Effects of Ozone and Sulfur Dioxide on the Host-Pathogen Relationship of Scotch Pine and *Scirrhia acicola*

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ABSTRACT

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Scotch pine seedlings from five seed sources were inoculated with *Scirrhia acicola* 5 days before or 30 min after fumigation for 6 hr with 533 $\mu g/m^3$ (0.20 ppm) of sulfur dioxide, 399 $\mu g/m^3$ (0.20 ppm) of ozone, or the same levels of ozone and sulfur dioxide combined. After 8 wk, seedlings inoculated 5 days before fumigation had more lesions incited by *S. acicola* than those inoculated 30 min after fumigation. Ozone caused more needle injury than sulfur dioxide did. The amount of injury caused by the combined gases was a greater-than-additive effect. The degree of infection varied significantly among the seed sources.

Pollutants in ambient air exist as a mixture of potentially phytotoxic compounds (13,22). Direct effects of ozone and sulfur dioxide singly (1,2,7,10,16,17,27,29,32) or combined (13,20,28–30,32) on trees and lesser vegetation have been studied extensively, and several fumigation studies have been done on the effects of the gases on pine foliage (5,6,9). Both decreased (31) and increased (3,13) fungal parasitism of plants exposed to sulfur dioxide have been reported. Similarly, ozone has been reported to increase (23,24,26), decrease (12,13,15,21), or have no effect on (25) the incidence of fungal infection.

Ham studied the effects of sulfur dioxide on brown spot needle blight of loblolly pine (11). He found that *Scirrhia acicola* (Dearn.) Siggers grew normally and produced viable conidia on agar after exposure to 1 ppm of sulfur dioxide for 4 hr. Scotch pine is not seriously jeopardized by this pathogen, but because of its ease of production, growth rate, moderate susceptibility to *S. acicola*, and sensitivity to air pollutants, it was used to test the role of air pollutants in pathogenesis of brown spot needle blight and to determine how *S. acicola* influences symptom expression to ozone and sulfur dioxide.

MATERIALS AND METHODS

Scotch pine seeds from five sources were planted in flats in sterile quartz sand and covered with sterile peat and vermiculite (50% each, v/v). Seed sources and planting dates are described in Table 1. Seeds were provided by Dr. James H. Brown, Department of Forestry, Ohio Agricultural Research and Development Center, Wooster, and conidial isolates of *S. acicola* were contributed by A.G. Kais, U.S. Forest Service, Gulfport, MS.

Seedlings were grown in single-cell containers by the method of Weidensaul and McClenahen (33). Seedlings were grown in the greenhouse and subirrigated every second day with Ingestadt's solution (18). A photoperiod was maintained for 18 hr until plants were 6 mo old, then increased to 24 hr, to insure young, pollutant- and fungus-susceptible tissue before inoculation and fumigation. Natural sunlight was supplemented with fluorescent (Vitalite) lighting (Durotest Corp., North Bergen, NJ) at an intensity of 8,600 lux. To increase humidity, seedling trays (30.5 × 61 cm) were covered with clear polyethylene film (0.1 mm thick) 7 days before and for 10 days after fumigation. Kais observed increased infection with high humidity (19).

Conidial isolates of *S. acicola* were maintained on malt agar in petri dishes. Spore suspensions were prepared by rinsing the cultures with 10 ml of sterile distilled water. Spores were counted with a hemacytometer and adjusted to a concentration of 2.5×10^5 conidia per milliliter. Approximately 3 ml of the spore suspension was atomized on 200 7- to 8-mo-old seedlings with fully expanded but not hardened needles. Inoculation was done either 5 days before or 30 min after fumigation.

Seedlings, except those used for controls, were fumigated at the same time for 6 hr with 399 $\mu g/m^3$ (0.20 ppm) of ozone, 533 $\mu g/m^3$ (0.20 ppm) of sulfur dioxide, or 399 $\mu g/m^3$ of ozone plus 533 $\mu g/m^3$ of sulfur dioxide. Five seedlings were used for each of the 60 possible treatment combinations. Ozone was produced by an Orec

