Effect of Puccinia graminis f. sp. tritici on Ozone Injury in Wheat

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ABSTRACT

Significantly less ozone injury occurred in mesophyll cells in the substomatal areas of wheat leaves inoculated with urediospores of *Puccinia graminis* f. sp. *tritici* than in noninoculated leaves. The protective effect was present in inoculated areas when plants were exposed to ozone 2, 3, or 4 hr after the start of urediospore incubation. The mesophyll cells under stomata with appressoria attached were rarely injured. Mesophyll cells under stomata without appressoria attached were also protected in areas of the leaf that were inoculated. Noninoculated areas were not protected. These results suggest that a diffusible substance is produced by germinating spores and infection structures that results in protection of localized areas of wheat leaf tissue from ozone injury.

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Ozone (O_3) is regarded as the most important plant pathogenic component of photochemical air pollution. The injurious effects of O_3 on plants are described in review articles (2, 7). The effects of environmental factors that interact to affect the severity of O_3 injury to plants were reviewed by Heck (4). Certain chemicals that alter plant sensitivity to O_3 were summarized by Reinert & Spurr (8).

Well-developed infections by several types of plant parasites are also known to affect plant sensitivity to O_3. The chlorotic areas surrounding lesions of *Pseudomonas phaseolicola* on kidney bean leaves were not injured by O_3 exposures that severely injured adjacent areas of the leaves (6). Tobacco leaves displaying symptoms of tobacco mosaic virus infection were protected from O_3 exposures that caused extensive injury to leaves on plants not infected (1). Infection of hollyhock and bean by *Puccinia helianthi* and *Uromyces phaseoli* gave localized protection of their respective hosts from injury caused by ozonated olefins (10). The protective effect which extended 1 to 2 mm beyond the limits of the fungus hyphae was first observed when exposures occurred 3 days after infection. No reports exist on the possible protective effects of plant pathogens on plants during the early phases of the infection process or before an infection is established.

In studies to determine effects of O_3 on the uredial cycle of the wheat stem rust fungus, *Puccinia*

graminis Pers. f. sp. *tritici* Eriks. & Henn., localized areas surrounding the pustules were not injured by O_3 doses that were sufficient to severely injure noninfected areas (3). In this paper we report the results of experiments performed to determine whether germinating spores, infection structures, or new infections of *P. graminis* protect wheat leaves from O_3 injury.

MATERIALS AND METHODS.—Wheat seeds, *Triticum aestivum* L. 'Lee' and 'Kota', were sown in a 2:1 mixture of gravel and Jiffy Mix (W. R. Grace Co., Travelers' Rest, S.C.) in 7.5-cm diameter styrofoam pots. Plants were watered with quarter-strength Hoagland's solution with nitrogen increased to half-strength. Plants were grown in a walk-in growth chamber at 29 C during 12 hr of daylight (6 AM - 6 PM) and at 15 C during 12 hr of darkness. Light intensity was 4,200 to 4,500 ft-c and the RH was 70-75%.

Race 15B of *P. graminis* was maintained on susceptible Kota seedlings. In all O_3 exposure experiments, a dry cotton swab was used to inoculate the lower surface of the first foliar leaves of 7-day-old Lee plants with fresh urediospores.

Plants were exposed to carbon-filtered air or to O_3 in two identical 90 X 90 X 120-cm exposure chambers (5) at 4,200 to 4,500 ft-c (6 AM - 6 PM), 25 C, and 50% RH. Ozone was produced by passing charcoal-filtered air over an ultraviolet lamp. Ozone concentrations were monitored with Mast ozone meters (Mast Development Co., 2212 E. 12th St., Davenport, Iowa 52803), and all values were corrected to a 1% neutral KI standard. After each exposure, plants were placed in the center of the growth chamber at 20 C, 50% RH, and 4,200 to 4,500 ft-c (6 AM - 6 PM).

For microscopic observations, leaves were fixed and stained in alcoholic lactophenol cotton blue (9). Analyses of variance were performed on the results.

