# The Influence of Soil Fertility and Water Stress on the Ozone Response of Hybrid Poplar Trees

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## ABSTRACT

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Experiments were conducted to determine the role of soil fertility and water stress on the ozone response of hybrid poplar trees. Rooted cuttings of hybrid poplar clone #388 (*Populus maximowiczii* × *P. trichocarpa*) were grown in a filtered-air greenhouse in 4.73-L plastic pots containing a greenhouse potting mix. A slow release 18-6-12 NPK fertilizer was added to obtain four soil fertility levels. Although the nitrogen content of the foliage increased significantly, after 2 mo of growth, neither linear growth nor percent dry weight was affected by these amendments. Trees with a foliar content of approximately 2.69% N were found to be more susceptible to ozone fumigation of 196 $\mu g/m^3$ for 6 hr than were trees with 1.53, 3.12, or 3.47% N. In the water stress experiment, withholding water from the test plants for 6–9 days reduced the relative water content in the foliage to 7–21%, respectively, below that of control plants, and protected the trees from a 6-hr fumigation at 196 $\mu g/m^3$. Visible water stress symptoms were not evident in plants from which water had been withheld for 6 days. These results are discussed as they pertain to susceptibility of urban trees to ozone damage.

*Additional key words:* nitrogen, resistance.

Soil fertility and water stress have been shown to consistently alter the ozone response of herbaceous crop plants (5,21). The significance of varied soil fertility on the ozone response of plants can only be properly evaluated when suboptimal, optimal, and supraoptimal amounts of the nutrient in question are considered (12–14). In this framework some seemingly contradictory results can be better resolved (21). Generally, optimal N, P, or K levels enhance ozone injury; suboptimal or supraoptimal amounts suppress this response in herbaceous species. Our understanding of the influence of soil nutrition on the ozone response of trees is meager at best (19). Since nutrient stress is a much more common

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problem in urban trees than in trees growing in natural forest ecosystems, it is important to understand the $O_3$ × nutrition interaction. This is especially true because there is a greater opportunity to manipulate the nutrient regimes of urban trees (20).

It is commonly observed that herbaceous plants suffering from water stress are less damaged by ozone than those with an ample supply of water (5,21). Rich et al (17) demonstrated that ozone enters leaves primarily via the stomata and subsequently showed that soil water stress can protect plants from ozone injury by increasing stomatal resistance (16). Little is known about water stress and the ozone response in trees (19). However, an extensive literature exists concerning stomatal oscillations in various tree species and their relationship to specific water balance strategies (6). On this basis one might predict that various tree species would respond differentially to a given degree of water stress and thus to ozone. This may partially explain why certain tree species are more susceptible to ozone than others (4).

The following experiments represent our analysis of the influence of soil fertility and water stress on the ozone response of hybrid poplar trees.

## MATERIALS AND METHODS

**Cultural conditions.** Rooted cuttings of hybrid poplar clone #388 *(populus maximowczii* × *Populus trichocarpa)* that were pruned to a uniform height of 8 cm were planted on 5 May in 4.73-L plastic pots containing a greenhouse potting mix (loam:peat:vermiculite, 1:1:1, v/v) and grown in a filtered-air greenhouse. Osmocote (18-6-12), a slow release fertilizer, was added to the pots at four rates: no amendment (c), 16 g/pot (×/2), 32 g/pot (×) and 64 g/pot (2×). The amount recommended by the manufacturer is two teaspoons (32 g/pot). There were 12 trees per treatment and two trees per pot for a total of 48 individuals in the experiment. Fertilizer was applied on 8 June and 4 July.

For the water stress experiment rooted cuttings of hybrid poplar clone #388, were placed in a filtered-air greenhouse and were grown for 2 mo in 4.73-L plastic pots containing a greenhouse potting mix containing loam, peat moss, and vermiculite (1:1:1, v/v). On 29 August, when the trees were 105–120 cm in height, water was withheld from eight individuals (severe stress) while 16 remaining plants received approximately 2,000 ml of water daily. Starting on 1 September, water was withheld from another group of eight individuals (mild stress) while the remaining eight individuals received 2,000 ml of water daily (no stress) until 6 September.

**Ozone fumigations.** After 46 days of treatment, the trees in the fertility experiments were fumigated in a 6 $m^3$ greenhouse chamber with 196 $\mu g/m^3$ for 6 hr at 26 C and 78% RH from 0930 to 1530 hours on 24 July. Ozone was evolved by passing a metered stream of pure dry oxygen through a commercial ozone generator (Instrument Development Co., Salt Lake City, UT 84101). The resulting ozone was introduced into a charcoal-filtered air stream that passed through a mixing chamber before entering the fumigation chamber. Air in the chamber was exchanged every 45 sec. Ozone concentrations were continuously monitored by an ozone meter (Mast Development Co., Davenport, IA 52802) that had been calibrated by the buffered potassium iodide method (8).

