EFFECTS OF GENOTYPE ON THE RESPONSE OF POPULUS TREMULOIDES MICHX. TO OZONE AND NITROGEN DEPOSITION

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ABSTRACT. Elevated O_3 concentrations and N deposition levels co-occur in much of eastern United States. However, very little is known about their combined effects on tree growth. The effects of three O_3 treatments: charcoal-filtered air, non-filtered air and O_3 added at the rate of 80 ppb for 6 hr d^{-1} 3 d per week), four N deposition levels (0, 10, 20 and 40 kg ha^{-1} yr^{-1}), and their interactions on growth of two Populus tremuloides clones in open-top chambers at two sites 600 km apart in Michigan were examined. Our results revealed a highly significant fertilization effect of the N treatments, even at the 10 kg ha^{-1} yr^{-1} rate. Ozone alone induced foliar injury, but not significant growth reductions. There was an indication that O_3 decreased growth at the 0 N level, but this decrease was reversed in all N treatments by the N fertilization effect. Further study is needed to more fully understand the combined effects of N deposition and O_3.

1. INTRODUCTION

The Great Lakes Region is being subjected to a gradient of air pollutants that may be adversely affecting the health of the area's extensive forests. Two pollutants known to co-occur in the region are O_3 and acidic deposition in the form of N and S. During 1986-1990, we have participated in a research effort to characterize the pollution gradient and to attempt to detect its impact on the forest ecosystem. As a part of this research, we established open-top chambers at two sites, approximately 600 km apart, to study the effects of O_3 on the growth and biomass of trembling aspen (Populus tremuloides Michx.) and sugar maple (Acer saccharum Marsh), two of the region's principal forest tree species.

The objective of this study was to examine the interaction of ozone and nitrogen deposition on the growth and biomass allocation of two trembling aspen (Populus tremuloides Michx.) clones.

2. MATERIALS AND METHODS

2.1 Plant Materials

During March, 1989, softwood cuttings were rooted from sucker sprouts on greenhouse-grown trembling aspen plants representing two

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genotypes (see Table I). Rooted plants were planted in 2.5 cm wide by 15 cm deep plastic pots ("Ray Leach" cells) in a 1:1:1 peat:perlite:packaged topsoil. These plants were grown in the greenhouse under a 16-hr photoperiod until mid-May when they were placed outside under 50% shade cloth. Plants were transplanted to 30 cm wide by 25 cm deep plastic pots in the above-mentioned soil mix and placed on the ground in open-top chambers. Plants were thoroughly watered on a daily basis as needed during the growing season.

TABLE I. Origin and relative sensitivity of two trembling aspen clones used in this study.

	O_3 Sensitivity		
Clone	Foliage	Stem Biomass	Origin
253	Sensitive	Intermediate	Leelanau County, Mi.
259	Sensitive	Sensitive	Porter County, Ind.

2.2 Experimental Design and Analysis

This study utilized three O_3 treatments x 4 N treatments x 2 clones in a factorial design with six replicates and one tree per clone per N treatment in each plot. The three O_3 treatments were charcoal-filtered air, non filtered air and O_3 added to charcoal-filtered air at the concentration of 80 ppb for 6 hr d^{-1}, 3 d per week. Open non-chambered plots were used to determine chamber effects. The 4 N treatments were 0, 10, 20 and 40 kg ha^{-1} yr^{-1}. Two thirds of the N load was added at the beginning of the growing season and the remaining one third was delivered in equal biweekly allocations. This was similar to the seasonal pattern of N deposition in northern Michigan. Nitrogen was added as 0.1 N nitric acid at pH 2.0 and was added just prior to the daily watering. The experiment was duplicated at two sites: The Mathie Botanical Garden at Ann Arbor, Michigan and the Ford Forestry Center at Alberta, Michigan. Since our chambers had open tops, our plants were also subject to ambient N deposition. The experiment was conducted from June 10 to September 15 at Ann Arbor and June 15 to September 15 at Alberta.

Standard analyses of variance were used to test for O_3 treatment, N and clonal differences and for interactions. Differences between treatment means were tested for significance using various mean separation tests.