RESULTS.—No symptoms resembling O_3 injury were observed in inoculated or noninoculated control plants exposed to carbon-filtered air. Among plants exposed to O_3, the first observable symptom of O_3 injury was the collapse of one or more mesophyll cells adjacent to substomatal cavities 1 - 2 days after exposure. The collapsed cells stained darkly and were easily identified when compared to noncollapsed mesophyll cells (Fig. 1). The mesophyll cells adjacent to a substomatal cavity will hereafter be called a "site". The measure of O_3 injury is reported here as

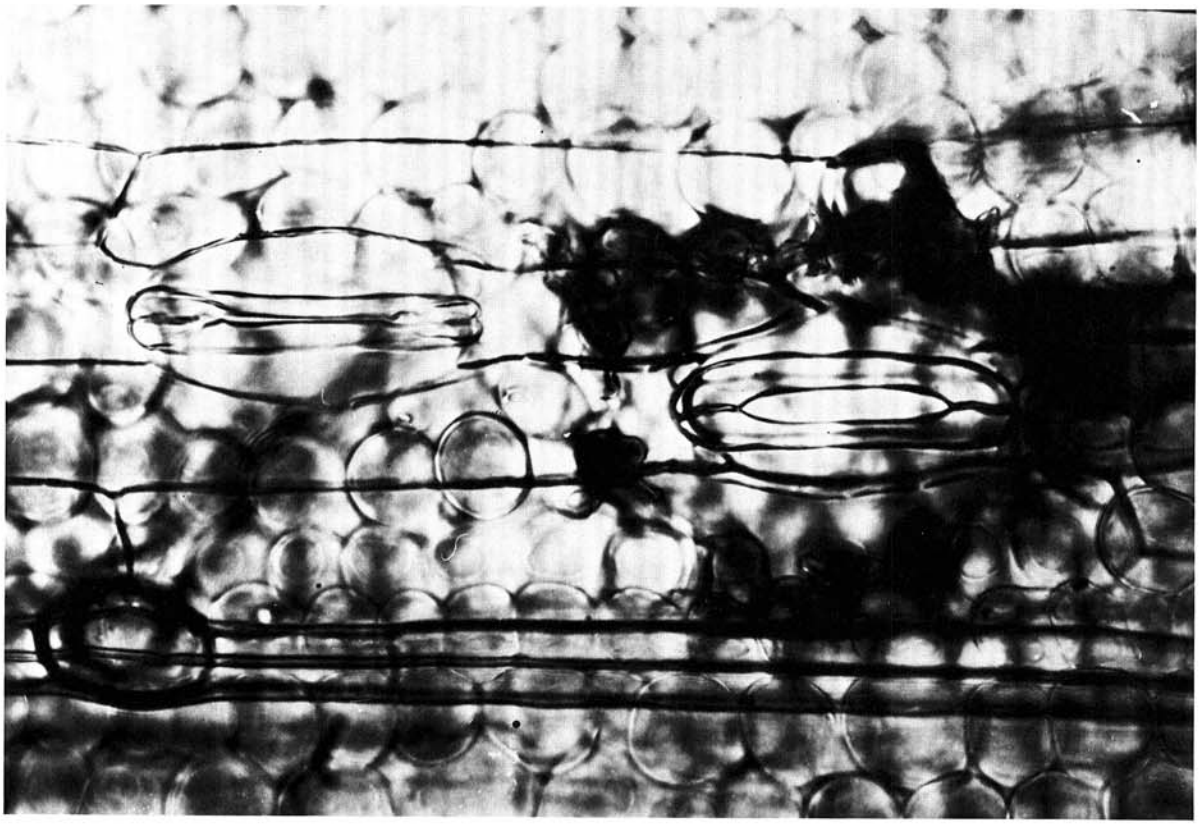

Fig. 1. Abaxial surface of a wheat leaf exposed for 5 hr to 30 pphm ozone. The mesophyll cells under the stoma on the right are collapsed (injured site) and absorbed more stain than the uninjured mesophyll cells under the stoma on the left (uninjured site).

TABLE 1. Effect of *Puccinia graminis* infection structures on the percentage of substomatal mesophyll sites injured by ozone[a]

Number of exposures	Percentage of substomatal mesophyll sites injured in each infection category[b]		
	Appressorium and infection hyphae absent	Appressorium present	Appressorium and infection hyphae present
3	43.2[c]	0.8	4.1
4	41.2[c]	3.7	0.0

[a] Plants were exposed to 24 pphm O_3 for 6 hr on 3 or 4 days (one pphm O_3 = 19.6 $\mu g/m^3$ at 760 mm of Hg and 25 C).
[b] For each exposure, the mesophyll cells in the substomatal area of 50 stomata were observed on each of five leaves in each of two experimental replicates.
[c] Significantly more injury (α = 0.01) occurred to sites where no infection structures were present than when an appressorium or an appressorium and infection hyphae were present.

the percentage of sites containing one or more collapsed mesophyll cells.

