## The Relative Susceptibility of Eighteen Coniferous Species to Ozone

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## ABSTRACT

Eighteen species of 2- to 6-year-old coniferous tree seedlings were exposed to 10 pphm O<sub>3</sub> for 8 hr or to 25 pphm O<sub>3</sub> for 4-8 hr. Virginia pine, jack pine, European larch, Austrian pine, Scotch pine, eastern white pine, eastern hemlock, Japanese larch, and pitch pine were susceptible. Arborvitae, balsam fir, Douglas fir, white fir, red pine, black hills spruce, Colorado blue spruce, Norway spruce, and white spruce were resistant to the highest dose. The incidence of sensitive plants within

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susceptible species populations ranged from 6-69%. Variable symptom response was observed among different species, among individual plants of the same species, and among different branches and needles of the same plant. Chlorotic mottle and tip necrosis or complete needle necrosis were the most commonly observed symptoms. Susceptible species were sensitive from the 4th through 13th weeks after budbreak. Seedlings in the dormant condition were resistant.

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At the present time in the USA, ozone  $(O_3)$  is thought to be the most important photochemical air pollutant affecting plants. Ozone develops in the atmosphere over urban areas as a result of photochemical reactions among a variety of compounds. Oxides of nitrogen and various hydrocarbons are important in many of these reactions. Although  $O_3$  develops principally in the atmosphere over cities, its effects are not confined to the urban area.

Ozone was initially demonstrated to be a phytotoxicant in 1958 when Richards et al. (11) showed that it was the cause of "stipple" of grape. The following year, Heggestad & Middleton (6) reported O<sub>3</sub> as the cause of weather fleck of tobacco. In a review of the literature on the influence of O<sub>3</sub> on plants, Rich (10) listed 57 different species as susceptible to O3. Only one forest tree genus, Pinus, was among the 57 plants listed. The only information available to date on the influence of O3 on conifers is a series of studies on the influence of O<sub>3</sub> on eastern white pine (Pinus strobus L.) and ponderosa pine (Pinus ponderosa Laws.). Ozone has been reported to cause needle tip necrosis (1, 7) or banding (2, 3) of the former species, and chlorotic mottle of the older needles of the latter species (9). With this information in mind, a study was set up to determine the relative susceptibility of 18 coniferous species to O<sub>3</sub>, and the relationship between phenological condition or season and sensitivity. A portion of these data was presented earlier (4, 13, 14).

MATERIALS AND METHODS.—Eighteen species of 2- to 6-year-old coniferous tree seedlings, potted in a 3:1:1 topsoil-peat-sand or a 3:1 peat-perlite mixture, were used in this study. The plants were

maintained either on greenhouse benches, where they received charcoal-filtered air and were watered regularly, or in outside beds, where they received ambient air and were watered and fertilized regularly. Prior to exposure, five plants/species were placed in the darkness in a controlled environment chamber for 1 hr at a temperature of 23 C and a relative humidity in the range of 72-85%. The predark period has been shown to enhance symptom development. Four seedlings/species were transferred to the exposure chamber, and one plant/species was left in the original chamber as a check.

Most species under study were exposed to 25 parts/hundred million (pphm) O<sub>3</sub> for 8 hr at 2-week intervals beginning 4 weeks after needle emergence. Phenological differences among species, the availability of fumigation chamber space, the availability of greenhouse space, and other factors made it impossible to establish a rigid 2-week interval fumigation schedule for all species. Plants were also exposed to concentrations of 10 pphm O<sub>3</sub> for 8 hr, and 25 pphm for 4 hr. Virginia pine (*Pinus virginiana* Mill.) seedlings were exposed to these doses from the 1st through the 16th week of growth.

