The Influence of Plant Age on the Sensitivity of Virginia Pine to Ozone

Donald D. Davis and Francis A. Wood

Former Special Fellow, currently Assistant Professor and Extension Air Pollution Specialist, Department of Plant Pathology and Center for Air Environment Studies; and Research Associate, Center for Air Environment Studies, and Professor, Department of Plant Pathology, respectively, The Pennsylvania State University, University Park 16802.

Contribution No. 667, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 19 April 1972 as Journal Series Paper No. 4192. Contribution No. 255-72, Center for Air Environment Studies.

We acknowledge the Office of Manpower Development, National Air Pollution Control Administration of the U.S. Public Health Service for providing Grant No. AP 00436 and special fellowship No. 5 F3 AP 33, 925; and the School of Forest Resources for providing space in the Forestry Resources Laboratory.

Accepted for publication 8 September 1972.

ABSTRACT

Virginia pine seedlings in the cotyledon, primary needle, and secondary needle stages were injured after exposure to 25 pphm O_3 for 2 hr. All three needle types reached maximum sensitivity to O_3 at approximately 3-5 weeks following initiation of needle growth, after which time the sensitivity declined. Cotyledons became resistant at 16 weeks; primary needles were still sensitive at the oldest age studied; secondary needles became resistant at 18 weeks. The basal portions of the cotyledons were more sensitive than the tip or mid portions. The youngest primary and secondary needles were injured most severely at the needle tip, whereas older needles were more severely injured at the base. The three needle types showed similar symptoms, mainly chlorotic mottle and tissue necrosis, as well as similar dosage-response curves. Dormant 3-year-old plants exposed to O_3 in December were highly resistant.

Phytopathology 63:381-388

Additional key words: air pollution, forest pathology.

Conifer species exposed to O_3 during the period of 4-14 weeks after the beginning of needle growth were sensitive for varying periods of time (6). Eastern white pine was recently reported to have a period of prime sensitivity for about 6 weeks, starting 1 week after needle emergence; by 12 weeks the seedlings were fairly resistant (2, 3). Other than these reports, little is known about the sensitivity of conifers to O_3 from time of emergence to maturity. Data of this nature are required if we are to gain a more thorough understanding of the phytotoxic nature of O_3.

Virginia pine (*Pinus virginiana* Mill.) was found to be the most susceptible of 18 coniferous species exposed to O_3 (6), and was selected for further studies. Our objectives were to compare symptom expression and relative sensitivity of cotyledons, primary needles, and secondary needles; and to determine the influence of both plant and needle age on O_3 sensitivity.

A summary of this information has been presented in abstract form (4, 5).

MATERIALS AND METHODS.–Virginia pine seedlings in the cotyledon and primary needle stages (plants less than 1 year old) were grown from seed in the greenhouse. Three-year-old plants with secondary needles were purchased from a nursery and maintained in pots in outside beds. All seedlings were potted in a 3:1 peat:perlite mixture and were watered uniformly.

Ozone was generated as previously described (6) and was transferred to the exposure chamber through Teflon tubing, where it mixed with charcoal-filtered air of the chamber. The exposure chamber was a modified version of a commercially available chamber (Environmental Growth Chamber Co., Chagrin Falls, Ohio) and has been described elsewhere (21). During exposures, temperature, relative humidity, and O_3 levels were monitored as previously reported (6). Oxidant values of Mast (Mast Development Co., Davenport, Iowa) meters were compared to values obtained from the determination of oxidants by the neutral buffered-potassium iodide method. Meter efficiencies were 80 to 85% during these studies.

Plants in the cotyledon and primary needle stages were moved directly from the greenhouse and exposed. Three-year-old plants were brought from the outside beds and conditioned for 2 days at 27 C, 80% relative humidity, and a 12-hr photoperiod of 2,400 ft-c beginning at 6 AM. This conditioning was done to partially alleviate any outside environmental influence that might obscure the effect of age on the plants' sensitivity. Dormant plants exposed in December were not preconditioned.

During exposure, temperature was maintained at 24 C, relative humidity at 75%, and light intensity at 2,400 ft-c.

To determine the influence of plant age on susceptibility to O_3, seedlings were exposed to 25 pphm O_3 for 4 hr at various stages of growth. Plants with cotyledons were exposed at 1, 3, 4, 8, 12, and 16 weeks after seed coat shedding. Primary needles were fumigated 1, 3, 4, 12, and 16 weeks after needle emergence. Three-year-old plants with secondary needles were exposed to O_3 every week throughout the growing season, beginning with the first needle emergence in late May and extending into October. Ten seedlings were used in each exposure.