2.3 Pollutant Dispersement and Monitoring

Three m-diameter, 2.3 m tall open-top chambers without rainfall exclusion tops as described by Heagle et al. (1973) were used in this project. Ozone was generated from compressed air that passed a series of water traps to remove N compounds and then into a Griffen Model 0.5A Ozone Generator. Ozone in the chambers and open plots was monitored in a time-shared fashion with a TECO Model 1A Ozone Analyzer that was calibrated weekly with a Monitor Labs Model 8500 Ozone Calibrator.

2.4 Measurements

Heights to the nearest cm and diameters to the nearest .01 mm of plants were recorded at the beginning of the field fumigation season and measured biweekly for the remainder of the experiment. Percentage of leaves showing injury was scored biweekly. Leaf, stem and root biomass were determined on a dry-weight basis at the end of the experiment.

3. RESULTS

Analysis of variance for height, diameter and biomass (Tables II and III) showed that differences occurred between the two sites. At the Alberta site, significant variance could be attributed to N deposition and clone but not to O_3. The only significant interaction that occurred was the O_3 x clone interaction for stem biomass.

At the Ann Arbor site, significant differences occurred for N and clone (except for stem biomass and height) but not for O_3 and no significant interactions occurred.

TABLE II. Summary of significance levels of various O_3 and N deposition treatments and interactions for several growth parameters for Populus tremuloides plants growing at Alberta, Michigan.

	Growth Parameters				
	Stem Biomass	Leaf Biomass	Root Biomass	Height	Diameter
O_3 Treatment	0.686*	0.795	0.378	0.885	0.668
N Deposition Level	0.000	0.000	0.000	0.000	0.000
Clone	0.001	0.029	0.000	0.000	0.001
O_3 x N	0.503	0.156	0.413	0.173	0.166
O_3 x Clone	0.040	0.150	0.204	0.323	0.257
N x Clone	0.483	0.274	0.221	0.819	0.699
O_3 x N x Clone	0.900	0.441	0.607	0.602	0.686

*Significance levels less than 0.05 and 0.01 indicate significant and highly significant treatment differences, respectively.

TABLE III. Summary of significance levels of various O_3 and N deposition treatments and interactions for several growth parameters for Populus tremuloides plants growing in Ann Arbor, Michigan.

	Growth Parameters				
	Stem Biomass	Leaf Biomass	Root Biomass	Height	Diameter
O_3 Treatment	0.230*	0.660	0.305	0.481	0.914
N Deposition Level	0.000	0.000	0.018	0.000	0.000
Clone	0.526	0.001	0.042	0.784	0.006
O_3 x N	0.210	0.163	0.282	0.736	0.229
O_3 x Clone	0.170	0.775	0.227	0.695	0.752
N x Clone	0.247	0.831	0.982	0.858	0.903
O_3 x N x Clone	0.257	0.487	0.355	0.296	0.772

*Significance levels less than 0.05 and 0.01 indicate significant and highly significant treatment differences, respectively.

The combined growth responses of the two clones across the three O_3 treatments and the open plot are shown in Tables IV and V. Two major differences occurred between the Alberta and Ann Arbor, Michigan sites. First, the trees at the Alberta site grew more vigorously than did those at the Ann Arbor site. Second, while there were no significant differences between open plots and chambers at Ann Arbor, there was a significant chamber effect at Alberta.

The combined growth responses for plants in the various O_3 treatments, across the N deposition treatments, show that N significantly affected all growth parameters at both sites (Tables VI and VII). Generally, there was a trend toward increased growth and biomass accumulation as the N deposition increased. However, stem and root biomass were less at the highest N treatment as compared to the medium N treatment at the Alberta, Michigan site.

The growth responses of the two clones across the four N treatments, comparing charcoal-filtered air and O_3-added air, are shown in Tables VIII and IX. There appeared to be a trend of O_3-induced reduction of growth for both clones at the 0 N treatment at Alberta, Michigan but this trend was not present at any of the three N-added treatments. Large growth differences between clones are detectable at the Alberta site (where the two clones grew more vigorously and as shown in Figures 1 and 2). No such differences were found at the Ann Arbor site.

Ozone-induced symptoms occurred on both clones at both sites and were evident on the majority of the leaves by the end of the growing season (Table X). From these data, it appears that Clone 259 was more

TABLE IV. Growth response of Populus tremuloides Clones 253 and 259 to different O_3 treatments at Alberta, Michigan. The numbers shown are means across all N treatments ± the standard error.

