Ozone Injury Increases Infection of Geranium Leaves by Botrytis cinerea

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ABSTRACT

Detached and attached, inoculated and noninoculated, ozone-injured and noninjured leaves from the lower, middle, and terminal regions of plants of geranium cultivars Enchantress and White Mountain were observed for infection by *Botrytis cinerea*. Previous exposure to ozone did not appreciably influence the susceptibility of leaves of either geranium cultivar to infection by *B. cinerea*, unless there was visible ozone injury. Ozone-injured, necrotic tissues on older attached and detached geranium leaves of both cultivars served as infection courts for *B. cinerea*. Phytopathology 60:669-670.

Only limited attention has been given to the influence of air pollutants on the development of plant diseases caused by fungi, bacteria, and viruses (3). Needle and leaf diseases of forest trees and plants were reduced in areas where sulfur dioxide levels were high (9). Cement-kiln dust on sugarbeet leaves may predispose them to infection by *Cercospora beticola* (10). Air pollution injury can predispose Ponderosa pine to bark beetle infestations (11). Prior exposure to different levels of fluoride influenced the number of tobacco mosaic virus lesions that developed on inoculated Pinto bean leaves (13). Infection of ozone-injured potato leaves by *Botrytis cinerea* was more extensive than on noninjured leaves (8). Infection of Pinto bean and sunflower leaves by *Uromyces phaseoli* and *Puccinia helianthi* provided protection against natural smog and ozonated olefin damage (14). Suppression of oat crown rust by ozone has also been reported (5).

Infection courts for *Botrytis cinerea* Pers., the cause of a common geranium (*Pelargonium hortorum* Bailey) disease in Massachusetts, usually consist of senescent or necrotic tissues (1, 2, 7). Since ozone can cause necrotic flecks on the fully expanded leaves of many plants (6), including geraniums (4), we decided to investigate the role of ozone injury in infection of geranium leaves by *B. cinerea*. This paper reports the results of these investigations.

MATERIALS AND METHODS.—Two-month-old plants of geranium cultivars Enchantress and White Mountain were placed in a greenhouse chamber in May 1969. All plants were exposed to 0.07-0.10 ppm ozone for 10 hr/day. Ozone was produced by a Welsbach generator, and levels were monitored with a Mast meter. The light level was maintained at 1,500 ft-c; the relative humidity at about 75%, and a temperature range of 27-32 C was maintained during exposure periods. Plants used as nonexposed controls were maintained in ozone-free, carbon-filtered air.

Spores were obtained from 10-day-old potato-dextrose agar (PDA) cultures of an isolate of *B. cinerea* obtained from an infected geranium leaf. Washed spore suspensions used as inoculum ranged from 7,000-8,000 spores/ml.

Eight leaves, with visible ozone injury, from lower and middle plant regions, and eight leaves, without visible ozone injury, from lower, middle, and terminal plant regions were obtained from plants of both cultivars previously exposed to ozone. Eight visibly noninjured leaves were obtained from the lower, middle, and terminal plant regions of nonexposed control plants. All leaves were dipped in sterile distilled water and placed on sterile glass rods in sterile moist chambers with petioles immersed in sterile distilled water. Half of the leaves were uniformly sprayed with a spore suspension of *B. cinerea* and half were not. All leaves were incubated at 22 C for 96 hr in diffuse natural daylight.

Observations of incubated leaves were made after 24, 48, 72, and 96 hr. Macroscopically visible symptoms were the criteria for determining whether leaf infection had occurred. Estimated per cent leaf infection was determined at each observation period. Average values were determined for each leaf sample. PDA, and PDA acidified with lactic acid to pH 4.2, were used for isolations from infected tissues.

Similar attached leaves in each of the lower, middle, and terminal regions of exposed and control plants of both cultivars were tagged and wet with tap water. Half the leaves were uniformly sprayed with a spore suspension of *B. cinerea* and half were not. All leaves were enclosed in loosely-tied, wet plastic bags. All plants were incubated at 20 C in diffuse incandescent light (12 hr/day) for 96 hr.

Observations and evaluations were made as previously described for detached leaves.

