Sensitivity of Eastern White Pine Clones to Acute Doses of Ozone, Sulfur Dioxide, or Nitrogen Dioxide

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ABSTRACT

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Long-term pollutant fumigations were conducted to determine the relative sensitivity of three eastern white pine (*Pinus strobus*) clones to ozone (O_3), sulfur dioxide (SO_2), or nitrogen dioxide (NO_2) based on foliar symptom expression, needle length, chlorophyll content, and dry weight of first-year secondary needles. Plants were exposed to 0.00, 0.10, 0.20, or 0.30 μl per liter of air of each pollutant 4 hr daily for 35 consecutive days. Sensitivity of the white pine clones to pollutants varied with the type of pollutant, pollutant concentration, and plant response chosen as the indexing parameter. Results based on NO_2 exposure differed with most indexing parameters from those obtained from O_3 or SO_2 exposure. White pine clones used in this study were less sensitive to NO_2 than to O_3 or SO_2 at the same concentrations. Pollutant-induced foliar symptoms could not be used to distinguish the type of causal pollutant. Neither was the amount of foliar injury consistently useful in determining clonal sensitivity rankings to each pollutant. Further work is needed to define plant responses to each pollutant before such plant materials can be used as bioindicators for monitoring ambient pollutants.

Differential sensitivity to gaseous pollutants among plant species and within the same species has been reported in both the ambient air and environmentally controlled fumigations (3–5). Several researchers have suggested that characteristic plant responses to a specific pollutant are an indicator for determining the type and concentration of a specific atmospheric pollutant (3,6). However, ambient air contains a mixture of pollutants in various ratios; generally, a plant is not exposed to a single pollutant. Thus, the interpretation of plant responses to pollutants under field conditions is more complicated than under controlled fumigation conditions where only the interspecific or intraspecific plant sensitivity to a single pollutant has been emphasized (5,9). It is clear that before plant bioindicator systems can be used in ambient conditions for pollutant monitoring, there is a need to determine plant responses to various individual pollutants. Limited information is available concerning the responses of plant species to different pollutants.

In southwestern Virginia, the Radford Army Ammunition Plant (RAAP), a known source of NO_2 and SO_2, has affected surrounding forests for several years (11). Three of the major phytotoxic gaseous pollutants, O_3, SO_2, and NO_2, have been identified in the ambient atmosphere near this facility (12). Field studies and indoor fumigations were conducted to evaluate the effect of these pollutants on forest trees (10,11).

The purpose of this study was to determine the responses of three selected eastern white pine (*Pinus strobus* L.) clones, growing in the vicinity of the RAAP, to O_3, SO_2, or NO_2. The specific objectives of this study were: to determine the sensitivity of the clones to O_3, SO_2, or NO_2 on the basis of foliar symptom expression, needle length, needle chlorophyll content, and needle dry weight; and to compare the sensitivity of those clones to these pollutants.

MATERIALS AND METHODS

Three clones of eastern white pine with differing pollutant sensitivity (determined by field observations and controlled fumigation experiments [10,11]) were selected and used in this study. The clones were designated either sensitive (clone II-1), intermediately sensitive (clone III-2), or insensitive (clone IV-2) to pollutant stress. The preparation and cultural practices used for growing 2-yr-old ramets with the same needle age have been reported in a previous study (17). First-year needles were 21–25 days old when plants were exposed to pollutants.

During the summers of 1979 and 1980, four ramets of each clone were exposed to either 0.00, 0.10, 0.20, or 0.30 μl of O_3, SO_2, or NO_2 per liter of air concurrently for 4 hr daily for 35 consecutive days. Except for daily pollutant exposure, plants were maintained in a greenhouse supplied with charcoal-filtered air.

Fumigations were conducted in continuously stirred tank reactors (CSTR) similar to those designed by Heck et al (7). Pollutants were monitored continuously 50 cm above the CSTR chamber floor and maintained within $\pm$ 0.01 μl/L of the desired concentrations during fumigations. Temperature, relative humidity, and illumination in the chambers was 30 ± 2 C, $65 \pm 5\%$, and 360–410 $\mu E/m^2/sec$ photosynthetically active radiation, respectively.

