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Quantifying Foliar Responses of White Ash to Ozone and Simulated Acid Precipitation: An Assessment Proposal for Forest Exposure Studies

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Abstract

Seedling populations represent an important linkage for assessing the effect of air pollution on forests. This study examines the foliar responses of white ash seedlings to ozone and acid precipitation as a means of identifying atmospheric deposition effects on forests. With white ash seedlings, regression analysis revealed significant increases in foliar stippling with rising ozone concentrations from 7 to 15 pphm. No stippling occurred in the absence of ozone or with acid rain treatment alone. However, exposure to ozone plus acid rain resulted in higher levels of foliar stipple as treatment concentrations increased. Until the inherent problems relating to the determination of the potential effects of air pollution on forests are resolved, the use of quantitative indices of injury to foliage and related growth impairments on susceptible and tolerant seedlings appears useful for assessing long-term ambient pollution effects on seedlings, trees, and forests.

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Introduction

Quantifying of genetic tolerance to atmospheric deposition is one of the more pressing problems for forest management. Although sufficient gene pools exist for selection and breeding, tree populations are continually affected by injurious concentrations of ambient pollution. High levels of indigenous pollutants have damaged world forests (Dochinger 1971) and long-term, ambient pollution over large expanses of forests in the United States has had adverse effects (Dochinger 1972; Miller and Millican 1971).

Intraspecific variation in responses to SO_2 and O_3 among plant sources has been demonstrated with Norway spruce (Rohmeder and others 1962), eastern white pine (Dochinger and Seliskar 1970; Houston 1974), ponderosa pine (Karpen 1970), American sycamore (Santamour 1969), red maple (Townsend and Dochinger 1974) and white ash (Jensen and Dochinger 1989). In addition, significant interclonal variation in tolerance to SO_2 was demonstrated by hybrid poplars derived from the same parentage (Dochinger and others 1972). This range of genetically controlled variability within species influences the feasibility of breeding or selecting pollutant-tolerant trees appear very promising.

The objectives of this study were to evaluate (1) quantitatively, the foliar responses of white ash (*Fraxinus americana*) seedlings to ozone and acid precipitation and (2) the use of white ash seedlings as biological indicator plants for long-term assessment of their foliar-growth impairment by air pollution under ambient conditions. This information will aid in examining the effects of atmospheric deposition in order to measure the degree of stress in forests.

Experimental Methods

Plant Culture and Treatment Conditions

In the spring of 1986, 200 one-year-old seedlings of white ash were chosen for uniformity and potted in a commercial soil mix (Metro-mix 500)[1] in 15-cm plastic pots. Approximately 14 days later, 63 plants that had broken dormancy were selected for study. Each plant was randomly assigned to one of nine treatment combinations: three ozone and three acid rain treatments. Each treatment combination was replicated 7 times with one seedling per replication.

All seedlings were watered with 300 ml of tap water before their fumigation.After ozone (O_3) treatment for 2 days, the seedlings were placed in acid rain chambers where they received 1.25 cm of simulated rain. The rain treatment plus the initial watering were adequate for the water requirements early in the study. Subsequently, with an increase in plant size, 600 ml was applied weekly. The nutrient needs of the seedlings were met initially, and again after 4 weeks, by the addition of 100 ml of soluble fertilizer (Peters 20-20-20 General Purpose Fertilizer) to each pot.

[1]The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or Forest Service of any product or service to the exclusion of others that may be suitable.

Atmospheric Deposition Treatments

Atmospheric deposition treatments consisted of three simulated acid rains and three ozone fumigations. Solutions of pH 3.0, 3.5, and 4.2 constituted the acid rain portion of study treatment. Rain was prepared with an automatic titrator which added an acid solution of 2 parts 1M H_2SO_4 and 1 part 1M HNO_3 dropwise to deionized water containing background ions (Table 1) until the desired pH was attained. Composition of rain was prepared to simulate an average summer rainfall (Table 2).

Table 1.—Composition of simulated acid rain

Acid rain mix before pH adjustment[a]	
Compound[b]	uM/L
$MgSO_4 \cdot 7H_2O$	3
$CaSO_4 \cdot 2H_2O$	7
$(NH_4)_2SO_4$	5
NH_4NO_3	6
NH_4Cl	9
KNO_3	2
$NaNO_3$	3

[a]pH adjustments made with an acid solution containing 2 parts 1M H_2SO_4 and 1 part 1M HNO_3; pH 4.2 required .01 ml/liter, pH 3.5 .11 ml/liter and pH 3.0 .54 ml/liter

[b]Constituents added to deionized water.

Table 2.—Comparisons of ions present in average summer rainfall in Central Pennsylvania to those present in simulated acid rain (mean figures, 1977-81)

Ion	Summer rain 1977–1981	Simulated acid rain
	uEq/L	
SO_4	128	30[a]
K	2	2
NO_3	46	11
Na	3	3
NH_4	25	25
Ca	14	14
Cl	9	9
Mg	6	6

[a]Before acid solution was added

The acid rains were applied once a week in plastic chambers equipped with 1.2 m-diameter revolving circular discs (2 rpm). Each application of 1.25 cm of rain lasted about 45 minutes.