Ozone was generated by passing pure oxygen through a commercial (ultraviolet light) ozonator. The desired  $O_3$  concentration was maintained by regulating either the amperage or oxygen flow through the  $O_3$  generator. The gas was transferred to the exposure chamber through glass tubing with Tygon connections or Teflon tubing. Once inside the chamber, the  $O_3$  mixed with incoming charcoal-filtered air

The exposure chamber was a modified version of a commercially available chamber (Environmental

TABLE 1. Species resistant or susceptible to 25 pphm O<sub>3</sub> for 8 hr

Susceptible species	Resistant species				
Hemlock, eastern	Arborvitae				
(Tsuga canadensis [L.] Carr.)	(Thuja occidentalis L.)				
Larch, European	Fir, balsam				
(Larix decidua Mill.)	(Abies balsamea [L.] Mill.)				
Larch, Japanese	Fir, Douglas				
(Larix leptolepis [Sieb. & Fucc.] Gord.)	(Pseudotsuga menziesii [Mirb.] Franco.)				
Pine, Austrian	Fir, white				
(Pinus nigra Arnold)	(Abies concolor [Gord. & Clend.] Lindl.)				
Pine, eastern white	Pine, red				
(Pinus strobus L.)	(Pinus resinosa Ait.)				
Pine, jack	Spruce, Black Hills				
(Pinus banksiana Lamb.)	(Picea glauca var. densata Bailey)				
Pine, pitch	Spruce, Colorado blue				
(Pinus rigida Mill.)	(Picea pungens Engelm.)				
Pine, Scotch	Spruce, Norway				
(Pinus sylvestris L.)	(Picea abies [L.] Karst.)				
Pine, Virginia	Spruce, white				
(Pinus virginiana Mill.)	(Picea glauca [Moench] Voss.)				

Growth Chamber Co., Chagrin Falls, Ohio) and has been described elsewhere (15).

Exposures were conducted at a temperature of 21 C, a relative humidity of 75%, and a light intensity of ca. 1,400 ft-c. During exposures, O<sub>3</sub> levels were monitored continuously with a Mast (Mast Development Co., Ames, Iowa) meter connected to a strip-chart recorder. Oxidant values of the Mast meter were compared to the determination of oxidants by the neutral-buffered potassium iodide method. Meter efficiencies during these fumigations ranged from 90 to 97%. When available, a second meter was used as a check. Continuous and intermittent temperature measurements were made, using copper-constantan thermocouples connected to a 24-point recorder, and relative humidity was monitored using lithium chloride sensors and a single-pen strip-chart recorder.

After exposure, plants were placed in controlled environment chambers maintained at 21-C day and 15-C night temperatures, and a constant relative humidity of 75%. A 12-hr photoperiod of ca. 1,400 ft-c beginning at 7 AM was utilized. The seedlings were held in this chamber ca. 1 week or until visible symptom development ceased, at which time only the current foliage was examined. Older needles were not evaluated.

RESULTS.—Relative susceptibility of species.—Nine of the 18 species were susceptible to an exposure of 25 pphm  $O_3$  for 8 hr (Table 1). The susceptible species were subsequently exposed to 25 pphm  $O_3$  for 4 hr, and 10 pphm for 8 hr. Austrian pine, jack pine, Virginia pine, and European larch were injured by 25 pphm  $O_3$  for 4 hr; the first three species were also injured by 10 pphm for 8 hr. The nine resistant species also are listed in Table 1.

The percentage of plants within the seedling populations that were susceptible to 25 pphm O<sub>3</sub> for 8 hr is shown in Table 2. The incidence varied from 6-69%. It was lowest in populations of eastern hemlock, Japanese larch, and pitch pine, and highest in populations of Virginia pine. There was a direct correlation between the incidence of susceptible individuals in the population and symptom severity on individual plants. Individual plants of susceptible species such as European larch, Austrian pine, jack pine, and Virginia pine often showed the most severe chlorotic mottle and necrosis; individual plants of less susceptible species, such as eastern hemlock or Japanese larch, usually developed light symptoms.

Susceptible species were ranked in order of decreasing susceptibility (Table 3). The

TABLE 2. The incidence of O<sub>2</sub>-susceptible plants among conifer seedling populations exposed to 25 pphm O<sub>2</sub> for 8 hr

Species	Plants exposed	Plants susceptible	Plants susceptible	
	no.	no.	%	
Hemlock, eastern	32	2	6	
Larch, European	28	10	36	
Larch, Japanese	32	2	6	
Pine, Austrian	44	10	23	
Pine, eastern white	73	12	16	
Pine, jack	60	21	35	
Pine, pitch	32	2	6	
Pine, Scotch	16	5	31	
Pine, Virginia	48	33	69	

TABLE 3. Relative susceptibility of 9 coniferous species

Species	Susceptibility value <sup>a</sup>				
Pine, Virginia	217				
Pine, jack	105				
Larch, European	72				
Pine, Austrian	69				
Pine, Scotch	31				
Pine, eastern white	16				
Hemlock, eastern	6				
Larch, Japanese	6				
Pine, pitch	6				

<sup>a</sup> Based on per cent population susceptible X severity factor.

