EVIDENCE FOR A CADMIUM AND OZONE INTERACTION ON POPULUS TREMULOIDES[1]

by Bruce B. Clarke and Eileen Brennan

In many highly populated areas, such as New Jersey, trees are periodically exposed to elevated ozone (O_3) levels during the growing season. More recently, evidence has been found for increased contamination of the environment by cadmium (Cd), a heavy metal which is emitted by various industrial processes, by vehicular traffic, and by the application of sewage sludge to agricultural and forest soils (Beavington 1975; Lagerwerff and Specht 1970; Sidle and Kardos 1977; Sommers 1977). Cadmium *per se* is also phytotoxic at certain concentrations (Page *et al.* 1972). Since two pollutants can act synergistically, as in the case of O_3 and sulfur dioxide (Reinert *et al.* 1975), we examined the possibility for an interaction between Cd and O_3 on trees. Quaking aspen, *Populus tremuloides* Michx., was chosen as the test plant because it is one of the most sensitive tree species to O_3 (Karnosky 1976).

Experimental Method

Cuttings of quaking aspen clone 6, rooted as described by Karnosky (1976), were obtained from the Cary Arboretum, Millbrook, New York 12545. Rooted ramets, 7 to 10 cm in length, were planted in 5 L plastic pots containing washed sand on 4 August. Plants received dilute nutrient solution (Shive and Robbins, 1937) for four weeks. On 5 September plants were separated into four treatment groups, each containing eighteen randomly selected individuals and placed outdoors in New Brunswick, New Jersey, exposed to whatever pollutants existed in ambient air. Ambient temperatures ranged from 20-29°C during the day and 12-23°C at night. The oxidant concentration of the air was measured continuously with a Mast Sensor (Clarke *et al.* 1978). Symptoms of Cd toxicity and O_3 injury were under daily surveillance. Each group received one of the following treatments: (1) complete nutrient solution, (2) complete nutrient solution amended with 1.0 ug Cd/ml as $CdCl_2$, (3) complete nutrient solution amended with 5.0 ug Cd/ml as $CdCl_2$ and (4) complete nutrient solution amended with 10 ug Cd/ml as $CdCl_2$. Solutions were poured on the sand daily for 30 days, in volumes sufficient to attain field capacity (750-1000 mls).

Plants were ozonated, after 30 days of Cd treatment, in a $6m^3$ glass enclosed fumigation chamber located within a greenhouse. Ozone was produced by passing pure dry O_3 through a commercial O_3 generator and mixed with a stream of charcoal filtered air. A complete air change occurred every 45 seconds (Leone *et al.* 1966). Chamber temperatures were maintained between 18-23°C and relative humidity at 75-80%. Uniform exposure to the O_3-air mixture was ensured by placing plants on a rotating table. Ozone levels in the chamber were monitored with a Mast Ozone Meter (Mast Development Co., Davenport, Iowa 52802) and calibrated by the neutral buffered KI method (Jacobs, 1960). Plants were watered to field capacity ½ h before fumigation. Two O_3 exposures were included in this experiment. The lower O_3 fumigation was conducted utilizing 0.2 ppm O_3 from 1130 hr to 1400 hr on 6 October and the higher exposure with 0.3 ppm O_3 from 1100 hr to 1400 hr on 7 October. Each fumigation contained five replicates per treatment. Control plants were placed in an identical chamber under similar conditions in the absence of O_3.

Ozone toxicity symptoms on aspen foliage were recorded 48 hr after fumigation. Plants were rated according to a foliar injury index (Table 2). Only leaves greater than 2 cm long were scored. Trees were separated into leaf, stem and root fractions and weighed. Tissue was dried in a forced air oven at 70°C, reweighed, and ground through a

[1]This research was supported by McIntire-Stennis funds at the New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey 08903.

Table 1. Cadmium concentration of aspen tissue in plants receiving various levels of cadmium daily for 30 days.