In the first experiment, plants were inoculated and then incubated in a moist chamber at approximately 25 C for 17 hr (3 PM - 8 AM). Plants were then exposed to carbon-filtered air or 24 pphm O_3 for 6 hr/day (9 AM - 3 PM) on each of the 4 days after inoculation. Leaves were randomly selected immediately after the 3rd and 4th days of exposure and prepared for microscopic observation. The substomatal area of 50 stomata in the inoculated region on each of five first foliar leaves were observed for each treatment. The percentage of injured and noninjured sites was determined when (i) no evidence of an appressorium or an infection (the presence of infection hyphae) was present at the stoma (65% of the stomata), (ii) an appressorium was attached to the stoma but no evidence of infection was seen (23% of the stomata), and (iii) an appressorium and infection were both present (12% of the stomata). This experiment was replicated two times.

In the situation where no appressoria or infections were present, 42% of the sites were injured (Table 1). When appressoria, with or without infections, were present, less than 5% of the sites were injured. The results were similar whether plants received three or four 6-hr exposures (Table 1).

A second experiment was performed three times to determine (i) if germinating spores or resulting appressoria would reduce O_3 injury when O_3 exposures began 1, 2, 3, or 4 hr after the start of urediospore incubation, and (ii) to determine whether reduction in O_3 injury would extend to sites where appressoria were not attached to the stomata.

Inoculations were made at 7 AM in an area extending 2-4 cm from the leaf tip. Inoculated plants were divided into four groups and placed in a moist chamber at approximately 25 C at 8 AM, 9 AM, 10 AM, or 11 AM to receive 4, 3, 2, or 1 hr, respectively, of incubation under dew conditions. Noninoculated controls for each incubation time consisted of plants brushed with a dry cotton swab and of plants not brushed with a dry cotton swab. At 12 AM, all plants were removed from the moist chamber and immediately exposed to carbon-filtered air or 30 pphm O_3 for 5 hr. Leaves were fixed and stained 48 hr after exposure.

Spore germination began about 1 hr after incubation started and after 3 hr, most of the spores had germinated. Appressorium formation began 2 hr after the start of incubation; after 4 hr appressoria were attached to 10-20% of the stomata. About 10% of the appressoria that formed during 3 hr of incubation produced infection hyphae and this value increased to about 40% after 4 hr of incubation. The percentage of injured sites was determined in the controls and in the inoculated area of inoculated plants for each infection category, as outlined in Table 2.

Germination and infection was not affected by the O_3 exposure. The percentage of injured sites in the inoculated and in the noninoculated plants exposed to O_3 was not significantly different after 1 hr of incubation (Table 2). When inoculated plants were incubated for 2, 3, or 4 hr, however, there was significantly less O_3 injury in the inoculated plants than in the noninoculated plants, even when appressoria were not attached to the stomata. When appressoria were attached to stomata, injury caused by O_3 rarely occurred (Table 2). The fact that sites without appressoria over the stomata were protected indicates the operation of some mechanism other than mechanical exclusion of O_3.

A third experiment was performed twice to further demonstrate the effect of appressoria and infections in preventing O_3 injury to sites where appressoria and infections were absent and to determine: (i) whether the protection extended more than 1-2 cm in either direction in the leaf, and (ii) whether protection occurred in more than one area of the leaf.

Leaves were inoculated in one of three areas, either 0-2, 2-4, or 4-6 cm from the leaf tip. Two types of noninoculated control plants, mentioned above, were included. All plants were then placed in a moist chamber for 4 hr and immediately exposed to either carbon-filtered air or 30 pphm O_3 for 5 hr. The percentage of mesophyll sites injured by O_3 when stomata did not have appressoria attached was

TABLE 2. Effect of *Puccinia graminis* on the percentage of substomatal mesophyll sites injured by O_3 when plants were incubated for 1 to 4 hr prior to exposure[a]

Incubation time (hr)	Percentage of substomatal mesophyll sites injured in each infection category			
	Noninoculated control[b]	Germinating spores[b]	No appressorium over stomata[c]	Appressorium attached to stomata[c]
1	28.7	24.6		
2	27.7	19.3[d]		
3	38.1		19.4[e]	1.6[e]
4	34.3		14.6[e]	0.6[e]

[a] Plants were exposed to 30 pphm O_3 for 5 hr immediately after incubation. Leaves were fixed and stained 48 hr after exposure.