"susceptibility value" was obtained by multiplying the incidence of susceptible members of each species population by a severity factor. A severity factor of 1 indicated that the species was injured only by  $O_3$  doses of 25 pphm for 8 hr; a factor of 2 indicated injury at 25 pphm for 4 hr; and a factor of 3 indicated injury after exposure to 10 pphm for 8 hr. In this way, both the threshold dose needed to cause injury and the incidence of susceptible members in the species population were expressed in one value. Those species which were not injured by 25 pphm  $O_3$  for 8 hr were considered resistant.

Description of symptoms.-Symptoms observed most commonly were chlorotic mottle or chlorosis of needle tips, chlorosis or necrosis of an area 1-2 cm in length ca. 1 cm above the needle base, ivory-colored flecks scattered across the needle surface, necrosis of the needle tips, necrotic bands at various points along the needles, necrosis of the entire needle, resin exudation on primary needles, and dark brown-to-black, glossy narrow bands at various points along the 2-year needles (especially of Virginia pine). Occurrence of the various symptom types is presented by species in Table 4. Often one or a combination of the symptom types listed in Table 4 were on a given plant; this was especially true of the exposures of highly susceptible plants to high O<sub>3</sub> concentrations (25 pphm for 8 hr). There was a tremendous amount of variation in symptom development from tree to tree within a given species,

TABLE 4. Distribution of symptom type by species<sup>a</sup>

from branch to branch on a given tree, and from needle to needle within the same fascicle. Needles on terminal branches were generally more susceptible than were needles on side branches. This was especially true of the older seedlings (5-6 years old). Current foliage was more sensitive than older foliage, and plants in dormant condition were generally resistant. The most severe symptoms usually developed on the needle surface that faced the light source in the exposure chamber; this was true for both two- and three-sided needles. In some instances, when needles were twisted, the most severe symptoms would develop on all three sides of the needle, but again only at points where a given face was exposed to the chamber ceiling lights.

The most common symptom on eastern hemlock was an ivory fleck on the distal half of the dorsal face of the needle. This symptom differed from a mottle in that the individual flecks were discrete and did not tend to merge together as did the various chlorotic areas characteristic of a mottle symptom.

European larch exhibited tip necrosis, entire needle necrosis, and needle twisting. There was a positive correlation between the severity of needle necrosis and the degree of needle twisting. Both spur shoot and primary needles were susceptible.

Tip necrosis was the most common symptom observed on Japanese larch. Initially, water-soaked areas developed approximately one-quarter of the needle length from the base of the needle; these water-soaked areas subsequently turned brown and collapsed, and tip necrosis developed.

Austrian pine showed chlorotic mottle of the needle tips, necrosis of the needle tips, and development of necrotic bands near the needle tips. In some instances, the area of chlorotic mottle developed into necrotic bands within 48 hr. The bands were usually from 0.5 to 2.0 cm long, and only one necrotic band developed/needle. The degree of tip necrosis varied, depending upon the length of the needle at the time of exposure.

Tip chlorosis of the current needles was the only symptom observed on Eastern white pine. The symptoms developed on needles that were 3-5 cm long and there was a moderate amount of twisting associated with the injury.

Species	Chlorotic				Necrotic					
	Tip mottle	Tip	Area near needle base	Ivory fleck	Tip	Bands	Area near needle base	Needle		
Hemlock, eastern				+						
Larch, European					++			+		
Larch, Japanese					+			0.5%		
Pine, Austrian	++				+	+				
Pine, eastern white		+			- 5	1000				
Pine, jack	++				+		+			
Pine, pitch	+	+			1.4					
Pine, Scotch	++				++					
Pine, Virginia	+		++		+		++	+		

a + = Symptom infrequent; + + = symptom common.

