 years) at two of the three sites along the O_3 gradient and where daily one-hour maximum concentrations were in the range of 96 to 125 ppb. Clonal differences in O_3 sensitivity were consistent with our OTC rankings. Elevated CO_2 (200 ppm over ambient and applied during daylight hours during the growing season) reduced visible foliar symptoms for all three clones from 31 to 96% as determined by symptom development in elevated O_3 versus elevated O_3 + CO_2 treatments. Degradation of the epicuticular wax surface of all three clones was found at the two elevated O_3 gradient sites. This degradation was quantified by a coefficient of occlusion which was a measure of stomatal occlusion by epicuticular waxes. Statistically significant increases in stomatal occlusion compared to controls were found for all three clones and for all treatments including elevated CO_2, elevated O_3, and elevated CO_2 + O_3. Our results provide additional evidence that current ambient O_3 levels in the Great Lakes region are causing adverse effects on trembling aspen. Whether or not elevated CO_2 in the future will alleviate some of these adverse effects, as occurred with visible symptoms but not with epicuticular wax degradation, is unknown.

Key words: ozone, carbon dioxide, FACE, aspen, greenhouse gases, climate change, gradients

1. Introduction

Global atmospheric concentrations of the greenhouse gases carbon dioxide (CO_2) and tropospheric ozone (O_3) are increasing at the rate of 1 to 2% per year (Keeling *et al.*, 1995; Mohnen *et al.*, 1993). While elevated CO_2 tends to enhance tree photosynthesis and growth and to increase water use efficiency (Ceulemans and Mosseau, 1994), O_3 is a highly phytotoxic gas that impacts forest trees (Barnard *et al.*, 1991; Chappelka and Samuelson, 1998; Hogsett *et al.*, 1997). Treshow (1970) and Treshow and Stewart

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(1973) were the first to demonstrate that trembling aspen (*Populus tremuloides* Michx.) is sensitive to O_3. The strong genetic control involved in aspen's response to O_3 was first shown by Karnosky (1976, 1977). Subsequent aspen studies have: documented decreased growth under ambient O_3 conditions (Wang *et al.*, 1986); developed dose response relationships for growth responses (Karnosky *et al.*, 1992a, b; Karnosky *et al.*, 1996; Karnosky *et al.*, 1998); characterized the physiological mechanisms for O_3 responses of aspen (Gagnon *et al.*, 1992; Coleman *et al.*, 1995a, b; Coleman *et al.*, 1996); documented population differences in O_3 tolerance (Berrang *et al.*, 1986, 1989, 1991); and implicated superoxide dismutase in O_3 tolerance (Sheng *et al.*, 1997; Karnosky *et al.*, 1998).

In contrast to their response to O_3, *Populus* species generally respond positively to increases in CO_2 concentrations. Increases in individual leaf area, whole crown leaf area, leaf area duration, and leaf area index have been observed in *Populus* trees grown in elevated CO_2 (Ceulemans *et al.*, 1994; Curtis *et al.*, 1995). Aspen grown in twice ambient CO_2 had greater photosynthetic rates and a greater whole plant photosynthesis because of increased photosynthetic rates in the lower half of the crown (Kubiske *et al.*, 1997).

Given that both tropospheric O_3 and CO_2 are likely to continue to increase into the foreseeable future, the need for a better understanding of the complex interaction of CO_2 and O_3 on forest ecosystems is obvious (Volin and Reich, 1996). However, relatively little research has been done to characterize the interacting effects of O_3 and CO_2 for trees (Barnes and Wellburn, 1998). Kull *et al.* (1996) reported that elevated CO_2 (150 ppm over ambient) increased O_3 susceptibility of two aspen clones exposed to O_3 as determined by photosynthetic responses. However, subsequent evaluation of growth responses of the same aspen clones suggested that elevated CO_2 (150 ppm over ambient) did not exacerbate the O_3 effects as predicted from Kull's work, but it also did not ameliorate the adverse effects of O_3 on plant growth over a three-year time period with plants growing in natural soil in OTCs (Karnosky *et al.*, 1998). In contrast, Volin and Reich (1996) and Volin *et al.* (1998) reported that elevated CO_2 (300 ppm over ambient) ameliorated O_3 effects for young aspen plants grown for 58 or 101 days in pots in growth chambers.