Dormant 3-year-old plants were exposed to O_3 in December at 5 C and 65% relative humidity, or at 24 C and 75% relative humidity. Preliminary results indicated that dormant plants were highly resistant; consequently plants were exposed to relatively high doses. Dosages of 10 or 25 pphm O_3 for 24 or 48 hr, or 50 pphm for 1, 2, or 4 hr were used, and four seedlings were exposed to each dose.

After 5 weeks of growth, 80 seedlings of each leaf type were exposed to 25 pphm O_3 for 1-8 hr to establish dosage-response curves. Eighty plants in the cotyledon and 80 plants in the primary needle stage were placed in the exposure chamber at 9 AM, and 10 plants of each needle type were removed at the end of each hour. Due to the large size of the 3-year-old plants, the number of seedlings in the exposure chamber was kept constant throughout the dosage study to maintain fairly uniform conditions within the chamber (this was not deemed necessary for the small primary and cotyledonous plants). Forty-five 3-year-old seedlings were kept in the exposure chamber at all times during the exposure, which began at 9 AM. At the end of each hour, five plants were removed from the chamber, and five different

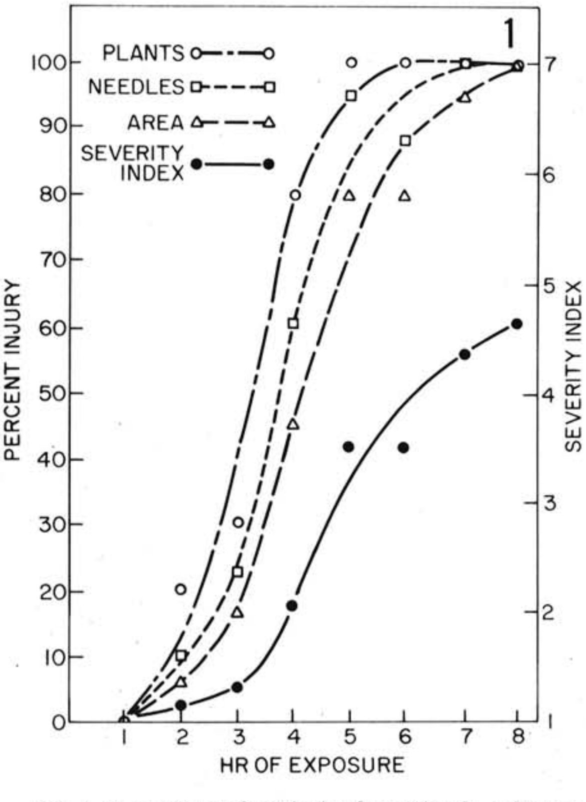


Fig. 1. Comparison of methods of assessing O_3 injury on Virginia pine using data from the exposure of secondary needles of 80 3-year-old seedlings to 25 pphm O_3 for 1-8 hr.

plants were placed into the chamber until after 8 hr of exposure when all plants were removed.

Following exposure, all plants were placed in controlled environment chambers at a constant 27 C, 80% relative humidity, and a 12-hr photoperiod of 2,400 ft-c beginning at 6 AM. Plants were maintained under these conditions for 1 week, at which time symptoms were evaluated.

Symptoms on individual needles were evaluated based on a severity index of 1-7. A value of "1" indicated no visible injury; "2" depicted a very slight chlorotic mottle; "3", a slightly more severe mottle; and so forth to "7", which indicated tissue necrosis and collapse. An average severity index was calculated for each plant, based on all cotyledons or primary needles. Secondary needle evaluation was based on a sample of 40 needles taken from the first flush of current needles on the terminal branch of each seedling. Only current growth was evaluated.

In selected studies, severity indices were also taken at the base, middle, and tip of each primary needle and cotyledon to determine whether there were differences in sensitivity at various portions of the same needle. Since pine needles grow from the base (16), these areas represented the youngest, the middle-aged, and the oldest tissues, respectively. Also, the position of the primary needles on the plant was noted; needles located at the top of the plant are the youngest; those at the bottom, the oldest.

Several methods were utilized to evaluate the severity of injury on 80 3-year-old plants exposed to O_3 during a dosage-response study. The percent needles injured was determined by a count of the number of sampled needles that exhibited symptoms. The percent of needle area injured indicates the extent of tissue injury measured in millimeters along the longitudinal axes of individual needles. The severity index was described above. In each case, an average value indicating the amount of injury was obtained for each plant, and an average value was determined for the 10 plants utilized in each exposure period. The percent plants injured simply indicated the number of plants showing O_3 symptoms.

Smooth curves were fitted to data using a plotting procedure based on the method of least squares. This method is nonstatistical and was utilized only as an aid in interpreting trends in the data. Analyses of variance were performed, and probabilities of 95% or greater were recorded; probabilities less than this were listed as nonsignificant. Means were statistically separated by the Scheffé method.

