

Chapter 8

Ozone affects the fitness of trembling aspen

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Abstract

Trembling aspen (*Populus tremuloides* Michx.) is sensitive to tropospheric ozone (O₃) as determined by visible foliar symptoms, accelerated foliar senescence and premature abscission, degradation and change in composition of epicuticular waxes, decreased photosynthesis and chlorophyll, and decreased

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aboveground and belowground growth. The species is highly variable in O₃ responses as some clones are similar in sensitivity to Bel W3 tobacco and other clones are tolerant to moderate levels of O₃. We have, therefore, hypothesized and presented evidence for natural selection of O₃-tolerance in aspen populations. This hypothesis has been criticized, however, as improbable because O₃ is thought to be a fairly weak selection force and because changes in tree population are thought to occur over very long time periods, longer than tropospheric O₃ has been known to be a problem. To shed more light on this argument, in 1994 we established a set of research field trials using clones of known origin and previously determined O₃ sensitivity at three sites in the Lake States region with differing O₃ profiles (Rhineland, Wisconsin—low O₃; Kalamazoo, Michigan—moderate O₃; and Kenosha, Wisconsin—high O₃). In this paper, we present evidence of changes in the relative volume d²h growth of clone 259 (O₃-sensitive) compared to clone 216 (O₃-tolerant) of -0.1%, -44.2%, and -62.8% at the low, medium and high O₃ sites at age 5. In addition, relative survival of the clone 259 compared to 216 was -11.0%, -6.8%, and -38.4% at the low, moderate, and high O₃ sites. Actual survival rates at the high O₃ site were 78.2% for clone 216 and only 48.2% for clone 259. Our results suggest that very rapid and significant changes in competitive ability and fitness can occur under ambient levels of O₃ in the lower Great Lakes region for aggrading forests. These results are consistent with the hypothesis that O₃ is inducing natural selection for O₃ tolerance in aspen.

1. Introduction

Ozone (O₃), a widespread and highly phytotoxic secondary air pollutant, has long been known to impact the growth of trembling aspen (*Populus tremuloides* Michx.) (Wang et al., 1986) by degrading chlorophyll (Gagnon et al., 1992), decreasing rubisco (Noormets et al., 2001), decreasing photosynthesis (Coleman et al., 1995), altering carbon allocation (Coleman et al., 1996), causing visible foliar symptoms and premature senescence (Karnosky et al., 1996), altering leaf epicuticular waxes (Mankovska et al., 1998), and predisposing trees to disease pests (Karnosky et al., 2002; Percy et al., 2002). It is also well known that trembling aspen is highly variable in response to O₃ (Karnosky, 1976) and that the response variation is under strong genetic control (Karnosky, 1977). Genetic control appears to be exerted on gas exchange rates (Noormets et al., 2001) and an antioxidant formation in response to O₃ exposure (Sheng et al., 1997; Karnosky et al., 1998; Noormets et al., 2000; Wustman et al., 2001).

Evidence for differences in O₃ tolerances among various populations differing in O₃ exposures has been presented by Berrang et al. (1986, 1989, 1991) who made the prediction that O₃ was altering the competitive ability of trembling aspen genotypes in areas of high O₃ exposure in North America. This