Trees deprived of water for 0, 6, or 9 days were exposed to 196 $\mu g/m^3$ ozone for 6 hr at 24.5 C and 62% RH from 0930 to 1530 hours on 6 September. The fumigation chamber and the method of ozone evolution and measurement were identical to those described above.

**Foliar and statistical analysis.** In the fertility experiment prior to fumigation a 5-g sample of fresh leaves (FW) was taken from each tree and dried in a forced-air oven at 70 C for 48 hr for percent dry weight (% DW) and Kjeldahl nitrogen analysis. The height of each plant was measured at this time. Two days following the fumigation, all plants were observed for symptoms of ozone injury. The percentage of injured leaves on each plant and the percentages of injured area on each leaf were recorded. From the data, an index of injury was determined as follows: ([percent injured leaves per plant] + [mean of maximum and minimum percent leaf injury])/20. A value of 0–10 was obtained, with zero equal to no damage and 10 equal to total damage. Both the severity and the amount of foliar injury are important in any index of ozone injury (3) and the above system satisfies both criteria and is relatively simple to use. The total nitrogen content of the foliage was determined by the micro-Kjeldahl technique and is expressed as the percent nitrogen on a dry weight basis (22). All data were subjected to analysis of variance (ANOVA) and, when appropriate, compared by using Tukey's test for significance at the $P = 0.05$ level (15).

In the water stress experiment, beginning 0.5 hr prior to fumigation at 0900 hours on 6 September, the diffusive resistance of the abaxial surface of four leaves from each plant was determined with a Lambda LI-20S diffusive resistance sensor and a LI-60 diffusive resistance meter (Lambda Instruments Co., Lincoln, NE 68504). Kanemasu et al (10) described the use and calibration of this porometer. In addition, diffusive resistance was measured during the fumigation (at 1240 hours) and after the fumigation was completed at 1540 hours.

Three leaves (the fifth to seventh fully expanded leaves) were harvested from each plant for water content measurement prior to fumigation. Water content was measured by using a number of 6-mm diameter leaf disks weighing a total of 0.1 g fresh weight (FW). The disks were floated on distilled water in petri dishes that were kept in the dark for 4 hr. The turgid weight (TW) of the disks was determined after blotting them dry on paper towelling, and the dry weight (DW) of the disks was determined after they had been dried in a forced-air oven at 70 C for 48 hr. All weights were determined with a Mettler H-10 analytical balance. The following water content measures were determined from the above data:

TABLE 1. The effect of four nutrient regimes on selected growth parameters and on ozone injury in hybrid poplar clone #388

| | Fertilizer (grams 18-6-12 per 4.73-L pot) | | | |
|---|---|---|---|---|
| Parameter | 0 | 16 | 32 | 64 |
| Total height (cm) | 57.6 a[z] | 50.1 a | 52.6 a | 54.7 a |
| Foliar dry wt (%) | 24.2 a | 25.6 a | 23.7 a | 24.3 a |
| Foliar nitrogen (%) | 1.53a | 2.69 b | 3.12 c | 3.47 d |
| Ozone injury rating (0–10 scale) | 1.46 a | 4.27 b | 2.05 a | 0.5 a |

[z]All means for each parameter followed by the same letter do not differ according to Tukey's test for significance, $P = 0.05$. Level means are based on data from 10 replicates.

TABLE 2. Foliar water status [x] and corresponding ozone injury index of hybrid poplar deprived of water for variable periods prior to ozonation[y]

| Water stress | RWC | %DW | %W | Ozone injury index |
|---|---|---|---|---|
| None | 86.4 a[z] | 26.9 a | 73.1 a | 6.4 a |
| Mild, 6 days | 79.6 a | 32.3 b | 67.7 b | 0.3 b |
| Severe, 9 days | 63.4 b | 33.9 b | 66.0 b | 0.0 b |

[x]RWC = relative water content = 100 (Fresh weight − dry weight)/(turgid weight − dry weight).
WSD = water saturation deficit = 100 − RWC; %DW = % dry weight; and % W = % water.
[y]196 $\mu g/m^3$ ozone for 6 hr.
[z]Numbers in vertical columns followed by the same letter do not differ according to Tukey's test for significance, $P = 0.05$. Means are based on data from eight replicates.