RESULTS.—*Ozone injury on leaves.*—Upper-surface interveinal and marginal necrotic flecks were observed on some of the lower and middle leaves of Enchantress after 15 days' exposure to ozone. Marginal yellowing and burning were observed on some of the lower and middle leaves of White Mountain after 30 days exposure to ozone. Leaves in the terminal regions of plants of either cultivar were not injured by ozone.

Infection of inoculated detached leaves.—Infection of flecked lower and middle leaves of Enchantress was observed as small, dark, wet spots originating in flecked areas after 24-hr incubation (Table 1). These infections spread rapidly, and after 96 hr, 80-95% of these leaves were infected and showed abundant growth and sporulation of *B. cinerea*. Infection of inoculated lower and middle unflecked leaves from plants exposed to ozone and similar leaves from control plants was observed after 48 and 72 hr, and resulted in 10-25% infection

TABLE 1. Ozone injury and infection of detached leaves of Enchantress and White Mountain geraniums by *Botrytis cinerea*

Leaf origin and presence or absence of visible ozone injury	% Infection after incubation for 24-hr periods[a]							
	Enchantress[b]				White Mountain[b]			
	24	48	72	96	24	48	72	96
Exposed to ozone[c]								
Lower, injury visible	5	30	60	95	10	40	80	100
Lower, injury absent	0	2	10	20	0	0	0	5
Middle, injury visible	2	25	50	80	5	30	75	90
Middle, injury absent	0	0	5	15	0	0	0	2
Nonexposed to ozone[d]								
Lower	0	2	15	25	0	0	0	10
Middle	0	1	5	10	0	0	0	5

[a] Avg 8 leaves/origin inoculated with 7,200 spores *B. cinerea*/ml and incubated at 22 C in diffuse natural daylight.
[b] Ozone injury on Enchantress is expressed as leaf flecks, on White Mountain as burned leaf margins.
[c] 0.07-0.10 ppm ozone, 10 hrs./day: 15 days for Enchantress, 30 for White Mountain.
[d] Maintained in ozone-free, filtered air.

after 96 hr. These infections originated near the leaf margins. Noninjured terminal leaves from exposed and nonexposed plants were not infected.

Infection of lower and middle leaves of White Mountain with burned margins was observed as dark, wet areas in or along burned margins after 24-hr incubation (Table 1). After 96-hr incubation, 90-100% of these leaves were infected and showed abundant growth and sporulation of *B. cinerea*. Infection of inoculated lower and middle noninjured leaves from plants exposed to ozone and similar leaves from control plants was observed after 96 hr, and resulted in infection of 5-10% of the leaf areas. Noninjured terminal leaves from exposed and nonexposed plants were not infected.

Infection of noninoculated detached leaves.—Natural *B. cinerea* infections were observed in flecked areas on noninoculated, ozone-injured lower and middle leaves of Enchantress after 48-hr incubation, and resulted in 25-30% infection after 96 hr. Natural *B. cinerea* infections were also observed in or along burned margins on noninoculated, ozone-injured lower and middle leaves of White Mountain after 48-hr incubation, and resulted in 50-60% infection after 96 hr. *B. cinerea* was isolated from infected tissues from both cultivars. All other leaves were not infected.

Infection of attached leaves.—Examination of inoculated and noninoculated leaves on exposed and nonexposed plants of both cultivars showed that the results obtained were similar to those obtained with detached leaves from comparable plants. Infection originated in ozone-injured leaf tissues, and was extensive only on ozone-injured leaves.

All noninfected detached and attached leaves from both cultivars were turgid and green after 96-hr incubation.

DISCUSSION.—Extensive infection of inoculated and noninoculated leaves by *B. cinerea* occurred only on those leaves with visible ozone injury. Ozone-injured necrotic tissues on detached and attached older geranium leaves of both cultivars provided suitable infection courts for *B. cinerea*. These results are similar to those previously reported by Manning et al. (8) on increased infection of ozone-injured potato leaves by *B. cinerea*. These results also lend support to the idea that air pollution-damaged plants may be more susceptible to infection by weak parasites (12, 13).

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