Reaction of Erysiphe graminis f. sp. hordei to Low Levels of Ozone

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Cooperative investigations of the Environmental Protection Agency, the USDA, and the North Carolina State University. Journal Series Paper No. 3572 of the North Carolina State University Agricultural Experiment Station, Raleigh.

Supported in part by NSF Grant No. 19650.

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The authors thank J. G. Moseman for the culture of powdery mildew and barley seeds.

Accepted for publication 13 April 1972.

ABSTRACT

Barley, *Hordeum vulgare*, and powdery mildew, *Erysiphe graminis* f. sp. *hordei*, were exposed to ozone at concentrations that often occur in polluted ambient air. The effects of ozone on germination of conidia, on plant infection by conidia, on growth of mycelia, and on sporulation were determined. When sporulating colonies were exposed to ozone, the percentage infection by exposed conidia was significantly reduced. Ozone exposures during spore incubation also significantly reduced infection. Germination of conidia was not significantly reduced by the same exposures which inhibited infection. Colony and spore mass length was significantly increased by ozone exposures which caused chlorosis of barley leaves.

Phytopathology 62:1144-1148.

Additional key words: air pollution.

Some injurious effects of ozone (O_3), the major plant-pathogenic component of photochemical oxidant pollution, on higher plants have been documented in several comprehensive reviews (3, 7, 14, 17). The effect of O_3 on in vitro cultures of several plant-pathogenic fungi have also been reported (9, 10, 11, 18, 19, 20). Until recently, very little has been published on the effects of O_3 on the interactions between plants and parasitic fungi. Plant injury caused by low O_3 concentrations increased the amount of infection of potato (13) and geranium (11) leaves by *Botrytis cinerea*. Infection of geranium petals by *B. cinerea* was not affected by 4-hr O_3 exposures that began after inoculation, but the disease development was often inhibited (12). White pine needles injured by O_3 were more susceptible to infection and invasion by *Pullularia pullulans* than were normal needles (1). Ozone injury before inoculation retarded infection of white pine by *Lophodermium pinastri* but O_3 injury after infection increased invasion (1).

The only report of O_3 effects on an obligate fungus parasite showed that low doses inhibited the growth of uredia of *Puccinia coronata* var. *avenae*,

but did not affect germination or infection by urediospores (6). There are no reports on the effects of O_3 on the host-parasite interactions of the powdery mildew fungi. Because the hyphae of the powdery mildew fungi grow on the host surface, in direct contact with ambient air, they are a logical subject for studies to determine the effects of air pollutants on parasitism. Ozone in a polluted atmosphere could influence any stage of the life cycle of powdery mildew, and thereby alter its prevalence and pathogenicity. To determine whether this might be true, we studied the effects of O_3 on all stages of the asexual growth cycle of *Erysiphe graminis* (DC.) Merát *hordei* Em. Marchal on barley, *Hordeum vulgare* L. Concentrations of O_3 representing O_3 levels experienced in the United States were used.

MATERIALS AND METHODS.—Investigations were conducted in the greenhouse and growth chamber facilities of the North Carolina State University Phytotron (5). Barley was grown in the greenhouse at 26-30 C in a mixture of gravel and Jiffy Mix in 9-cm styrofoam pots. Plants were watered on the five weekdays with quarter-strength Hoagland's solution with nitrogen increased to half-strength, and on weekends with deionized water. One to 6 hr prior to the start of each experiment, plants were moved to a walk-in growth chamber at 20 C, 65% relative humidity (RH) and 4,200-4,500 ft-c during a 12-hr (6AM-6PM) photoperiod using incandescent and fluorescent light.

Manchuria (C.I. 2330) and two nearly isogenic selections from the cross Psaknon (C.I. 6350) X 14^4 Manchuria were used. Conidia of *E. graminis hordei* (isolate CR 3 of race 3) from 6- to 8-day-old infections on Manchuria were used to inoculate the upper surface of the first foliar leaves of 7-day-old plants at 3 PM. Inoculations were made by the rolling method (16), or by the brushing of plants with leaves bearing sporulating colonies.