Treatment	Stem (g)	Leaf (g)	Root (g)	Height (cm)	Diameter (mm)
Open Plot	4.3±0.5 b[1]	6.7±0.5 b	13.9±1.0 b	53.4±3.1 b	6.5±0.2 b
Filtered	8.9±1.2a	11.4±1.0a	21.8±1.4a	76.5±5.0a	7.7±0.2a
Non-Filtered	7.6±0.8a	11.0±1.1a	17.6±1.5ab	67.8±4.5ab	7.2±0.2a
O_3	6.7±0.8ab	9.5±0.9ab	19.5±1.6a	65.2±3.7ab	7.3±0.3a

[1] Treatments listed in a column with the same letter are not significantly different at the 0.05 level as determined by the Duncan's multiple range test. Treatments without letters after them were not significantly different.

TABLE V. Growth response of Populus tremuloides Clones 253 and 259 to different O_3 treatments at Ann Arbor, Michigan. The numbers shown are means across all N treatments ± the standard error.

Treatment	Stem (g)	Leaf (g)	Root (g)	Height (cm)	Diameter (mm)
Open Plot	1.8±0.1	4.2±0.2	9.2±0.4b[1]	33.4±1.8	5.1±0.1
Filtered	1.9±0.1	4.8±0.3	10.0±0.4ab	38.9±1.8	5.0±0.1
Non Filtered	1.6±0.1	4.7±0.3	11.0±0.5a	36.2±1.7	5.0±0.1
O_3	1.8±0.1	4.7±0.3	10.2±0.5ab	34.6±1.0	4.9±0.1

[1] Treatments listed in a column with the same letter are not significantly different at the 0.05 level as determined by the Duncan's multiple range test. Treatments without letters after them were not significantly different.

TABLE VI. The influence of N deposition treatment on Populus tremuloides trees growing at Alberta, Michigan. The numbers shown are means across all O_3 treatments ± the standard error.

N Deposition Treatment	Stem (g)	Leaf (g)	Root (g)	Height (cm)	Diameter (mm)
High	7.5±0.9a[1]	10.7±0.8a	18.4±1.3a	67.8±4.0a	7.3±0.2a
Medium	9.4±1.6a.	13.0±1.0a	22.4±1.6a	78.6±4.3a	8.1±0.2a
Low	6.9±1.0ab	9.5±0.9a	18.8±1.3a	68.5±4.2a	7.5±0.3a
0	3.8±0.7 b	5.6±0.7 b	13.1±1.2 b	48.3±3.5 b	5.8±0.3 b

[1] Treatments listed in a column with the same letter are not significantly different at the 0.05 level as determined by the Duncan's multiple range test. Treatments without a letter after them were not significantly different.

TABLE VII. The influence of N deposition treatment on Populus tremuloides trees grow- at Ann Arbor, Michigan. The numbers shown are means across all O_3 treatments ± the the standard error.

N Deposition Treatment	Stem (g)	Leaf (g)	Root (g)	Height (cm)	Diameter (mm)
High	2.3±0.1a[1]	5.7±0.3a	11.5±0.6a	41.7±1.9a	5.5±0.1a
Medium	2.1±0.1a	5.3±0.3ab	10.8±0.4a	41.1±1.6a	5.3±0.1a
Low	1.9±0.1a	4.7±0.2 bc	10.0±0.4a	36.4±1.5 b	5.2±0.1a
0	0.9±0.0 b	2.9±0.1 c	8.4±0.4 b	24.9±1.0 c	4.0±0.1 b

[1] Treatments listed in a column with the same letter are not significantly different at the 0.05 level as determined by the Duncan's multiple range test. Treatments without a letter after them were not significantly different.

TABLE VIII. Growth response of two Populus tremuloides clones to different N and O_3 treatments at Alberta, Michigan. The numbers shown (g) are means of six replicates ± the standard error.

