Austrian Pine Injury Traced to Ozone and Sulfur Dioxide Pollution

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ABSTRACT

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A foliar disorder of Austrian pine observed in New Jersey since 1967 was found to result from a mixture of ozone and sulfur dioxide. In fumigation chamber studies, a mixture of 0.2 ppm ozone and 0.1 ppm sulfur dioxide for 6 hr reproduced symptoms observed in the field. Grafted material originating from trees that exhibited either a sensitive or resistant response in the field gave the same differential response in the experimental fumigation. Needles of resistant trees had higher stomatal resistance and higher soluble sugar content than needles of susceptible trees.

A foliar disorder of Austrian pine (*Pinus nigra* 'Austriaca' Arnold) presumably caused by air pollution has occurred in varying degrees yearly in New Jersey since first reported by Brennan and Davis (1). Characteristically, a brown to orange necrotic area, separated from healthy green tissue by a narrow purple band, appears at the tip of the current season's needles. Histologically, both stele and mesophyll cells are affected (9). In nature, approximately 20% of a population is generally symptomatic. In this paper we present evidence that a mixture of sulfur dioxide (SO_2) and ozone (O_3) is responsible for the injury and that certain characteristics are associated with the resistant genotype.

MATERIALS AND METHODS

Austrian pine trees were exposed during the summer months to gaseous air pollutants in a dynamic fumigation chamber described earlier (7). Because New Jersey has a history of oxidant plant injury (2), we first considered O_3 and then SO_2 as causal agents because they represent a community type of pollutant, and finally a mixture of O_3 and SO_2.

Initially, 2-yr-old Austrian pine seedlings of unknown parentage were used for fumigation studies. The seedlings purchased from Princeton Nursery were potted in loam soil and maintained in a greenhouse with charcoal-filtered air until experimental fumigations. Eventually, 2- and 5-yr-old grafted seedlings obtained originally from mature trees representative of many that have consistently exhibited either a susceptible (typical foliar symptom) or resistant response in situ were used in the various fumigations. Inasmuch as grafted seedlings of both ages yielded similar results, only data for the 5-yr-old grafts will be included.

O_3 fumigations. O_3 was evolved by passing a metered stream of pure dry oxygen through a commercial ozone generator. The resulting O_3 was introduced into a charcoal-filtered air stream that passed through a mixing chamber before entering the 6-m^3 fumigation chamber. Air in the chamber was exchanged every 45 sec. Gas concentrations were monitored continuously by an ozone meter calibrated by the buffered KI method (6).

Seven 2-yr-old Austrian pine trees were fumigated at each of the following dosages: 400 (0.20 ppm), 600 (0.30 ppm), and 1,400 (0.70 ppm) $\mu g/m^3$ for 3, 6, and 6 hr, respectively. The exposures were made in midsummer at 24 C and 50% relative humidity. Seven susceptible (S) and seven resistant (R) grafted trees were exposed to 200 (0.10 ppm) and 400 (0.20 ppm) $\mu g/m^3$ for 6 hr under similar environmental conditions.

Throughout this study tests with 2-yr-old seedlings of unspecified origin were repeated at least three times because of the variability in responses observed in the field. Tests with grafts gave such uniform results that they were repeated only once.

SO_2 fumigations. SO_2 was introduced from a commercial cylinder (3% SO_2) into a stream of charcoal-filtered air that entered the chamber. Concentrations were monitored by the colorimetric method of West and Gaeke (11). Two-year-old seedlings were fumigated with SO_2 at 1,308 (0.5 ppm), 2,617 (1.0 ppm), or 5,234 (2.0 ppm) $\mu g/m^3$ for 6 hr at 24 C and 70% relative humidity. The number of trees in each exposure ranged from 4 to 17. Grafted stock was also exposed to SO_2 at 262 (0.1 ppm), 524 (0.2 ppm), or 2,617 (1.0 ppm) $\mu g/m^3$ for 6 hr, six replicate trees per treatment.

Fumigations with O_3 and SO_2. Experiments with O_3 and SO_2 combined were conducted in the chamber described earlier, and the gases were monitored as before, except that the ozone meter was equipped with a chromium trioxide scrubber to remove SO_2 interference. Nineteen 2-yr-old Austrian pine seedlings were fumigated with 400 (0.20 ppm) $\mu g/m^3$ O_3 combined with 1,832 (0.7 ppm) $\mu g/m^2$ SO_2 for 4 hr. Grafted stock (6 R, 6 S) were fumigated with the following combinations of O_3 and SO_2 for 6 hr: 200 (0.1 ppm), 262 (0.1 ppm) $\mu g/m^3$; 200 (0.1 ppm), 524 (0.2 ppm) $\mu g/m^3$; 400 (0.2 ppm), 262 (0.1 ppm) $\mu g/m^3$; and 400 (0.2 ppm), 524 (0.2 ppm) $\mu g/m^3$.

Two days after each fumigation, test plants were observed for foliar damage and compared with foliage of trees injured in the field. After the initial observations, the plants were observed every 2 days during the next 2 wk for changes in symptom development.

Resistance mechanisms. After determining the pollutants responsible for injury, we considered two factors that might determine resistance or susceptibility of the grafted material. The relative stomatal diffusive resistance of the current season's needles of R and S grafted stock was measured with a ventilated-diffusive resistance porometer (10) before and after fumigation with the SO_2/O_3 mixture that injured S seedlings. Total reducing sugars were also measured (12).