Ozone, SO_2, or NO_2 was supplied and monitored as previously reported (17). Pollutant monitors were calibrated every 2 wk according to U.S. Environmental Protection Agency quality assurance guidelines (13–15).

First-year needles were examined each day until symptoms appeared. Thereafter, foliar injury estimates were recorded weekly in 5% increments (0–100% scale). Needle length of the five oldest fascicles of each ramet was measured weekly to the nearest millimeter. Needle dry weight and chlorophyll content of first-year needles were determined at the end of the experiment. Needle dry weight of the five oldest fascicles of each ramet was determined after freeze-drying fresh tissues for 24 hr. Needle chlorophyll content was measured following the procedures of Wood and Bachelard (16). Absorbance of chlorophyll solutions was measured at 645 nm and 663 nm to determine the concentrations of chlorophyll *a* and *b*, respectively, which were calculated according to the following formulae (16):

$$\text{chlorophyll } a \text{ (mg/L)} = 12.7\ (A_{663\text{ nm}}) - 2.69\ (A_{645\text{ nm}})$$
$$\text{chlorophyll } b \text{ (mg/L)} = 22.9\ (A_{645\text{ nm}}) - 4.68\ (A_{663\text{ nm}})$$

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in which $A_{663\ nm}$ indicated the absorbance at 663 nm and $A_{645\ nm}$ indicated the absorbance at 645 nm.

The study was conducted as a randomized complete block design with years representing blocks. Pollutant treatments were randomly distributed daily among CSTR chambers to minimize chamber effects. Four ramets from each clone/chamber were used. An analysis of variance (ANOVA) was performed on data representing each parameter measured to determine clonal response to increasing concentrations of each pollutant.

RESULTS

Latent period for initial symptoms. In the sensitive clone, foliar symptoms induced by exposure to O_3 or SO_2 at 0.10, 0.20, or 0.30 μl per liter of air appeared in week 1 and those induced by NO_2 at the same pollutant concentration appeared in week 2.

In the intermediately sensitive clone, foliar symptoms induced by O_3 or SO_2 at 0.10 μl/L appeared in weeks 2 and 3 (in weeks 1 and 2 at higher concentrations) and those induced by NO_2 at 0.20 and 0.30 μl/L appeared in weeks 3 and 4, respectively. Nitrogen dioxide at 0.10 μl/L did not result in foliar injury on white pine.

In the insensitive clone, foliar symptoms induced by exposure to O_3 or SO_2 at 0.30 μl/L appeared in week 4. These were the only two tested pollutant concentrations that resulted in foliar symptoms.

Foliar injury. Exposure to O_3 caused first-year needles of eastern white pine to develop general chlorosis, pigmented mottling, necrotic banding, and necrotic tip-burn (Fig. 1). Among the three clones, premature defoliation only occurred in the sensitive clone. At the end of the 10-wk experiment, 0, 20, 30, and 40% of the needle area in the sensitive clone was injured by exposure to O_3 at 0.00, 0.10, 0.20, and 0.30 μl/L, respectively; compared to 0, 15, 15, and 15%, respectively, in the intermediately sensitive clone; and 0, 0, 0, and 5%, respectively, in the insensitive clone.

Exposure to SO_2 caused first-year needles of eastern white pine to develop chlorosis, chlorotic mottling, necrotic banding, and necrotic tip-burn. Premature defoliation occurred in the sensitive clone only. At the end of the 10-wk experiment, 0, 50, 55, and 65% of the foliar area in the sensitive clone was injured by 0.00, 0.10, 0.20, and 0.30 μl/L SO_2, respectively; compared to 0, 25, 35, and 30%, respectively, in the intermediately sensitive clone; and 0, 0, 0, and 5%, respectively, in the insensitive clone.

Exposure to NO_2 caused first-year needles of eastern white pine to develop mottling and necrotic tip-burn. Premature defoliation was not observed in any of the tested clones. At the end of the 10-wk experiment, 0, 10, 15, and 20% of the foliar area in the sensitive clone was injured by exposure to 0.00, 0.10, 0.20, and 0.30 μl/L NO_2, respectively; compared to 0, 0, 5, and 15%, respectively, in the intermediately sensitive clone. Nitrogen dioxide, at tested concentrations, did not induce any foliar symptoms in the insensitive white pine clone.

