Age of Pine Seedlings with Primary Needles Affects Sensitivity to Ozone and Sulfur Dioxide

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ABSTRACT

Seedlings of Virginia *(Pinus virginiana)*, shortleaf *(P. echinata)*, slash *(P. elliottii* var. *elliottii)*, and loblolly *(P. taeda)* pines at ages 2, 4, 6, 8, and 10 wk were exposed to individual dosages of ozone and sulfur dioxide. Exposures were 2 hr at 655.0 ± 65 $\mu g/m^3$ for SO_2 and 477.5 ± 48 $\mu g/m^3$ for O_3 (25 ± 2.5 parts per hundred million). The two gases were equally injurious to all species, and all species were equally sensitive to each gas. Maximum sensitivity of the seedlings to the two gases, however, occurred at different ages. For ozone the greatest sensitivity was 2 wk or younger, and for sulfur dioxide at 8 to 10 wk or older.

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Additional key words: air pollution, relative sensitivity, primary needle stage.

The present investigation was initiated as part of an effort to accelerate the screening of large numbers of southern pine seedlings for resistance to ozone and sulfur dioxide. In the course of this work it became apparent that sensitivity of seedlings in the primary needle (PN) stage was affected by aging. Since it appeared that the greatest sensitivity to the two gases occurred at different ages, it was regarded as essential that the research reported herein be completed before screening could proceed.

MATERIALS AND METHODS.–Seedlings of Virginia (*Pinus virginiana* Mill.), shortleaf (*P. echinata* Mill.), slash (*P. elliottii* Engelm. var. *elliottii*), and loblolly (*P. taeda* L.) pines were grown in a mixture of forest soil, sand, and peat moss (6:2:1, v/v) and potted in 10- × 10- × 10-cm plastic pots. The soil mixture was not sterilized and fertilizer was not added. Approximately 10 seedlings were grown in each pot. Plantings were made at 2-wk intervals to produce seedlings of five age groups (approximately 2, 4, 6, 8, and 10 wk) that could be fumigated simultaneously.

Seedlings were maintained in the greenhouse before and after exposure to the pollutants. Greenhouse temperatures ranged from 19 to 32 C. Humidity was raised and maintained above 60% in the greenhouse with mist from pneumatic nozzles, with only occasional short periods below 40%. Seedlings were carefully watered to prevent undue soil moisture stress. The greenhouse was not supplied with filtered air; consequently, there was slight visible injury from phytotoxicants in ambient air.

All seedlings were exposed in a single fumigation chamber described earlier (3) for 2 hr at concentrations of 655.0 ± 65 $\mu g/m^3$ for SO_2 and 477.5 ± 48 $\mu g/m^3$ for O_3 (25 ± 2.5 parts per hundred million for both gases). Chamber conditions during fumigations were controlled at 27 C and 70% relative humidity. Each of the four pine species was exposed at five different ages to each gas on three different dates. Four pots of 10 seedlings each were fumigated simultaneously on each data as replicates for each pine species-age treatment. Fumigations were conducted on the following dates: ozone–8/26/69, 1/29/70, 2/18/70; sulfur dioxide–8/28/69, 2/4/70, 2/20/70.

Sulfur dioxide concentrations were monitored and controlled by a Davis sulfur dioxide analyzer calibrated by the permeation tube method (2). Ozone concentrations were monitored and controlled by a Mast ozone analyzer calibrated by the neutral buffered-potassium iodide method (1).

Seedlings were examined and data recorded 1 wk after fumigation. Foliar symptoms were rated 0, light, or heavy, according to the degree of injury. Light injury was disregarded because of slight injury by ambient air pollution. Injury symptoms on cotyledons did not differ from those observed on primary needles. An analysis of variance consisting of a randomized block design with a factorial arrangement of treatments was used. Linear and quadratic orthogonal comparisons were made in order to test for an age effect.

RESULTS AND DISCUSSION. – *The effect of age on sensitivity.* – The greatest sensitivity to ozone and sulfur dioxide in PN seedlings of the four species tested did not occur at the same age (Fig. 1). The seedlings tested were most sensitive to ozone at 2 wk of age, or possibly earlier, and most sensitive to sulfur dioxide at 8 to 10 wk, or older. An analyses of variance showed the curves to be linear and negative for ozone and linear and positive for sulfur dioxide. This information has interesting implications in other investigations. For example, with mixed gas fumigations, the need of which is universally recognized (6), the type and amount of injury probably would be greatly affected by the age of foliage at the time of fumigation. At 2 wk of age one would expect injury to be quite different from that at 8 to 10 wk. Although statistical analysis showed the age effect to be linear, in reality a curve covering a longer time span would probably be quadratic, particularly for sulfur dioxide. With this gas, one would eventually expect to reach an age at which no