Two novel methods for studying the effects of O_3 on aspen ecosystems are described in this paper. First, aspen clones differing in O_3 sensitivity were established in plantations at three locations differing in their ambient O_3 characteristics (41 to 70.4 ppmh in SUM0 [the sum of daylight hourly O_3 concentrations] from June 1 to August 30, 1996). Second, a Free-Air CO_2 and O_3 Enrichment (Aspen FACE) experiment near Rhinelander, Wisconsin, was established where aspen clones differing in O_3 and/or CO_2 sensitivity were planted in a FACE system capable of accurately and reliably dispensing CO_2 and/or O_3.

2. Methods

2.1. THE O_3 GRADIENT STUDIES

In 1993, we established three types of aspen plantations at each of three locations (Rhinelander, Wisconsin - low O_3; Kalamazoo, Michigan - intermediate O_3; and Kenosha, Wisconsin - high O_3) along a natural O_3 gradient in the Lake States (Fig. 1).

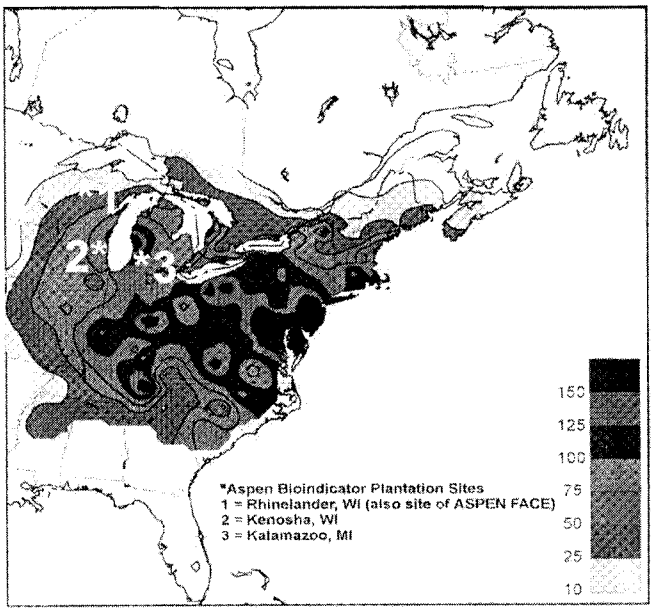

Fig. 1. Spatial distribution of average hours with ozone >82 ppb (1986-1993) (from: Dann and Summers, 1997) and locations of aspen plantations (1, 2, 3).

At each location, we established three experiments. The first was a "common garden" experiment of 7 aspen clones differing in O_3 sensitivity (clones 1, 253 and 259 = O_3 sensitive; clones 10, 216, 221, and 271 = O_3 tolerant [Karnosky, 1976; Karnosky *et al.*, 1992a]). This experiment consisted of individual trees of each clone planted at 2 m x 2 m spacing and there were 10 replicates at each site. In the second experiment, we established a "growth and yield" trial with O_3 tolerant clone 216 and O_3 sensitive clone 259 planted in 16 tree (4 x 4) blocks at 2 m x 2 m spacing between trees and with six replicates. The final experiment at each site was a "competition trial" between clones 216 and 259 where trees were planted at 0.5 m x 0.5 m between trees and 100 tree (10 x 10) blocks were established in either pure clonal blocks or mixed clonal blocks (the O_3 sensitive and tolerant clones were alternately spaced in this part of the plantation). Again, six replicates were used at each site. Two border rows of clone 271 were established around each of the three plantations at each site.