The most common symptoms on jack pine were chlorotic mottle of the needle tips, necrosis of the needle tips, and the necrosis of areas near the needle base. In some instances, chlorotic mottle of the needle tip preceded the development of a necrotic needle tip. The degree of tip mottle and tip necrosis was positively correlated with needle length.

Pitch pine exhibited a chlorotic mottle of the needle tips which in some instances extended to within 1-2 cm of the needle base. On some of the needles, the area of chlorotic mottle became necrotic within 2 days after exposure.

Chlorotic mottle of the needle tips and necrosis of the needle tips were commonly observed on Scotch pine. The bases of affected needles usually remained green, and in some instances the area of chlorotic mottle eventually developed into a necrotic area. Needles of different sizes were affected, and as with many of the other species, the degree of needle involvement was correlated with needle length.

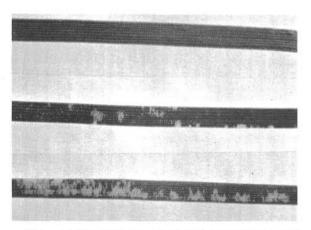


Fig. 1. Chlorotic mottle of Virginia pine needles. The upper needle represents a healthy check.

The most common symptoms on Virginia pine were chlorotic and necrotic areas 1-2 cm in length located ca. 1 cm above the base of the needle. In addition, chlorotic mottle of the needle tips (Fig. 1), necrosis of the needle tips, and over-all needle necrosis were also common. Virginia pine produces primary needles early in the spring prior to the

emergence of the fascicular needles. The primary needles were sensitive to  $O_3$ , and showed any one or a combination of the above symptoms. In addition, resin exudation, often from opaque, water-soaked necrotic areas, was sometimes observed on these needles.

Relationship of age to sensitivity.—Table 5 shows the relative susceptibility of the various conifers in relation to time after needle emergence. The horizontal bars represent the period of time during which the tissue was susceptible. Resistant species failed to develop symptoms at any age. Most species were resistant to this dose after about 13 weeks. Virginia pine was susceptible from 4 to 15 weeks after needle emergence.

Shaded plants of most pine species were generally more sensitive to  $O_3$  than those grown in full sunlight. The shaded or suppressed plants usually had longer, thinner needles than the nonshaded plants. Also, plants grown in the greenhouse were consistently more sensitive than those grown outside.

Many of the susceptible species were also exposed to  $O_3$  during the dormant season. At this time, the plants were resistant, and only Virginia pine developed symptoms after being subjected to 25 pphm  $O_3$  for 24 hr.

DISCUSSION.-The exposure of seedlings of 18 coniferous species illustrated definite interspecific differences in O3 susceptibility. Generally, the pines and larches were the most susceptible, while the spruces and firs were resistant. Within populations of O<sub>3</sub>-susceptible species, individual plant response varied, often ranging from no observable symptoms to complete tissue necrosis. This intraspecific variation in susceptibility appeared to be inherent, not due to environmental factors. Variable symptom response was also observed among different branches of the same plant, among different needle fascicles on the same branch, and even among needles of the same needle fascicle. These findings suggest that factors such as the microclimate of individual needles play an important role in determining the sensitivity of specific tissues.

Chlorotic mottle and necrosis of various portions of needles were the symptoms observed most frequently. The mottle was similar to that reported on ponderosa pine (9), and the needle necrosis was

TABLE 5. Relationship between age of current foliage and susceptibility to 25 pphm O<sub>3</sub> for 8 hr

Species	Age of current foliage in weeks										
	4	5	6	7	8	9	10	11	12	13	14
Larch, European	rch, European										
Larch, Japanese Pine, Austrian	_										
Pine, eastern white Pine, jack											
Pine, pitch Pine, Scotch Pine, Virginia											-117

a Horizontal bars represent the period of time the tissue was susceptible.

similar to the tip necrosis reported on eastern white pine (1, 7). Thus, it appears that chlorotic mottle and tip necrosis may be common symptoms of  $O_3$  injury to coniferous species. However, these symptoms are not unique to  $O_3$ -induced diseases. This causes some confusion in attempts to evaluate symptoms in the field, since other factors of the environment, such as insects, viruses, drought, frost, salt, nutrient deficiencies, other pollutants, and herbicides may cause symptoms similar to those caused by  $O_3$ . Hence, field evaluations of symptoms which have not been duplicated under artificial conditions must be treated with caution. Field evaluations are also complicated, as symptoms other than typical may be produced.

