Moderate Dosages of Ozone Enhance Infection of Onion Leaves by *Botrytis cinerea* but not by *B. squamosa*

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ABSTRACT

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Exposure of onion plants to moderate chronic dosages of ozone under controlled conditions resulted in predisposition of the older leaves to enhanced infection by *Botrytis cinerea*. More lesions per square centimeter of leaf surface were induced by *B. cinerea* on the two oldest nonsenescing leaves of plants of ozone-sensitive onion cultivars Autumn Spice and Fiesta inoculated after 4-hr exposure to 0.16 ppm (314 μg/m³) of ozone (a dosage rarely, if ever, encountered in New York) than were induced on the corresponding leaves of nonexposed plants. This effect occurred in both the presence and absence of macroscopically visible, ozone-induced leaf injury. Similar results were obtained following inoculation with *B. cinerea* of plants exposed or not exposed to the relatively high chronic dosage of 0.12 ppm (235 μg/m³) of ozone for 5 hr per day for 4 days. More lesions per square centimeter of leaf surface were induced by *B. squamosa* on the two oldest nonsenescing leaves of exposed than on nonexposed plants (cultivar Autumn Spice) but only after exposure to the relatively high acute dosage of 0.25 ppm (490 μg/m³) of ozone for 4 hr (a dosage rarely, if ever, encountered in New York). Exposure of onion plants (cultivar Autumn Spice) infected with *B. cinerea* or *B. squamosa* to the relatively high chronic dosage of 0.14 ppm (274 μg/m³) ozone for 5 hr per day for 5 days had no detectable effect on expansion of preestablished lesions induced by these pathogens.

Additional key words: *Allium cepa*.

Leaves of onion (*Allium cepa* L.) plants grown on organic soil at Bradford, Ontario, Canada, and injured by ambient ozone were reported by Wukasch and Hofstra (23) to be more susceptible to *Botrytis* spp. than those not so injured. They observed that more lesions from which *Botrytis* spp. could be isolated occurred on leaves of onion plants grown in nonfiltered open-top chambers containing ambient ozone than on leaves of similar plants grown in open-top chambers with activated charcoal filters and, therefore, relatively free of ozone (23). Onions are an economically important crop in New York where both Botrytis leaf fleck and Botrytis leaf blight (caused by *Botrytis cinerea* Pers. and *B. squamosa* Walker, respectively) occur and where the latter often reaches economically important levels. Ozone concentrations greater than those generally considered as background are routinely detected throughout New York (1). Therefore, it was decided to examine in greater detail the potential for ozone to predispose onion plants to enhanced levels of Botrytis leaf fleck and Botrytis leaf blight under more extensively controlled conditions. Similar ozone-induced predisposition of potato (*Solanum tuberosum* L.) and geranium (*Pelargonium hortorum* Bailey) leaves to *B. cinerea* was correlated with the presence of ozone-induced necrosis on those leaves (15,16). Such necrosis apparently served as an infection court for the fungus. However, predisposition of broad bean (*Vicia faba* L.) leaves to *B. cinerea* by ozone was not correlated with the presence of ozone-induced necrosis (14). In either case, variation in the sensitivity of plant cultivars to ozone could be expected to influence the degree of predisposition. The study reported here was conducted to determine if (i) the potential for ozone-induced predisposition of onion leaves to *B. cinerea* was influenced by the cultivar, (ii) a single acute exposure of onion plants to ozone could predispose leaves to infection by *B. cinerea* or *B. squamosa* when inoculations were conducted before development of ozone-induced necrosis, (iii) chronic exposure of onion plants to ozone for 3–5 days could predispose leaves to infection by *B. cinerea* or *B. squamosa* when inoculations were conducted after the final exposure period, (iv) ozone-induced necrosis on onion leaves increased or decreased lesion induction by *B. cinerea* or *B. squamosa*, and (v) chronic ozone exposure of onion plants previously infected by *B. cinerea* or *B. squamosa* influenced expansion of lesions induced by these pathogens. The relative sensitivity of eight onion cultivars to ozone was first established. Several of these cultivars that differed in sensitivity to ozone were used in experiments to resolve the first three considerations. One ozone-sensitive cultivar was used in experiments to resolve the latter two considerations.

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MATERIALS AND METHODS

Cultural conditions. Onion plants were grown from seed in 10.16-cm (4-in.)-diameter plastic pots filled with a greenhouse soil and maintained in a growth chamber with a 16-hr photoperiod (~25,000 lux) and a 24/18 C day/night temperature regime. All plants were between 8 and 10 wk old when utilized. Before an experiment, plants were placed for 4 days under a wooden frame covered with shade cloth that screened out ~40% of the total light. This shading period enhanced plant sensitivity to ozone. Cultivar Autumn Spice was used except as noted.

Ozone exposures and exposure chamber. Acute exposures of plants to ozone were generally for 4 hr at ozone concentrations of 0.16, 0.18, 0.20, or 0.25 ppm (314, 353, 392, and 490 μg/m³, respectively). Chronic exposures were either 0.08 or 0.12 ppm (157 and 235 μg/m³, respectively) for 5 hr per day for up to 5 consecutive days. Ozone concentration fluctuated as much as ± 0.03 ppm (59.5 μg/m³) during some exposures.