RESULTS.–*Symptoms.*–The most common symptoms on Virginia pine cotyledons, primary needles, and secondary needles were chlorotic mottle and tissue necrosis of the tip or entire needle. All three needle types showed similar symptoms; the only deviation was an occasional resinosis of the primary needles.

Figure 1 illustrates the results of several methods utilized to evaluate symptom severity. These methods provided similar results, with the percent area injured yielding lower severity readings than the others.

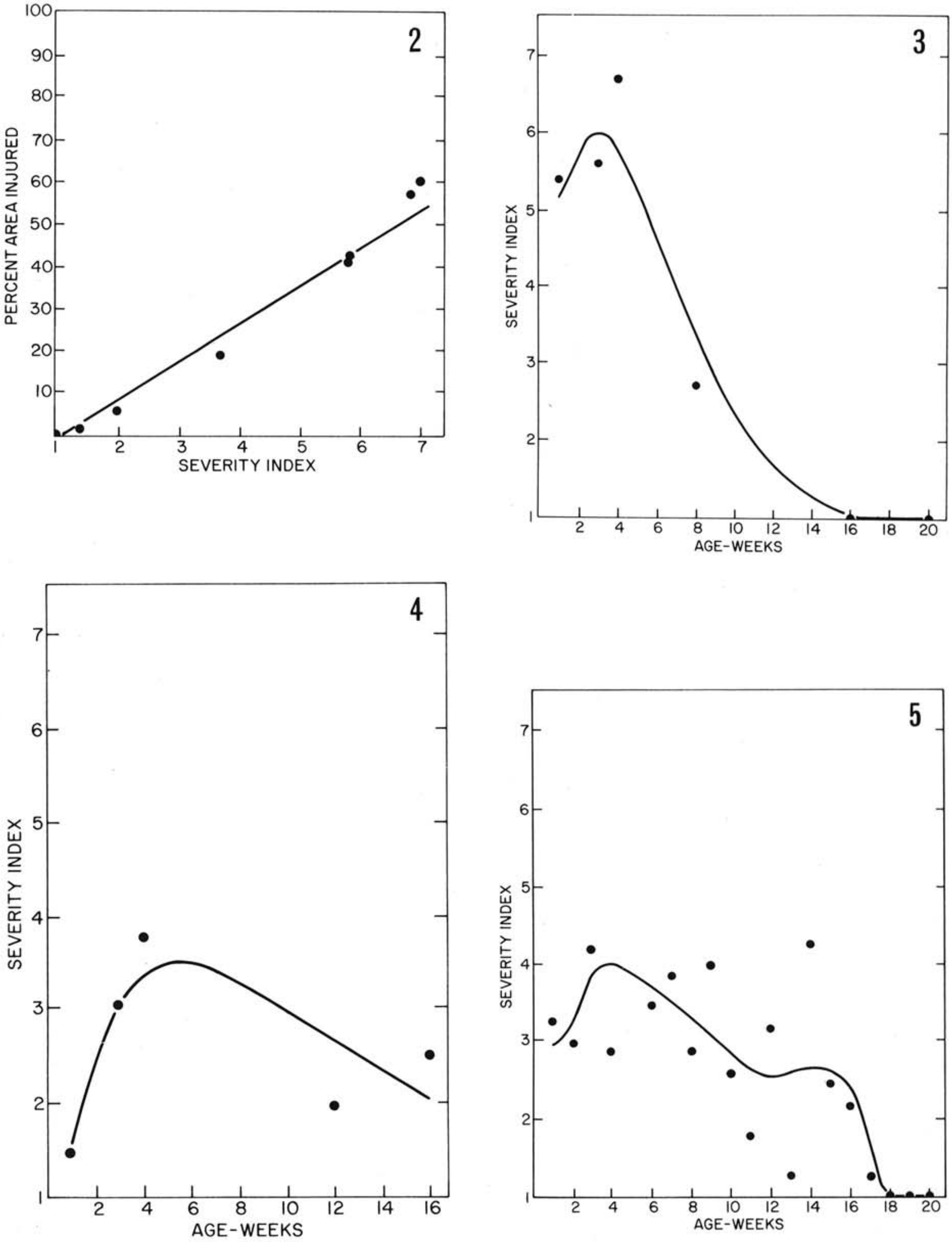


Fig. 2-5. 2) Relationship between severity index and percent of Virginia pine needle area injured. **3)** Influence of age on the sensitivity of Virginia pine cotyledons exposed to 25 pphm O_3 for 4 hr. **4)** Influence of age on the sensitivity of Virginia pine primary needles exposed to 25 pphm O_3 for 4 hr. **5)** Influence of age on the sensitivity of Virginia pine secondary needles on 3-year-old plants exposed to 25 pphm O_3 for 4 hr.