Needle length, chlorophyll content, and needle dry weight. After long-term exposure to O_3, SO_2, or NO_2, the amount of reduction in needle length varied with the type of pollutant, pollutant concentration, and white pine clone (Table 1). Significant clone × pollutant concentration interactions occurred indicating that clonal response to these pollutants, in terms of needle length, was dependent upon pollutant dose. Response of white pine needle length varied among different clones as well as among pollutant

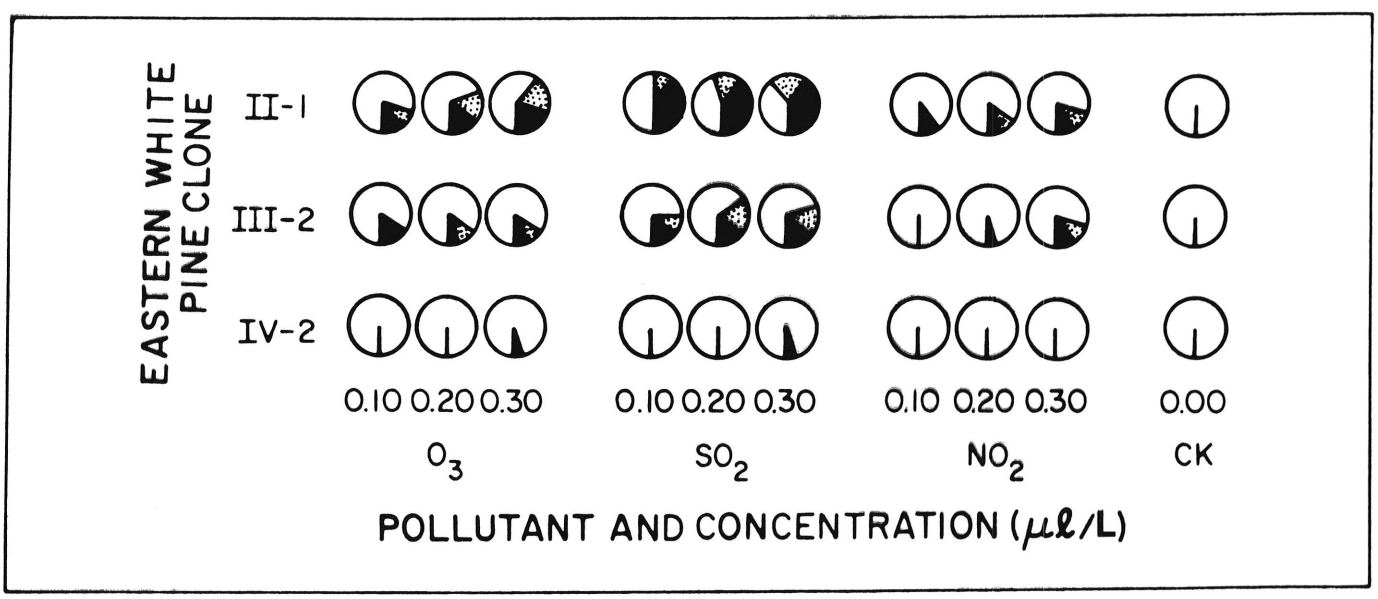

Fig. 1. Type and percentage of visible symptoms on first-year secondary needles of eastern white pine after exposure to 0.00, 0.10, 0.20, and 0.30 μl of O_3, SO_2, or NO_2 per liter of air for 4 hr daily for 35 consecutive days. ⊠ Chlorosis, ■ necrosis, and □ unaffected needle surface. Each circle area is 100%. Clone II-1 = sensitive, III-2 = intermediately sensitive, and IV-2 = insensitive.

