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A COMPARISON of HEIGHT GROWTH and LEAF PARAMETERS of HYBRID POPLAR CUTTINGS GROWN in OZONE-FUMIGATED ATMOSPHERES

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Abstract

Hybrid poplar cuttings were fumigated with an ozone dosage of 15 ppm-hours. One treatment was a steady fumigation at 0.2 ppm while the second fumigation fluctuated between 0.1 and 0.3 ppm. No significant differences were found in cutting height, leaf area, leaf width, and leaf dry weight, but significant differences were found in chlorophyll content and carbohydrate content.

THE STUDY

OZONE is a phytotoxic pollutant that often slows the growth rate of woody species (Jensen 1973, Jensen and Dochinger 1974, Jensen and Masters 1975). Associated with reduced growth rates, the fumigated plants often exhibit leaf injury, a chlorosis, fewer leaves, or premature senescence. These observations strongly suggest that leaf development may play an important role in determining the response of plants to air pollutants (Craker and Starbuck 1972, Ting and Dugger 1968, Ting and Mukeryi 1971, Tingey et al. 1973).

Three leaf characteristics that may be important are leaf size, chlorophyll content, and carbohydrate content. Leaf size is important because it has often been correlated with plant growth (Watson 1952). Chlorophyll may be important because of its involvement in photosynthesis, and carbohydrates because without them growth would be impossible. This study compares growth of hybrid poplar cuttings with these three leaf parameters in clean and ozone-polluted air.

MATERIALS AND METHODS

The basal ends of 6-inch cuttings of hybrid poplar clone 207 (*Populus deltoides* Bartr. × *P. trichocarpa* Torr and Gray) were soaked in a solution of 50 ppm IBA overnight. The cuttings were then potted in a 1:1 mixture of sand and soil in 15-cm plastic pots and placed on the greenhouse bench. After the cuttings had broken bud, 45 uniform cuttings were selected and 15 assigned to each of the following treatments: 1) control, 2) 0.2 ppm ozone for 7.5 hours per day (steady fumigation), and 3) 0.1 ppm, 0.3 ppm, 0.2 ppm, 0.3 ppm, and 0.1 ppm ozone each for 1.5 hours per day (fluctuating fumigation). In both ozone treatments, the dosage (time × concentration) was equal. Treatments were applied 5 days a week.

The cuttings were treated in chambers described by Jensen and Bender (1977). The light intensity was 2,500 footcandles and the photoperiod was 16 hours. Temperature was maintained at 25° ± 2°C and the relative humidity was over 60 percent during the treatment period.

After 6 weeks, total height of all cuttings and the length and width of all leaves of five preselected cuttings in each treatment were measured. The leaves used for leaf measurements were counted and divided into three equal groups: top, middle, and bottom. The leaves were removed by groups, the area measured with an electronic area meter, and a 0.5-g sample frozen for chlorophyll determination. The remaining tissue was dried to constant weight. Chlorophyll content was determined according to the AOAC procedure (1960).

The remaining 10 seedlings in each treatment were randomly divided into five pairs and the leaves counted and divided into three groups per seedling as above. The leaves in each pair-group were removed and cut into small sections, and two 0.5-g samples frozen for carbohydrate analysis. It was necessary to combine the leaves from two seedlings to have enough leaf tissue for the two analyses.

Carbohydrate content was measured by refluxing a 0.5-g sample in 50 ml of 80 percent alcohol for 10 minutes. The sample and liquid were then transferred to a Virtis homogenizer and homogenized for 30 seconds. The mixture was next poured into a Soxhlet extraction thimble and the liquid filtered off and stored. The residue in the thimble was extracted with 100 ml of 80 percent ethanol for 30 minutes, 100 ml of 50 percent ethanol two times, each for 30 minutes, and finally with 100 ml of 20 percent ethanol for 45 minutes. The liquid from each extraction was combined with the

liquid from the reflux treatment and evaporated to dryness in a rotary-flash evaporator. The residue from the evaporation was dissolved in 10 percent ethanol and an aliquot was analyzed for reducing sugar with Nelson's reagent (Nelson 1944). A second aliquot sample was digested with invertase and analyzed for sucrose.