The percent area affected and the severity index were the only methods of the four which give quantitative injury data on individual needles. Percent area affected is often used with broadleaved species (13, 14, 19). However, it is time-consuming to measure the degree of injury in this way. The severity index was linearly related to the percent area affected (Fig. 2), and therefore was utilized for all studies.

Influence of plant age.—Figures 3-5 illustrate the influence of plant age on the sensitivity of Virginia pine cotyledons, primary needles, and secondary needles exposed to 25 pphm O_3 for 4 hr. As shown, all three needle types reached maximum sensitivity approximately 3-5 weeks after emergence, after which the sensitivity declined.

The severity indices of 1-, 3-, and 4-week-old cotyledons were statistically similar and were significantly greater than those 8-20 weeks old, which were also statistically similar. The 4-week-old primary needles were injured significantly more than only those seedlings at the 1- or 12-week age. The remaining indices were similar. The secondary needles of the 3-year-old plants had significantly greater values at the 4-week stage than at the 13-, 17-, 18-, 19-, or 20-week age. The indices of the remaining ages were statistically similar.

Dormant 3-year-old seedlings exposed to O_3 in December were relatively resistant. Symptoms were not observed on plants exposed to 10 pphm O_3 for 24 or 48 hr. One of four plants exposed to 25 pphm O_3 for 24 hr at 5 C and 65% relative humidity was injured, and two seedlings exposed for 48 hr showed symptoms. Plants exposed to 25 pphm O_3 for 24 hr at 24 C and 75% humidity were not injured, whereas one of those exposed for 48 hr showed symptoms. Symptoms were not observed on seedlings exposed to 50 pphm O_3 for 1 or 2 hr under either environmental regime. One of the four plants exposed to 50 pphm for 4 hr at 5 C and 65% humidity was injured, whereas two of those plants exposed to this same dose at 24 C and 75% humidity were injured.

Although the sensitivity of the previous year's needles was not evaluated, these needles were sensitive to O_3. Chlorotic mottle and tip necrosis were present throughout the growing season on these needles after exposure to O_3.

TABLE 1. Sensitivity of various portions of cotyledons of Virginia pine at different ages exposed to 25 pphm O_3 for 4 hr

Age (weeks)	Cotyledon portion		
	Base	Mid	Tip
1	5.4	4.5	3.8
3	5.4	5.2	5.2
4	6.7[a]	6.7[a]	5.2
8	2.6	2.3	1.9
12	1.0	1.0	1.0
16	1.0	1.0	1.0
20	1.0	1.0	1.0

[a] Significantly different from tip value at the 95% level of confidence.

TABLE 2. The sensitivity of various portions of primary needles of Virginia pine at different ages exposed to 25 pphm O_3 for 4 hr

Age (weeks)	Needle portion		
	Base	Mid	Tip
1	1.0	1.0	1.4
3	1.8	2.9	3.3[a]
4	2.3	2.9	3.6[a]
12	1.8	1.7	1.7
16	2.2	1.8	1.6

[a] Significantly different from base and mid values at the 95% level of confidence.

Influence of tissue age.—Table 1 illustrates the difference in sensitivity of the base (youngest), middle, and tip (oldest) tissues of individual cotyledons of various ages. The basal or middle portion appeared to be the most sensitive tissue of the cotyledon throughout the first 8 weeks of growth. However, the basal, as well as the middle needle portion, had significantly more injury than the tip portion only at 4 weeks of age. The three portions of the cotyledons at all other ages had statistically similar indices. Twelve- to 20-week-old cotyledons were resistant.

Similar comparisons were made for the various portions of the primary needles injured by O_3. As shown in Table 2, the tips (oldest) of the very young needles were most severely injured by O_3, but at 12-16 weeks the basal third (youngest) was the most sensitive portion of the needle. However, only the 3- and 4-week-old needles had statistically different indices among the needle portions, with the tips showing more injury than the basal or middle portions.

These comparisons were not made for secondary needles, but it was generally observed that the young secondary needles were most severely injured at the tips, whereas older needles showed more widespread symptoms.

Influence of needle age.—The sensitivity of primary needles located at different positions on ten 4-week-old plants and ten 16-week-old plants is shown by Fig. 6. Each needle of the 4-week-old plants was evaluated. Due to the large number of needles, only every fourth needle on the 16-week-old plants was rated. At the very early stages of growth (less than 4 weeks), there were few primary needles with little variation in sensitivity among them. However, at 4 and 16 weeks of age, the oldest needles at the base of the plant (positions 20 and 23, respectively) were more sensitive than the youngest needles (position 1) at the top (Fig. 6).