Plants were exposed to O_3 in a series of four identical 90- X 90- X 120-cm exposure chambers (8) installed in the growth chamber. Exposure was at 25 C, RH 65%, and 4,200-4,500 ft-c. When exposures occurred at night, the temperature was 20 C. Each exposure chamber was equipped with an electric-arc ozonizer that produced O_3 from air filtered through activated charcoal. Ozone concentrations were monitored with Mast O_3 meters, and all values were corrected to a 2% neutral KI standard. Plants were removed from the exposure chambers to the center of the growth chamber between exposures and after the last exposures.

For microscopic examination, infected leaves were fixed and stained in 2% lactophenol-cotton-blue (21), or the fungus was stained by carefully placing 2% lactophenol-cotton-blue on the leaf surface. In all germination and infection experiments, 10 plants were inoculated for each treatment, and 100 spores/

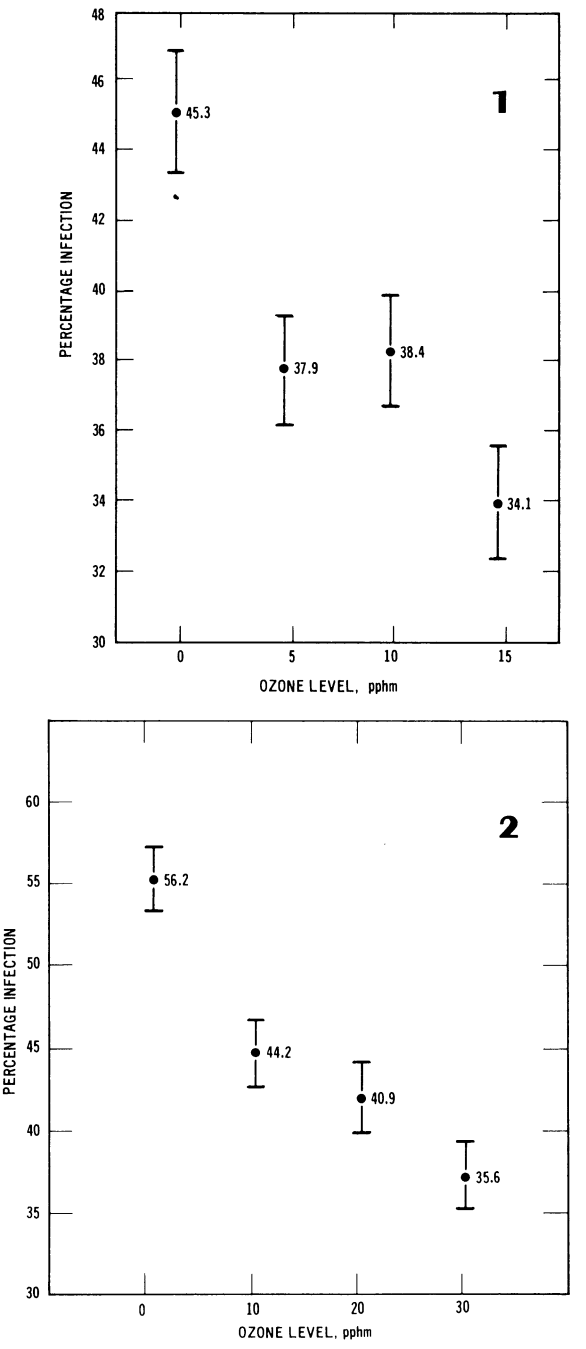

Fig. 1-2. 1) Effect of ozone exposures on the percentage of germinated *Erysiphe graminis* conidia that caused infection of barley leaves. Sporulating colonies were exposed to 0, 5, 10, or 15 pphm ozone for 6 hr/day on the 7th, 8th, and 9th days after inoculation. Each value is the mean of a total of 6,000 germinated spores on a total of 60 leaves in six inoculations. The 95% confidence interval about each mean is indicated by the horizontal bars. **2)** Effect of ozone exposures on the percentage of germinated *Erysiphe graminis* conidia that caused infection of barley leaves. Conidia were exposed for 24 hr during incubation to 0, 10, 20, or 30 pphm ozone. Each value is the mean of a total of 3,000 germinated spores on a total of 30 leaves in three inoculations. The 95% confidence interval about each mean is indicated by the horizontal bars.

leaf were observed in taking measurements. In all colony growth experiments, three colonies on each of four leaves/treatment were used in taking measurements.