Fumigation with 0, 7, or 15 pphm O_3 was applied in CSTR chambers (Heck and others 1978). The seedlings were exposed to ozone for 7 hours per day (0900 to 1600h) for 2 consecutive days every week. Ozone was generated with a corona discharge-type ozone generator and verified with a thermoelectron ozone monitor. The latter was calibrated daily with a Monitor Labs 8500 calibrator.

During the course of this 8-week study, plants were transferred from a maintenance greenhouse into the fumigation chambers in another greenhouse. They were fumigated on Monday and Tuesday. On Wednesday morning, the plants were removed from the fumigation chambers and placed in the rain chambers. After this treatment, the seedlings were returned to the maintenance greenhouse.

Data Collection and Analysis

Every week, the leaflets of each leaf on all seedlings were rated for foliar injury. The classic symptom of ozone injury on broadleaf species is stipple. It is characterized on white ash by pin-size spots of purplish pigmentation on the adaxial surface of the leaves. As injury intensifies, the stipples usually coalesce and finally develop into massive tan-colored necrotic lesions,eventually resulting in premature leaf loss. The quantity of stipple produced on each leaflet was assigned a rating of 0, 1, 5, 10, 25, 50, 75, 90, 95, 99, or 100 percent injury. To determine the overall stipple injury rating for each seedling, ratings for individual leaflets were tabulated and then divided by the total number of leaves evaluated. Foliar injury ratings were adjusted by arc sine transformation procedures before regression analysis.

Results

Visible foliar symptoms occurred on white ash plants exposed to ozone and pH 3.0 acid rain only. Acid rain injury was characterized by small (0.5 mm diameter), circular-like, necrotic lesions which, initially, appeared on the adaxial surfaces and occasionally became bifacial lesions on maturing foliage primarily. These foliar symptoms were noted during the 4th week of treatment and were not recorded since they occupied less than 1 percent of leaf surface.

Ozone symptoms were discernible on older leaves usually after 3 weeks of fumigation, although foliar stipple was at times observed weekly on immature leaves. In the six ozone-acid rain treatments, stippling of white ash leaflets was observed on 63, 34, and 31 percent of the 7 pphm ozone, pH 3.0-, 3.5-, and 4.2-treated seedlings, respectively. For the 15 pphm ozone, pH 3.0-, 3.5-, and 4.2-treated plants, 86, 84, and 59 percent, respectively, were noted.

An increasing ozone concentration from 7 to 15 pphm and rising acidity produced the most significant amount of stippling of white ash leaflets. Both pH 3.0 and 3.5 at 15 pphm O_3 accounted for the most foliar pigmentation across all other treatments (Fig. 1). In addition, a significant variation in leaflet stipples was observed after exposure to pH 3.0 in comparison to pH 4.2 rain at 7 pphm. Over the 8-week period of study, a gradual increase was observed in the quantity of foliar stippling resulting from the various O_3 acid rain treatments. No foliar stippling was discernible in the non-ozone treatments.

Discussion

Seedlings are a critical link with future forests; therefore, they are an important population for exposure studies in the laboratory and in the forest environment. The primary purpose of this study was to evaluate the possibility of quantifying white ash foliar symptoms to injury from ozone and acid rain. The initial assessment of treatment effects on foliar symptoms indicated that a percentage rating of visible foliar injury is feasible and provides reliable, repeatable results.

These findings may offer a possible basis for relating the percentage of visible foliar injury to growth impairments for laboratory and field assessments. Based on previous study with white ash, this species was reported to be very sensitive to O_3 and acid rain. Regression analysis also accounted for significant variation in leaf dry weight, height, leaf area, and new-growth dry weight with an increase in ozone concentration from 7 to 15 pphm. In addition, all growth variables decreased with an increase in acidity from pH4.2 to 3.0. The decreases ranged from 6 percent for height to approximately 20 percent for leaf dry weight, leaf area, and new-growth dry weight (Jensen and Dochinger 1989).

Similar observations are reported by other researchers. Across all acid rain treatments, 9-week-old white ash biomass was suppressed by the application of ozone. Significant pollutant × pH interactions occurred in leaf area, total plant dry weight, and root-shoot ratios with decreasing pH and O_3 treated seedlings (Chappelka and others 1988).

For all treatments, increasing O_3 concentrations resulted in significant linear decreases in root, leaf, and total dry weights; total stem; increased root-shoot ratio; and mean relative growth rates of the total plant, stem, root, and leaves in 5-week-old white ash. Visible symptoms from O_3 (15 pphm). simulated rain (pH 3.0), or O_3 plus simulated acid rain (15 pphm and pH 3.0) were observed on 61, 61, and 54 percent of the seedlings in all treatments, respectively. The combined effect of O_3 and acid rain resulted in significantly less root dry weight and root-shoot ratio at 5 pphm O_3, and significantly less relative root growth rate and root-shoot ratio at 10 pphm O_3 as rain pH decreased (Chappelka and Chevone 1986).