Weeds were controlled around all trees by herbicide application in the first season and then mowing in subsequent years until weed competition was no longer a problem. All trees were measured annually and also observed at least once per season for visible foliar symptoms. All three sites were old field sites that had not been in agricultural use for several years previously. The sites all have sandy loam soils with relatively high fertility. Temperature and rainfall patterns from the three sites suggest that they are not widely different in summer climate (Table I). Each site was enclosed in a 3 m tall deer fence. O_3 has been monitored independently and continuously at the Kenosha site and Rhinelander site by the Wisconsin Department of Natural Resources. The Kalamazoo site was monitored for O_3 by the Pharmacia-Upjohn Company about 0.5 km from our aspen site. The O_3 data was quality assured and was part of the AIRS (Aeometric Information and Retrieval Systems of the U.S. Environmental Protection Agency) network. A summary of the O_3 values for 1995-1997 are shown in Table II.

TABLE I

Comparisons of monthly mean temperatures (°C) and seasonal total rainfall (cm) at our three aspen sites along a natural O_3 gradient (from: http://www.acdc.noaa.gov/online).

Average Monthly Temperatures (deg. C)

	1995			1996			1997		
	Kala	Keno	Rhine	Kala	Keno	Rhine	Kala	Keno	Rhine
June	23.38	21.57	21.98	22.38	17.95	18.00	21.97	17.98	19.60
July	25.37	23.37	21.83	22.95	20.75	17.88	23.05	20.61	19.55
Aug	25.89	23.92	21.78	24.35	22.33	20.08	21.68	19.26	16.81

June through August Rainfall (cm)

Date	Kalamazoo	Kenosha	Rhinelander
1995	26.58	19.64	20.17
1996	30.48	39.10	35.43
1997	29.58	38.17	24.12

TABLE II

Summary of O_3 values at the three locations where we have aspen bioindicator plots for 1995 to 1997.

	1995			1996			1997		
Site	1-hr Max (ppb) (June-Aug)	SUM 0 ppmh (June-Aug) 8 a.m.-8 p.m.	SUM 06 ppmh (June-Aug) 24 hr.	1-hr Max (ppb) June-Aug)	SUM 0 ppmh (June-Aug) 8 a.m.- 8 p.m.	SUM 06 ppmh (June-Aug) 24 hr.	1-hr Max (ppb) (June-Aug)	SUM 0 ppmh (June-Aug) 8 a.m.-8 p.m.	SUM 06 ppmh (June-Aug) 24 hr.
Rhinelander, WI	79	37.1	4.1	85	41.0	2.5	80	40.6	4.1
Kalamazoo, MI	125	48.0	20.6	106	47.3	17.3	96	53.8	25.7
Kenosha, WI	120	59.5	29.8	122	70.4	15.1	112	58.7	10.7

We evaluated foliar injury on all leaves on a subset of the trees representing all the clones at the O_3 gradient sites. Foliage from a subset of the trees representing all clones at the O_3 gradients sites was collected for wax composition analyses in August of 1995, 1996, 1997, and 1998. Leaf segments (5 mm x 10 mm) were air dried, gold-coated on a cold stage, and examined under a JSM 6400 SEM. Two hundred stomates per clone, per site were evaluated for occlusion.

2.2 THE ASPEN FACE STUDY

The Forest Atmospheric Carbon Transfer and Storage (FACTS 2) Free-Air CO_2 and O_3 Enrichment (Aspen FACE) facility was located at the USDA Forest Service, Harshaw Experimental Farm near Rhinelander, Wisconsin. The study encompassed 32 ha of land with twelve 30 m diameter treatment rings spaced 100 m apart within a deer-fenced area. The 12 rings were composed of 3 control rings, 3 rings with elevated O_3, 3 rings with elevated CO_2, and 3 rings with elevated O_3 + elevated CO_2. Ozone was administered during the daylight hours according to the 1.5x profile for the lower Great Lakes region as described by Karnosky *et al.* (1996). This profile was a modified ambient profile developed from the 1987 O_3 data for Washtenaw County, Michigan. It was modified to more closely fit the 6-year averages documented by Pinkerton and Lefohn (1987). We only fumigated during appropriate weather conditions, i.e. we avoided fumigating on cool days (<15°C) or during rain, fog, or dew events. Ozone was monitored by Thermo Environmental Instruments Inc. Model 49C monitors adapted for rapid response times (4 sec) and calibrated weekly. CO_2 was administered during daylight hours at 560 ppm, which was about 200 ppm above the ambient level of CO_2. CO_2 was monitored with Licor Inc. LI-6252 CO_2 analyzers and calibrated weekly.

