Ozone and Botrytis Interactions in Onion-Leaf Dieback: Open-Top Chamber Studies

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ABSTRACT

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Paired open-top chambers were used to study interactions between *Botrytis* spp. and ozone in field-grown onions. Charcoal filters removed 35 to 65% of the ambient ozone, resulting in six-fold reduction of onion leaf dieback and a 28% increase in onion yield compared with unfiltered chambers. Symptoms of leaf injury appeared soon after ozone levels exceeded 294 $\mu g/m^3$ (0.15 ppm) for 4 hr. Lesions caused by *Botrytis* were few because no dew formed in the chambers. However, when leaves were wetted with foggers, inoculation with mycelial suspensions of *B. squamosa* in late August produced significantly more lesions and leaf dieback in the unfiltered chamber. *Botrytis squamosa*, *B. cinerea*, *B. allii*, and several genera of secondary fungi were isolated from these lesions. *Botrytis squamosa* was recovered from lesions only, whereas *B. cinerea* and *B. allii* were associated more generally with onion leaf tissue regardless of lesions.

Additional key words: Yield loss, onion, blast, open-top chambers.

Onion leaf dieback and flecking have been variously attributed to several parasitic and nonparasitic agents (4, 5, 6, 12). Tip burn, which develops rapidly in the absence of detectable fungi, has been called "blast" and is generally associated with severe drying conditions following moist conditions (12). A close relationship may also exist between onion-leaf dieback and elevated levels of ozone in the atmosphere (5). Ozone injury was accompanied by flecking, sometimes penetrating to the lacuna.

Several *Botrytis* spp. that infect onion leaves can produce various flecking and tip dieback symptoms (6, 10, 21). These symptoms also have been called blast (21). Thus the "blast" syndrome involves several distinct diseases. Hancock and Lorbeer (6), however, on the basis of symptomological and etiological differences, distinguished *Botrytis squamosa* leaf blight and *Botrytis cinerea* leaf fleck from "blast".

Exposure of some plants to ozone predisposes them to *Botrytis cinerea* infection (16, 17, 18). The similarity of symptoms on onion attributed to either *Botrytis* spp. or ozone suggests that both agents are involved, or that they interact. The present study was made to evaluate the relative contributions of both *Botrytis* spp. and ozone to leaf dieback of onions.

Since many environmental parameters can influence the degree of injury caused by various agents, it was decided to conduct a study on pollutant-disease interactions using open-top chambers over a stand of onions in the field. The use of paired chambers, one equipped with a charcoal filter to remove ozone and the other not, provided a means of reducing ozone levels in the field with minimum effect on other environmental parameters (8, 15). Ozone injury and its effect on the incidence of *Botrytis* infection could be investigated by comparing the filtered air treatment with those receiving ambient ozone levels in the chamber and in the open field.

MATERIALS AND METHODS

Onions (*Allium cepa* L. 'Autumn Spice') were sown in organic soil at the Ontario Ministry of Agriculture and Food Muck Research Station at Bradford. Forty to 50 seeds were planted per meter of row. Rows were 0.43 m apart. Normal herbicide, fungicide, insecticide, and fertilizer treatments were applied until experimentation began.

When plants had reached the second true leaf stage (12 June), cylindrical open-top chambers (2.7 m in diameter), one with and one without an activated charcoal filter, were placed over uniform areas of the existing crop. The chambers, built according to prototypes developed at the Boyce Thompson Institute (15), were spaced to avoid between-chamber shading. A third chamberless control plot (2.7 m in diameter) was located 3 m from the chambers. The original chamber design was modified in several ways. Corrugated "Clear-Lite Supreme" (Atlas Asbestos Company, Montreal, Quebec) fiberglass panels were used in wall construction, and 1.3-cm grid nylon mesh covered the open top. A corrugated plastic pipe 15.2 cm in diameter with 2-cm-diameter holes cut at 3-cm intervals, was oriented around the inner base of the chamber with the holes directed inward and perpendicular to the chamber walls. Air flow through this pipe was more uniform than with the long flat polyethylene tubes originally employed. This prevented air stagnation while increasing blower output due to decreased pressure (K. M. King and P. M. Smith, *personal communication)*].

An aspirated psychrometer, shielded mercury thermometers, a light meter with a visually corrected filter, and a hot-wire anemometer were used to measure relative humidity, temperature, light intensity, and air velocity, respectively. A calibrated chemiluminescence ozone analyzer (Bendix Model 8003 Ozone Analyzer, Bendix Process Instruments Division, Roncevette, WV 24970) was used to monitor external ambient and chamber ozone levels from 7 July to 1 August. Supplementary data, from 13 August until 19 September, was obtained from Atmospheric Environment Service (Environment Canada) chemiluminescence monitoring in close proximity to the Muck Research Station.