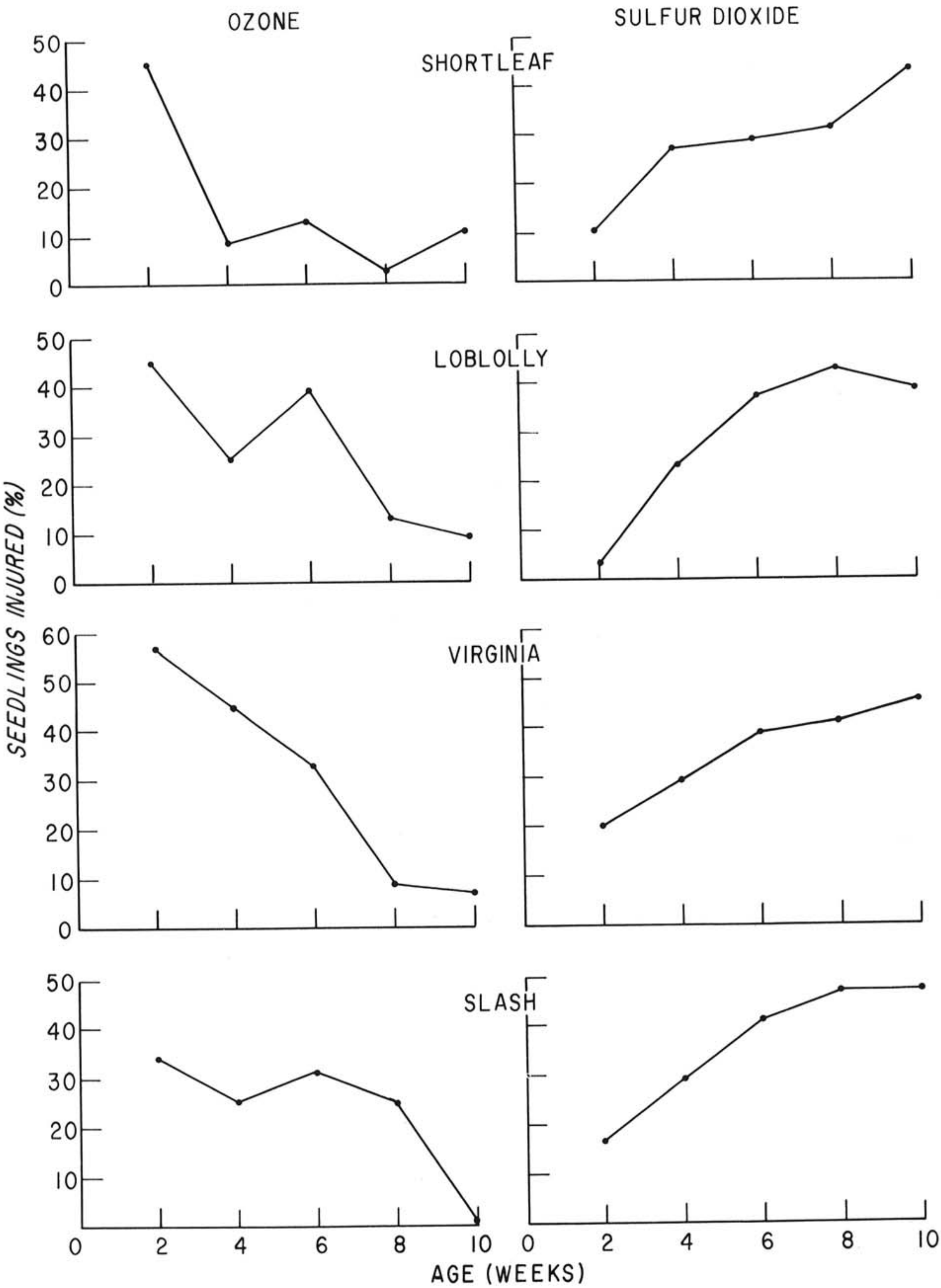
OZONE
SULFUR DIOXIDE
SHORTLEAF
LOBLOLLY
VIRGINIA
SLASH
SEEDLINGS INJURED (%)
AGE (WEEKS)
0
10
20
30
40
50
60
2
4
6
8

Fig. 1. The sensitivity of four southern pine species at five ages to ozone and sulfur dioxide at 25 pphm for 2 hr.

new individuals were injured and the curve would flatten out; this would probably be the best age at which seedlings could be screened for resistance. Important questions raised by these data are whether there is a similar variation in sensitivity of secondary needle (SN) stage seedlings as needles mature, and whether there is a similar variation in sensitivity of broad-leaved plants.

The relative sensitivity of species. – This analysis did not show any difference in sensitivity between any of the species for either gas. There were rather wide confidence bands in the analysis, however, indicating a need for more refinement in experimental techniques. After some refinement of methods, particularly in more critical maintenance of air and soil temperatures, photoperiod, and light intensity in the greenhouse before fumigation, some differences, such as a higher sensitivity of Virginia pine and a lower sensitivity of slash pine to ozone, would probably become apparent.

In a similar study on PN seedlings of white, jack, and red pines (4), differences in sensitivity were found, but no age effect was detected. Jack pine was found to be most sensitive to ozone, but there was little or no difference in sensitivity between red and white pines. In exposure to sulfur dioxide, red pine appeared to be more tolerant than white or jack pine. The probable reason why no age effect could be detected was that only three age groups were tested and these covered a relatively short age span, only 4 wk; consequently, no statistical difference between the groups was detected. In other research (5) on SN seedlings, Virginia pine was found to be highly sensitive, although it was not compared with shortleaf, loblolly, or slash pines.

Somewhat more injury of all species was produced by the August fumigations of both gases than by the January or February fumigations. This differential needs to be investigated further, however, since there were only three fumigations employed for each gas.

In the future, fumigations carried out to screen trees for resistance to sulfur dioxide should probably be conducted in conformity with the national secondary standards for air quality. Currently, the maximum allowable ground-level concentration of sulfur dioxide for a 3-hr avg is 0.5 ppm, a somewhat higher concentration than was used in the present study. The current national standard for ozone, 0.08 ppm for a 1-hr average, is well below the exposure level used in this work. If automobile emission control efforts are successful, ozone levels in future work of this nature could be lower when the plant species is highly sensitive and displays little genetic variability.

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