The Aspen FACE gas dispensing apparatus was modified from that developed by Lewin *et al.* (1994) and Nagy *et al.* (1994). In order to accommodate both CO_2 and O_3 from our vertical vent pipes, we modified our gas injection system in two ways compared to that system used at the FACTS I (Duke) experiment with loblolly pine (Hendrey *et al.*, 1998). First, to accommodate a larger flow rate to dilute O_3 concentrations coming out of the vertical vent pipe (to minimize O_3 concentrations near the vertical vent pipes), we changed from a hole configuration to a slot. We oriented the vertical vent pipe so the slot was pointed away from the center of the ring and placed a set of baffles to redirect air coming from the vertical vent pipes into the FACE ring. Finally, we added a flashing on top of the baffles to direct air down and into the ring (Walklate *et al.*, 1996). Together, these modifications improved the stability and distribution of both CO_2 and O_3 in our FACE rings and minimized CO_2 usage. CO_2 was distributed from the central storage tanks to each FACE ring through copper pipe. O_3 was generated from pure oxygen with a Praxair O_3 generator capable of producing 18-30 lbs. of O_3 per day. The O_3 was dispensed to the FACE rings through stainless steel tubing. Three controlling microcomputers were used to control the opening and closing of the valves at each ring and to control release of CO_2 and/or O_3 into the rings. CO_2 and O_3 were dispensed from the upwind side of each ring and monitored at plot centers.

Each Aspen FACE ring was divided into two halves by a central walkway. One half of each ring was planted at 1 m x 1 m with 1-year-old plants of five trembling aspen genotypes differing in O_3 sensitivity (8L, 216 and 271 = O_3 tolerant; and 42E and 259 = O_3 sensitive). Each clone was planted in two-tree plots randomly located in the one half ring. This resulted in 30 to 35 trees per clone being in the sweet spot where CO_2 and O_3 were close to target values (±10% for 1 minute averages). The other one half of the ring was divided into two sections: one planted with aspen clone 216 (O_3 tolerant) and sugar maple (*Acer saccharum* Marsh.) seedlings intermixed at 1 m x 1 m spacing, and the other with aspen clone 216 and paper birch (*Betula*

papyrifera Marsh.) intermixed at 1 m x 1 m spacing. There are five border rows of aspen around each FACE ring. An irrigation system was used to supplement rain events during the first two growing seasons. It was used on four occasions in 1997 and six times in 1998 after periods of extended dry conditions to assure establishment of the trees. Soil tensiometers were used to evaluate when watering was needed. All trees were planted from 1-year-old stock in July, 1997 and treatments with CO_2 were run from August 15 to September 30, 1997. CO_2 treatments resumed on May 1, 1998 and O_3 treatments started on May 15, 1998. Both CO_2 and O_3 treatments were stopped on October 12, 1998. The treatments were timed to have CO_2 exposures from budbreak to budset for trembling aspen.

We thoroughly characterized all plants in our FACE study. They were all measured at the time of planting and at the end of the first growing season. A subset of intensively sampled trees were used for characterizing seasonal growth, crown architecture, and physiological characteristics. We sampled foliage from sample leaves of clones 216, 259 and 271 for SEM in August of 1997 and 1998 as previously described. Visible foliar symptoms were scored on June 1, July 1, August 1, and September 1, 1998.