Visual assessments of leaf necrosis and incidence of Botrytis spp. were made 14, 21 and 30 July, 13 and 25 August, and 19 September. The youngest five leaves of ten randomly-sampled onion plants were rated for percentage necrotic leaf area using a modified Horsfall-Barratt (21) scheme, where: 0 = 0%, .5 = 0-1%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-94%, 9 = 94-97%, 10 = 97-99%, 11 = 99-100%, and 12 = 100% injury. Intensity of *Botrytis* was assessed on the same sample by counting the number of lesions per leaf. Analysis of variance was performed on both data sets, and Tukey's *w* procedure (23) served as the multiple comparison test for treatment means. Due to the non-normal distribution of percentage leaf necrosis with values below 20%, these data were arcsin-square root transformed, then analyzed.

Lesions caused by *Botrytis* were rare on onions by mid-August in either chamber when compared with control plots. Since air injection fans were running continuously, it was thought that air turbulence within the chamber at night prevented dew formation and, hence, spore germination and infection by *Botrytis*. Timers were installed on 13 August to shut down the air injection system from 2100 hours to 0900 hours in an effort to increase leaf wetness, but no significant amount of *Botrytis* infection occurred within either chamber by 19 August. Probably chamber walls prevented the infra-red radiation necessary for cooling the onion leaves to the dewpoint temperature (25). A fogger was thus installed at 1.2 m height within each chamber on 25 August, and onions were uniformly fogged at least 20 min in the evening after blowers were shut off. Dew was simulated until 3 September on days when no natural precipitation occurred.

By 25 August, onions had matured in the control plot, but not in the chambers. Plants were harvested by taking four replicate, meter-long samples from the same location in each chamber. Eight samples were taken from the control plot. Samples were cured for ten days, topped, weighed, and analysis of covariance was computed on bulb weights with plant density as the covariate (23).

The effect of oxidant air pollution on infection by *Botrytis squamosa* was studied using isolate number 66-6b (obtained from J. W. Lorbeer, Cornell University, Ithaca, New York). Mycelium of a 2-wk-old culture [grown on potato-dextrose agar (PDA) at 20 C in the dark] was homogenized in a physiological saline solution (8.5 gm NaCl/liter sterile distilled water), and adjusted to 0.50 optical density at 450 nm. Plants in each chamber were sprayed with 200 ml of the suspension with 0.1 ml of Tween-20 surfactant per 100 ml on 25 August, and another 100 ml on 27 August, using an aerosol atomizer.

On 19 September, five 1-m-long samples from each chamber were assessed for injury and harvested for final yield as before. In addition, samples of leaf tissue were taken from both chambers to characterize fungi present on and in the lesions. Leaf pieces (1 cm^2), with and without *Botrytis* lesions, were either surface-sterilized or washed and plated separately on acidified (pH 5) PDA. Fifty pieces were rinsed three times in sterile distilled water, and 55 pieces were dipped sequentially for 15 sec into each of 70% ethanol, 0.6% sodium hypochlorite, and sterile water before plating.

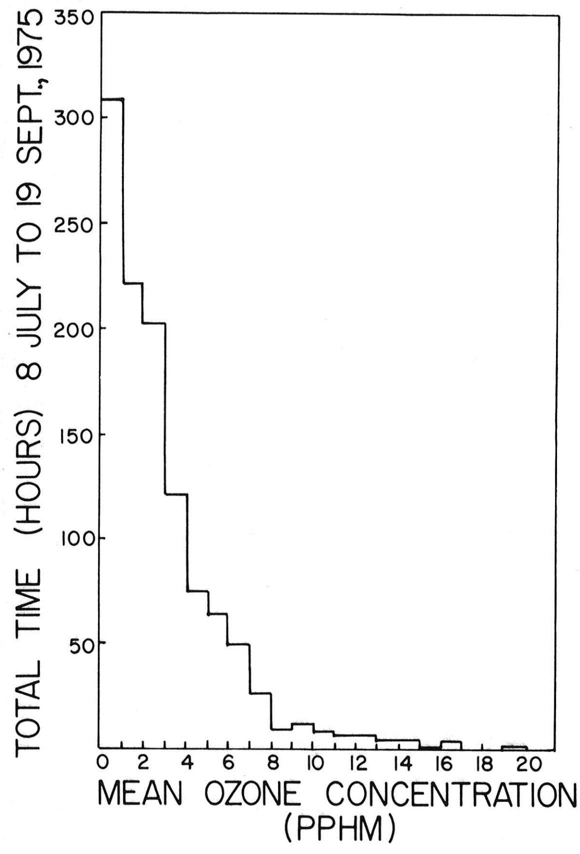

Fig. 1. The relative frequency of mean hourly ozone concentrations from 8 July to 19 September at Bradford, Ontario, 1975.