Model 03V5-0 ozone generator (Ozone Research and Equipment Corp., Phoenix, AR). Sulfur dioxide was metered from a gas cylinder containing an analyzed mixture of 1.5% SO_2, 19.8% O_2, and 78.7% N_2. Pollutant levels were monitored serially from six chambers through constantly conditioned Teflon sample lines and electrically operated solenoid valves that directed samples to a UV ozone meter (Model 1003-AH, Dasibi Environmental Corp., Glendale, CA) and a conductometric-type sulfur dioxide analyzer (Calibrated Instruments, Inc., New York, NY). Temperature (25.5 C) and relative humidity (73%) were constant throughout fumigation. Wet- and dry-bulb temperatures were monitored with copper-constantan thermocouples in each Teflon chamber (101.6-cm cube). Relative humidity was calculated from wet-bulb depression measurements. Twelve 60-W Vitalite fluorescent tubes with a sunlight spectrum were used above each chamber to provide 7,700 lux at plant height. Ambient air contaminants were removed with brominated charcoal filters, and "clean air" was exchanged five times per minute. Ozone and sulfur dioxide were introduced into the clean-air line with electrically operated micrometer needle valves (Hoke Inc., Cresskill, NJ). Because no environmental differences except pollutant levels existed among chambers, duplication of a given gas treatment in more than one chamber was unnecessary.

Seedlings were fumigated when 28–34 wk old and were examined for symptoms of fungal infection 8 wk after fumigation. Although there were total age differences (ie, different planting dates) among provenances, the age of sensitive foliage was the same for all. The number of fungal lesions was totaled, and the extent of pollutant-induced needle injury on the top 12.7 cm of each seedling leader was measured.

Data were subjected to an analysis of variance, and treatment means were compared by Duncan's new multiple range test. Square root and arc sine transformations of numbers of lesions and percentage needle injury by pollutants, respectively, were used.

RESULTS

Typical brown spot lesions developed during the 8-wk incubation period. They appeared as yellow spots, approximately 1 mm in diameter, that later changed to light brown. Some lesions coalesced, forming irregular oblong areas. Individual lesions also developed into yellow bands (bar spots). The number of lesions was affected significantly by the time of inoculation relative to fumigation. Genetic differences among seed sources apparently accounted for the observed variability in *S. acicola* infection. There was little difference in numbers of infections among nonfumigated inoculated plants, but seedlings grown from Pennsylvania or England seeds were more severely infected than those grown from Greece or Norway seeds (Table 1). For nonfumigated seedlings, the severity of brown spot was the same within any seed source at each inoculation time.

Except for the controls, seedlings inoculated 5 days before fumigation were more heavily infected than those inoculated 30 min after (Table 2). Nonfumigated seedlings from Norway and Greece seeds showed more resistance to infection than the others regardless of inoculation time, but there was no seed source × time-of-inoculation interaction. The amount of brown spot disease did not differ significantly among seedlings from Czechoslovakia, England, and Pennsylvania seeds (Table 1). There were significantly more brown spot lesions on seedlings fumigated with sulfur dioxide alone or combined with ozone than on controls when inoculation was done 5 days before fumigation. When inoculation was done 30 min after fumigation, seedlings exposed to sulfur dioxide alone had more lesions than those exposed to ozone alone or combined with sulfur dioxide, but no significant differences were noted between treated seedlings and controls (Table 2).

Needle injury caused by the gases was significant, and symptoms of injury by ozone or sulfur dioxide were similar and appeared primarily as tip burn. Ozone caused more injury than sulfur dioxide (Table 3). A significant synergistic effect resulting from combined ozone and sulfur dioxide was consistent among all seed sources, whether seedlings were inoculated or not. *S. acicola* did not alter the response of plants to the gases, and there was no significant differential sensitivity among seed sources to the gases alone or combined.

DISCUSSION

S. acicola conidia require 14–52 hr for germ tube development, and germ tubes may meander for some time on the needle surface before entering the host via stomata. Sulfur dioxide at 0.20 ppm would not be expected to stimulate stomatal closure, particularly at a relative humidity above 50–60%. Ozone, on the other hand, can induce stomatal closure at low concentrations and rather high humidity. *S. acicola* hyphae probably were well established in host

TABLE 1. Scotch pine seed sources and susceptibility of nonfumigated seedlings to infection by *Scirrhia acicola*

Seed origin	Latitude (°)	Longitude (°)	Elevation (m)	Planting date	Lesions per tree (no.)
Czechosolvakia	48.9 N	16.2 E	396–457	4/11/77	2.00 ab
Greece	41.2 N	23.5 E	1,372	4/11/77	1.14 b
England (East Anglia)	51.2 N	0.8 E	213	3/23/77	2.72 a
Norway	59.3 N	8.8 E	100	2/23/77	1.34 b
Pennsylvania (Nye Branch)	40.6 N	79.1 W	366	2/23/77	2.80 a

[a]Data are averages of 10 trees. Values followed by the same letter are not different at $P = 0.01$ according to Duncan's new multiple range test.