Response Parameter	N	TREATMENT: Charcoal Filtered Clone 253	Charcoal Filtered Clone 259	O_3[1] Clone 253	O_3[1] Clone 259
Stem Biomass	High	12.4± 4.8	4.2± 0.6	11.5± 3.6ab	4.1± 1.5ab
	Medium	17.7± 5.0	8.1± 2.7	12.3± 2.1a	6.4± 1.9a
	Low	10.3± 3.2	5.9± 1.4	9.0± 2.4ab	5.3± 1.2ab
	Zero	8.4± 3.5	4.2± 1.4	3.8± 1.4 b	1.1± 0.5 b
Leaf Biomass	High	14.1± 2.8ab[2]	8.9± 1.1ab	13.8± 3.1a	8.5± 1.9
	Medium	18.1± 4.0a	13.5± 3.6a	15.1± 2.4a	13.3± 3.4
	Low	11.2± 2.4ab	9.5± 2.4ab	8.7± 2.2ab	7.9± 2.4
	Zero	10.4± 3.2 b	5.7± 1.6 b	5.9± 1.4 b	2.9± 1.1
Root Biomass	High	24.2± 1.9	16.4± 2.6	30.5± 4.4a	9.9± 1.4 b
	Medium	29.4± 6.1	24.6± 5.4	30.4± 4.3a	20.8± 2.6a
	Low	23.3± 2.1	21.8± 2.6	22.9± 5.0ab	17.8± 1.6a
	Zero	19.3± 3.9	15.3± 4.0	16.1± 2.3 b	6.9± 1.6ab
Height Growth	High	91.8±15.9	58.5± 4.5ab	81.0±10.9ab	51.1± 3.6 bc
	Medium	111.1±16.6	75.6±10.4a	90.8±10.2a	76.0± 9.2a
	Low	80.8±16.8	70.4±10.5ab	69.4±10.4ab	64.8± 3.4ab
	Zero	74.2±13.8	41.8± 6.2a	51.5± 8.2 b	36.7±10.6 c
Diameter Growth	High	8.6± 0.8	6.8± 0.3ab	7.6± 0.8	6.1± 0.2ab
	Medium	9.4± 0.6	8.0± 0.9a	9.1± 0.4	8.1± 0.5a
	Low	7.6± 0.8	7.5± 0.9a	9.1± 1.3	7.4± 0.5ab
	Zero	7.1± 1.1	6.5± 0.6 b	6.0± 0.6	4.8± 1.2 b

[1] Ozone was added at the rate of 80 ppb for 6 hr d^{-1}, 3 d per week, during the during the growing season.

[2] Treatments listed in a column with the same letter are not significantly different at the 0.05 level as determined by the Duncan's multiple range test. Treatments without letters after them are not significantly different.

TABLE IX. Growth response of two Populus tremuloides clones to different N and O_3 treatments at Ann Arbor, Michigan. The numbers shown (g) are means of six replicates ± the standard error.

Response Parameter	N	TREATMENT: Charcoal-Filtered Clone 253	Charcoal-Filtered Clone 259	O_3[1] Clone 253	O_3[1] Clone 259
Stem Biomass	High	1.8± 0.6	1.9± 0.3ab	3.4± 0.3a	1.8± 0.3
	Medium	2.3± 0.4	2.2± 0.3a	1.9± 0.3 b	1.7± 0.3
	Low	1.8± 0.3	1.3± 0.2ab	2.1± 0.2 b	1.9± 0.2
	Zero	0.8± 0.3	0.9± 0.2 b	0.7± 0.1 c	1.0± 0.1
Leaf Biomass	High	4.3± 1.3	6.5± 0.5a	6.9± 0.9a	5.8± 0.7
	Medium	5.1± 0.8	7.2± 0.9ab	4.2± 0.6a	5.3± 1.0
	Low	4.2± 0.7	4.8± 0.6 bc	4.6± 0.6ab	4.8± 1.2
	Zero	2.6± 0.4	3.3± 0.2 c	2.5± 0.2 b	3.7± 0.5
Root Biomass	High	11.1± 3.0	12.3± 1.6ab	15.8± 2.2	9.7± 1.1
	Medium	13.4± 1.6	12.8± 1.0a	10.1± 1.2	9.5± 1.2
	Low	11.2± 1.7	10.0± 1.2ab	11.6± 1.5	9.3± 0.9
	Zero	9.7± 1.6	8.0± 1.2 b	10.9± 0.5	8.8± 1.3
Height Growth	High	36.1±10.4	40.4± 3.3a	52.7± 3.6a	38.5± 4.1
	Medium	43.6± 5.4	42.7± 3.1a	40.0± 4.8ab	38.0± 5.3
	Low	39.8± 4.5	33.1± 3.2ab	36.3± 7.0ab	29.7± 2.3
	Zero	26.7± 2.4	27.7± 1.6 b	22.2± 3.7 b	26.6± 3.1
Diameter Growth	High	4.9± 0.4ab[2]	5.7± 0.2a	5.6± 0.2a	5.5± 0.3
	Medium	5.3± 0.3a	5.6± 0.2ab	4.8± 0.2a	5.2± 0.4
	Low	4.9± 0.3ab	5.0± 0.3ab	5.3± 0.3a	5.3± 0.2
	Zero	3.9± 0.2 b	4.6± 0.1 b	3.9± 0.1 b	3.8± 0.3