18

The minimum dose of O<sub>3</sub> needed to cause injury on trees has been reported for only a few species. Ponderosa pine showed symptoms only after fumigation with 50 pphm O<sub>3</sub> for 9 hr/day for 9-18 continuous days (9). In contrast, eastern white pine has been injured by doses as low as 6.5 pphm for 4 hr (1), and white ash showed symptoms after exposure of 10 pphm for only 0.5 hr (12). By comparison, the Bel W-3 tobacco variety, which is often used as an O<sub>3</sub> monitor, is injured by concentrations as low as 10 pphm for 2 hr (8). Nine of the 18 species used in our study were injured by 25 pphm O<sub>3</sub> for 8 hr; four species, by 25 pphm for 4 hr; and three species, by 10 pphm for 8 hr. The continuous air monitoring program of the National Air Surveillance Network of the National Air Pollution Control Administration reported total oxidant levels for 1966 in Philadelphia which were in the range of the concentrations of O<sub>3</sub> used in this study. Because at times O<sub>3</sub> is probably the major constituent of the "total oxidant", it seems that periods of suitable concentration and duration of this pollutant exist frequently enough in urban and adjacent areas to cause damage to woody plants.

The incidence of  $O_3$ -sensitive plants within each susceptible species population ranged from 6 to 69%. An examination of plants growing in an area of high ambient oxidant levels would probably reveal a low incidence of members of a susceptible species. The low incidence may be due to the most susceptible plants or species being lost due to selection pressure against these individuals by  $O_3$ .

Our selection of eastern white pine was relatively resistant to  $O_3$ . This is in contrast to previous reports (1, 2, 3). Other factors, such as genetic variation in resistance to  $O_3$  within the species, or differences in the temperature or relative humidity regimes before, during, and after exposure, could account for these differences.

In general, the period of time when conifers are susceptible to  $O_3$  is much longer than the period of susceptibility for many other plants. Virginia pine was susceptible during the major portion of its growing season (Table 5). In contrast, bean plants are most susceptible when the leaves are from 7 to 14 days old (5). Many conifers also retain their needles throughout the dormant season, and the same needles may be exposed to ambient levels of air pollution throughout the year for as many years as the tree retains the needles.

The age of current tissue also influenced the degree of symptom development. Early in the growing season, only the tips of the current needles were sensitive to  $O_3$ . Later, the entire needle, with the exception of the needle base, was sensitive. These results are similar to those of Linzon (7) concerning the susceptibility of eastern white pine needles to  $O_3$ .

Although O<sub>3</sub> develops principally in the atmosphere over cities, its effects are not confined to the urban area. For example, there is evidence that O<sub>3</sub>, which is thought to be responsible for the decline in ponderosa pine in the San Bernadino Mountains east of Los Angeles, originates in the pollution cloud over Los Angeles, and under certain meteorological conditions is carried to the mountains ca. 75 miles away (9). Therefore, it is possible that the O<sub>3</sub> which develops in the atmosphere over the megalopolis of the northeastern USA might drift into forests adjacent to the megalopolis for equivalent distances. Consequently, decisions regarding future plantings in urban areas of the Northeast and the management of forests adjacent to the megalopolis should take into consideration the relative susceptibility of the various species to O<sub>3</sub>. Our data indicate that the spruces, firs, arborvitae, and red pine are resistant, and should be used wherever possible. It should also be kept in mind that our data are based on the susceptibility of 2- to 6-year-old seedling populations, and that there may or may not be a correlation between seedling sensitivity and adult plant sensitivity. However, these do represent the best data available at the present

The ultimate control of air pollution will probably be achieved by elimination of sources, which can best be accomplished by legislation based on air quality standards. As trees form an integral part of the urban way of life, and as some of our most economically and aesthetically desirable species are susceptible to ambient levels of  $O_3$ , it is important that the results of studies such as these be considered in the establishment of air quality standards.

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