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GENETIC CONTROL OF RESPONSES TO INTERACTING TROPOSPHERIC OZONE AND CO_2 IN *POPULUS TREMULOIDES*

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ABSTRACT

We exposed trembling aspen (*Populus tremuloides* Michx.) clones differing in tropospheric ozone (O_3) tolerance in various open-top chamber studies for three growing seasons, and examined the effects of O_3, CO_2, and O_3 + CO_2 on growth and physiological processes. Ozone in the range of 80 ppm hr (Sum 00) per growing season decreased height, diameter, and stem and leaf biomass slightly in a tolerant clone but severely in a sensitive clone. Elevated CO_2 (150 ppm over ambient) did not compensate for the O_3 effects.

Antioxidant enzyme analysis showed elevated SOD levels in the tolerant clone but not in the sensitive clone following O_3 exposure. Northern blot analysis indicated that the chloroplastic and cytosolic Cu/Zn SOD's were significantly increased in response to O_3 in the tolerant but not the sensitive clone. Currently, we are conducting molecular analysis to determine the functional significance of SOD's in regulating O_3 tolerance in aspen. © 1997 Elsevier Science Ltd

INTRODUCTION

Global atmospheric CO_2 is increasing rapidly and preindustrial CO_2 concentrations are expected to double by the end of the next century [1]. Tropospheric ozone (O_3), a secondary pollutant generated from nitrogen oxides and hydrocarbons, is also increasing globally, especially in and around major metropolitan areas [2]. Thus, in the future, trees in much of the world will be exposed simultaneously to elevated CO_2 and O_3. While CO_2 generally stimulates tree growth [3] and O_3 generally decreases tree growth [4], there is little information available on the impacts of interacting O_3 and CO_2 on forest tree growth and productivity. Trembling aspen (*Populus tremuloides* Michx.) is a good model species to examine the effects of these two pollutants, as it is highly responsive to both O_3 [5, 6, 7, 8, 9, 10, 11] and CO_2 [12]. Furthermore, we have identified a wealth of genetic variation in the response of trembling aspen to air pollutants, and we have isolated O_3-sensitive and tolerant clones [9, 10, 11, 13, 14].

In this report we (1) compare the influence of O_3 on three years growth of two aspen clones differing in O_3 sensitivity; (2) examine the potential for elevated CO_2 to ameliorate the negative O_3 effects; and (3) examine the underlying mechanisms of O_3 tolerance in aspen.

MATERIALS AND METHODS

Two aspen clones differing in visible foliar injury [14] and in growth responses [9, 10, 11] were chosen for study. Clone 216 is relatively O_3 tolerant and clone 259 is O_3 sensitive. Rooted cuttings of each clone were initiated in the greenhouse in February, 1991. In June, 1991, the rooted cuttings were moved outdoors where they were transplanted into 40 cm tall, 15 cm diameter pots in an artificial soil mix [11]. In October, 1991, the trees were transplanted into native soil in open-top chambers at the Ford Forestry Center in Alberta, Michigan. The open-top chambers were standard 3.1 m diameter x 2.5 m tall chambers as described by Heagle *et al.* [15]. The chambers were modified with frustems to decrease wind incursion. The chamber height was extended to 5.0 m for the third growing season. Trees were planted in a circle inside the chambers so all trees were equidistant from the chamber edge and from the center of the chamber with 6 sensitive and 6 tolerant trees per chamber. Trees were weeded, watered, and sprayed with pesticides on a minimal "as need" basis.

Three replicate chambers were utilized for each of the following treatments: simulated ambient O_3 (1X), twice simulated ambient O_3 (2X), elevated O_3 plus elevated CO_2 (2X + CO_2), and charcoal-filtered air. Open-plot circles of trees were planted to test for chamber effects. The 1X treatment was a modified ambient developed to mimic the O_3 levels in the southern Great Lakes region [11]. The 2X treatment was meant to mimic conditions under relatively high areas such as southeastern Wisconsin. The 2X + CO_2 had the same O_3 profile as the 2X but also had 150 ppm CO_2 above ambient during the growing season. Fumigations ran each year from approximately June 1 to August 30.

Heights and diameters of the treated trees were measured yearly. Six trees (3 sensitive and 3 tolerant) from each chamber were harvested at the end of the first growing season and 4 trees (2 sensitive and 2 tolerant) were harvested at the end of the second growing season. The final 2 trees per chamber were harvested at the end of the third growing season.