Figure 7 illustrates the influence of both primary needle position and needle portion on the sensitivity of ten 4-week-old plants to O_3. As shown, there was least influence of needle portion at the top and base of the plant. At needle positions 7 and 9-13, the needle tips had significantly greater indices than the basal portions, and were greater than the middle portions at the 9, 10, 12, and 13 needle positions.

A similar set of data is shown in Fig. 8 for the 16-week-old plants. Again, the greatest influence of needle portion was observed on the middle-aged needles. In contrast to the results from the 4-week-old plants, the basal needle tissues of the 16-week-old plants were most sensitive, whereas the tip portions of the needles were least sensitive. However, no significant differences were noted among indices of various needle portions.

Dosage response.–The response of 10 plants of each needle type exposed to 25 pphm O_3 for 1-8 hr is shown in Fig. 9. All three needle types were sensitive to O_3, with cotyledons most sensitive and secondary needles most resistant. The threshold time needed to cause injury at 25 pphm O_3 was approximately 2 hr. After 5-8 hr at this concentration, severe injury was noted on all needle types.

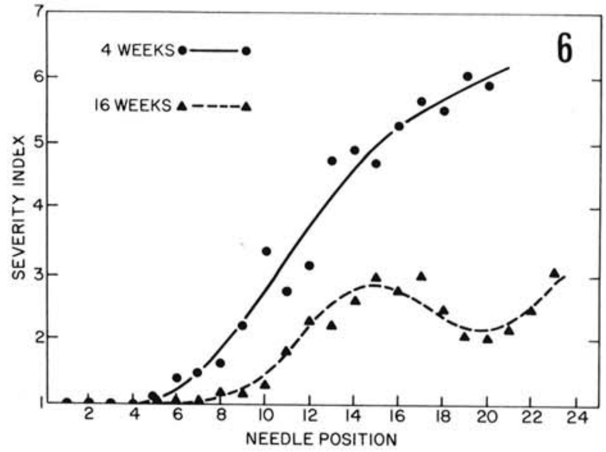
Injury to the cotyledons was significantly greater than that on the primary needles only at the 7- and 8-hr exposures. Cotyledon indices were significantly greater than those of the secondary needles at the 2-, 3-, 4-, 5-, and 6-hr exposures. Ratings on primary needles were significantly greater than those on the cotyledons at the 2-hr exposure, and were greater than those of the secondary needles at the 2-, 3-, 4-, 7-, and 8-hr exposures.

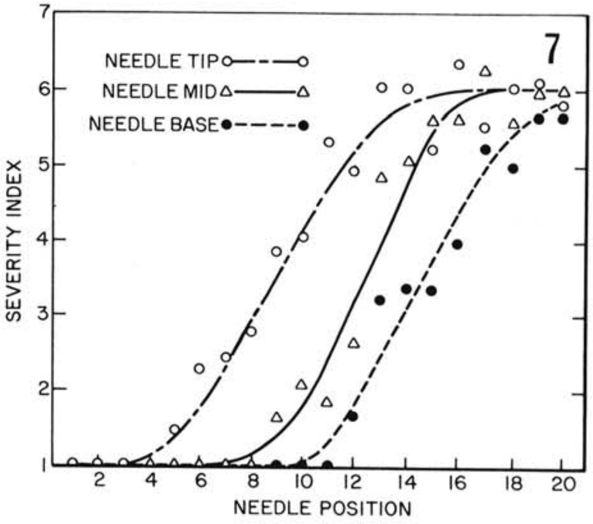
The various time-concentration threshold levels needed to cause injury are shown in Fig. 10. These data were taken from various studies conducted at 24 C, 75% relative humidity, and 2,400 ft-c of light.

DISCUSSION.–*Symptoms*.–Symptoms produced on Virginia pine cotyledons, primary needles, and secondary needles exposed to O_3 were similar and were like those reported on other conifers (1, 2, 3, 12, 15). Thus, chlorotic mottle and tip or needle necrosis may represent typical O_3 symptoms on the various needle types of many conifers.

The results obtained using the severity index of 1-7 were closely correlated with the other symptom evaluation systems used. This method allows a rapid quantitative measurement of injury on individual needles. Data recorded in this manner may be more useful than simply knowing the percent of plants or percent of needles injured. The percent area injured also yielded comparable data, but was more cumbersome to record and evaluate. Costonis & Sinclair (2, 3), after investigating O_3 injury to eastern white pine, reported a severity rating system quite similar to that used throughout these studies.

