# Effect of Ozone on the Wheat Stem Rust Fungus

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ABSTRACT

The effects of low concentrations of ozone on various phases of the uredial cycle of the wheat stem rust fungus were studied. Daily 6-hr ozone exposures of infected plants significantly decreased the growth of hyphae and the number of urediospores produced. The mean spore production over a 17-day period was 90, 82, and 50% of the controls when plants were exposed for 6 hr/day for 17 days to 6, 12, and 18 pphm ozone, respectively. Ozone exposures 24 to 48 hr before inoculation injured the plants and reduced penetration and infection. When plants were inoculated immediately after exposure and before injury developed, no reduction in penetration and infection occurred. Germination and infection of wheat plants by spores produced on exposed plants was not affected when these spores were used to inoculate plants not previously exposed.

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*Additional key words*: air pollution, *Puccinia graminis*, sporulation, infection.

Little is known of the effects of air pollution on rust fungi. Field observations indicate that sulfur dioxide decreased the incidence of several species (4, 6). Also, Sharp (7) found that urediospores of *Puccinia striiformis* West. germinated poorly in ambient air when high concentrations of negative ions were present.

Ozone ($O_3$) injury to many plant species is now widespread in the USA (5). The concentrations of $O_3$ that are injurious to green plants may also affect the incidence and severity of important plant parasitic rust fungi. A recent report (2) indicates that low concentrations of $O_3$ do affect rust fungi. Heagle (2) found that growth of uredia of crown rust, *Puccinia coronata* var. *avenae* (Fraser & Led.) was significantly decreased by $O_3$, but germination and infectivity of the urediospores produced on plants exposed to $O_3$ was not decreased. This is the only report of effects of $O_3$ on the rust fungi.

The purpose of the present research was to determine effects of ambient concentrations of $O_3$ on various stages in the uredial cycle of wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn.

MATERIALS AND METHODS.—Experiments were performed in the laboratory and growth chamber facilities of the Southeastern Plant Environment Laboratory at North Carolina State University, Raleigh. Wheat, *Triticum aestivum* L. 'Lee' and 'Kota', were grown in a mixture of gravel and Jiffy Mix (W. R. Grace Co., Travelers Rest, S.C.) in 7.5-cm diam styrofoam pots. Plants were maintained at 29 C and 70-75% relative humidity (RH) during 12 hr of light (4,200-4,500 ft-c) (6 AM-6 PM) and at 15 C during 12 hr of darkness. The plants were watered with 0.25-strength Hoagland's solution with nitrogen increased to 0.5-strength.

*P. graminis*, race 15 B, was maintained on the cultivar Kota. Unless otherwise indicated, the lower surface of the first foliar leaves of 7-day-old plants of the cultivar Lee was inoculated by spreading freshly collected spores with a cotton swab. Inoculated plants were incubated for 17 hr (3 PM - 8 AM) in a moist chamber at ca. 25 C.

Lee plants were exposed to $O_3$ in four identical 90 X 90 X 120 cm chambers (3) installed in a second growth chamber. Light intensity and day-length were the same as described above, but the temperature was 20 C; and the RH, 50%. The environment within exposure chambers was similar to the growth chamber except the temperature was 25 C.

Activated-charcoal-filtered air was drawn over an ultraviolet lamp to produce $O_3$. Ozone was monitored during exposures with Mast $O_3$ meters (Mast Development Co., 2212 E. 12th St., Davenport, Iowa 52803), and all values were corrected to a 1% neutral KI standard. Exposures were for 6 hr in the light from 9 AM to 3 PM. After each exposure, plants were moved from the exposure chambers to the center of the second growth chamber.

For microscopic examination, leaves were fixed and stained in alcoholic-lactophenol-cotton-blue (8). Analyses of variance were performed on the results. Tukey's test (1) was employed to identify significantly different means. Specific methodology is provided with the results of each of three experiments.

RESULTS.—*Infectivity and growth of hyphae.*—The first experiment was performed twice to determine the effects of $O_3$ on infection and early

TABLE 1. Effect of ozone on the growth of *Puccinia graminis* mycelia within host leaf tissue

| No. ozone exposures[b] | Hyphal length ($\mu$) per ozone concentration (pphm)[a] | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| 2 | 32 | 35 | 36 | 29 |
| 3 | 81 | 68 | 62 | 55[c] |
| 4 | 121 | 109 | 94 | 57[c] |

[a] Each figure is the mean of 50 colonies. Five colonies on each of five leaves/treatment were measured in each of two replicates.

[b] Infected plants were exposed for 6 hr/day on the 4 days after inoculation.

[c] According to the analysis of variance, colonies in exposed plants were significantly smaller after three and four exposures than in control plants (1% level of confidence). Tukey's test indicated that colonies in plants exposed to 24 pphm were significantly smaller than colonies exposed to 0 pphm $O_3$ after four exposures (5% level of confidence).

colony growth when exposures occurred after incubation. Plants were inoculated, incubated, and immediately exposed to 0, 6, 12, or 24 pphm $O_3$ (1 pphm $O_3$ = 19.6 $\mu g/m^3$ at 760 mm of Hg and 25 C) for 6 hr/day for 4 days.