RESULTS AND DISCUSSION

The response of Austrian pine seedlings and grafted stock to the various pollutants is presented in Table 1. O_3 at 600 $\mu g/m^3$ for 6 hr was required to cause even a slight mottle on a few needles of 2-yr-old seedlings of unspecified origin. At 1,400 $\mu g/m^3$, needles became severely bleached or mottled, but tip necrosis occurred only once. Grafted material (R and S) exhibited no symptoms after a 6-hr O_3 exposure to 200 or 400 $\mu g/m^3$, levels that more realistically might occur in New Jersey (*unpublished*).

The ozone toxicity symptom induced on Austrian pine was unlike that observed in the field, and the concentration needed to elicit a symptom was greater than that in ambient air in New Jersey. Therefore, O_3 was eliminated as the cause of the problem. This explains our field observations, in that Austrian pine injury did not occur during ozone episodes when sensitive crops such as bean,

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Table 1. Response of resistant (R) and susceptible (S) Austrian pine seedlings and grafted stock to 6-hr pollutant exposures

Pollutant concentration ($\mu g/m^3$)	Plant material	Replicates	Symptoms None	Symptoms Atypical	Symptoms Typical[a]
O_3					
400[b]	Seedling	7	X		
600	Seedling	7		X	
1,400	Seedling	7		X	
200	Graft	7	X (R,S)		
400	Graft	7	X (R,S)		
SO_2					
1,308	Seedling	4	X		
2,617	Seedling	17		X	
5,234	Seedling	17		X	
262	Graft	6	X (R,S)		
524	Graft	6	X (R,S)		
2,617	Graft	6		X (R,S)	
$O_3 + SO_2$					
400:1,832[c]	Seedling	19			X
200:262	Graft	6	X (R,S)		
200:524	Graft	6	X (R,S)		
400:262	Graft	6	X (R)		X (S)
400:524	Graft	6	X (R)		X (S)

[a] Symptoms typical of those observed in field injury.
[b] 3-hr duration.
[c] 4-hr duration.

Table 2. Relative stomatal resistance and total soluble sugars[z] in Austrian pine resistant (R) and susceptible (S) grafted seedlings before, immediately after, and 2 days after 6-hr fumigation with 0.2 ppm O_3 and 0.1 ppm SO_2

Graft	Before	Immediately after	2 days after
	Stomatal resistance (sec/cm)		
R	20.6 a	52.8 c	52.8 c
S	19.8 a	34.6 b	34.6 b
	Total soluble sugar (%, dry weight)		
R	6.22 a	8.9 d	8.9 d
S	5.98 a	6.4 b	6.8 c

[z] Each value is the mean of eight replicate trees. All values were compared by Tukey's test for significance at the $P = 0.05$ level.

tobacco, potato, and Eastern white pine displayed symptoms of O_3 toxicity.

SO_2 fumigation concentrations of 2,617 $\mu g/m^3$ and above caused the tips of the needles to become necrotic in the 2-yr-old seedlings, and there was great variability in the amount of injury among individuals. In the grafted material, both R and S trees exhibited necrotic tip burn without the purple band typically observed in the field. SO_2 was eliminated as the cause of injury in the field because the R and S trees did not respond differentially and the concentration required to produce the injury was two orders of magnitude higher than ambient SO_2 values in New Jersey (*unpublished*).

When 2-yr-old seedlings were exposed to a mixture of O_3 and SO_2, 5 of 19 individuals developed tip necrosis of the current year's needles. When grafted material was exposed to a mixture of 400 $\mu g/m^3$ O_3 with 262 or 524 $\mu g/m^3$ SO_2, R trees were never injured and S trees were always injured. The symptom was similar to that observed in the field; tip necrosis limited by a purple band consistently occurred on 10–25% of the current year's needles of S grafts. The pollutant concentrations required to reproduce the tip burn symptoms were within the range of those found in New Jersey but slightly higher than the normal; however, a higher dose of any pollutant is generally required to injure plants in fumigation chambers than in the field (8). We conclude that an O_3 and SO_2 mixture was responsible for Austrian pine injury in the field.

Data relating to possible resistance mechanisms in R vis-à-vis S grafted material are presented in Table 2. Unfumigated R and S trees had comparable stomatal resistance values, but during an O_3 and SO_2 fumigation, resistance increased in the R plants, almost to that of plants held in darkness for 24 hr, viz. 60 sec. The situation is similar to that observed in O_3-resistant onion plants (5). The soluble sugar content of unfumigated R and S plants was similar, but after fumigation with the pollution mixture, more sugar was detected in R than in S plants. Whether this is a result of injury in the S plants or a cause of resistance in the R plants is not known because sugars were not measured in control plants at all three dates. The influence of carbohydrate content on injury from the O_3 and SO_2 mixture has not been established experimentally, but plants with low levels of soluble sugars are more susceptible to O_3 injury than are plants with high sugar contents (4).

In 1970, six 2-yr-old trees that had exhibited an S or R response to ambient air pollution were transplanted to the horticultural farm. Although the needles continued to be symptomatic to some degree each year, tree height and retention of needles have apparently not been affected. This study with Austrian pine presents additional evidence for a synergistic action of two pollutants on a coniferous species in the northeastern United States. Earlier, chlorotic dwarf of eastern white pine was also attributed to a mixture of O_3 and SO_2 (3).

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