All exposures to ozone were conducted in a previously described mist chamber (11) that was converted into an ozone exposure chamber (Fig. 1) described previously (19). The chamber was divided in half by a removable Plexiglas partition. One side was used as an ozone-free control chamber, and the second side served as an exposure chamber. Ozone levels within the chamber were constantly monitored and recorded by a Mast ozone meter and strip chart recorder (Mast Development Company, Davenport, IA 52803). The efficiency of the ozone meter was established by using a Dasibi model 1003-PC ozone generator and monitor calibrated according to Environmental Protection Agency specifications (5) and the ozone concentrations monitored during the experimental exposures were adjusted accordingly. This procedure generally was conducted before initiation of each group of experiments.

Plants were placed in this chamber ~16 hr before initiation of an exposure period and maintained at 60% RH, 24/18 C day/night temperatures, and 16,000 lux (at plant height) between 0600 and 2100 hours. Exposures generally were initiated at 1000 hours.

Inoculum production. The isolates of *B. cinerea* used in this study included C-80-1 from blighted onion leaves collected near Elba, NY, and C-80-2 and C-80-3 from blighted onion leaves and florets, respectively, collected in Orange County, NY. The isolates of *B. squamosa* were S-80-6 from blighted onion florets collected in Orange County, B-SS-2 from M. L. Lacy at Michigan State University, and 24-1, a single-ascospore isolate from an apothecium of the perfect state of *B. squamosa* produced naturally in New York. Virulence of all isolates of *B. cinerea* or *B. squamosa* was maintained by routine transfer of single conidia reisolated from infected onion leaves to potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI 48232) in 9-cm-diameter plastic petri dishes.

Conidia of uniform age of both *B. cinerea* and *B. squamosa* for use in inoculation of onion plants were produced by following a procedure previously described (19). Conidial suspensions for inoculation were adjusted to ~30,000 and 8,000 conidia per milliliter for *B. cinerea* and *B. squamosa*, respectively.

Inoculation and incubation procedures. In all inoculations, individual leaves of intact onion plants (up to three per plant) were sprayed with a conidial suspension of either *B. cinerea* or *B. squamosa* until their surfaces were uniformly covered with fine droplets. This was accomplished by using a Preval aerosol atomizer (Precision Valve Corp., Yonkers, NY 10702). After inoculation, the plants were placed in the ozone exposure chamber and incubated at 100% RH for 48 hr under a 15-hr photoperiod (16,000 lux) and 24/18 C day/night. Except where described otherwise, disease was assessed after the 48-hr incubation period by counting the numbers of lesions per leaf and calculating the numbers of lesions per square centimeter of leaf surface for each leaf. The oldest nonsenescing leaf of each plant was designated leaf 1 and the next two successively younger leaves were designated leaves 2 and 3. Data from leaves 1, 2, and 3 were analyzed separately because both the susceptibility of leaves to *B. cinerea* or *B. squamosa* and their sensitivity to ozone varied with leaf age. Uninoculated control plants were included in each experiment.

Sensitivity of onion cultivars to ozone. Onion cultivars Autumn Spice (Stokes Seeds Inc., Buffalo, NY 14240), Excel, Fiesta, Yellow Granex (Moran Seed Company, El Macero, CA 95618), Downing Yellow Globe, Early Yellow Globe, Buccaneer, and Sentinel (Joseph Harris Co. Inc., Rochester, NY 14624) were rated for relative sensitivity to ozone. Twenty plants of each cultivar were exposed to 0.20 ppm ozone for 4 hr and then placed in an environmentally controlled growth chamber for 4 days. Each leaf of each plant then was given a leaf injury value based on the percentage of the leaf surface exhibiting ozone-induced injury. Leaves exhibiting no injury were given an injury value of 0 and, leaves that exhibited injury on 1–10, 11–20, 21–40, or 41–60% of their surfaces were given values of 1, 2, 3, or 4, respectively. The leaf injury values for the leaves of each plant then were summed and the sum divided by the number of injured leaves for that plant. This resulted in a mean injury value for the injured leaves of that plant. This value then was multiplied by the fraction of leaves injured on that plant (eg, by 3/5 if three of five leaves exhibited injury) to obtain a plant injury value. The mean plant injury values for each cultivar were compared by statistical analyses.

Statistical analyses. Differences between the treatment means for ozone injury values in the cultivar sensitivity trial were statistically evaluated ($P = 0.05$) by using a Kruskal-Wallis test (10). In all other experiments, statistical contrasts (22) were conducted following analyses of variance and were used to evaluate pertinent comparisons between treatment means. A difference between two treatment means was considered statistically significant when the P-value obtained from the statistical contrast of those means was $\leqslant 0.05$.

RESULTS

Symptoms of ozone injury, Botrytis leaf fleck, and Botrytis leaf blight. Macroscopic injury induced by ozone generally developed as small, white, necrotic specks on onion leaves. Individually these specks usually did not exceed 0.5 mm in diameter. Collectively they formed stippled areas that occasionally developed into necrotic patches as large as 5 cm in length and 1 cm in width (Fig. 2A). The stippled areas most commonly were observed on adaxial surfaces of more distal portions of the leaves. Tip necrosis sometimes was