Plant age (cotyledons).–The early age at which cotyledons were sensitive to O_3 was possibly related to their early role of supporting the young plant. The





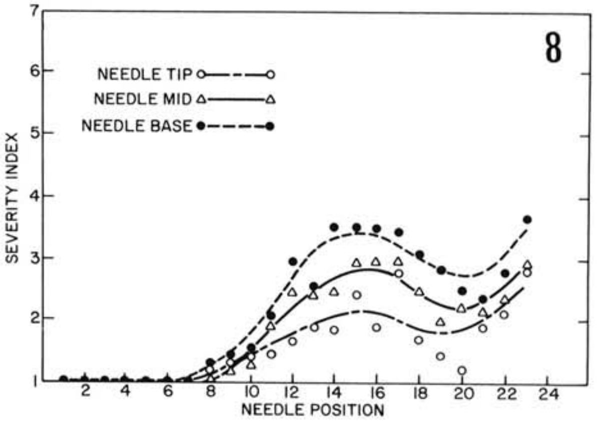


Fig. 6-8. 6) Influence of primary needle position on sensitivity of 4- and 16-week-old Virginia pine seedlings exposed to 25 pphm O_3 for 4 hr (position 1 represents the youngest needle, located at the plant apex; positions 20 and 23 represent the oldest needles located at the plant base of the 4- and 16-week-old plants, respectively). **7)** Influence of primary needle position and needle portion on 4-week-old Virginia pine seedlings exposed to 25 pphm O_3 for 4 hr (position 1 represents the youngest needle, located at the plant apex; position 20 represents the oldest needle located at the plant base). **8)** Influence of primary needle position and needle portion on 16-week-old Virginia pine seedlings exposed to 25 pphm O_3 for 4 hr (position 1 represents the youngest needle, located at the plant apex; position 23 represents the oldest needle located at the plant base).

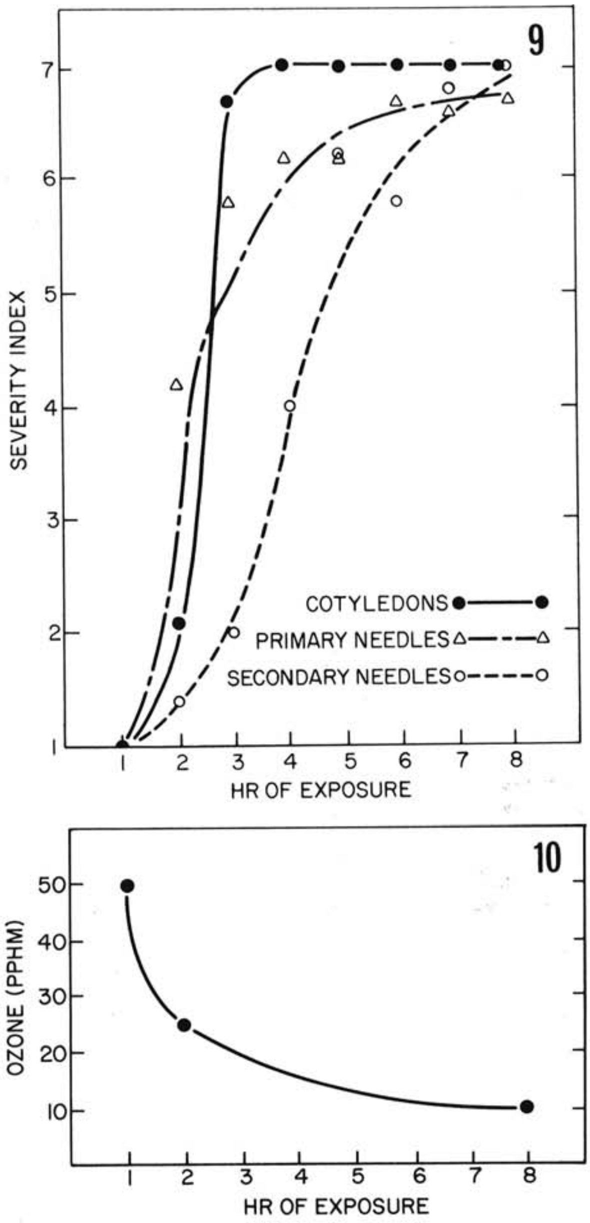


Fig. 9-10. 9) Dosage response of Virginia pine cotyledons, primary needles, and secondary needles exposed to 25 pphm O_3. **10)** Threshold doses of O_3 needed to cause visible symptoms on secondary needles of 3-year-old Virginia pine seedlings.

cotyledons possess stomata, are photosynthetic (16), and support the plant until the juvenile needles become photosynthetically functional. Thus, as soon as gas exchange begins, uptake of O_3 and subsequent injury is possible. Also, Dugger et al. (7) showed that the sensitivity of pinto bean leaves to O_3 was correlated with sugar carbohydrate level within the leaf. Injury only occurred when the sugar carbohydrate level was between 1 and 4 mg/g fresh wt of tissue. A similar phenomenon may also be operating within the Virginia pine cotyledons. As these tissues become older, the carbohydrates may be utilized until a low level is reached, after which the tissues become resistant to O_3.