Analyses of variance were performed on the results of each experiment, and treatment separations were identified according to Tukey's method (4).

RESULTS.—*Conidia germination and infection.*—In experiment 1, plants were exposed to 0, 5, 10, or 15 pphm O_3 for 6 hr (9AM-3PM) on the 7th, 8th, and 9th days after inoculation. Prior to each exposure, plants with sporulating colonies were shaken for discard of mature conidia. After each exposure, the new conidia which matured during exposure were used to inoculate unexposed plants. Percentage germination and the percentage of germinated conidia which produced normal infections (infections containing functional secondary hyphae) were determined after 42 hr of incubation in the absence of O_3. This experiment was repeated once.

Percentage germination was not significantly affected by ozone, but the conidia from colonies exposed to 5, 10, or 15 pphm O_3 consistently produced significantly fewer infections than those not exposed (Fig. 1). In this and in all subsequent experiments, host plant genotype did not affect the reaction of *E. graminis* to O_3.

Experiment 2 was performed to determine whether percentage infection would be further reduced if O_3 exposures occurred during early colony growth as well as during sporulation. Infected plants were exposed for 6 hr (9AM-3PM) to 0, 5, 10, or 15 pphm O_3 on 8 consecutive days after inoculation. Prior to the 8th exposure, plants were shaken to discard mature conidia. After the 8th exposure, the new conidia which matured during exposure were used to inoculate unexposed plants. The percentage of germinated conidia that produced normal infections was determined after 42 hr. This experiment was repeated once.

The increased number of O_3 exposures did not produce a further reduction in percentage infection.

TABLE 1. Effect of ozone on *Erysiphe graminis* colony length

Number of exposures[a]	Colony length (in microns) per ozone concentration (pphm)[b]			
	0	5	10	15
2	331 h	278 j	297 ij	302 i
4	1298 k	1358 i	1326 j	1405 h
6	2606 j	2747 i	2773 h	2773 h
8	4111 j	4130 j	4255 i	4395 h

[a]Plants and colonies were exposed to ozone for 6 hr/day at concentrations of 0, 5, 10, or 15 pphm ozone on the 8 days after inoculation. (One pphm O_3 = 19.6 $\mu g/m^3$ at 760 mm of Hg and 25 C).

[b]Means followed by the same letter in each row are not significantly different at the .05 level according to Tukey's test. Each figure is the mean of a total of 24 colonies on eight leaves in experiment 2.

The results were similar to those in experiment 1 (Fig. 1).

Experiment 3 was designed to determine whether O_3 exposure during the spore incubation period would affect germination and infection. Inoculated plants were immediately exposed to 0, 10, 20, or 30 pphm O_3 for 24 hr. Immediately after exposure, half the plants were stained to determine percentage germination. The remaining plants were used to determine percentage infection as previously described. This experiment was conducted 3 times.

The relatively high doses of O_3 failed to significantly affect germination, but plant tissues were often severely injured at both 20 and 30 pphm. The number of normal infections, however, was significantly reduced by all O_3 concentrations (Fig. 2), and the results were similar to previous experiments.

In all experiments, germination of exposed conidia appeared to be normal. Prior to penetration, however, the germ tube protoplasm of conidia that failed to infect appeared to be released at the tip of the germ tubes, indicating germ tube rupture.

Colony growth and sporulation.—The rate of colony growth, the number and size of cells, and amount of sporulation are indicators of colony vigor. To determine whether these indicators are affected by O_3, infected plants were exposed as described in experiment 2. Leaves were sampled, fixed, and stained immediately following the 2nd, 4th, 6th, and 8th exposures. To determine effects on cell division and elongation, the number and length of individual cells in the longest hypha in individual colonies was measured after the 2nd exposure. Colony lengths (the distance between the tips of the most extended hypha at two opposite ends of each colony) were measured after 2, 4, 6, and 8 exposures. To measure O_3 effects on sporulation, the number of conidiophores, with conidia and without conidia, was determined per colony after four exposures. The length of the colony which contained spore-bearing conidiophores was measured after eight exposures. This experiment was repeated once.