We sampled foliage at various times from potted plants of the same clones growing in open-top chambers under a square wave exposure of 100 ppb for 6 hours per day and 5 days per week to examine the role of various antioxidant systems in affecting O_3 sensitivity. Fumigations were conducted from June 1 to August 30, 1992. Recently mature leaves of both clones were collected from on August 26, 1992. They were placed in liquid nitrogen and stored in a low-temperature freezer until biochemical analyses were performed.

Biochemical analyses for superoxide dismutase (SOD) and glutathione were analyzed, and northern blot analyses were done as described by Sheng *et al.* [16].

RESULTS AND DISCUSSION

The total three-year exposures were 60, 161, 249, and 262 ppm hours for the control, 1X, 2X, and 2X + CO_2 treatments, respectively. The control levels are typical of those in very pristine environments while the 1X and 2X doses are in the range of seasonal exposures typical in much the eastern half of the U.S. [17].

Visible foliar symptoms consisting of bifacial necrosis, general chlorosis, and premature senescence all occurred in all O_3 treatments and with both clones. However, symptom development was sooner and more severe in the sensitive than in the tolerant clone and was sooner and more severe as the O_3 dose increased over the treatments. Decreases in photosynthesis were detectable in all O_3 treatments and were most severe in the 2X O_3 + CO_2 [18].

The trends in growth and productivity are illustrated in Figure 1 which shows total stem biomass of the two clones over the three growing seasons. Significant stem biomass reductions were evident in both clones in the second and third year for all O_3 treatments when compared to controls. By the end of the third growing season, the most severe effects were seen in the sensitive clone in the 2X and 2X + CO_2 treatments where a nearly 70% decrease in total stem biomass was evident. Elevated CO_2 did not compensate for O_3 impacts as 2X and 2X + CO_2 treatments were not significantly different for either clone.

The largest impact in above-ground growth was on diameter growth (Table 1). Similar results have been found by Matyssek *et al.* [19] and Karnosky *et al.* [9, 10, 11]. Our results suggest that O_3 at doses currently occurring over much of the eastern U.S. could be negatively impacting tree growth in trembling aspen and other O_3 sensitive species. Furthermore, the great differential in growth caused O_3 for two clones of otherwise similar growth rates could have a major impact on their relative competitive ability as suggested by Berrang [13, 14].

Superoxide dismutase activity increased almost two-fold in response to O_3 and to O_3 + CO_2 treatments in the tolerant clone (216), but did not increase in response to O_3 in the sensitive clone (259), as shown in Figure 2. In contrast, glutathione was not significantly affected by O_3 treatments for either clone (Figure 3), but trends of increasing glutathione with O_3 were evident in the tolerant clone while they appeared to decrease with elevated O_3 in the sensitive clone. Subsequent northern blot investigation of the SOD response in our plants has shown that the Mn SOD and cytosolic Cu/Zn SOD transcripts were responsible for the increase in

SOD levels [18]. The SOD's appear to be highly conserved as we obtained a 95% homology of sequence with aspen and pea chloroplastic Cu/Zn SOD's [20].

Reports by Badiani *et al.* [21] and Polle *et al.* [22] suggest that CO_2 may decrease antioxidant production and thereby make plants more susceptible to O_3. However, that did not appear to be the case for SOD or glutathione with aspen. Our increased O_3 sensitivity [18] when aspen are grown under elevated CO_2 remains unexplained, and currently is the subject of a new experiment using a free air CO_2 exposure (FACE) system in Rhinelander, Wisconsin, USA.

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Figure 1. Total stem dry weights of an O_3 tolerant aspen clone (216) and an O_3 sensitive clone (259) exposed for three growing seasons (1992–94) to charcoal–filtered air (CF), or a modified ambient (1X), twice modified ambient (2X) or twice modified ambient plus 150 ppm CO_2 over ambient (2X + CO_2) in open–top chambers.