TABLE 1. Summary of analyses of variance of eastern white pine needle length (millimeters) response to O_3, SO_2, or NO_2 exposure at 0.00, 0.10, 0.20, and 0.30 μl/L[a]

		Type of pollutant					
		O_3		SO_2		NO_2	
Source of variation	d.f.	Mean square	*F*-value[b]	Mean square	*F*-value	Mean square	*F*-value
Clone (Cl)	2	5,691.97	1,133.53**	6,537.54	852.46**	944.66	28.91**
Concentration (Conc)	3	1,305.21	259.93**	1,195.34	155.89**	522.29	15.99**
Cl × Conc	6	154.46	30.76**	748.96	97.66**	78.16	2.39*
Error	83	5.02		7.67		32.67	

[a] Detailed description of experiment referred to in the text.
[b] * = significant, $P = 0.05$; and ** = significant, $P = 0.01$.

concentrations within clones. For all pollutant exposures, the variation in needle length due to clone was always greater than that due to concentration.

Concentration of both chlorophyll *a* and *b* in needles was affected by pollutant exposures (Tables 2 and 3). Nitrogen dioxide was found to have the least injurious effect among the three pollutants. It is interesting to note that chlorophyll *a* and chlorophyll *b* reacted differently to the same doses of NO_2 among clones. Chlorophyll *a* concentration was affected only by increasing NO_2 concentrations, whereas chlorophyll *b* content exhibited a dependence on both NO_2 concentration and white pine clone. Analyses of variance indicated that chlorophyll *a* and *b* responded similarly to O_3 and SO_2 exposures. A significant difference was observed in the interaction of clone × pollutant concentration regardless of the type of pollutant or type of chlorophyll measured.

Needle dry weight was affected differently from content of chlorophyll *a* and *b* when exposed to the same doses of NO_2 (Table 4). A significant interaction between clone and pollutant concentration was observed with needle dry weight. Significant interactions between white pine clone and pollutant concentration also occurred with needle dry weight in O_3 and SO_2 exposures.

DISCUSSION

Eastern white pine sensitivity to different air pollutants was shown to be dependent upon the particular white pine clone, the type of pollutant, the pollutant concentration, and the kind of plant response selected as the criterion for sensitivity indexing. Long-term exposure to pollutants resulted in a reduction of various growth responses and the extent of reduction reflected intraspecific variation. In this study, under uniform cultural practices utilizing clonal material, eastern white pine exhibited considerable variation in sensitivity to pollutants.

When applied in the same dosages, SO_2 and O_3 caused a greater adverse effect on white pine growth than NO_2. These results are in agreement with those of Bennett and Hill (1,2) who found that NO_2 was the least phytotoxic pollutant to alfalfa plants among O_3, SO_2, and NO_2. Our results indicate that different pollutants cause different amounts of visible injury to white pine and also demonstrate that, under the concentrations tested, the sensitivity of white pine to NO_2 is less common than the sensitivity to O_3 and SO_2. In most cases, there does appear to be a close relationship between the extent of white pine foliar injury and the amount of pollutant dose the plant received. However, the types of foliar injury induced by O_3, SO_2, or NO_2 were not distinguishable in most clones. The effects of chronic exposures of O_3, SO_2, and/or NO_2 on eastern white pine (17) differ from those caused by the exposures of these pollutants at higher concentrations in our experiments. The relationships between pollutant dose and foliar injury is complex and can vary among and between the type and concentration of pollutant and white pine clone. Therefore, foliar injury severity alone is an inadequate criterion for describing white pine sensitivity

TABLE 2. Summary of analyses of variance of eastern white pine chlorophyll *a* (microgram per gram of needle dry weight) response to O_3, SO_2, NO_2 exposure at 0.00, 0.10, 0.20, and 0.30 μl/L[a]

		Type of pollutant					
		O_3		SO_2		NO_2	
Source of variation	d.f.	Mean square	*F*-value[b]	Mean square	*F*-value	Mean square	*F*-value
Clone (Cl)	2	691.64	79.48**	1,164.70	147.31**	54.28	2.25 NS
Concentration (Conc)	3	855.51	98.31**	2,701.53	341.68**	382.01	15.83**
Cl × Conc	6	272.22	31.28**	129.68	16.40**	40.57	1.68 NS
Error	83	8.70		7.91		24.13	

[a] Detailed description of experiment referred to in the text.
[b] NS = not significant; and ** = significant, $P = 0.01$.