The number and length of cells in the longest hyphae were not significantly affected by two 6-hr exposures. Colonies were significantly smaller on exposed plants than on nonexposed plants after two exposures (Table 1). After 4, 6, and 8 exposures, however, colony lengths on plants exposed to 5, 10, or 15 pphm O_3 were usually significantly larger than on those not exposed (Table 1).

Ozone did not significantly affect the number of conidiophores or the number of conidiophores bearing conidia after four exposures (Table 2). The length of the spore-bearing area per colony after eight exposures, however, was significantly larger at 15 pphm than at the lower concentrations (Table 2). Leaves of plants exposed to 10 and 15 pphm O_3 were often partially chlorotic after six exposures, whereas those at 0 and 5 pphm remained relatively green.

DISCUSSION.—The powdery mildews are ectoparasitic; their hyphae are exposed to ambient air. Because they are ectoparasites, oxidant air pollution, toxic to higher plants, might also be expected to

TABLE 2. Effect of ozone on asexual reproduction of *Erysiphe graminis* after eight exposures

Sporulation measure	Effect per ozone concentration (pphm)[a]			
	0	5	10	15
Conidiophores (number)[b]	57 h	65 h	58 h	56 h
Conidiophores with conidia (number)[b]	39 h	44 h	40 h	34 h
Length of spore mass (in microns)[c]	3,191 i	3,168 i	3,259 i	3,435 h

[a]Means followed by the same letter are not significantly different at the 5% level according to Tukey's test. Each value is the average of a total of 24 colonies on eight leaves in experiment 2.

[b]Colonies were sampled after exposure to 0, 5, 10, or 15 pphm O_3 on the 4 days after inoculation.

[c]Colonies were sampled after exposure to 0, 5, 10, or 15 pphm O_3 on the 8 days after inoculation.

affect powdery mildews. On the other hand, the exposed nature of the powdery mildews requires tolerance to variations in meteorological conditions. Our studies show that once infection is established, the vegetative growth of *E. graminis hordei* is tolerant to O_3 and is even slightly stimulated by O_3 levels commonly found in many urban areas. This tolerance suggests either a lack of penetration of hyphae by O_3 or a physiological resistance of hyphal protoplasm to O_3. We feel that the O_3 fails to penetrate the hyphae, possibly owing to decomposition from reaction with the hyphal cell walls. If O_3 penetrates powdery mildew hyphae, injury should result because O_3 is one of the strongest oxidizing agents known. That O_3 injures plant cells is well documented.

Our results show that *E. graminis* is adversely affected during certain phases of high metabolic activity when cell walls may be more easily penetrated by O_3. The percentage of spores which successfully infected host cells was reduced when maturing or germinating spores were exposed to O_3. The cause of the increased sensitivity at these stages is probably due either to easier O_3 penetration through conidial papillae or immature cell walls, toxic effects of O_3 on metabolic activities unique to these stages, or a combination of these factors. Buxton et al. (2) and Moseman & Greeley (15) reported that short periods of ultraviolet irradiation inhibited germination and infection of *E. graminis*. In their studies as in ours, infectivity of the fungus was more easily altered than was germination, suggesting that a common mechanism may be involved.

Colony growth was not inhibited. Instead it was slightly stimulated, even when host leaves became partially chlorotic. The stimulation may have resulted indirectly from action of O_3 in causing injury to the host plant or directly from action on the mildew. It is possible that the lack of inhibition was due to the fact that haustoria invade only host epidermal cells which are visibly affected less by O_3 than are mesophyll cells.

These experiments have provided some clues to the influence of oxidant pollutants on the epidemiology of barley powdery mildew in the field. Ozone-induced growth stimulation might increase sporulation and increase disease incidence. On the other hand, O_3-induced inhibition of penetration could reduce the incidence of this disease. The effects of oxidant pollutants on germination, infection, and invasion by other groups of fungi in the field also remain unknown. It is possible that many diseases caused by fungi would be much more sensitive to pollutant-caused disruptions than the relatively resistant barley powdery mildew. Any inhibitory or stimulatory effect of air pollutants on fungi, whether parasitic or saprophytic species, could have far-reaching effects on the incidence and severity of plant diseases and on the orderly progression of organic decay cycles.