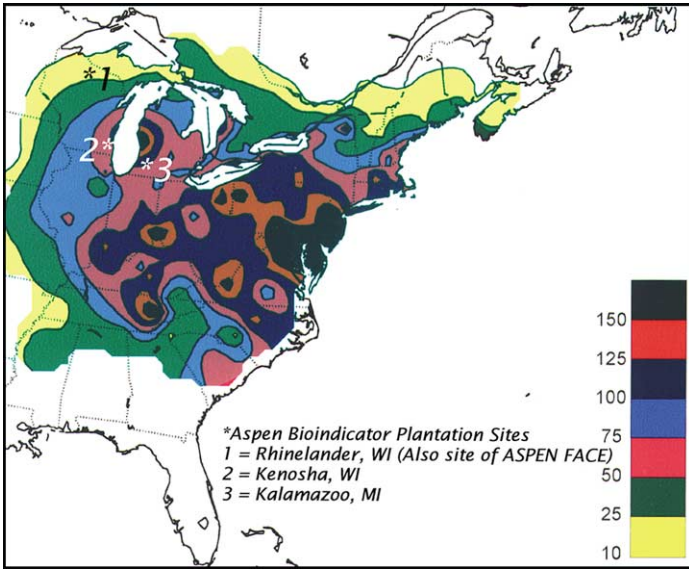


Figure 1. Spatial distribution of average hours with ozone > 82 ppb (1986–1993) (from Dann and Summers, 1997) and locations of aspen plantations (1 = Rhinelander, WI; 2 = Kenosha, WI; 3 = Kalamazoo, MI).

research was challenged by other researchers who speculated that O₃ was not a strong enough selective pressure in most areas (Taylor et al., 1994; Taylor and Pitelka, 1992) and that trees had not been exposed to O₃ for a long enough time period to evolve O₃ tolerance (Barrett and Bush, 1991).

This study was designed to address the question as to whether or not O₃ could impact the growth and survival of trembling aspen genotypes differing in O₃ tolerance and growing in a competitive environment over the first five years after planting.

2. Methods

In 1994, we established three types of aspen plantations at each of three locations (Rhinelander, Wisconsin—low O₃; Kalamazoo, Michigan—moderate O₃; and Kenosha, Wisconsin—high O₃) along a natural O₃ gradient in the Lake States (Fig. 1).

At each location, we established three experiments (Karnosky et al., 1999). The first was a “common garden” experiment of 7 aspen clones differing in O₃ sensitivity (clones 1, 253 and 259 = O₃-sensitive; clones 10, 216, 221, and



Figure 2. The Kenosha, WI site is shown here with the Wisconsin Department of Natural Resources air quality monitoring shed (foreground), the growth and yield plot (background) and the competition plot (right).

271 = O₃-tolerant (Karnosky, 1976; Karnosky et al., 1992)). This experiment consisted of individual trees of each clone planted at 2 m × 2 m spacing and there were 10 replicates at each site. In the second experiment, we established a “growth and yield” trial with O₃-tolerant clone 216 and O₃-sensitive clone 259 planted in 16-tree (4 × 4) blocks at 2 m × 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 × 0.5 m between trees and 100-tree (10 × 10) blocks were established in either pure clonal blocks or mixed clonal blocks (the O₃-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. Fig. 2 shows the Kenosha, WI, 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 1). Each site was enclosed in a 3 m tall deer fence.

O₃ has been monitored independently and continuously at the Kenosha site and Rhinelander site by the Wisconsin Department of Natural Resources. The

Table 1. Comparisons of monthly mean temperatures (°C) and seasonal total rainfall (cm) at our three aspen sites along a natural gradient (from: <http://www.acdc.noaa.gov/online>)

Average monthly temperatures (°C)															
1995		1996		1997		1998		1999							
Rhine	Kala	Keno	Rhine	Kala	Keno	Rhine	Kala	Keno	Rhine	Kala	Keno	Rhine	Kala	Keno	
June	21.98	23.38	21.57	18.00	22.38	17.95	19.60	21.97	17.98	16.89	19.67	18.72	18.17	20.78	19.06
July	21.83	25.37	23.37	17.88	22.95	20.75	19.55	23.05	20.61	20.33	22.33	22.22	22.22	23.72	25.44
Aug	23.92	25.89	23.92	20.08	24.35	22.33	16.81	21.68	19.26	20.56	22.33	22.67	18.72	19.89	21.22

Date	June through August rainfall (cm)		
	Rhineland	Kalamazoo	Kenosha
1995	20.17	26.58	19.64
1996	35.43	30.48	39.10
1997	24.12	29.58	38.17
1998	20.60	16.0	26.80
1999	34.70	25.17	30.43

Table 2. Summary of O₃ values at the three locations where we have aspen bioindicator plots for 1995 to 1999