[1] Ozone was added at the rate of 80 ppb for 6 hr d^{-1}, 3 d per week, during the growing season.

[2] Treatments listed in a column with the same letter are not significantly different at the .05 level as determined by the Duncan's multiple range test. Treatments without letters after them are not signficantly different.

sensitive to O_3 early in the season, but that both were quite sensitive to O_3, based on visible foliar injury, by the end of the growing season.

TABLE X. Symptom development as indicated by percentage of leaves showing necrosis on Populus tremuloides leaves grown in open-top chambers with 80 ppb O_3 for 2 d per week, 6 hr d^{-1} at Alberta, Michigan.

Clone Number	7/6/89	7/20/89	8/3/89	8/17/89	8/31/89
253	0	6.9	11.1	18.1	66.7
259	0	13.5	24.0	45.0	70.8

4. DISCUSSION

Research on the interaction of O_3 and N has shown that nitrogen can enhance (Brewer et al., 1961; Leone et al., 1966; Ormrod et al., 1973; Pell et al., 1990) or not affect (Elkiey and Ormrod, 1981) the sensitivity of plants to O_3, depending on the plant species and environmental conditions. Increased nutrient input from acidic precipitation has been shown to stimulate growth (Irving, 1983; Keane and Manning, 1988) and to either decrease O_3 impact (Keane and Manning, 1988), increase O_3 impact (Chappelka and Chevone, 1988) or to not affect O_3 impact (Norby and Luxmoore, 1983; Norby et al., 1985; Rebbeck and Brennan, 1984; Reich et al., 1985). Considering that vegetation in much of North America is simultaneously subjected to elevated O_3 and N deposition, there is clearly a need for more research on the interaction of these two pollutants.

The most striking result of our study was the fertilization effects of N deposition, even though we added N at a pH of 2.0. Trembling aspen growth was significantly enhanced by all three N-added treatments.

We found a trend toward a O_3 effect at the 0 N level at the Alberta site. This is consistent with season-long O_3 fumigations which we have run for the past 3 years, where sensitive clones have consistently shown a 20 to 50% reduction in stem biomass accumulation in season-long fumigations (Karnosky and Scholz, 1990). The same two clones used in this O_3-x-N study were reduced by 15% (Clone 253) and 33% (Clone 259) in 1990 (Karnosky et al. 1991) in season-long exposures to twice ambient (where ambient was modified from the Upper Great Lakes profile, determined by Pinkerton and Lefohn, 1987).

The trend toward an O_3-induced reduction in growth was lost as N deposition was added as a combined effect. All N deposition treatments resulted in increased aspen growth and also appeared to mask over the O_3 effect.

We did find a significant amount of visible foliar injury and premature leaf senescence and abscision even in the N treatments, indicating that multiple season exposures may have been needed to detect growth responses to O_3 in this study. It is likely that the late season foliar injury and leaf abscision reduced carbohydrates in overwinter storage, which may affect the subsequent year's growth.

Our aspen plants grew more vigorously at the Alberta site than at Ann Arbor. This was probably due to the high temperature stress that the Ann Arbor trees experienced during the time these trees were being transplanted in early June. The transplant shock was much less apparent at Alberta where plants resumed vigorous growth shortly after transplanting. While we cannot rule out genotype by environment interactions that may have occurred in this study, we believe that the growth differences between the two sites were primarily due to environmental conditions around transplanting time. In two previous seasons of O_3 exposures at these two sites, our plants have previously grown more vigorously at the Ann Arbor site. Clone 253 has previously outgrown Clone 259 at both sites.

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