After the 2nd, 3rd, and 4th day of exposure, leaves from each treatment were examined microscopically. To measure infection, the percentage of appressoria that produced infection pegs and infection hyphae was determined for 50 appressoria on each of five primary leaves/treatment. A measure of colony size (the distance from the end of the guard cell of the stoma where infection originated to the tip of the most extended hypha) was taken for five colonies on each of five leaves/treatment.

The postinoculation exposures did not inhibit the production of infection pegs and infection hyphae, and did not cause the death of young rust colonies. No marked effects of $O_3$ exposure on hyphal growth were seen after two exposures (Table 1). After three and four exposures, colonies in all exposed leaves were significantly smaller than colonies in leaves exposed to charcoal-filtered air (0 pphm $O_3$). When significantly different means were identified by Tukey's test, only the colonies exposed 4 times to 24 pphm $O_3$ were significantly smaller than colonies in the other treatments (Table 1).

The second experiment was performed twice to determine whether exposure of the plants to $O_3$ before inoculation would reduce infection. Plants were exposed once for 6 hr to 0, 6, 12, or 24 pphm $O_3$. Plants from each treatment were then divided into three groups and inoculated with urediospores as follows: (i) immediately after exposure; (ii) 24 hr after exposure; or (iii) 48 hr after exposure. Inoculated plants were incubated for 17 hr. Leaves from each group of plants were observed microscopically 48 hr after inoculation. The percentage of spores that germinated, the percentage of appressoria that produced infection pegs, substomatal vesicles, and infection hyphae, and the length of hyphae were determined.

Ozone injury symptoms in leaves were not visible until 24 hr after exposure; injury was fully developed after 48 hr. The first microscopic evidence of $O_3$ injury was the collapse of mesophyll cells adjacent to substomatal cavities. With increasing $O_3$ concentration and time, more mesophyll cells died and injury appeared macroscopically as small (<1 mm diam) white or tan necrotic areas. In plants exposed to 24 pphm ozone, ca. 10% of the leaf surface was injured after 48 hr. Plants exposed to lower concentrations had approximately 1-2% injury.

Spore germination varied by less than 2% from treatment to treatment. There were no significant $O_3$ effects on infection when inoculations occurred immediately after exposure to $O_3$ (Table 2). However, when inoculations occurred 24 or 48 hr after exposure and when injury symptoms were present, less penetration and infection occurred in exposed than in unexposed plants (Table 2). The treatment differences were consistent at the 5 and 10% level of significance.

TABLE 2. Effect of preinoculation exposures to ozone on the percentage of appressoria of *Puccinia graminis* that produced infection pegs, substomatal vesicles, and infection hyphae

| Time of inoculation hr after exposure[a] | % Infection pegs/pphm $O_3$[b] | | | | % Substomatal vesicles/pphm $O_3$ | | | | % Infection hyphae/pphm $O_3$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 6 | 12 | 24 | 0 | 6 | 12 | 24 | 0 | 6 | 12 | 24 |
| 0-1 | 80[c] | 82 | 85 | 88 | 78[c] | 78 | 80 | 84 | 67[c] | 64 | 68 | 70 |
| 24-25 | 61[d] | 57 | 43 | 53 | 59[e] | 54 | 38 | 48 | 51[e] | 46 | 31 | 40 |
| 48-49 | 64[d] | 45 | 57 | 40 | 61[d] | 42 | 55 | 37 | 49[d] | 35 | 47 | 30 |

[a] Six-hr exposure period.

[b] Each value is the mean of 500 appressoria. Fifty appressoria on each of five leaves/treatment in two replications were measured.

[c] No significant difference between exposed and unexposed treatment means according to analyses of variance.

[d] Differences between exposed and unexposed treatment means were significant at ($\alpha$. 10) according to analyses of variance.

[e] Differences between exposed and unexposed treatment means were significant at ($\alpha$. 05) according to analyses of variance.

Hyphae were rarely observed near injured mesophyll cells. Occasionally, hyphae extended through an injured area to colonize healthy cells. Because colonies in injured areas were scarce, hyphal measurements were made only in noninjured areas. The length of hyphae in leaf areas not visibly injured was not significantly affected by preinoculation $O_3$ exposure of the leaves.