3. Results and Discussion

Visible foliar symptoms for aspen have not been detected at the clean-air (Rhinelander) gradient plot or in the control and elevated CO_2 treatments in the FACE experiment. However, visible foliar symptoms were seen in five out of six years (no symptoms were seen in 1998) at the higher O_3 (Kalamazoo and Kenosha sites) and in the O_3 and O_3 + CO_2 FACE treatments in 1998. Symptomatic leaves were those showing black bifacial necrosis, chlorosis, or upper leaf surface black or red stipple (Karnosky *et al.*, 1996). Young leaves did not show symptoms and recently mature leaves (LPI 6-10, Larson and Isebrands, 1970) showed most pronounced symptoms. Older leaves (LPI 15-20 or more) often showed multiple symptoms and secondary pests so they were more difficult to diagnose.

Foliar symptom data for a typical year at the gradient plots is shown in Table III and at the FACE site is shown in Table IV. At both of the higher O_3 gradient sites and in the FACE experiment, clone 259 was the most sensitive clone. This is consistent with previous research from OTCs (Karnosky *et al.*, 1996). Relatively, symptoms were worse in the O_3 treatments in the FACE experiment. The seasonal 12-hr. daylight O_3 doses that induced the symptoms ranged from 47.3 to 70.4 ppmh at the gradient sites and from 57.6 to 60.7 ppmh (AOT 40 values at the FACE study were 25.8 to 30.7 ppmh) at the FACE experiment (Table V). These data are consistent with previous OTC results where visible foliar injury was seen on 50% of all leaves on O_3-sensitive clones at 50 to 60 ppmh (Karnosky *et al.*, 1996). Elevated CO_2 in the FACE experiment resulted in a decreased amount of visible foliar symptoms in all three clones. This is consistent with the results of Volin and Reich (1996) and Volin *et al.* (1998) who found that elevated CO_2 ameliorates the effects of O_3 on photosynthesis and growth in aspen. It is contrary to our previous results which suggested no amelioration (Kull *et al.*, 1996) in OTCs. The level of CO_2 was higher in our FACE experiment (ambient + 200 ppm) versus our OTC work (ambient + 150 ppm). In

addition, the soil at the FACE site is considerably higher in N than that in our OTC site. Either of these two factors could have caused the difference between our OTC and FACE results with the same clones.

TABLE III

Summary of the percentage of leaves per tree showing visible injury (% L.I.) for O_3 and presence of sooty mold fungus (*Alternaria* spp.) on three aspen clones scored in August, 1996 at the three O_3 gradient sites. Means are of 20 trees per clone. Clones with the same first letter were not different ($p < 0.05$) from one another within each site and clones with the same second letter were not different ($p < 0.05$) between sites as determined by the Student-Newman-Keuls multiple comparisons test.

Location	Clone	% L.I.[1] (O_3) ± S.E.	% L.I.[1] (Sooty mold) ± S.E.
Rhinelander	216	0 ± 0a, x	0 ± 0 a, x
	259	0 ± 0a, x	0 ± 0 a, x
	271	0 ± 0a, x	0 ± 0 a, x
Kalamazoo	216	8.2 ± 1.8 a, y	0 ± 0 a, x
	259	34.7 ± 2.8 b, y	21.0 ± 3.3 b, y
	271	-	-
Kenosha	216	5.0 ± 1.9 a, y	10.6 ± 2.6 a, y
	259	43.1 ± 4.2 c, y	55.6 ± 4.8 b, z
	271	16.5 ± 1.8 b, y	13.0 ± 1.8 a, y

[1]Percentage of leaves affected by O_3 or sooty mold fungus.

TABLE IV

Percent leaves showing visible injury on August 1, 1998 for three trembling aspen clones varying in O_3 tolerance in the O_3 or O_3 + CO_2 treatments of the FACTS 2 (Aspen FACE) project in Harshaw, Wisconsin. Each replicate is the mean of at least 30 trees. Clones with the same first letter were not different ($p < 0.05$) from one another for that treatment and clones with same second letter were not different between treatments as determined by the Student-Newman-Keuls multiple comparisons test.