TABLE 2. Effects of inoculation time relative to fumigation on severity of infection of Scotch pine by *Scirrhia acicola*

Fumigation treatment[a]	Total lesions, all seed sources[b]	
	Inoculation 5 days before fumigation	Inoculation 30 min after fumigation
Sulfur dioxide	193 e	152 cd
Ozone	163 de	100 ab
Sulfur dioxide + ozone	195 e	99 a
Control	147 cd	125 abc

[a]SO_2 at 533 $\mu g/m^3$ (0.20 ppm); O_3 at 399 $\mu g/m^3$ (0.20 ppm); all fumigations, 6 hr.

[b]Data are averages of 25 trees. Values followed by the same letter are not different at $P = 0.05$ and are compared within and between columns according to Duncan's new multiple range test.

TABLE 3. Extent of needle injury caused by ozone and/or sulfur dioxide on noninoculated Scotch pine seedlings

Fumigation treatment[a]	Needle injury[b] (% length)
Sulfur dioxide	4.40 a
Ozone	9.82 b
Sulfur dioxide + ozone	20.61 c
Control	0.85 a

[a]SO_2 at 533 $\mu g/m^3$ (0.20 ppm); O_3 at 399 $\mu g/m^3$ (0.20 ppm); all fumigations, 6 hr.

[b]Data are averages of 25 trees combined from five seed sources. Values followed by the same letter are not different at $P = 0.01$ according to Duncan's new multiple range test.

tissue inoculated 5 days before fumigation and were not seriously affected by the gases. Once established in host tissue, fungal hyphae are resistant to gaseous toxicants (13). This could explain why much more infection was observed in seedlings inoculated 5 days before fumigation than in those inoculated 30 min after. The experimental conditions in this study maximized the potential for fungal infection.

There was a greater likelihood that conditions were more favorable to *S. acicola* infection after sulfur dioxide treatment than after treatment with ozone alone or combined with sulfur dioxide. The greater number of lesions found on seedlings fumigated with sulfur dioxide could be related to stomata being open after fumigation, since, at low levels, sulfur dioxide has less influence on stomatal behavior than ozone has.

How the time of inoculation relative to pollutant exposure affects the extent of parasitism of pine species by fungi is not known. Susceptibility of eastern white pine to fungal parasites and to air pollutants is genetically controlled (1,4,8). Since there were no differences in infection severity within a given seed source at any inoculation time for nonfumigated tissue, the primary reason for infection differences is assumed to be related to gas treatments and not to changes in host resistance. One study reported that *S. acicola* grew normally and produced viable conidia on agar after exposure to 2,265 $\mu g/m^3$ (1 ppm) of sulfur dioxide for 4 hr (11), which supports the suggestion that infection is influenced by host responses to sulfur dioxide. Other studies have shown that ozone can increase or decrease fungus sporulation and growth (14,15).