TABLE 3. Leaf diffusive resistance before, during, and after ozonation of hybrid poplar deprived of water for variable periods of time

| | Leaf diffusive resistance (sec $cm^{-1}$) | | |
|---|---|---|---|
| Water stress | Before fumigation | During fumigation | After fumigation |
| None | 1.5 a[z] | 1.5 a | 1.9 a |
| Mild, 6 days | 1.5 a | 1.5 a | 3.9 b |
| Severe, 9 days | 14.8 c | 14.6 c | 14.8 c |

[z]All values followed by the same letter do not differ according to Tukey's test for significance, $P = 0.05$. Means are based on data from eight replicates.

$$\text{Relative water content (RWC)} = \frac{(FW - DW) \times 100}{(TW - DW)}$$

$$\text{Water saturation deficit (WSD)} = \frac{(TW - FW) \times 100}{(TW - DW)}$$

$$\text{Percent water (\%W)} = \frac{(FW - DW) \times 100}{FW}$$

Barrs (1) gave a thorough description of the meaning of these measurements relating to water stress. Two days after fumigation all plants were rated for ozone damage symptoms by using the method described above. All values were subjected to ANOVA and compared, when appropriate, by using Tukey's test for significance at the $P = 0.05$ level (15).

## RESULTS

The results of the soil fertility study are summarized in Table 1. Because of the loss of a few replicates the total sample size was reduced to 10 per treatment. The four nutrient levels employed had no effect on plant height or percent dry weight of the foliage, however, the percent nitrogen of the leaf tissue differed significantly between all nutrient levels. Hybrid poplar plants grown at one-half the recommended dosage of Osmocote (18-6-12) were more severely injured by ozone fumigation than those that received any other treatment level. The nitrogen content in the foliage corresponding to the greatest degree of ozone injury was 2.69%. Although the difference was not statistically significant, hybrid poplar grown at the recommended dosage of Osmocote were more severely injured by ozone than were unfertilized control plants. The highest fertility regime suppressed ozone damage, although not significantly at the $P = 0.05$ level, and nutrient toxicity symptoms were becoming evident.

In the water stress experiment, the values for the four criteria of plant water status in relation to ozone damage on hybrid poplar are presented in Table 2. The plants subjected to severe water stress had shown an epinastic response on the day of ozone treatment. In addition, water content in the foliage of these trees was significantly less than in the control. The diffusive resistance of leaves of the severely stressed group also was significantly higher than that of the control or mildly stressed plants (Table 3). In the mildly stressed plants the RWC and WSD did not differ significantly from the controls at $P = 0.05$, but did differ at $P = 0.10$, and the %DW and %W values differed from the controls at $P = 0.05$. In addition, the diffusive resistance of the mildly stressed plants did not differ from the controls until after the fumigation (Table 3). Both levels of water stress protected the trees from ozone damage (Table 2).

## DISCUSSION

Mineral nutrition has a profound effect on gas exchange rates in trees. Keller (11), reported that individuals of *P. nigra* with a foliar concentration of about 2.5 – 3.5% N had the highest net photosynthetic rates and plants with 2.5 – 3.1% N had the highest transpiration rates. He also found that optimal nitrogen fertilization enhanced the overall productivity of *P. nigra* plants when net photosynthesis was compared with dark respiration. His results approximate the values for nitrogen in the foliage of hybrid poplar clone #388 that were found to lead the enhanced foliar ozone damage.

Bowersox and Ward (2) reported that nitrogen fertility was highly correlated with growth rates of hybrid poplar (including clone #388) in the field in Pennsylvania. At maximum growth, first-year foliage contained 1.76% – 2.73% N, on a dry weight basis, with a mean of 2.26%. In our experiment the nutrient level corresponding to the highest percent nitrogen value reported by Bowersox and Ward (2) was found to predispose plants to the greatest amount of ozone injury. Thus, the levels of nitrogen tested in our experiment are in the same range as those found in the field.

In the water stress experiment, the RWC values reported for the control plants are similar to those found by Smart and Bingham (18) for apple leaves and by Jarvis and Jarvis (9) for birch and aspen leaves. The classification of the mild and severe groups corresponds with Hsiao's (7) concept of water stress as measured by RWC. An 8–10% drop in RWC has been regarded as mild stress and a greater than 20% drop in RWC, as severe stress (7).

The high diffusive resistance in the severly stressed plants indicates that the stomata were closed and that this prevented damage to these trees. Rich and Turner (16) found that the stomata of mildly water-stressed bean plants closed more rapidly than those of bean plants receiving an ample supply of water. This also appears to happen with hybrid poplar trees. The different diffusive resistance values between the control and mild treatment groups after fumigation and the respective ozone injury ratings of these plants suggest that the level of water stress may have been greater in the mild treatment group during fumigation. It is significant that hybrid poplar trees that are slightly water stressed are not very susceptible to ozone damage. Thus, as in herbaceous plants, water stress protects hybrid poplar from ozone injury.

The results from this study reveal that edaphic factors have a profound influence on the ozone response of hybrid poplar clone #388. The commonplace problems that urban trees face such as low nutrient availability, restricted root growth, soil compaction, rapid soil drying, and abnormally high foliar heat burdens all lead to nutritional and water stress problems on these trees. Such conditions may suppress sensitivity to ozone damage in trees growing in an urban state like New Jersey.

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