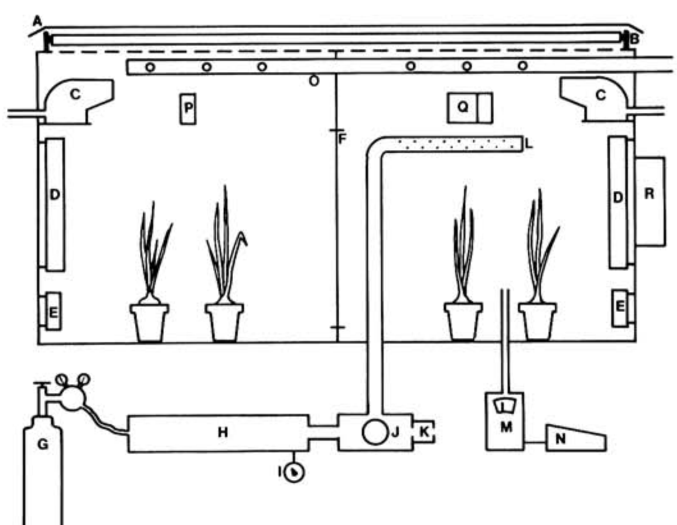

Fig. 1. Schematic drawing of environmental control chamber and ozone generating and monitoring system. A, mylar-lined ceiling; B, lamps; C, humidifiers; D, cooling coils; E, heating element; F, removable Plexiglas partition; G, oxygen tank; H, ultraviolet lamp; I, rheostat; J, mixing box with blower fan; K, charcoal filter; L, Pyrex tube for ozone dispersal; M, ozone meter; N, strip chart recorder; O, exhaust pipe; P, circulating fan; Q, control unit with circulating fan; and R, control panel.

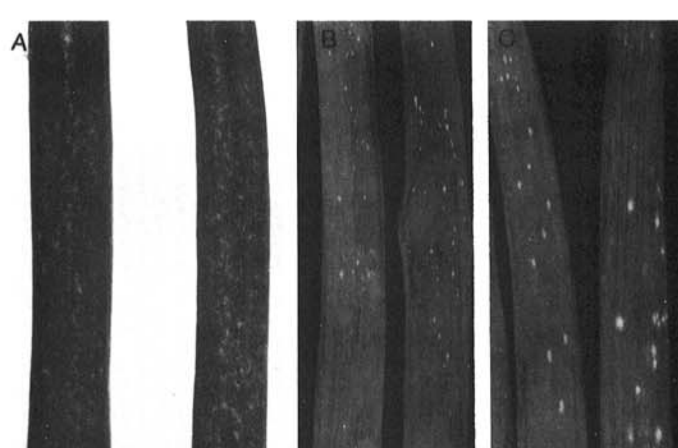

Fig. 2. Typical symptoms of ozone injury, infection by *Botrytis cinerea*, and infection by *B. squamosa* on onion leaves. **A,** Necrotic stippling on leaves of plants which developed after exposure of the plants to 0.18 ppm ozone for 4 hr. **B,** Lesions (flecks) induced by *B. cinerea* after 48 hr of incubation. **C,** Lesions induced by *B. squamosa* after 48 hr of incubation.

observed on leaves of plants exposed to ozone, but it was not readily distinguishable from tip necrosis which occurred on leaves of plants not exposed to ozone. The amount of tip necrosis that occurred in our experiments was not quantified. Infection of leaves by *B. cinerea* or *B. squamosa* was expressed macroscopically as spindle-shaped necrotic lesions (Fig. 2B,C). The lesions induced by *B. cinerea* after 2 days of incubation did not exceed 1 × 3 mm and averaged ~0.5 × 1 mm. These lesions occasionally exhibited a white halo around the white or rarely tan necrotic center and usually did not penetrate transversely through the leaf to the lacuna. In comparison, the lesions induced by *B. squamosa* after 2 days of incubation averaged ~2 × 4 mm, usually exhibited a white to greyish halo around the tan necrotic center, and generally penetrated transversely through the leaf tissue to the lacuna. Although lesions of *B. squamosa* continued to expand and eventually coalesced, such expansion generally had not occurred by the time data were taken.

Sensitivity of onion cultivars to ozone. The eight onion cultivars tested fell into three ranks of relative sensitivity to ozone (Table 1) as determined by visual assessment of leaf injury. The cultivars Autumn Spice and Fiesta were ranked "sensitive," as they exhibited significantly ($P = 0.05$) more injury than any of the other cultivars. The cultivar Sentinel was ranked "intermediate" as it exhibited significantly ($P = 0.05$) less injury than Autumn Spice or Fiesta but more than any of the other cultivars. Downing Yellow Globe, Yellow Granex, Buccaneer, Excel, and Early Yellow Globe were ranked as "tolerant" as they exhibited significantly ($P = 0.05$) less injury than the other cultivars.

Lesion induction on leaves exhibiting macroscopically visible ozone injury. Twenty onion plants exhibiting ozone-induced necrotic flecking on the second oldest nonsenescing leaf were selected from a set of plants that had been exposed for 4 hr to 0.18 ppm ozone 96 hr previously. The plants were randomly divided into two subsets of 10, and then 10 nonexposed plants were added to each subset. Leaf 2 of each plant in one subset then was inoculated with isolate C-80-3 of *B. cinerea* and leaf 2 of the plants in the other subset was inoculated with isolate S-80-6 of *B. squamosa*. All plants then were incubated as described and the numbers of lesions that developed per square centimeter of inoculated leaf surface were determined after 3 days. On plants exposed to ozone, the numbers of lesions that developed within areas of visibly injured leaf tissue and those within areas of not visibly injured leaf tissue were assessed separately. This experiment was conducted twice with similar results (Table 2). Significantly ($P = 0.05$) larger numbers of lesions were induced by *B. cinerea* on leaves of exposed plants than on leaves of nonexposed plants regardless of the presence or absence of macroscopically visible injury caused by ozone. No significant differences were observed in lesion induction by *B. squamosa* on exposed versus nonexposed leaves. Lesions induced by either species generally were observed on areas of leaves free from stippling caused by ozone or between individual necrotic specks within an injured area and only occasionally appeared to arise from necrotic specks.