TABLE 3. Summary of analyses of variance of eastern white pine chlorophyll *b* (micrograms per gram of needle dry weight) response to O_3, SO_2, or NO_2 exposure at 0.00, 0.10, 0.20, and 0.30 μl/L[a]

		Type of pollutant					
		O_3		SO_2		NO_2	
Source of variation	d.f.	Mean square	*F*-value[b]	Mean square	*F*-value	Mean square	*F*-value
Clone (Cl)	2	88.29	19.38**	101.79	18.68**	51.13	4.15*
Concentration (Conc)	3	147.84	32.45**	572.50	105.05**	49.03	3.98*
Cl × Conc	6	35.04	7.69*	69.29	12.71**	12.15	0.99 NS
Error	83	4.56		5.45		12.31	

[a] Detailed description of experiment referred to in the text.
[b] NS = not significant; * = significant, $P = 0.05$; and ** = significant, $P = 0.01$.

TABLE 4. Summary of analyses of variance of eastern white pine needle dry weight (milligram per millimeter of fascicle) response to O_3, SO_2, or NO_2 exposure at 0.00, 0.10, 0.20, and 0.30 μl/L[a]

		Type of pollutant					
		O_3		SO_2		NO_2	
Source of variation	d.f.	Mean square	*F*-value[b]	Mean square	*F*-value	Mean square	*F*-value
Clone (Cl)	2	13,692.08	460.82**	6,795.79	231.99**	5,691.58	37.33**
Concentration (Conc)	3	4,030.07	135.64**	11,494.07	392.38**	1,231.58	8.08**
Cl × Conc	6	1,764.13	59.37**	1,062.56	36.27**	567.07	3.72**
Error	83	29.71		29.29		152.47	

[a] Detailed description of experiment referred to in the text.
[b] ** = significant, $P = 0.01$.

to pollutants.

White pine response to a pollutant is believed to be related to plant genotype. Our data clearly support the concept that white pine sensitivity to a pollutant varies intraspecifically and that different pollutants could have different effects on growth of white pine clones. Across all clones, a comparable reduction in needle dry weight, needle chlorophyll contents, and needle length occurred, especially in O_3 and SO_2 exposures. Since biomass production is a function of a number of assimilatory apparatuses, such as chlorophyll content, leaf area, etc., it is reasonable to suggest that plant biomass production is one of the better indexing criteria to reflect plant sensitivity to pollutants. Linzon (9) showed that first-year white pine needles contributed more to tree vigor than did those of two other needle age classes. Swieboda (12) found that 80% of the needle chlorophyll in Scotch pine was synthesized in the first year of plant growth. The adverse effects of air pollutants on chlorophyll content of first-year needles may have contributed to the biomass decrease observed in this study.

Regardless of which plant response was measured, the sensitive clone was consistently more severely affected than the insensitive clone. This suggests that air pollutants in current-day ecosystems may actively select for the insensitive individuals and against the sensitive specimens. Species characteristics that are now preserved and contribute to genetic diversity could be lost if the reproduction of pollutant-sensitive trees is either reduced or eliminated.

Based on our results, several factors may contribute to the conflicting information in the literature concerning plant sensitivity to pollutants (3,4,8): the nonuniformity of plant materials, such as different clones or cultivars; different cultural practices; different indexing criteria used as parameters to assess plant sensitivity; different pollutant types; and different pollutant concentrations, durations, and seasonal or daily timing of exposures.

Our data indicate that the extent of foliar symptoms, which has been used extensively in the past to determine plant sensitivity to pollutants, is inadequate as the sole parameter (3,5). Rather, other growth responses should also be considered when developing sensitivity rankings.

Since different pollutants can induce similar plant responses, results of this study indicate that the relative sensitivity of eastern white pine to pollutants could only be objectively determined by cross-testing various plant responses. In practice, plant response to pollutants should be further defined before plant bioindicator systems can provide reasonable accuracy in identifying pollutant species and/or concentration in the ambient air.

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