		Ozone				Ozone + CO_2			
Clone	O_3 Tolerance	Rep 1	Rep 2	Rep 3	$\bar{x}$	Rep 1	Rep 2	Rep 3	$\bar{x}$
216	tolerant	17.8	12.7	24.7	18.4 b, y	5.2	4.5	8.1	5.9 b, x
259	sensitive	55.3	51.1	57.8	54.7 c, y	33.1	37.7	41.9	37.6 c, x
271	tolerant	11.5	4.5	30.0	15.3 a, y	0	0	1.6	0.5 a, x

We found evidence of a secondary pathogen on trees impacted by O_3 at two of our O_3 gradient plots. A sooty mold fungus (*Alternaria* spp.) occurred on a large number of leaves on the O_3 sensitive clone 259 at both the Kalamazoo and Kenosha sites. We believe this may have been associated with the degradation of the leaf epicuticular waxes visible under SEM (Figure 2). Epicuticular wax deposits on all three clones were unaltered at the Rhinelander site but modified at the Kalamazoo and Kenosha sites. Waxes were platelike rather than crystalline and stomates were occluded by amorphous deposits on leaves collected from these sites. Effects appeared to be most dramatic at the Kenosha site in all three years and enhanced on the most O_3 sensitive clone (259). This modification resulted from changes in wax synthesis induced by O_3 (Percy *et al.*, 1998).

TABLE V

Mean ozone concentration (ppb) for the 7 a.m. to 7 p.m. time interval and AOT40 (ppmh) for 24 hrs per day for ambient air and the 3 O_3 and 3 O_3 + CO_2 treatments of the FACTS 2 (Aspen FACE) project in Harshaw, Wisconsin, for the period from May 1 to August 31, 1998.

		May		June		July		August		Season	
Treat-ment	Rep	Mean (ppb)	AOT 40 (ppmh)	Mean (ppb)	AOT 40 (ppmh)	Mean (ppb)	AOT 40 (ppmh)	Mean (ppb)	AOT 40 (ppmh)	Mean (ppb)	AOT 40 (ppmh)
O_3	1	52	5.6	55	6.7	57	7.6	59	6.9	54.7	26.8
O_3	2	54	6.5	57	7.8	58	8.3	58	8.1	56.3	30.7
O_3	3	53	6.2	55	6.9	56	7.1	59	7.0	54.7	27.2
O_3+CO_2	1	52	6.1	55	7.3	55	7.1	64	9.2	54.0	29.7
O_3+CO_2	2	53	6.3	57	7.9	57	7.8	62	8.3	55.7	30.3
O_3+CO_2	3	50	5.2	55	6.7	57	7.4	57	6.5	54.0	25.8
Ambient		41	2.2	33	0	30	0	29	0.2	34.6	2.4

To quantify the degree of epicuticular wax degradation, we examined leaves from the three aspen clones (216, 259, 271) in the four Aspen FACE treatments and at the highest O_3 (Kenosha) gradient plot (Figure 3). We developed a coefficient of occlusion based on the degree of stomates covered by amorphous wax deposits with 0 = no occlusion and 4 = total occlusion. The Kenosha site had the highest coefficient of occlusion for all three clones. This was particularly interesting because no visible foliar symptoms were present in 1998 at the Kenosha site. This shows the value of epicuticular waxes as a very sensitive bioindicator of O_3 and/or CO_2 effects. At the FACE site, we saw significantly increased stomatal occlusion with all three treatments (CO_2 + O_3, CO_2, and O_3) as compared to controls. While O_3 has been commonly reported to induce wax degradation (Percy *et al.*, 1990) and stomate occlusion (Huttunen, 1990), we believe this is the first report of increased wax occlusion of stomates in elevated CO_2.

We compared the relative photosynthetic rates under control (ambient CO_2) and elevated CO_2 treatments in the FACE treatments in 1997 (Table VI). As can be seen, elevated CO_2 significantly increased the maximum light saturated photosynthesis. We compared aspen from our FACE site to OTC data (Coleman *et al.*, 1995), field-grown seedlings (Roden and Pearcy, 1993), and to potted plants grown in the biotron (Volin *et al.*, 1998). Our FACE rates of photosynthesis were much higher than our previous OTC results. In addition, the biotron-grown plants (Volin *et al.*, 1998) were the least productive photosynthetically. The differences could be due to different light intensities. Light levels are generally lower in the biotron (Olsyk *et al.*, 1986) and light is also decreased significantly (about 12% lower on average) in OTCs (Heagle *et al.*, 1996). The N levels in the various studies could also have affected the photosynthetic rates as photosynthesis is linearly related to N levels in aspen (Coleman *et al.*, 1998).