Lesion induction on leaves inoculated before development of macroscopically visible injury caused by ozone. Two sets of 12 plants were exposed to 0.16 ppm ozone for 4 hr and two similar sets were not. Leaves 1, 2, and 3 of plants from one exposed and one nonexposed set were inoculated with suspensions of conidia of *B. cinerea* immediately after the exposure period. The remaining exposed and nonexposed sets were similarly inoculated 24 hr after the exposure period. The numbers of lesions that developed per square centimeter of inoculated leaf surface were assessed after a 48-hr incubation period. This experiment was conducted twice with similar results for each of three different isolates of *B. cinerea* (Table 3). Significantly larger numbers of lesions were induced by all three isolates on leaf 1 of exposed plants than on leaf 1 of nonexposed plants (Table 3). Similar results occurred on leaf 2 of exposed plants with the exception of those inoculated with isolate C-80-2. In this case, no significant differences in lesion numbers were observed on leaf 2 of exposed and nonexposed plants. There were no significant differences between the numbers of lesions induced by any of the three isolates on leaf 3 of exposed and nonexposed plants.

Similar experiments were conducted with isolates B-SS-2 and S-80-6 of *B. squamosa*. There were no significant differences between numbers of lesions on the corresponding leaves of exposed and nonexposed plants when the ozone dosage was 0.16 ppm ozone for 4 hr on either 1 or 2 consecutive days (*unpublished*). In other similar experiments, sets of 12 plants were exposed to 0.20 or 0.25 ppm ozone for 4 hr and then immediately inoculated with suspensions of conidia of isolate S-80-6. For each set of exposed plants, there was a corresponding set of nonexposed control plants inoculated with isolate S-80-6. These experiments were repeated twice with similar results (Table 4). There were no significant differences in the numbers of lesions induced on any of the inoculated leaves of nonexposed plants compared to the numbers of lesions induced on the corresponding leaves of plants exposed to 0.20 ppm ozone for 4 hr. Significantly more lesions occurred on leaves 1 and 2 of plants exposed to 0.25 ppm ozone for 4 hr than on the corresponding leaves of nonexposed plants.

Another experiment was conducted to determine if the increase in the numbers of lesions caused by *B. cinerea* on plants exposed to 0.16 ppm ozone for 4 hr could be prevented by use of an onion cultivar relatively tolerant of ozone. Sets of 15 plants of the cultivars Fiesta (ozone-sensitive) and Downing Yellow Globe (ozone-tolerant) were exposed to 0 or 0.16 ppm ozone for 4 hr. Leaves 1 and 2 of each plant then were inoculated with a suspension of conidia from isolate C-80-3 and incubated for 48 hr. This experiment was conducted only once. Significant increases in the numbers of lesions occurred on leaves 1 and 2 of exposed plants of cultivar Fiesta compared to the corresponding leaves of nonexposed plants of this cultivar (Table 5). The number of lesions on leaves 1 and 2 of Downing Yellow Globe plants exposed to ozone and the number on corresponding leaves of nonexposed plants were not significantly different.

Lesion induction on leaves inoculated immediately after chronic exposure to ozone. Sets of 10 plants each were exposed to 0 or 0.08 ppm ozone for 5 hr per day for either 3, 4, or 5 consecutive days.

TABLE 1. Sensitivity of onion cultivars to injury by exposure to ozone at 0.20 ppm for 4 hr

Cultivar	Injury rating[a,b]	Relative sensitivity
Autumn Spice	1.26 a	Sensitive
Fiesta	1.19 a	Sensitive
Sentinel	0.88 b	Intermediate
Buccaneer	0.41 c	Tolerant
Yellow Granex	0.35 c	Tolerant
Excel	0.34 c	Tolerant
Early Yellow Globe	0.30 c	Tolerant
Downing Yellow Globe	0.21 c	Tolerant

[a] Injury ratings are the average of individual plant injury values for 20 plants of each cultivar. Values followed by different letters are significantly different ($P = 0.05$) as determined by a Kruskal-Wallis test (SAS Institute, Cary, NC 27511).

[b] Leaves exhibiting no injury were given an injury value of 0. Those that exhibited injury on 1–10, 11–20, 21–40, or 41–60% of their surfaces were given values of 1, 2, 3, or 4, respectively.

TABLE 2. Average numbers of lesions induced by *Botrytis cinerea* or *B. squamosa* per square centimeter of leaf surface of onion plants inoculated 4 days after exposure to 0 or 0.18 ppm ozone for 4 hr[a,b]

Pathogen	Exposed and stippled tisssue	Exposed but not stippled tissue	Nonexposed tissue
B. cinerea	0.85 a	0.60 a	0.21 b
B. squamosa	1.35 a	1.36 a	1.82 a

[a] Contrasts between values within rows followed by different letters had significance levels $<P = 0.05$ as determined by statistical contrasts performed using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511).