Starch content was determined by transferring the residue from the Soxhlet extraction to a boiling flask and refluxed for 1 hour with 0.2N H_2SO_4 (Smith et al. 1964). The extract was neutralized and analyzed for reducing sugar with Nelson's reagent.

All the variables were subjected to an analysis of variance with a completely randomized design.

RESULTS AND DISCUSSION

No significant differences were found in cutting height, leaf area, leaf width, and leaf dry weight (Table 1). These results precluded an attempt to compare leaf size with height growth.

Leaves exposed to the steady fumigation were longer than those in the fluctuating fumigation and in the control. These results suggest that even though the pollutant dosage may be the same, the method by which the dose is applied may influence plant development. Similar observations have been reported by Temple (1972) and must be considered in interpreting the interaction between plants and air pollution.

Chlorophyll content varied with leaf location and treatment (Table 1). The amount of chlorophyll increased basipetally regardless of treatment. The chlorophyll content of the leaves in the fluctuating fumigation was significantly higher than in the other treatments.

The smaller amount of chlorophyll in the top third of the plants is due to new leaves being formed in this zone. As the leaves stop expanding, the chlorophyll content becomes stable and remains uniform in the middle and bottom portions of the cuttings.

The significantly higher chlorophyll content of the cuttings in the fluctuating fumigation associated with slightly but not significantly smaller leaf area may have played a role in the growth of these cuttings. The photosynthesis rate may have been higher with the increased chlorophyll content so that the amount of photosynthate available for growth remained the same.

The distribution of carbohydrates in the leaves is shown in Table 2. The leaves in the fluctuating fumigation had a significantly reduced sugar content and significantly lower starch content. The leaves in all three treat-

Table 1.—Height, leaf size, leaf dry weight, and chlorophyll content of hybrid poplar cuttings fumigated with ozone, by treatment.

Parameter	Control	Steady fumigation	Fluctuating fumigation
Height (mm)	345a	329a	318a
Leaf width (mm)	58a	58a	56a
Leaf length (mm)	90a	104b	88a
Leaf area (cm^2/cutting)	576a	561a	518a
Leaf dry weight (g/cutting)	3.69a	3.77a	3.21a
Chlorophyll (mg/g fresh weight)			
Top	0.590a	0.573a	1.441b
Middle	1.042a	1.039a	2.275b
Bottom	1.298a	1.137a	2.855b
Average	0.977a	0.916a	2.190b

Values followed by the same letter in the same row are not significantly different at the 0.01 probability level, according to Duncan's Multiple Range test.

ments had similar levels of sucrose. The leaves in the top third of the cuttings always had the lowest content of carbohydrates whereas the bottom third had the highest reducing sugar and sucrose content. The leaves in the middle section had the highest starch content.

As with the leaf size measurements, no relationships were found between the carbohydrate content and height. The higher concentration of reducing sugars and low concentration of starch in fluctuating fumigation suggest that the carbohydrates are stored or mobilized differently in these cuttings than in the cuttings in the control and steady fumigation treatments. The sugars apparently were not stored in the leaves as starch but accumulated as reducing sugars.

Table 2.—Carbohydrate content of leaves of hybrid poplar cuttings fumigated with ozone, by treatment (mg/g fresh weight)

Cutting section	Reducing sugar	Sucrose	Starch
CONTROL			
Top	17.7	16.5	19.6
Middle	20.6	20.0	44.3
Bottom	28.5	27.4	33.4
Average	22.3a	21.3a	32.4ab
STEADY FUMIGATION			
Top	17.1	17.9	28.8
Middle	20.7	21.4	53.2
Bottom	24.2	25.7	44.8
Average	20.6a	21.7a	42.3b
FLUCTUATING FUMIGATION			
Top	19.9	13.8	12.8
Middle	28.9	23.3	30.6
Bottom	35.9	28.5	26.8
Average	28.2b	21.8a	23.4a

Values followed by the same letter in the same column are not significantly different at the 0.01 probability level, according to Duncan's Multiple Range test.

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