Effect of Ozone on Parasitism of Corn by Helminthosporium maydis

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ABSTRACT

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Corn plants inoculated with *Helminthosporium maydis* race T were exposed to ozone (O_3) for 6 hr/day on various days before, after, or both before and after inoculation. Lesion length and sporulation were affected by O_3 concentration and timing of the exposures. Lesions were larger on plants exposed to O_3 both before and after inoculation than on nonexposed plants. Sporulation was increased by exposure to 0.12 μliters/liter of O_3 on 6 days before inoculation and decreased by exposures to 0.18 μliters/liter on 6 days after inoculation.

Additional key words: air pollution, sporulation.

Ozone (O_3) can inhibit infection, invasion, or sporulation by several obligate parasites (2, 3, 5, 6, 9). However, *Botrytis cinerea* is the only fungus reported to cause more disease on certain hosts exposed to O_3 (10, 11). Because oxidant air pollution is so widespread (4, 8), there is a need to determine the effects of O_3 on parasitism by other fungi. In this study, I examined the effects of exposure to O_3 on parasitism of corn, *Zea mays* L., by *Helminthosporium maydis* Nisikado, race T.

MATERIALS AND METHODS

Experimental plants.—All experiments were conducted in phytotron facilities of the Southeastern Plant Environment Laboratory at North Carolina State University, Raleigh, N. C. (1). Corn hybrid Wf9 × W64A with Texas male-sterile cytoplasm was grown in a mixture of gravel and Jiffy Mix (5) in 10-cm diameter plastic pots. Plants were grown for 7 days after seeding in a controlled environment chamber at 26 C (light) and 20 C (dark) and 70-75% relative humidity (RH) during a 12-hr light period with fluorescent and incandescent light at 45,000-48,000 lux (0600 hours to 1800 hours). On the 8th day after planting, all plants were placed in another chamber where conditions were the same except that the temperature was constant at 20 C. Plants remained in this chamber except during 6-hr exposures on designated days to O_3 and three 16-hr periods in a mist chamber. Plants were watered on the five weekdays with quarter-strength Hoagland's solution with nitrogen at half-strength, and on weekends with deionized water.

Inoculum.—An isolate of *H. maydis*, obtained from corn in North Carolina and identified as race T by its reaction on corn containing N or T cytoplasm, was used. Leaves of corn cultivar Dixie Darling with sporulating lesions were harvested at 4-wk intervals, dried, and used to obtain conidia for inoculum preparation. Experimental plants were inoculated when 14 days old by spraying with conidia suspended (about 250 conidia / ml) in a solution of 1% Tween-20 in deionized water.

Exposure to ozone.—Plants were exposed to 0, 0.6, 0.12, or 0.18 μliters/liter (ppm) of O_3 for 6 hr/day (0900 hours to 1500 hours) at 24 C, 45,000-48,000 lux, and 70-75% RH. Times of exposure were: (i) at 0, 1, 2, 5, 6, and 7 days before inoculation; (ii) at 1, 2, 5, 6, 7, and 9 days after inoculation; or (iii) both before and after inoculation on each exposure day listed above. Noninoculated and nonozonated controls were included for all treatments. Exposure and monitoring methods have been described previously (5, 7).

Incubation.— Immediately after inoculation, plants were incubated in darkness for 16 hr (1600 hours to 0800 hours) at 26 C in a mist chamber (1 min of mist each 30 min). To induce sporulation, infected plants were subjected to this same incubation regime on the 6th and 7th days after inoculation.

Measurement of lesion size and sporulation.—Eight days after inoculation, samples of individual lesions on the fifth foliar leaves were prepared for microscopic examination to determine lesion size and sporulation by: (i) firmly pressing a strip of plain cellophane adhesive tape over each lesion, (ii) marking the tape with a felt-tip pen at the ends of each lesion, and (iii) removing the tape strip (with adhering conidia and conidiophores) and placing the tape, adhesive side down, on a glass microscope slide. Before microscopic examination, a drop of lactophenol-cotton blue was placed between the tape and slide to facilitate observation of the spores. Thirty lesions were sampled for each treatment; the number of spores from each lesion, lesion length, and length and width of sporulating area (area covered by conidia or conidiophores) were measured.

The experiment was performed twice. Separate analyses of variance and LSD ($P = 0.05$) tests were performed on data within each exposure schedule.

RESULTS

Effect of O_3 on foliar injury and lesion size.—Ozone at 0.06 or 0.12 μliters/liter did not cause visible leaf symptoms or affect lesion size in any of the exposure regimes. Ozone at 0.18 μliters/liter caused a slight foliar chlorosis when exposures occurred before, after, or both before and after inoculation. Lesion size was not significantly affected by O_3 at 0.18 μliters/liter unless exposures occurred both before and after inoculation (Table 1).

Effect of O_3 on sporulation.—The effects of O_3 on the number of spores per lesion and on the number of spores per square millimeter of sporulating area were similar, but there was less variability within treatments for the latter measure (Table 1). For both measures, the effects depended on the O_3 concentration and the timing of exposure in relation to inoculation.