[b] Data are the average numbers of lesions observed for 20 replicates (one replicate per plant) and were collected from the second oldest nonsenescing leaf of each plant.

Leaf 2 of each plant within each set was inoculated with isolate C-80-3 of *B. cinerea* immediately after the final exposure period for that set. The numbers of lesions induced per square centimeter of inoculated leaf surface area were assessed after a 48-hr incubation period. Similar inoculation experiments were conducted with isolate C-80-3 of *B. cinerea* and isolate S-80-6 of *B. squamosa* after exposure of plants to an ozone concentration of 0.12 ppm for 5 hr per day for 3, 4, or 5 consecutive days. All experiments were conducted twice with similar results (Table 6). There were no significant differences between the numbers of lesions induced by either species of *Botrytis* on corresponding leaves of plants exposed to either 0 or 0.08 ppm ozone for any time period or between the numbers of lesions induced by *B. squamosa* on corresponding leaves of plants exposed to either 0 or 0.12 ppm ozone for any time period. Significant increases in the numbers of lesions induced by *B. cinerea* occurred on leaves of plants exposed to 0.12 ppm ozone for 5 hr per day on 4 consecutive days compared with the numbers of lesions induced on the corresponding leaves of nonexposed plants.

Lesion expansion in the presence of ozone. Experiments were conducted to determine if ozone influenced the expansion of lesions induced by either *B. cinerea* or *B. squamosa*. In one experiment, 100 plants were randomly divided into four sets of 25 plants each. Leaves 1 and 2 of each plant in one of these sets were inoculated with isolate C-80-1 of *B. cinerea*, and the leaves of each plant in a second set of 25 plants were inoculated with isolate C-80-3 of *B. cinerea*. Leaves 1 and 2 of each plant from the third and fourth sets of 25 plants were inoculated with isolates S-80-6 and 24-1, respectively, of *B. squamosa*. The inoculated plants were incubated for 48 hr, and then two groups of 40 plants were selected from the 100 plants so that each group contained 10 plants each infected by isolates C-80-1, C-80-3, S-80-6, or 24-1. One group of 40

TABLE 3. Average numbers of lesions induced by *Botrytis cinerea* per square centimeter of leaf surface of onion plants exposed to 0 or 0.16 ppm ozone for 4 hr and inoculated immediately or 24 hr after exposure[a]

Time of inoculation	Isolate	Leaf[b]	Ozone[c] Plus	Ozone[c] Minus	t-value[d]	Significance level (P)
Immediately after exposure	C-80-1	1	0.66	0.12	2.35	0.0145
		2	0.55	0.10	2.20	0.0162
		3	0.10	0.15	0.44	0.6541
	C-80-2	1	1.80	1.00	2.11	0.0173
		2	1.70	1.85	0.80	0.5430
		3	0.60	0.45	0.95	0.3840
	C-80-3	1	1.31	0.87	2.50	0.0130
		2	0.91	0.22	2.60	0.0110
		3	0.20	0.15	0.35	0.6953
24 hr after exposure	C-80-1	1	0.80	0.20	2.55	0.0125
		2	0.75	0.13	2.63	0.0093
		3	0.13	0.18	0.33	0.6982
	C-80-2	1	2.21	1.55	1.95	0.0344
		2	1.61	1.30	1.05	0.3324
		3	0.53	0.49	0.30	0.6994
	C-80-3	1	2.01	1.23	2.84	0.0078
		2	1.53	0.80	2.76	0.0088
		3	0.24	0.33	0.40	0.6432

[a] Statistical contrasts were performed using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511). Means within the plus ozone and minus ozone columns of each row were contrasted.
[b] Leaves 1, 2, and 3 refer to the first, second, and third oldest nonsenescing leaves of the plants.
[c] Data are the average numbers of lesions observed for 24 replicates (one plant per replicate) obtained by combining the results of two similar experiments that produced similar results.
[d] The t-value is the estimate divided by the standard error of that estimate. Each t-value represents the value for the contrast of the means from the plus and minus ozone columns of that row.

TABLE 4. Average numbers of lesions induced by *Botrytis squamosa* per square centimeter of leaf surface of onion plants inoculated immediately after exposure to 0.20 or 0.25 ppm ozone for 4 hr or not exposed[a]

Ozone concentration (ppm)	Leaf[b]	Ozone[c] Plus	Ozone[c] Minus	t-value[d]	Significance level (P)
0.20	1	2.23	2.54	0.78	0.3988
	2	2.54	2.94	0.90	0.3802
	3	2.05	1.60	1.10	0.1952
0.25	1	2.85	2.04	2.23	0.0280
	2	2.70	1.90	2.10	0.0327
	3	1.95	2.10	0.50	0.5432

[a] Statistical contrasts were made using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511). Means within the plus ozone and minus ozone columns of each row were contrasted.
[b] Leaves 1, 2, and 3 represent the first, second, and third oldest nonsenescing leaves on the onion plants, respectively.
[c] Data are the mean numbers of lesions observed for 24 replicates (one plant per replicate).
[d] The t-value is the estimate divided by the standard error of that estimate. Each t-value represents the value for the contrast of the means from the plus and minus ozone columns of that row.