	Cd Concentration/Aspen		
Cd Treatment	Stem	Leaf	Root
(ug/ml)	*ug/g*		
0	0.9 a*	1.5 a	1.3 a
1.0	25.2 b	36.8 b	69.2 b
5.0	59.5 c	70.9 c	135.5 c
10.0	95.8 d	80.6 c	249.9 d

*Means of eighteen replicates within a column sharing the same letter are not significantly different at the 5% level of Tukey's HSD test. Means in the same row underscored by the same line are not significantly different at the 5% level of probability.

40 mesh screen in a Wiley Mill. One gram samples were ashed at 550°C in a muffle furnace for 8 hr, digested in 3 N nitric acid and analyzed for Cd by means of a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. All data were statistically analyzed using an ANOVA and Tukey's HSD Multiple Comparison Test (Steel and Torrie 1960).

Cd tissue concentrations and Cd symptoms

As shown in Table 1, tissues harvested from plants receiving nutrient solution devoid of Cd contained about 1.0 ppm Cd. When the Cd concentration in the sand was increased, the Cd concentration in all tissue fractions increased significantly. Generally, the Cd gradient was roots > leaves > stems. This is a rather common distribution when Cd is administered via the roots.

Aspens treated with 1 ppm Cd developed a bifacial interveinal chlorosis which became increasingly more severe at the higher Cd treatments (Fig. 1). Actually, the 1 ppm Cd treatment had some beneficial effects; the trees had a greater number of leaves (Fig. 2) and the stem length was increased (Fig. 3).

Cd and O_3 Interaction

During the 30 days that the quaking aspens grew outdoors the oxidant concentration of ambient air ranged from 0.01 to 0.08 ppm, relatively low for the fall months in New Jersey. Trees treated with 10 ppm Cd exhibited significantly more oxidant injury on the foliage than trees receiving 0, 1, or 5 ppm Cd (Fig. 4).

Table 2. Foliar injury index used to rate ozone toxicity symptoms on tomato and aspen.

Numerical Rating	*Severity of injury*	*% Leaf area*
0	*No injury*	*0*
1	*Slight*	*0-10*
2	*Slight/moderate*	*10-25*
3	*Moderate*	*25-50*
4	*Moderate/severe*	*50-75*
5	*Severe*	*75-90*
6	*Death*	*90-100*

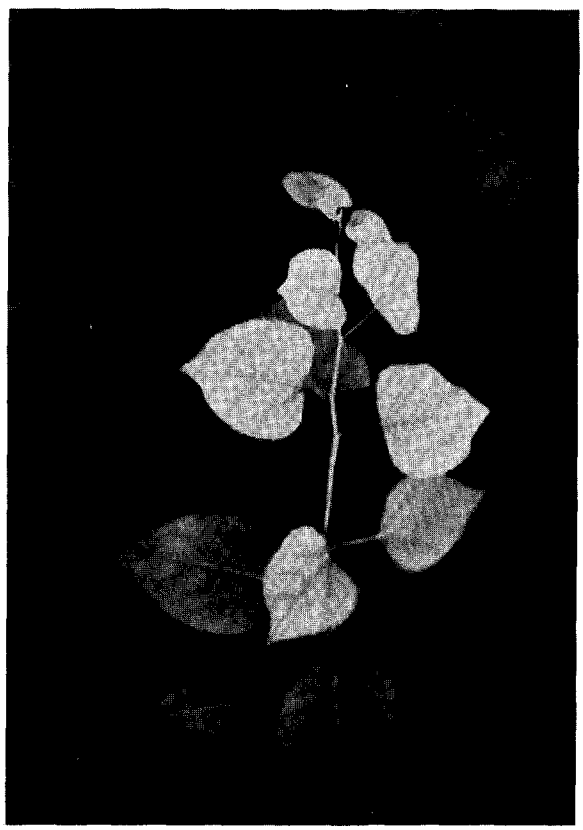


Figure 1. Interveinal chlorosis on the foliage of an aspen treated with 10 ppm Cd for 30 days.

When the aspen trees were subjected to a low O_3 exposure in a fumigation chamber (0.20 ppm O_3 for 2.5 hr), the results were comparable to that obtained in ambient air. Again, O_3 phytotoxicity

was more severe on the trees receiving 10 ppm (Fig. 5). Plants receiving no cadmium increments exhibited a light stipple of the adaxial leaf surface, but the tree treated with 10 ppm Cd had blotches along green veins and injury extending through to the abaxial surface (Fig. 6 and 7).