[b] Each mean is the average of 1,500 stomata. One hundred substomatal sites on each of five leaves per treatment were observed in each of three experimental repeats.

[c] Each mean is the average of 750 substomatal sites. Fifty sites on each of five leaves per treatment were observed in each of three replicates.

[d] Significantly less injury ($\alpha = 0.05$) occurred to substomatal sites on leaves with spores incubated for 2 hr than on leaves without spores.

[e] Significantly less injury ($\alpha = 0.01$) occurred to substomatal sites with or without appressoria attached to the stomata than in noninoculated leaves.

determined for each of the three leaf areas after 48 hr.

Ozone did not affect appressorium formation or infection. The amount of infection was the same in all three leaf areas and was similar to the 4-hr incubation period in Experiment 2. Rubbing the leaves with a dry cotton swab caused no apparent injury and did not affect the amount of O_3 injury in exposed plants. The percentage of sites injured by O_3 in each treatment is presented in Fig. 2. All three leaf areas were injured to about the same degree. When stem rust was present, there was always a significant reduction in the percentage of sites showing injury, regardless of the area inoculated. Inhibition of O_3 injury was about 50 percent in the inoculated areas, but the inhibition did not extend to noninoculated areas.

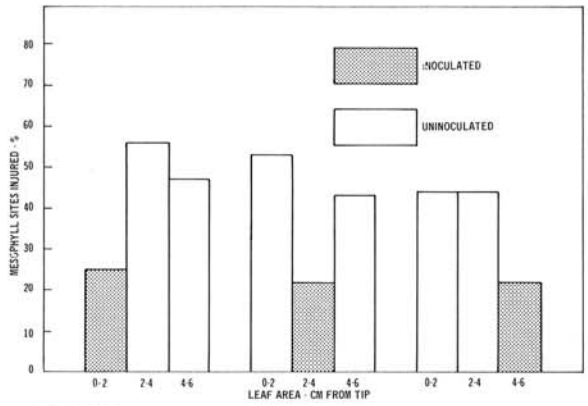

Fig. 2. Percentage of substomatal mesophyll sites injured by O_3 in leaf areas inoculated with *Puccinia graminis* or in adjacent noninoculated areas. Plants were exposed to 30 pphm O_3 for 5 hr. Each bar represents the mean injury in 2,000 substomatal mesophyll sites that did not have appressoria attached to the stomata. Two-hundred sites were observed on each of five leaves in each of two replications. The sites in the inoculated areas were injured significantly less ($\alpha = 0.05$) than those in noninoculated areas.

DISCUSSION.—*Puccinia graminis* protected wheat from O_3 injury when exposures began 2-4 hr after incubation started, prior to penetration, indicating an early interaction between the fungus and host tissues. Furthermore, the protection extended to nearby stomata that did not have appressoria over them. The protection may be caused by a diffusible substance that is present in germinating spores and infection structures and that acts directly or interacts with the host to reduce O_3 injury. The exact time at which the protection became effective was not determined. In these experiments, plants were taken from the moist chamber and immediately placed in the exposure chambers where water evaporated from the leaves within 30 min. The injurious effects of O_3 exposure were not visible until 24-48 hr after exposure. In the case of 2-hr dew periods, spores germinated, but infection did not occur. Therefore, protection must have been initiated within 2.5 hr. It is not known whether the protection was effective immediately, during exposure, or was concurrent with symptom development. When penetration and infection occurred, the early development of rust hyphae may have resulted in additional protection between the time of exposure and symptom development.

The mechanism by which plant pathogens protect their respective hosts from O_3 injury has not been determined. In addition to the work reported here, infections by a bacterium (6), a virus (1), and two species of rust fungi (10) have protected their respective hosts from pollutant injury showing that protection is common to several diverse types of pathogens. Whether or not a common mechanism of protection occurs is not known.

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