LITERATURE CITED

1. COSTONIS, A. C., & W. A. SINCLAIR. 1967. Effects of Lophodermium pinastri and Pullularia pullulans on healthy and ozone-injured needles of Pinus strobus. Phytopathology 57:807 (Abstr.).
2. BUXTON, E. W., F. T. LAST, & M. A. NOUR. 1957. Some effects of ultraviolet radiation on the pathogenicity of Botrytis fabae, Uromyces fabae, and Erysiphe graminis. J. Gen. Microbiol. 16:764-773.
3. DARLEY, E. F., & J. T. MIDDLETON. 1966. Problems of air pollution in plant pathology. Annu. Rev. Phytopathol. 4:103-118.
4. DIXON, W. J., & T. J. MASSEY. 1957. Introduction to statistical analysis, p. 440-442. McGraw Hill Book Co., Inc., New York, N.Y.
5. DOWNS, R. J. 1969. Phytotrons: Controlled environments for plant studies. Research/Development 20(11):30-33.
6. HEAGLE, A. S. 1970. Effect of low level ozone fumigations on crown rust of oats. Phytopathology 60:252-254.
7. HECK, W. W. 1968. Factors influencing expression of oxidant damage to plants. Annu. Rev. Phytopathol. 6:165-188.
8. HECK, W. W., J. A. DUNNING, & H. JOHNSON. 1968. Design of a simple plant exposure chamber. USDHEW, Public Health Service, Natl. Center for Air Pollution Control Publ. APTD-68-6.
9. HIBBEN, C. R., & G. STOTZKY. 1969. Effects of ozone on the germination of fungus spores. Can. J. Microbiol. 15:1187-1196.
10. KORMELINK, J. R. 1967. Effects of ozone on fungi. M.Sc. Thesis, University of Utah. 30 p.
11. MANNING, W. J., W. A. FEDER, & I. PERKINS. 1970. Ozone injury increases infection of geranium leaves by Botrytis cinerea. Phytopathology 60:669-670.
12. MANNING, W. J., W. A. FEDER, & I. PERKINS. 1970. Ozone and infection of geranium flowers by Botrytis cinerea. Phytopathology 60:1302 (Abstr.).
13. MANNING, W. J., W. A. FEDER, I. PERKINS, & M. GLICKMAN. 1969. Ozone injury and infection of potato leaves by Botrytis cinerea. Plant Dis. Reptr. 53:691-693.
14. MIDDLETON, J. T. 1961. Photochemical air pollution damage to plants. Annu. Rev. Plant Physiol. 12:431-448.
15. MOSEMAN, J. G., & L. W. GREELEY. 1966. Effect of ultraviolet light on Erysiphe graminis f. sp. hordei. Phytopathology 56:1357-1360.

16. NAIR, K., R. SADASIVAN, & A. H. ELLINGBOE. 1962. A method of controlled inoculations with conidiospores of Erysiphe graminis var. tritici. Phytopathology 52:714.
17. RICH, S. 1964. Ozone damage to plants. Annu. Rev. Phytopathol. 2:253-266.
18. RICH, S., & H. TOMLINSON. 1968. Effects of ozone on conidiophores and conidia of Alternaria solani. Phytopathology 58:444-446.
19. RICHARDS, M. D. 1949. Ozone as a stimulant for fungus sporulation. Phytopathology 39:20 (Abstr.).
20. TRESHOW, M., F. M. HARNER, H. E. PRICE, & J. R. KORMELINK. 1969. Effects of ozone on growth, lipid metabolism, and sporulation of fungi. Phytopathology 59:1223-1225.
21. WHITE, N. H., & E. P. BAKER. 1954. Host pathogen relations in powdery mildew of barley. I. Histology of tissue reactions. Phytopathology 44:657-662.