Site	1995	1996	1997	1998	1999	5-year
	Max 1-h (ppb)					
Rhineland	79	87	80	74	93	82.2
Kalamazoo	125	106	96	109	103	107.8
Kenosha	120	122	130	119	123	122.8
	Sum 06 (ppm h 8 a.m.–8 p.m. for June 1 to August 31)					
Rhineland	4.1	2.5	3.6	2.7	4.6	3.5
Kalamazoo	20.6	17.3	24.4	21.4	21.4	21.0
Kenosha	29.8	15.1	–	17.7	27.3	22.5

Kalamazoo site was monitored for O₃ by Pharmacia about 0.5 km from our aspen plots. The O₃ data was quality assured and was part of the AIRS (Aerometric Information and Retrieval Systems of the US Environmental Protection Agency) network. A summary of the O₃ values for 1995–1999 is shown in Table 2.

We evaluated foliar injury on all leaves on a subset of the trees representing all the clones at the O₃ gradient sites.

Heights (±10 cm), diameters (±1 mm), and survival were recorded yearly.

Table 3. Age 5 heights, diameters, and estimated volumes for an O₃-tolerant clone (216) and an O₃-sensitive clone (259) from three sites along an O₃ gradient (Rhineland, WI = low O₃; Kalamazoo, MI = moderate O₃; Kenosha, WI = high O₃). Values are means ± Standard error

Variable	Clone	Rhineland	Kalamazoo	Kenosha
Height (cm)	216	248 ± 6	383 ± 8	531 ± 10
	259	239 ± 4	326 ± 5	374 ± 7
Diameter (cm)	216	1.71 ± 0.08	2.26 ± 0.06	2.98 ± 0.06
	259	1.89 ± 0.07	1.86 ± 0.05	2.21 ± 0.05
Volume (D^2H)	216	1014 ± 114	2589 ± 122	5872 ± 309
	259	1012 ± 74	1444 ± 76	2182 ± 131
Ratio	216/259	1.001	1.793	2.691

3. Results

Maximum 1-hr O₃ concentrations averaged 82.2 ppb (Rhineland), 107.8 ppb (Kalamazoo) and 122.8 (Kenosha) over the 5-year (1994–1999) period (Table 2). During five years (1994–1999) after establishment, we have documented significant, repeatable and consistent site-to-site and clone-to-clone differences in visible O₃ symptoms, degradation of epicuticular waxes and changes in wax composition, and occurrence of pests. The Kenosha site has always had the most severe O₃ impacts.

3.1. Visible foliar symptoms

Visible foliar symptoms were seen on O₃-sensitive aspen clones each growing season by late July to early August at the Kalamazoo and Kenosha sites (Fig. 3). Visible foliar symptoms occurred on 5–8% of clone 216 leaves and 34.7–43.1% of clone 259 leaves (Karnosky et al., 1999). No visible foliar symptoms were detected at the Rhineland site. Visible foliar symptoms consisted of black bifacial necrosis, chlorosis, or upper leaf surface black stipple and premature leaf abscission. These are all classic O₃ symptoms on aspen (Karnosky, 1976; Wang et al., 1986; Karnosky et al., 1996). Visible symptoms were also seen on other O₃ bioindicator plants, including understory black cherry volunteer seedlings and on nearby milkweed plants, at the Kalamazoo and Kenosha sites, but not at Rhineland.

3.2. Growth

The trees grew at different rates at the three sites with the trees being the largest at the Kenosha site (Table 3). The ratio of the volume growth of the O₃-tolerant clone (216) to the volume growth of the O₃-sensitive clone changed from 1.001



Figure 3. Examples of visible foliar symptoms seen at the Kenosha, WI site. A: Black bifacial necrosis on clone 259. B, C: Clones 216 (B) and 259 (C) showing the differential premature leaf abscission due to O₃ following a late August O₃ event.

at Rhinelander to 1.793 (Kalamazoo) and 2.691 (Kenosha) indicating the larger detrimental effect of O₃ on the O₃-sensitive clone 259 than on the O₃-tolerant clone 216.