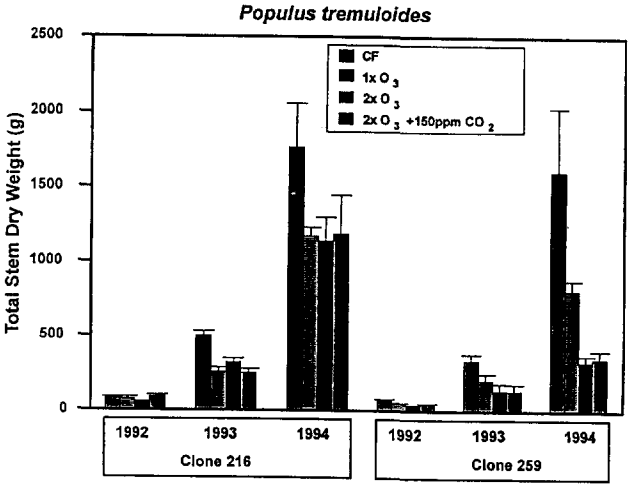

Table 1. Average (and standard errors) heights (cm) and diameters (mm) of an O_3-tolerant aspen clone (216) and an O_3-sensitive clone (259) exposed for three growing seasons (1992–94) to charcoal-filtered air (control) or a modified ambient (1X), twice modified ambient (2X) or twice modified ambient plus 150 ppm CO_2 over ambient (2X + CO_2) in open-top chambers.

HEIGHT (cm)

	Clone 216			Clone 259		
	1992 Year 1	1993 Year 2	1994 Year 3	1992 Year 1	1993 Year 2	1994 Year 3
Control	188.3 ± 6.3 a	334.6 ± 14.4 a	534.7 ± 56.5	137 ± 11.9	236.8 ± 18.6	441.7 ± 31.7 a
1X	144.8 ± 8.3 ab	259.9 ± 16.2 b	490.0 ± 30.6	124.4 ± 9.7	212.9 ± 11.1	348.7 ± 31.2 b
2X	152.4 ± 10.7 ab	292.1 ± 16.8 ab	489.0 ± 24.2	123.4 ± 8.0	216.1 ± 16.8	315.7 ± 21.9 b
2X + CO_2	151.9 ± 9.4 ab	264.9 ± 11.6 b	466.7 ± 33.7	117.4 ± 6.5	182.5 ± 7.5	290.0 ± 20.0 bc

DIAMETER (mm)

	Clone 216			Clone 259		
	1992 Year 1	1993 Year 2	1994 Year 3	1992 Year 1	1993 Year 2	1994 Year 3
Control	15.6 ± 0.12 a	29.4 ± 1.2 a	43.3 ± 4.9	13.6 ± 0.2 a	25 ± 1.9 a	41.6 ± 4.1 a
1X	13.1 ± 0.16 b	23.1 ± 0.8 ab	36.4 ± 1.0	12.1 ± 0.1 b	22 ± 1.1 ab	32.0 ± 0.6 b
2X	12.3 ± 0.13 c	24.6 ± 0.8 ab	35.7 ± 1.9	10.5 ± 0.1 c	18.9 ± 1.3 b	22.9 ± 1.5 c
2X + CO_2	13.6 ± 0.12 b	23.4 ± 1.4 ab	37.2 ± 2.4	10.8 ± 0.1 c	17.2 ± 1.1 b	23.0 ± 2.6 c

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Figure 2. Total superoxide dismutase activity in leaves of 1-year-old aspen exposed in open-top chambers to O_3, or O_3 + CO_2, or charcoal-filtered air. Leaves were collected on August 26, 1992 after 2 months' exposure to 100 ppb ozone for 6 hours per day, 5 days per week. Data shown represent the means from three different trees.

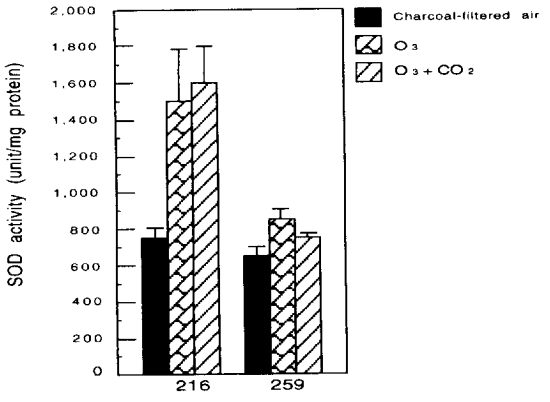

Figure 3. Content of total glutathione in leaves of 1-year-old aspen exposed in open-top chambers to O_3 or O_3 + CO_2 or charcoal-filtered air. Leaves were collected on August 26, 1992 after 2 months' exposure to 100 ppb ozone for 6 hours per day, 5 days per week. Data shown represent the means from three different trees.