When aspen trees were subjected to a higher O_3 dosage (0.30 ppm O_3 for 3 hr) in a controlled chamber, all the individuals were severely injured and there was no opportunity to observe a Cd-O_3 interaction (Fig. 8).

Conclusion

Relatively low levels of Cd, while not adversely affecting the growth of aspen, significantly intensified the degree of foliar injury resulting from low O_3 exposures. This is the first time this phenomenon has been demonstrated with a tree species, although it has been shown at higher Cd levels with some herbaceous plants in controlled fumigations (Czuba and Ormrod 1974). We believe that the Cd levels selected in this study are realistic since Cd concentrations as high as 1700 ppm have been reported in heavily polluted soils (Buchauer 1971).

This interaction may have practical importance when trees are growing near a point source of Cd such as a zinc or nickel smelter, in close proximity to a heavily traveled highway, or on soil amended with Cd contaminated sewage sludge. Further studies are needed to determine how long term O_3 and Cd exposures will effect tree physiology and productivity in the field.

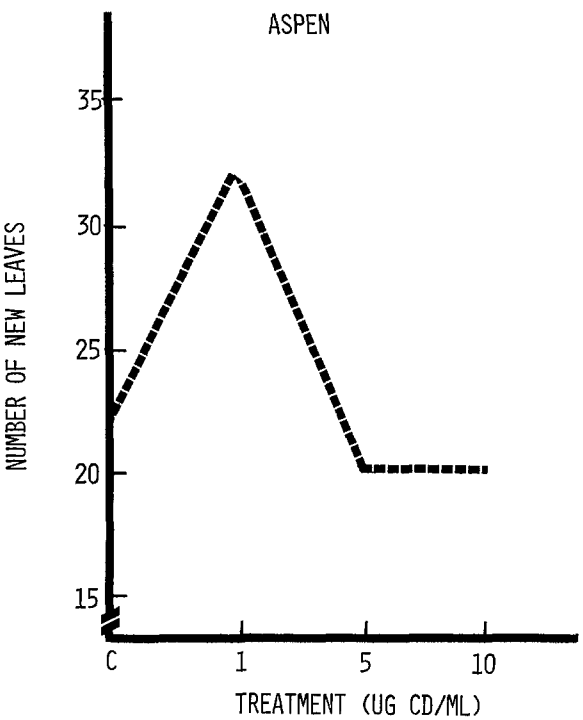


Figure 2. Number of new leaves produced on quaking aspen plants during 30 days of Cd treatment. Mean value for the 1 ug Cd/ml treatment was significantly different from other treatment means at 5% level of Tukey's HSD test.

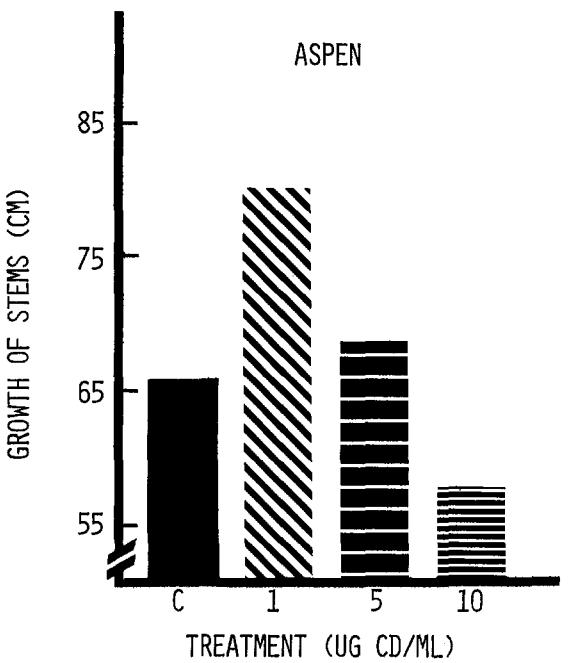


Figure 3. Stem length of quaking aspen plants after 30 days of Cd treatment.