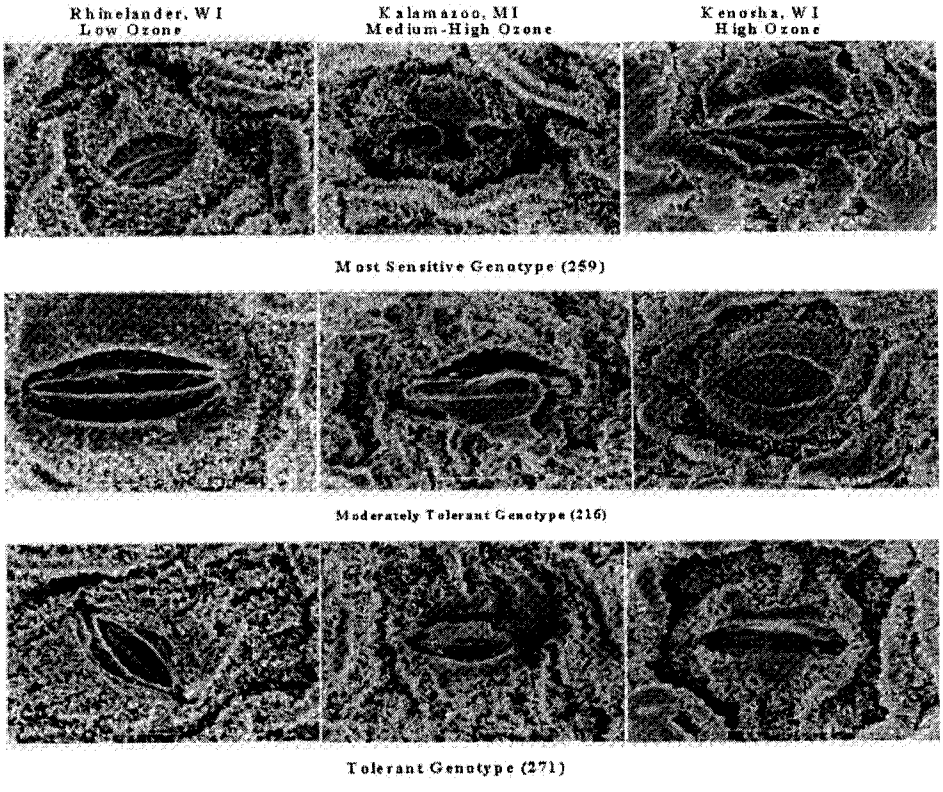

Fig. 2. Relationship between 1997 leaf surface wax structure and ozone levels in three aspen genotypes of differing O_3 sensitivities growing in 4-year-old plantations we established along a natural ozone gradient through Wisconsin and Michigan. *Note*: There was no visible foliar injury on the leaves in this composite photograph.

4. Summary and Conclusions

We present evidence suggesting that ambient O_3 concentrations in the lower Great Lakes region of the United States are inducing visible foliar symptoms and epicuticular wax degradation on trembling aspen. This evidence is from two novel experimental methods. First, we examined the response of three aspen clones varying in O_3 sensitivity and that had been established in replicated trials along a natural O_3 gradient. Second, we examined the response of the same three aspen clones in a Free-Air CO_2 and O_3 Enrichment (FACE) facility. The O_3-induced visible foliar symptoms were consistent among and between clones for the two experiments and consistent with our previous rankings. Elevated CO_2 in the FACE study decreased visible foliar injury suggesting an amelioration of O_3 effects by CO_2 for aspen. However, for epicuticular wax degradation, elevated O_3 at the two elevated O_3 sites and in the FACE experiment consistently degraded the aspen wax surface and this effect was not ameliorated by elevated CO_2. In fact, elevated CO_2 alone appeared to negatively impact aspen epicuticular waxes.