LITERATURE CITED

1. BERRY, C. R. 1961. White pine emergence tipburn, a physiogenic disturbance. U.S. For. Serv. Southeast For. Exp. Stn. Pap. 61. 8 pp.
2. BERRY, C. R., and L. A. RIPPERTON. 1963. Ozone, a possible cause of white pine emergence tipburn. Phytopathology 53:552-557.
3. CHIBA, O., and K. TANAKA. 1968. The effect of sulfur dioxide on the development of pine needle blight caused by *Rhizosphaera kalkoffii* Bubok (1). J. Jpn. For. Soc. 50:135-139.
4. COSTONIS, A. C. 1968. Relationship of ozone, *Lophodermium pinastri* and *Pullularia pullulans* to needle blight of eastern white pine. Ph.D. thesis, Cornell University, Ithaca, NY. 176 pp.
5. COSTONIS, A. C. 1970. Acute foliar injury of eastern white pine induced by sulfur dioxide and ozone. Phytopathology 60:994-999.
6. COSTONIS, A. C. 1973. Injury to eastern white pine by sulfur dioxide and ozone alone and in mixtures. Eur. J. For. Pathol. 3:50-55.
7. DAVIS, D. D., and R. G. WILHOUR. 1976. Susceptibility of woody plants to sulfur dioxide and photochemical oxidants. Ecological Research Series, USEPA, Corvallis, OR. EPA-600/3-76-102. 72 pp.
8. DOCHINGER, L. S. 1968. The impact of air pollution on eastern white pine: The chlorotic dwarf disease. J. Air Pollut. Control Assoc. 18:814-816.
9. DOCHINGER, L. S., F. W. BENDER, F. L. FOX, and W. W. HECK. 1970. Chlorotic dwarf of eastern white pine caused by an ozone and sulfur dioxide interaction. Nature (London) 225:476.
10. FARRAR, J. F., J. RELTON, and A. J. RUTTER. 1977. Sulfur dioxide and growth of *Pinus sylvestris*. J. Appl. Ecol. 114:861-876.
11. HAM, D. L. 1971. The biological interactions of sulfur dioxide and *Scirrhia acicola* on loblolly pine. Ph.D. thesis, Duke University, Durham, NC.
12. HEAGLE, A. S. 1970. Effect of low-level ozone fumigations on crown rust of oats. Phytopathology 60:252-254.
13. HEAGLE, A. S. 1973. Interactions between air pollutants and plant parasites. Annu. Rev. Phytopathol. 11:365-388.
14. HEAGLE, A. S., and L. W. KEY. 1973. Effect of ozone on the wheat stem rust fungus. Phytopathology 63:397-400.
15. HEAGLE, A. S., and A. STRICKLAND. 1972. Reaction of *Erysiphe graminis* f. sp. *hordei* to low levels of ozone. Phytopathology 62:1144-1148.
16. HECK, W. W. 1968. Factors influencing expression of oxidant damage to plants. Annu. Rev. Phytopathol. 6:165-188.
17. HECK, W. W., J. B. MUDD, and P. R. MILLER. 1977. Plants and microorganisms. Pages 437-585 in: Ozone and Other Photochemical Oxidants. National Academy of Sciences, Washington, DC. 719 pp.
18. INGESTADT, T. 1966. Experiments on constant internal nutrient concentrations. In: Fifth International Colloquium on Plant Analysis and Fertilizer Problems. Royal College of Forestry, Stockholm, Sweden.
19. KAIS, A. G. 1975. Environmental factors affecting brown-spot infection on longleaf pine. Phytopathology 65:1389-1392.
20. KARPEN, D. N. 1970. Ozone and sulfur dioxide synergism: Foliar injury to a ponderosa pine geographic race plantation in the Puget Sound Region. Plant Dis. Rep. 54:945-948.
21. KRAUSE, C. R., and T. C. WEIDENSAUL. 1978. Effects of ozone on the sporulation, germination, and pathogencity of *Botrytis cinerea*. Phytopathology 68:195-198.
22. MANNING, W. J. 1975. Interactions between air pollutants and fungal, bacterial, and viral plant pathogens. Environ. Pollut. 9:87-89.
23. MANNING, W. J., W. A. FEDER, and I. PERKINS. 1970. Ozone injury increases infection of geranium leaves by *Botrytis cinerea*. Phytopathology 60:669-670.
24. MANNING, W. J., W. A. FEDER, and I. PERKINS. 1970. Ozone and infection of geranium flowers by *Botrytis cinerea*. (Abstr.) Phytopathology 60:1302.
25. MANNING, W. J., W. A. FEDER, and I. PERKINS. 1972. Effects of *Botrytis* and ozone on bracts and flowers of poinsettia cultivars. Plant Dis. Rep. 56:814-816.
26. MANNING, W. J., W. A. FEDER, I. PERKINS, and M. GLICKMAN. 1969. Ozone injury and infection of potato leaves by *Botrytis cinerea*. Plant Dis. Rep. 53:691-693.
27. McCUNE, D. C., and T. C. WEIDENSAUL. 1978. Effects of atmospheric sulfur oxides and related compounds on vegetation. Pages 80-129 in: Sulfur Oxides. National Academy of Sciences, Washington, DC. 209 pp.
28. MENSER, H. A., and H. E. HEGGESTAD. 1966. Ozone and sulfur dioxide synergism; injury to tobacco plants. Science 153:424-425.
29. NIELSEN, D. G., L. E. TERRELL, and T. C. WEIDENSAUL. 1977. Phytotoxicity of ozone and sulfur dioxide to laboratory fumigated Scotch pine. Plant Dis. Rep. 61:699-703.
30. REINERT, R. A. 1975. Pollutant interactions and their effects on plants. Environ. Pollut. 9:115-116.
31. SAUNDERS, P. J. W. 1966. The toxicity of sulfur dioxide to *Diplocarpon rosae* Wolf causing blackspot of roses. Ann. Appl. Biol. 58:103-114.
32. TINGEY, D. T., and R. A. REINERT. 1975. The effect of ozone and sulfur dioxide singly and in combination on plant growth. Environ. Pollut. 9:117-126.
33. WEIDENSAUL, T. C., and J. R. McCLENAHEN. 1977. Influences of rooting media on containerized tree growth. Ohio Rep. 60:83-86.