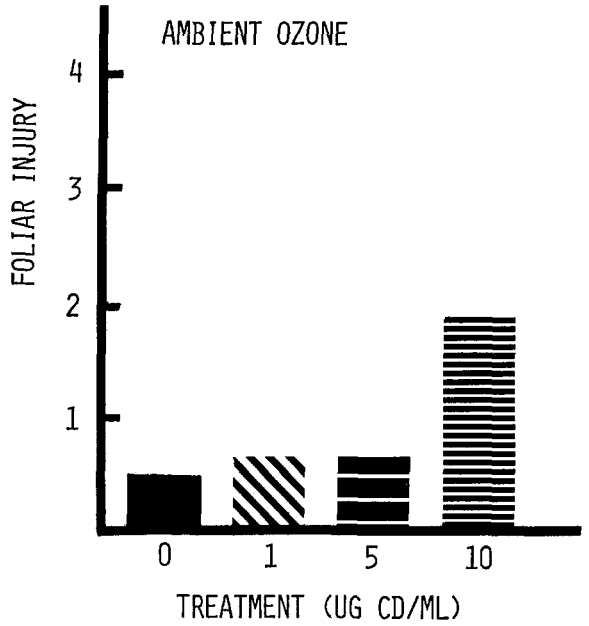


Figure 4. Oxidant injury on quaking aspen exposed to ambient oxidant levels during 30 days of Cd treatment. Mean value for the 10 ug Cd/ml treatment was significantly different from other treatment means at 5% level of Tukey's HSD test.

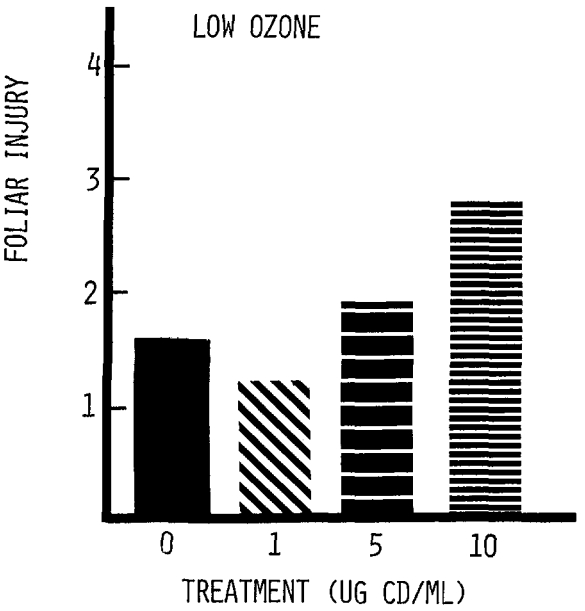
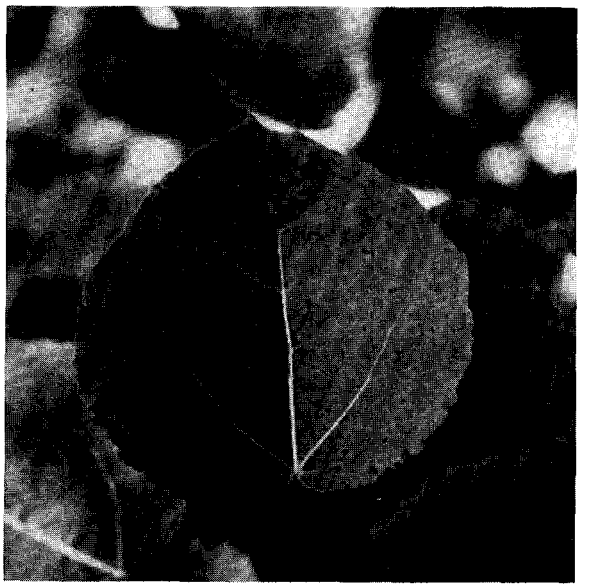


Figure 5. Foliar injury on Cd-treated aspen foliage resulting from a controlled-fumigation with 0.2 ppm O_3 for 2.5 hr. Mean value for the 10 ug Cd/ml treatment was significantly different from other treatment means at 5% level of Tukey's HSD test.



Figures 6 and 7. Typical ozone injury on the foliage of Cd-treated aspen resulting from a fumigation with 0.2 ppm O_3 for 2.5 hr.