TABLE 5. Average numbers of lesions induced by *Botrytis cinerea* per square centimeter of leaf surface of an ozone-tolerant and ozone-sensitive onion cultivar exposed to 0 or 0.16 ppm ozone for 4 hr and inoculated immediately after exposure[a]

Cultivar	Leaf[b]	Ozone[c] Plus	Ozone[c] Minus	t-value[d]	Significance level (P)
Fiesta (ozone-sensitive)	1	0.89	0.25	2.20	0.0180
	2	0.60	0.15	2.34	0.0140
Downing Yellow Globe (ozone-tolerant)	1	0.35	0.20	0.44	0.5881
	2	0.19	0.38	0.96	0.3977

[a] Statistical contrasts were performed using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511). Means within the plus ozone and minus ozone columns of each row were contrasted.
[b] Leaves 1 and 2 represent the first and second oldest nonsenescing leaves of each plant.
[c] Data are the average numbers of lesions observed for 15 replicates (one plant per replicate).
[d] The t-value is the estimate divided by the standard error of that estimate. Each t-value represents the value for the contrast of the means from the plus and minus ozone columns of that row.

plants then was exposed to 0.10 ppm (196 $\mu g/m^3$) ozone, and the other to 0 ppm ozone, for 6 hr per day, for 4 consecutive days. The daily exposures were conducted from 1000 hours to 1400 hours. One day after the final exposure period, the width and length of 10 lesions from each inoculated leaf were measured with a vernier caliper and multiplied to obtain an estimate of the area of each lesion. These areas were averaged for each leaf. A similar experiment was conducted in which the ozone concentration was 0.14 ppm (275 $\mu g/m^3$). Lesions of *B. squamosa* were consistently larger and those of *B. cinerea* were occasionally larger on leaf 1 than on leaf 2 (Table 7). There were no significant differences between the size of lesions on leaves of exposed plants and the size of lesions on leaves of nonexposed plants regardless of the pathogen, isolate, or concentration of ozone.

DISCUSSION

Symptomatology and terminology of onion leaf diseases involving *B. cinerea, B. squamosa,* ozone, and water stress. The nature and symptomatology of leaf diseases of onion grown on organic soils in the northeastern United States and eastern Canada involving *Botrytis* spp., ozone, and water stress were poorly understood until pioneering studies on these were completed during the period 1960–1980. A precise terminology and symptomatology for these diseases provided the basis for diagnosing and evaluating the interactions of these disorders as they occurred under field and controlled environmental conditions in the present study.

The pathogenesis of *B. cinerea* and *B. squamosa* on onion leaves and the injury of onion leaf tissue by ozone have been characterized and the resulting disorders described and named (2,6,24). The symptoms of these disorders and those induced by water stress (resulting from drought conditions following extended periods of moisture) are somewhat similar. The confusion caused by these similar symptoms and the overlapping terms published to describe these symptoms and name the disorders has needed clarification, which has been attempted in the present study. Diseases induced by *Botrytis* spp. and leaf tip dieback resulting from water stress have been called blast (12,21). Botrytis leaf blight and Botrytis leaf fleck have been distinguished on symptomological differences caused by *B. squamosa* and *B. cinerea*, respectively (6). Injury of onion foliage due to water stress has been called tipburn (3). Ozone-induced injury of onion also has been called tipburn (4).

Differentiation of the individual disorders can be achieved by comparison of their respective syndromes. Ozone injury of onion leaves includes tip necrosis and small necrotic specks that are typical of foliar injury induced by ozone on many plant species and that conventionally has been described as flecking. The necrotic lesions induced by *B. cinerea* on onion leaves are superficial (not penetrating to the lacuna) and nonexpanding and also have been

TABLE 6. Average numbers of lesions induced by *Botrytis cinera* or *B. squamosa* per square centimeter of leaf surface of onion plants exposed to 0, 0.08, or 0.12 ppm ozone for 5 hr/day on 3, 4, or 5 consecutive days and inoculated immediately after the final exposure period[a]

Species	Ozone concentration (ppm)	No. of exposures	Ozone[b] Plus	Ozone[b] Minus	t-value[c]	Significance level (P)
B. cinerea	0.08	4	0.64	0.72	0.43	0.6982
		5	0.74	0.62	0.63	0.6013
	0.12	3	0.49	0.64	0.58	0.6742
		4	0.92	0.45	2.10	0.0143
B. squamosa	0.12	4	2.40	2.86	0.89	0.5032
		5	2.15	2.00	0.60	0.5987

[a]Statistical contrasts were made using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511). Means within the plus ozone and minus ozone columns of each row were contrasted.
[b]Data are the mean numbers of lesions for 20 replicates (one plant per replicate) and were obtained from the second oldest nonsenescing leaves of each plant.
[c]The t-value is the estimate divided by the standard error of that estimate. Each t-value represents the value for the contrast of the means from the plus and minus ozone columns of that row.