*Urediospore production and infectivity of exposed spores.*—A third experiment was performed 3 times to determine the effects of daily $O_3$ exposures on the numbers of urediospores produced and on the germination and infection of fresh plants by urediospores produced on plants exposed to $O_3$. Thirty-six pots of plants were inoculated as evenly as possible by brushing them with plants bearing sporulating uredia. Immediately after incubation, plants were thinned to 10 plants/pot. Ninety plants (three samples consisting of three pots each) were randomly assigned to each treatment (exposure to 0, 6, 12, or 18 pphm for 6 hr a day for 18 days). The number of mature, dehiscent spores produced in each treatment was measured after 9, 11, 13, 15, and 17 days of exposure. Spores from each 30-plant sample were collected by gently tapping plants with a glass rod over a porcelain pan. Spores were then washed into a 125-ml Erlenmeyer flask by lightly spraying them with a 0.1% solution of Tween 20 (polyoxyethylene sorbitan monolaurate) in water. Three ml of alcoholic-lacto phenol-cotton-blue were added to each flask to prevent spore germination. The number of spores in six 0.01-ml portions of spore suspension were counted microscopically for each sample. Knowing the volume of spore suspension in each sample, we calculated the number of spores produced/sample for each treatment.

As a measure of $O_3$ injury, the percentage of chlorotic or necrotic tissue in the first foliar leaves was estimated after 10 and 17 days of exposure. Chlorosis after 10 exposures averaged about 5, 5, 5, and 50% for the 0, 6, 12, and 18 pphm $O_3$ treatments, respectively. Necrosis after 10 exposures was less than 1% in all treatments. After 17 exposures, necrosis was more extensive than chlorosis. Necrosis averaged ca. 15, 25, 30, and 65%, and chlorosis averaged about 10, 10, 20, and 15% in the 0, 6, 12, and 18 pphm $O_3$ treatments, respectively.

These methods of inoculation and sampling resulted in a fairly uniform number of spores measured in the three samples of a given treatment. For example, the average percentage variation about the mean of the three samples within a given treatment was less than 13% after 9 days.

Ozone significantly reduced the number of urediospores produced at all concentrations tested (Table 3). The significant $O_3$ effects at each collection time, according to Tukey's test, are presented in Table 3. In general, maximum numbers of spores were collected after 11 days in the $O_3$ treatments and after 13 days in the control. The early peak and rapid decline in sporulation among the plants exposed to $O_3$ may have been due to factors associated with $O_3$ injury. In general, sporulation decreased with increasing exposures and was less among plants that were injured by $O_3$.

TABLE 3. Effect of exposures to ozone on urediospore production by *Puccinia graminis*

| No. ozone exposures[a] | No. urediospores (millions)[b] per ozone concentration (pphm)[c] | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 18 |
| 9 | 4.2 h | 3.4 i | 3.3 i | 2.9 i |
| 11 | 8.6 i | 10.3 h | 8.8 i | 5.3 j |
| 13 | 9.4 h | 7.0 i | 6.7 i | 4.9 j |
| 15 | 7.4 h | 7.5 h | 7.5 h | 3.6 i |
| 17 | 6.4 h | 3.7 i | 3.0 j | 1.6 k |
| Mean | 7.2 h | 6.4 i | 5.9 j | 3.6 k |

[a] Infected plants were exposed for 6 hr/day on the 17 days after inoculation.

[b] Each figure is the average of nine samples; three samples were taken at each collection time in each of three replicates.

[c] Means in the same line, followed by different letters, are significantly different at the 5% level of confidence according to Tukey's test.

To determine the effect of $O_3$ on germination and infection, spores were collected after the 18th exposure. Percentage germination was determined by placing spores in distilled water on microscope slides and incubating them in a moist chamber for 24 hr. The percentage infection on 7-day-old plants not exposed to $O_3$ was determined 72 hr after inoculation as previously outlined.

Ozone had no measurable effect on the percentage germination and infection by spores collected after 18 exposures.

DISCUSSION.—This study and previous evidence (2) suggests that the $O_3$ component of photochemical air pollution could reduce the inoculum potential of cereal rusts in the field, where elevated $O_3$ concentrations occur. The results are relevant because some of the concentrations of $O_3$ used are commonly surpassed in the air surrounding many urban areas. For instance, we have found that the daily 6-hr average (9 AM-3 PM) $O_3$ concentration near Raleigh, N. C., in June 1971, exceeded 6 pphm on 9 days. In Riverside, Ca., in the Los Angeles basin, the peak $O_3$ concentrations occur later in the day than in most other areas. The daily 6-hr (12 PM-6 PM) average concentration in Riverside for June 1971 exceeded 6 pphm on 25 days and 12 pphm on 15 days (O. C. Taylor, *personal communication*).

The mechanism of $O_3$ action in inhibiting infection, hyphal growth, and urediospore production is not known. Ozone may act directly upon the fungus or indirectly through the host. Our data suggest that $O_3$ affects the rust fungus primarily through its effects on host mesophyll tissue. Retardation of infection and hyphal growth was not found unless evidence of mesophyll cell injury was observed. Reduction in sporulation was related to increased necrosis of host tissue, which probably decreased the suitable substrate for rust colonization.

No effects on infection or on rust morphology were observed when inoculated plants were exposed immediately after incubation, during penetration. This is further evidence of a lack of direct action by $O_3$ on the fungus. The lack of $O_3$ effects at the early stages of development may have been the result of the fact that the infection structures arising from appressoria are formed within the substomatal cavity of the host plant where they may be partially protected from exposure to $O_3$.

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