Figure 6. 0 ppm Cd treatment (top).

Figure 7. 10 ppm Cd treatment (bottom).

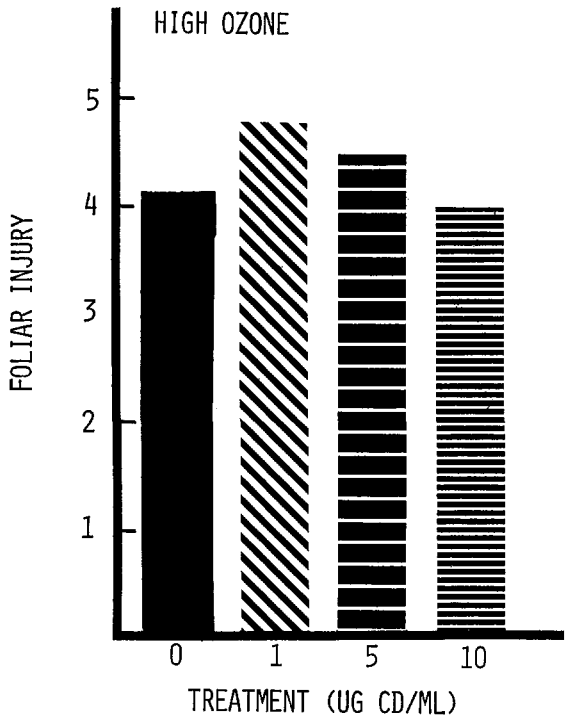


Figure 8. Ozone injury on Cd-treated aspen foliage resulting from a controlled fumigation with 0.3 ppm O_3 for 3 hr. Treatment means not significantly different at 5% level of Tukey's HSD test.

Literature Cited

Beavington, F. 1975. *Heavy metal contamination of vegetation and soil in domestic gardens around a smelting complex.* Environ. Pollut. 9:211-217.

Buchauer, M. 1971. Effects of zinc and cadmium pollution on vegetation and soils. Ph.D. Thesis, Rutgers University. pp. 328.

Clarke, B., M. Henninger, and E. Brennan. 1978. *The effect of two antioxidants on foliar injury, and tuber production in Norchip potato plants exposed to ambient oxidants.* Plant Dis. Reptr. 62:715-717.

Czuba, M., and D.P. Ormrod. 1974. *Effects of cadmium and zinc on ozone-induced phytotoxicity in cress and lettuce.* Can. J. Bot. 52:645-649.

Jacobs, M.B. 1960. The chemical analysis of air pollutants. Interscience, New York. pp. 430.

Karnosky, D.F. 1976. *Threshold levels for foliar injury to Populus tremuloides by sulfur dioxide and ozone.* Can. J. Forest Research 6:166-169.

Lagerwerff, J.V., and A.W. Specht. 1970. *Contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc.* Environ. Sci. Technol. 4:583-586.

Leone, I.A., E. Brennan, and R. Daines. 1966. *Effect of nitrogen nutrition on the response of tobacco to ozone in the atmosphere.* J. Air Pollution Control Assn. 16:191-196.

Page, A.L., F.T. Bingham, and C. Nelson. 1972. *Cadmium absorption and growth of various plant species as influenced by solution cadmium concentration.* J. Environ. Qual. 1(3):288-291.

Reinert, R.A., A.S. Heagle, and W.W. Heck. 1975. Plant responses to pollutant combinations. **In** Response of Plants to Air Pollution, eds. J.B. Mudd and J.T. Kozlowsky. 1976. Academic Press, N.Y. pp. 382.

Shive, J.W., and W.R. Robbins. 1937. Methods of growing plants in solution and sand cultures. N.J. Agr. Exp. Sta. Bull. 6361.

Sidle, R.C., and L.T. Kardos. 1977. *Transport of heavy metals in a sludge-treated forested area.* J. Environ. Qual. 6(4):431-437.

Sommers, L.E. 1977. *Chemical composition of sewage sludges and analysis of their potential use as fertilizers.* J. Environ. Qual. 6(2):225-231.

Steel, B.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, N.Y. pp. 99-111.

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