TABLE 7. Average sizes of lesions induced by *Botrytis cinerea* and *B. squamosa* on leaves of onion plants exposed to different concentrations of ozone for 6 hr/day for 4 consecutive days starting 1 day after their inoculation or not exposed[a,b]

Pathogen	Ozone concentration (ppm)	Isolate	Leaf[c]	Ozone Plus	Ozone Minus	t-value[d]	Significance level (P)
B. cinerea	0.10	C-80-1	1	0.60	0.75	0.70	0.5012
			2	0.49	0.44	1.10	0.3493
		C-80-3	1	0.51	0.53	0.44	0.6542
			2	0.31	0.42	0.94	0.4012
	0.14	C-80-1	1	0.45	0.60	1.42	0.2300
			2	0.30	0.42	1.30	0.2900
		C-80-3	1	0.62	0.54	1.18	0.3172
			2	0.40	0.31	1.38	0.2452
B. squamosa	0.10	S-80-6	1	3.30	3.76	0.90	0.4012
			2	2.20	2.30	0.45	0.6301
		24-1	1	5.64	4.52	1.50	0.1988
			2	3.64	2.70	1.20	0.2998
	0.14	S-80-6	1	5.40	4.35	1.38	0.2860
			2	3.75	3.00	1.44	0.2432
		24-1	1	4.95	5.64	0.90	0.3989
			2	3.68	4.20	1.06	0.3593

[a]Statistical contrasts were performed using the estimate option of an SAS general linear model program (SAS Institute, Cary, NC 27511). Means within the plus ozone and minus ozone columns of each row were contrasted.
[b]Data are the average sizes of lesions in square millimeters. Ten lesions from both leaves 1 and 2 of 10 plants were measured and their values averaged.
[c]Leaves 1 and 2 represent the first and second oldest nonsenescing leaves of each plant.
[d]The t-value is the estimate divided by the standard error of that estimate. Each t-value represents the value for the contrast of the means from the plus and minus ozone columns of that row.

described as flecks. Flecks induced by *B. cinerea* (Fig. 2B) and specks induced by ozone (Fig. 2A) need not be confused. Necrotic flecks induced by *B. cinerea* are elliptical, average ~2 mm in length at maturity, and generally occur singly or in small groups of a few lesions. Individual specks induced by ozone are more spherical and smaller and generally occur in great numbers giving the affected tissue a stippled or, in more severe cases, a bleached appearance. The lesions induced by *B. squamosa* (Fig. 2C) are deep (penetrating to the lacuna) and continue to expand and coalesce and thus are easily distinguishable from lesions induced by *B. cinerea* and those resulting from ozone injury. For clarity, the term "tipburn" is best reserved for necrosis of onion leaf tips related to water stress. In the current study, injury of onion leaves induced by the action of ozone has been referred to as ozone-induced injury.

The symptoms of Botrytis leaf blight, ozone-induced injury, and tipburn each include varying degrees of leaf tip necrosis. Tip necrosis of any one of these diseases can not in itself be differentiated as to cause. However, the presence or absence of symptoms other than tipburn can indicate which causal agent was involved. Tip necrosis induced by *B. squamosa* is associated with lesions that penetrate to the lacuna of the leaf and a subsequent blighting of approximately half to all of the leaf. Tip necrosis induced by ozone generally is accompanied by necrotic stippling. Tip necrosis induced by moderate dosages of ozone seldom involves more than 1 cm of the leaf tip, although that induced by high dosages may involve up to one third of the leaf. Tip necrosis of onion leaves due solely to water stress would not be accompanied by either ozone-induced stippling or the lesions induced by *B. cinerea* or *B. squamosa*.

Interactions of ozone, *B. cinerea*, and *B. squamosa*. Both *B. cinerea* and *B. squamosa* induced typical lesions on leaves of ozone-exposed and nonexposed onion plants (exposure before inoculation) in the current study. Frequently, the average number of lesions induced per square centimeter of leaf surface of exposed plants was significantly greater than that induced on nonexposed plants. Although ozone can predispose onion leaves to enhanced infection by *B. cinerea* and *B. squamosa*, it is emphasized that this predisposition results in an increase in the susceptibility of the normally susceptible onion leaves and is not mandatory for the infection of such leaves by either *B. cinerea* or *B. squamosa*. Both a relatively moderate acute dosage of ozone (0.16 ppm for 4 hr) and a relatively high chronic dosage (0.12 ppm for 5 hr per day for 5 days) predisposed onion leaves (cultivar Autumn Spice) to enhanced infection by *B. cinerea*, but only a relatively high acute dosage of ozone (0.25 ppm for 4 hr), and none of the chronic dosages tested were successful in predisposing leaves to enhanced infection by *B. squamosa*. Thus, it is likely under ambient ozone levels that field-grown onion plants would be predisposed more readily to enhanced infection by *B. cinerea* than by *B. squamosa*. In light of the inability of *B. cinerea* to cause extensive damage to onion leaves, such infection in itself should have little if any economic impact. If ambient ozone levels equal or exceed the relatively high acute dosage required to predispose onion leaves to enhanced infection by *B. squamosa*, the epidemiology of Botrytis leaf blight of onion could be affected significantly. Long-term chronic exposure of field-grown onion plants to low or moderate levels of ambient ozone could similarly alter the epidemiology of Botrytis leaf blight if such exposure were to have a greater effect on any stage of the disease cycle than occurred with the relatively short chronic exposure periods utilized during the current study. In reality, even the dosage of 0.16 ppm for 4 hr, defined as relatively moderate in this study, is unlikely to occur naturally in New York. In any event, Botrytis leaf blight of onion can be expected to seriously threaten onion production in New York during seasons in which temperature and moisture conditions conducive to the development of this disease occur, regardless of ambient ozone levels.

Numerous factors, including tissue age, influence a plant's sensitivity to ozone (9) and may govern the potential of ozone to predispose that plant to infection by plant pathogens. The ozone-induced predisposition of onion leaves to enhanced infection by *B. cinerea* and *B. squamosa* accordingly was conditioned by the relative age of the leaves inoculated and the cultivar of onion used. In instances where predisposition occurred, leaves 1 and 2 generally were predisposed but leaf 3 never was predisposed to either species of *Botrytis*. This was undoubtedly due to the greater sensitivity of mature than of immature leaf tissue to ozone (9). Leaves 1 and 2 were fully expanded or nearly so when exposed to ozone, and therefore, were composed of mature leaf tissue, whereas leaf 3 was not fully expanded at the time of exposure and, therefore, was composed of immature leaf tissue.