Primary needles.—The small amount of injury on the youngest primary needles may have been influenced by the small number of functional stomata present. As the primary needles begin supporting the plant, gas exchange increases, and the plant becomes more sensitive. Changes in carbohydrate levels may also be involved.

Due to their indeterminant growth in the greenhouse, young plants with primary needles continuously develop new needles. In recent studies in this laboratory, these needles were sensitive to O_3 up to at least 6 months of age. Therefore, in the southern part of the USA, where a longer growing season occurs, and also in greenhouse-grown conifers, the primary needles may remain sensitive several months longer than those grown outdoors in northern USA. During the fall and winter in northern USA, the primary needles probably become more resistant as do dormant secondary needles.

Secondary needles.—Stomatal movements have been reported to follow both inherent and environmentally controlled yearly rhythms. Seidman & Riggan (18) illustrated a seasonal pattern for plants grown in both a greenhouse and a growth chamber. In both cases stomatal opening was maximum in the spring, decreased slightly through the summer, and reached a low point in December, after which the amount of opening increased again as spring approached. The decrease in stomatal opening from spring to fall closely approximated the seasonal pattern of sensitivity of Virginia pine to O_3, and would reflect a decrease in respiration and the ability to take up O_3.

Krueger (11) found that the concentration of total carbohydrates in the current secondary needles of Douglas fir increased gradually from bud-break to mid-June, the latest date used. If this also occurs in Virginia pine, this increase would correlate with decreasing sensitivity of Virginia pine secondary needles to O_3.

Pharis (17) reported that with increasing maturation during 6 months of foliar growth, there was a definite decline of foliar moisture content of adequately watered sugar and ponderosa pines. Lowest levels occurred in the fall with the onset of cool weather. Also, Kozlowski & Clausen (10) reported that the moisture content of needles of eastern white pine, red pine, and eastern hemlock declined progressively from May throughout the growing season. The moisture content itself may be related to O_3 sensitivity. However, these investigators (10, 17) also realized that the decreasing percentage of foliar moisture was possibly related to increased carbohydrate translocation into the new needles, whereas the actual amount of moisture remained fairly constant. Thus, these studies indicate that the decreasing sensitivity of secondary conifer needles to O_3 may also be related to the needle carbohydrate content.

The reduction in foliar moisture percentage or the increase in carbohydrate levels may be related to increased cell wall thickness within the needles (17). The concomitant loss of cell wall elasticity and permeability may affect the uptake and translocation of O_3 and/or secondary toxic products within the needles. The internal concentrations of cell carbohydrates and photosynthates may also cause internal moisture stress which subsequently effect partial stomatal closure in conifers (9). This would also affect O_3 uptake by conifer needles.

As previously reported (6), secondary needles of Virginia pine seedlings were found to be sensitive to O_3 over a 12-week period, beginning after 4 weeks of growth. However, in the current study the seedlings were sensitive during the age period of 1-18 weeks. In the previous study seedlings were not preconditioned for 2 days at 24 C, 80% relative humidity, as they were in the current study, but were brought in directly from outside beds and exposed. Apparently the 2 days of preconditioning made the plants sensitive at an earlier age, and also extended the length of time that they were sensitive.

Tissue and needle age.—The pattern of sensitivity of the primary and secondary needles closely follows the pattern of development of stomata. The tip of the needle is the first area to develop functional stomata, and it is also the first area to be injured. As the basal part of the needle matures, stomata became functional and this area becomes sensitive. By this time the tip is relatively resistant. Thus, the "recently mature" tissues of individual needles appear to be most sensitive. These findings are similar to those of Menser et al. (13), who found young tobacco leaves to be most severely injured at the tips, whereas mature leaves showed more widespread injury. Stomatal functioning was suggested as a major factor controlling the injury pattern. Also, carbohydrate levels may be involved. The reason for the constant high sensitivity of the basal portion of the cotyledon is unknown.

Cotyledons, juvenile needles, and secondary needles were all injured by doses of O_3 as low as 25 pphm for 2 hr, an amount that commonly occurs in many of our polluted cities. Conifer seedlings in certain areas may therefore be under a constant stress or selection pressure due to O_3 from time of seedling emergence. This could result in an insidious impact on plantings and natural stands in high oxidant areas.