3.3. Fitness

Fitness involves the ability of plants to survive and to produce offspring. The trends in survival can be seen in Fig. 4. Survival at the low O₃ site was 95%

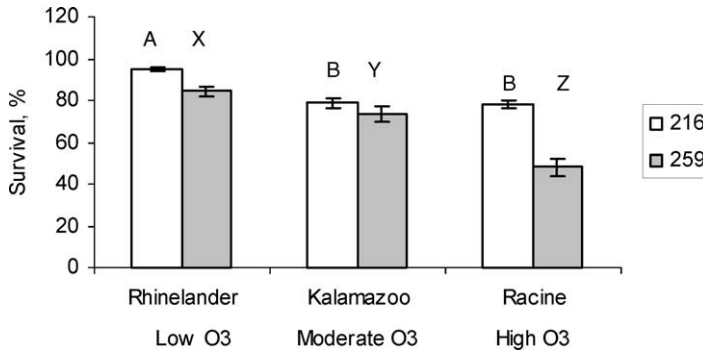


Figure 4. Survival at age 5 of two aspen clones differing in O₃ tolerance clone 216 = O₃ tolerant and clone 259 = O₃ sensitive. Rhinelander, WI = low O₃; Kalamazoo, MI = moderate O₃; Kenosha, WI = high O₃. The values shown with similar letters are not different at the 0.0001 level (Duncan's Multiple Range test via SAS).

(clone 216) and 84% (clone 259). Significant decreases in the survival of both clones were seen at the elevated O₃ sites and the largest difference in survival between the O₃-tolerant and O₃-sensitive clones came at the highest O₃ site.

4. Discussion

In this paper we present evidence linking elevated levels of ambient O₃ to differential survival of O₃-sensitive and O₃-tolerant aspen clones grown for five years along a natural O₃ gradient in the Lake States region. Age 5 survival data shows the same statistical trends as to the findings of visible foliar symptoms and height and diameter growth in that the O₃-tolerant clone (216) had less visible foliar symptoms, less growth impact and higher survival rates than did the O₃-sensitive clone (259).

These results are consistent with previous visible symptoms and growth studies in open-top chambers (Karnosky et al. 1992, 1996) and in Free-air CO₂ Enrichment (FACE) studies (Karnosky et al., 1999; Isebrands et al., 2001; McDonald et al., 2002) where clone 216 has always been more tolerant than clone 259.

We present evidence of a change in the ratio of volume growth of clone 216 from 1.01 at the low O₃ site to a maximum of 2.36 at the highest O₃ site. In short-term open-top chamber studies, we found an increase in the ratio of total biomass from 1.015 in charcoal-filtered air to 1.324 in 2 × ambient O₃ (Karnosky et al., 1996).

The impacts of O₃ on evolutionary processes of plants are poorly understood. Dunn (1959) reported effects of O₃ on southern California *Lupinus*

populations. Reiling and Davison (1992) found evidence of O₃-induced population changes in native herbaceous species. Karnosky (1981, 1989) presented evidence for a 10-fold higher mortality rate among O₃-sensitive *Pinus strobus* genotypes as compared to O₃-tolerant genotypes over a 15-year time frame in southern Wisconsin. Berrang et al. (1986, 1989, 1991) found evidence of population differences in O₃ tolerance and these were related to the O₃ concentrations of each population.

The question if evolutionary changes are occurring under ambient O₃ in natural populations remains a key genecological question (Pitelka, 1988; Davison and Barnes, 1998; Winner et al., 1991). In this paper, we provide evidence for a reduced fitness of an O₃-sensitive clone as determined by a 36% decrease in survival compared to the low O₃ site within five years from establishment. The spacing conditions (0.5 m × 0.5 m) are characteristic of aspen stands regenerating after a clear-cut or fire and the highest ambient O₃ levels reported in this study are typical of those found over much of the eastern one-half of the United States.

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