There were more spores on plants exposed to 0.06 or 0.12 μliters/liter of O_3 before inoculation; the number of conidia produced per lesion was 208 and 337%, respectively, of that on the controls (Table 1). These differences were significant only at the 0.12 μliters/liter O_3 concentration, but similar trends occurred at 0.06 μliters/liter O_3 both times the experiment was performed; there were no statistical interactions between experimental repeat and O_3 concentration. For both measures of sporulation, the 0.18 μliters/liter treatment was statistically different from the 0.12 μliters/liter treatment, but not statistically different from the controls (Table 1).

On plants exposed only after inoculation, the number of spores per lesion in the 0.06, 0.12, and 0.18 μliters/liter treatments was 98, 59, and 42% respectively, of that of the controls, but only on the plants exposed to the 0.18 μliters/liter treatment was the number of spores per lesion significantly different from the controls (Table 1). The number of spores per square millimeter of sporulating area also was decreased by postinoculation exposures, and both the 0.12 and 0.18 μliters/liter O_3 treatments were significantly different from the controls (Table 1).

Trends toward decreased sporulation also resulted from exposures both before and after inoculation (Table 1). The number of spores per square millimeter of sporulating area in the 0.12 and 0.18 μliters/liter treatments were significantly less than in the 0.06

TABLE 1. Effect of ozone exposure before, after, or both before and after inoculation on lesion size and sporulation of *Helminthosporium maydis* on *Zea mays*[a]

Exposure regime[a] (6-hr/day) on days (no.):	Ozone conc. (μliter/liter)	Lesion length (mm)	Spores per lesion (no.)	Spores per mm^2 of sporulating area[b]
Before inoculation 7, 6, 5, 2, 1, 0	0.00	14.5	87 b	8.2 bc
	0.06	14.8	181 b	12.6 b
	0.12	15.8	294 a	16.0 a
	0.18	14.3	74 b	6.3 c
After inoculation 1, 2, 5, 6, 7, 9	0.00	17.1	361 a	16.8 a
	0.06	16.1	352 a	14.5 ab
	0.12	17.2	213 ab	9.8 bc
	0.18	17.6	153 b	7.6 c
Both before and after inoculation (on all days listed above)	0.00	16.2 b	288	11.0 a
	0.06	15.8 b	304	12.6 a
	0.12	15.6 b	141	8.8 b
	0.18	18.1 a	176	6.8 b

[a]Plants were inoculated 14 days after seeds were planted. Data within each exposure regime were analyzed separately and direct comparison between regimes cannot be made. Each value is the mean of 30 lesions (15 lesions from 5-10 fifth-foliar leaves in each of two replicates) 8 days after inoculation. Values in a subcolumn followed by different letters are significantly different from each other (LSD, $P = 0.05$). Absence of letters in a subcolumn indicates no significant differences according to analyses of variance.

[b]Sporulating area is defined as the portion of the lesion with conidia or conidiophores present.

μliters/liter treatment and in the controls. However, the differences in spores per lesion were not statistically significant ($P = 0.10$) (Table 1).

DISCUSSION

Warren (12) reported that more growth and sporulation of *H. maydis* race T occurred on corn leaves at 30 C than at 22.5 C and that least growth and sporulation occurred at 15 C. In our experiment, the temperature regimes during lesion development were partially different, depending on timing of exposures. Plants exposed after or before and after inoculation were held at 24 C for six 6-hr exposures, and plants exposed only before inoculation were held at 20 C. This temperature difference during a period of rapid fungus growth in host tissue may explain why lesions were smaller and sporulation less on the nonozonated control plants in the group treated before inoculation than in comparable controls treated after, or both before and after, inoculation (Table 1). The results of each exposure regime therefore, have been analyzed separately. Indirect comparisions of the effects of different doses of O_3 in the three exposure regimes are possible only by using the growth and sporulation in nonozonated controls within each regime as relative reference points (Table 1).

There are no previous reports of the effects of O_3 on sporulation of *H. maydis*, but O_3 has been shown to stimulate or inhibit sporulation of facultative parasites growing on agar media (3). Results from this study show that small doses of O_3 can affect sporulation of *H. maydis* on host tissue, but the mode of action is unknown. The increased sporulation from exposure to 0.12 μliters/liter before inoculation was caused by an effect on the host, because the fungus was never exposed to O_3. Why the amount of sporulation after exposure to 0 and 0.18 μliters/liter was similar is not known. Exposures to O_3 after inoculation could have inhibited sporulation by a direct effect on the fungus inasmuch as conidiophores and conidia were being formed on leaf surfaces during two of the exposures (7 and 9 days after inoculation).

Ozone concentrations as high as those that affected sporulation occur intermittently over much of the Eastern United States (4, 8). The lowest O_3 dose used in this study (0.06 μliters/liter for 6 hr per day for 6 to 12 consecutive days) has been exceeded near Raleigh, North Carolina, on several occasions each summer during 1970 to 1975. The 0.12 or 0.18 μliters/liter concentrations have occurred in the Eastern USA, but not for the extended times that were used in this study. Since O_3 can either stimulate or inhibit the sporulation of *H. maydis* (depending on the O_3 concentration and developmental stage of the fungus during exposure), it is difficult to theorize on the net effect in a field situation in which all stages of parasitism would be present during an epidemic. Thus, further studies are needed to determine whether exposure to oxidants would increase or decrease the incidence and severity of the disease caused by *H. maydis*.

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