Genetic background, as expressed in cultivar variation, can also regulate sensitivity to ozone in plants (9). The cultivars Fiesta and Autumn Spice were the most sensitive of eight onion cultivars tested for response to ozone in this study. Plants of onion cultivars more tolerant of ozone were not predisposed to *B. cinerea* or *B. squamosa* by dosages that are thresholds for the predisposition of ozone-sensitive cultivars. The onion cultivars Downing Yellow Globe and Early Yellow Globe, ranked as tolerant to ozone, and Sentinel, ranked as intermediate, are commonly grown in New York. Autumn Spice and Fiesta generally are not grown in New York. The potential for onion plants grown in New York to be predisposed to *B. cinerea* or *B. squamosa* by ambient ozone in the past may have been tempered unintentionally by use of cultivars relatively tolerant of ozone. However, the sensitivity of certain plant cultivars to ozone is greater when the cultivars are grown in the field rather than under controlled conditions (8,17). The cultivars Downing Yellow Globe, Early Yellow Globe, and Sentinel, therefore, might be more sensitive to ozone when grown in the field than when grown under the growth chamber conditions in the current study. However, plants of the cultivars Downing Yellow Globe and Sentinel were not visibly injured by the levels of ambient ozone monitored in field plots established during the current study.

Field-grown onion plants similarly may have been protected in the past from ozone-induced predisposition to *B. cinerea* or *B. squamosa* by use of dithiocarbamate fungicides and the more recent use of chlorothalonil. These fungicides have antioxidant as well as fungistatic properties (18,24). However, the extent of protection from ozone that these compounds would afford field-grown onion plants has not been determined.

The mechanism regulating ozone-induced predisposition of onion leaves to enhanced infection by *B. cinerea* and *B. squamosa* has not yet been precisely elucidated. One hypothesis suggests that necrosis induced by ozone serves as an infection court for *B. cinerea* and *B. squamosa*. An infection court hypothesis has been suggested to explain the ozone-induced predisposition of potato and geranium to *B. cinerea* (15,16). In the present study, predisposition of onion leaves to *B. cinerea* was detected equally in regions of leaf surface exhibiting macroscopic necrotic specks induced by ozone and in regions of leaf surface of the same leaves not exhibiting such specks. In the former situation, lesions induced by conidia of *B. cinerea* generally did not appear to arise from individual necrotic specks induced by ozone. Also, predisposition to both *B. cinerea* and *B. squamosa* was detected in certain instances when onion leaves were inoculated immediately after the plants were exposed to ozone. Necrotic specks generally did not develop until ~36–72 hr after the exposure period. Lesions induced by either pathogen generally were visible macroscopically 24 hr after inoculation. Therefore, the presence of infection courts in the form of ozone-induced necrotic specks was not fundamental in the predisposition of onion leaves to enhanced infection by *B. cinerea* or *B. squamosa*. However, this does not preclude the possibility that a microscopic infection court of either a single necrotic cell or several contiguous necrotic cells was involved in the predisposition. An alternative hypothesis to the infection court mechanism of predisposition can be postulated. It is known that ozone can injure the cell membrane, causing it to leak (9), and also that lesion induction by *B. cinerea* and *B. squamosa* can be increased by adding nutrients to the inoculum (2). Therefore, lesion formation on leaves of onion plants exposed to ozone may have been stimulated by abnormally high nutrient concentrations at the leaf surfaces, which could have resulted from leakage of nutrients through cell membranes disrupted by ozone. Evidence in support of this hypothesis has been developed (20).

The potential for ozone to interact with *B. cinerea* and *B. squamosa* could result from the action of ozone directly on the pathogen or from the action of ozone on the host that may in turn influence development of the pathogen. In the current study, chronic exposure of plants infected with *B. cinerea* or *B. squamosa* to a maximum of 0.14 ppm ozone for 5 hr per day for up to 5 days had no detectable effect on expansion of preestablished lesions induced by these pathogens. Sporulation of *B. cinerea* and *B. squamosa* occurred only occasionally under the conditions of these experiments and did not appear to differ in frequency, extent, or density of conidial production on plants exposed to ozone compared to those not exposed. Heagle (7) detected decreased conidiation and an increase in the length of lesions induced by *Helminthosporium maydis* Nisikado race T on corn plants chronically exposed to ozone after infection by the pathogen. Krause and Weidensaul (13) observed that conidiation of *B. cinerea* on geranium plants also was decreased by exposure of infected plants to 0.15 ppm (294 $\mu g/m^3$) ozone for two 6-hr periods. The percentage germination of conidia that developed during those exposures also was decreased as was the ability of those conidia that germinated to infect geranium leaves (13). Such ozone-induced decreases in conidial production and/or viability could limit the enhancement of infection resulting from ozone-induced predisposition of onion leaves or could even decrease the amount of infection that occurs. Conversely, ozone-induced increases in lesion expansion could augment the total amount of disease observed, if such increases occur in response to levels of ozone greater than those tested in the current study and if those levels occurred under field conditions.

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