TABLE VI

Photosynthesis light response parameters (means ± se) of *Populus tremuloides* trees grown in the FACTS 2 experiment under ambient field conditions (ambient) and under free-air CO_2 enrichment (elevated). For comparison, other published results are shown for these same *P. tremuloides* clones grown under elevated CO_2 in OTCs (Coleman *et al.*, 1995b), and for unspecified, biotron-grown seedlings (Volin *et al.*, 1998) and unspecified field trees of *P. tremuloides* (Roden & Pearcy, 1993). Parameters are: light saturated photosynthesis rate (A_{sat}, μmol $m^{-2}s^{-1}$), dark respiration rate (Rd, μmol $m^{-2}s^{-1}$), light compensation point (LCP, μmol $m^{-2}s^{-1}$), apparent quantum yield (f, μmol mol^{-1}). Significant differences ($p < 0.05$) among treatments from the FACTS 2 experiment are indicated by (*) with n = 3 as determined by a simple t test in Systat.

	Volin *et al.*, 1998 (Biotron)		Ambient		FACTS 2 (FACE)	
	Ambient CO_2	Elevated CO_2	Coleman *et al.*, 1995 (OTCs)	Roden & Pearcy, 1993 (field)	Ambient CO_2	Elevated CO_2
A_{sat}	5.8	7.8	8.5 ± 0.6	c. 15	21.6 ± 1.1	30.8 ± 1.4 *
Rd			1.8 ± 0.1	1.7 ± 0.2	3.8 ± 0.2	2.9 ± 0.2 *
LCP			48 ± 6	71 ± 7	33 ± 3	21 ± 1 *
ϕ			51 ± 5	27 ± 2	68 ± 4	74 ± 6

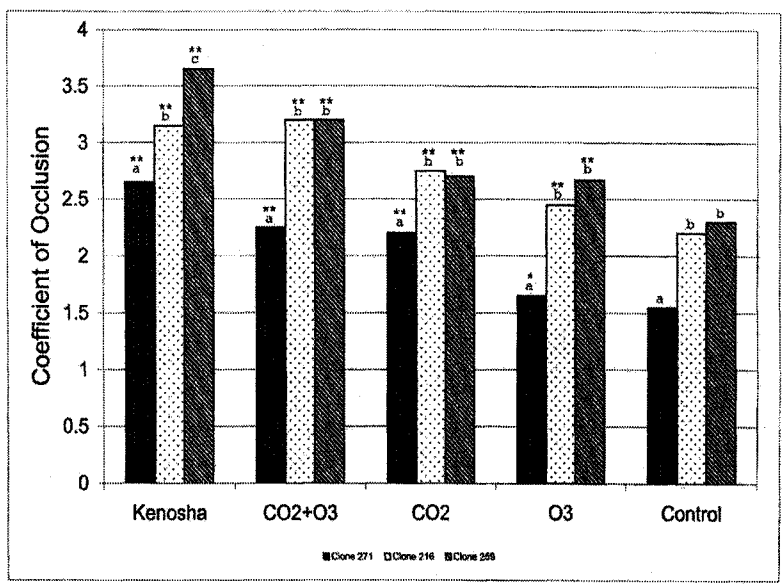

Fig. 3. Coefficient of occlusion as determined by scanning electron microscopy for the four treatments at the Aspen FACE (CO_2 + O_3, CO_2, O_3, and control) and at the Kenosha site for three trembling aspen clones sampled in August, 1998. 0 = no occlusion; 4 = almost totally occluded by leaf surface waxes. Each FACE treatment value was the mean of 100 stomates per clone for each of 3 replicates and the Kenosha data is from 100 stomates of each clone. Significant differences from control values are shown by *($p < 0.05$) and **($p < 0.01$) and significant differences ($p < 0.05$) between clones for a given treatment are indicated by letters as determined by the nonparametric Kolmogorov-Siminov test (Myslivec, 1957 and Smelko, 1991).

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