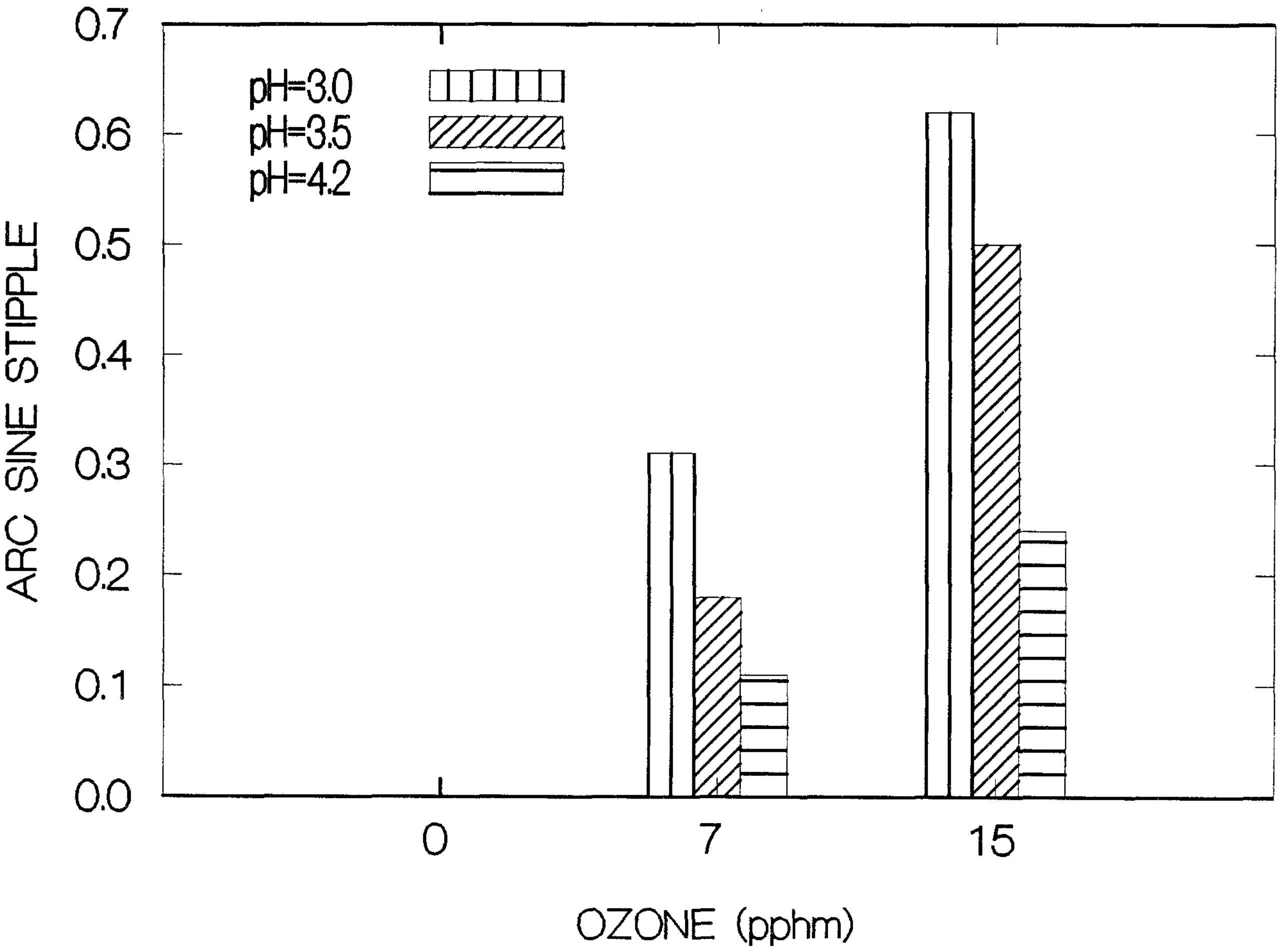

Figure 1.—Effects of pH/ozone on white ash foliar stippling.

Various measurements may be used to examine genetic differences in air pollution on plants. Height growth, as a percentage of growth exhibited by treated or control progenies or clones of similar parentage, is a useful parameter for discerning tolerance or sensitivity to pollution (Dochinger and others 1972; Townsend and Dochinger 1974). Tolerant lines generally show less growth depression than susceptible ones. Foliar injury, expressed as a percentage of each leaf injured or as a percentage of all leaves displaying symptoms, also may be used and is probably the most useful means for identifying air pollution effects on vegetation.

A number of causal stress agents, including air pollution, cause visible symptoms on plant foliage. These injurious responses can aid in identifying cause and effect, including effects on yield. Consequently, until the problems concerning the assessment of the effects of atmospheric deposition on forests, are resolved, utilization of indices of injury to foliage and growth impairment on susceptible and tolerant seedlings will be useful for determining the nature and extent of long-term ambient pollution.

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