Dosage response.—The dosage-response curves for cotyledons, primary needles, and secondary needles were similar. This suggests that young plants rather than older plants with secondary needles could be used in air pollution studies. The younger plants can be grown from seed at any time of the year, whereas it is often difficult to manipulate or break dormancy in older plants during the dormant season. Also, young plants in the cotyledon or juvenile needle stage require less space than older plants.

It is apparent from Fig. 10 that plants exposed to high concentrations of O_3 for a short time are injured more severely than plants exposed to a lower concentration for a longer time, both having equal "dose values" (dose = concentration $\times$ time). Thus, caution should be exercised when comparing amounts of injury based on "dose values".

The dosage-response curves indicate that the segment of the population of Virginia pine that we examined is very sensitive. However, it was not damaged by concentrations as low as those that cause injury to selected clones of eastern white pine (1, 3), tobacco (8, 13), and white ash (19). It is more susceptible, on a population basis, than many species of conifers and hardwoods (20).

LITERATURE CITED

1. BERRY, C. R., & L. A. RIPPERTON. 1963. Ozone, a possible cause of white pine emergence tipburn. Phytopathology 53:552-557.
2. COSTONIS, A. C., & W. A. SINCLAIR. 1969. Ozone injury to Pinus strobus. J. Air Pollution Control Assoc. 19:867-872.
3. COSTONIS, A. C., & W. A. SINCLAIR. 1969. Relationship of atmospheric ozone to needle blight of eastern white pine. Phytopathology 59:1566-1574.
4. DAVIS, D. D. 1970. The influence of age on the susceptibility of Virginia pine to ozone. Phytopathology 60:573 (Abstr.).
5. DAVIS, D. D., & F. A. WOOD. 1969. Effects of ozone on Virginia pine (Pinus virginiana). Phytopathology 59:1023 (Abstr.).
6. DAVIS, D. D., & F. A. WOOD. 1972. The relative susceptibility of eighteen coniferous species to ozone. Phytopathology 62:14-19.
7. DUGGER, W. M., W. C. TAYLOR, E. CARDIFF, & C. R. THOMPSON. 1962. Relationship between carbohydrate content and susceptibility of pinto bean plants to ozone damage. Amer. Soc. Hort. Sci. Proc. 81:304-315.
8. HEGGESTAD, H. E. 1966. Ozone as a tobacco toxicant. J. Air Pollution Control Assoc. 16:691-694.
9. HODGES, J. D. 1967. Patterns of photosynthesis under natural environmental conditions. Ecology 48:234-242.
10. KOZLOWSKI, T. T., & J. J. CLAUSEN. 1965. Changes in moisture contents and dry weights of buds and leaves of forest trees. Bot. Gaz. 126:20-26.
11. KRUEGER, K. W. 1967. Nitrogen, phosphorus, and carbohydrate in expanding and year old Douglas fir shoots. Forest. Sci. 13:352-356.
12. LINZON, S. N. 1967. Ozone damage and semimature-tissue needle blight of eastern white pine. Can. J. Bot. 45:2047-2062.
13. MENSER, H. A., H. E. HEGGESTAD, & O. E. STREET. 1963. Response of plants to air pollutants. II. Effects of ozone concentration and leaf maturity on injury to Nicotiana tabacum. Phytopathology 53:1304-1308.
14. MENSER, H. A., H. E. HEGGESTAD, O. E. STREET, & R. N. JEFFREY. 1963. Response of plants to air pollutants. I. Effects of ozone on tobacco plants preconditioned by light and temperature. Plant Physiol. 38:605-609.
15. MILLER, P. R., J. R. PARMETER, O. C. TAYLOR, & E. A. CARDIFF. 1963. Ozone injury to the foliage of Pinus ponderosa. Phytopathology 53:1072-1076.
16. MIROV, N. T. 1967. The genus Pinus. Ronald Press, N.Y. 602 p.
17. PHARIS, R. P. 1967. Seasonal fluctuation in the foliage-moisture content of well-watered conifers. Bot. Gaz. 128:179-185.

18. SEIDMAN, G., & W. B. RIGGAN. 1968. Stomatal movements: a yearly rhythm. Nature 217:684-685.

19. WILHOUR, R. G. 1971. Influence of ozone on white ash (Fraxinus americana). Ph.D. Thesis, The Pennsylvania State Univ. University Park, Pa. 91 p.

20. WOOD, F. A., & D. D. DAVIS. 1969. Sensitivity to ozone determined for trees. The Pennsylvania State Univ. Sci. Agri. 17:4-5.

21. WOOD, F. A., D. B. DRUMMOND, R. G. WILHOUR, & D. D. DAVIS. 1968. An exposure chamber for studying the effects of air pollution on plants. Phytopathology 58:504 (Abstr.).