RESULTS

Influence of chambers on the environment.—The temperature, relative humidity, light intensity, and air movement all were the same in filtered (F) and unfiltered (UF) chambers. Compared with the no chamber plot (NC), the chambers did not affect relative humidity or temperatures under cool or moderate weather conditions, but as reported (8, and K. M. King and P. M. Smith, *personal communication*) on bright, hot days, temperatures within chambers were elevated above ambient by 1.0 ± 0.5 C. Furthermore, although light intensity during cloud cover was comparable with that outside the chambers, enclosed plants received only from 65 to 90% of ambient light on sunny days depending on their location in the chambers. Maximum daily radiation was received at the chamber center, and the northern sector.

Initially, the charcoal filter removed up to 90% of the pollutant. However, downflux from the open tops of the chambers increased with the windspeed, and gave ozone levels at plant height which were between 35 and 65% of ambient ozone, as previously reported (8, K. M. King and P. M. Smith, *personal communication*). Ozone levels also increased with distance from the air injection manifold. Pollutant concentrations in the UF chamber closely approximated ambient levels except on still days when levels in the UF chamber were slightly depressed.

Ozone monitoring.—Ozone was monitored intermittently during the growing season, but in considerable detail during the high-oxidant periods in July. Major oxidant episodes were recorded on 13 July and again on 30 July to 2 August when hourly average ozone concentrations exceeded 294 $\mu g/m^3$ (15 pphm). Minor episodes [in excess of 157 $\mu g/m^3$ (8 pphm) ozone] occurred on at least five separate occasions, namely 7, 12, 15, 17, and 29 July. Levels at Bradford during July, August, and September exceeded 8 and 10 pphm for 51 and 31 hr respectively, representing 4.6% and 2.8% of the total (Fig. 1). A maximum average hourly concentration of 380 $\mu g/m^3$ (19.4 pphm) was observed on 30 July.

Leaf injury.—Slight ozone incited flecking and tipburn, as described by Engle et al. (5), were apparent on onions in the field and in the UF chamber on 14 July and throughout the growing season. Although differences in extent of injury between F and UF chambers were not significant on 14 July, sufficient levels of ozone had occurred by 21 July and thereafter so that injury was greater in the UF chamber. During most of August, ozone concentrations were less frequently phytotoxic than during July and this was reflected in less new injury within both chambers. The greater amount of leaf necrosis in the field by 25 August was due to the added effect of crop senescence.

Leaf necrosis, measured on the youngest five leaves only, fluctuated considerably during the growing season as a result of leaf development and sporadic ozone episodes. The amount of necrosis in the F air was consistently lower than that in the UF air and NC treatment, the difference being greatest after major ozone episodes. Differences between the UF air and the NC treatment were variable, but the NC treatment showed more injury toward the end of the season. *Botrytis* lesions were consistently more numerous in the open field than in either chamber.

With each assessment date regarded as a block containing three treatments, analysis of variance was performed on both the injury due to ozone and due to *Botrytis* as shown in Table 1. There was no significant difference between leaf necrosis in the UF and NC treatments taken over the entire season. Onion leaf dieback in F air was only one-sixth of that found in UF air, and leaves in the UF air were also visibly more chlorotic than in F air. Before inoculation with *Botrytis squamosa*, there was no significant difference in the incidence of *Botrytis* between chambers. Over twelve times the number of lesions were observed in the open field compared to plants in the chambers. Post inoculation infection levels were 237% higher in the UF air than in F air when an adequate leaf wetness period was provided. More frequent *Botrytis* sporulation on necrotic leaf tips, *Alternaria porri* (purple blotch) lesions and scallions (thick-necked, immature onions) were noticed in the UF than in the F chamber. Within-chamber onions required almost a month more to mature than field plants, and were characteristically taller than plants grown outside.

Fungal isolations.—The *Botrytis* sp. most frequently isolated from characteristic lesions was *B. squamosa*, though *B. allii* and *B. cinerea* also were found. Secondary fungi, like *Penicillium* spp., especially *P. decumbens*, *Stemphylium botryosum*, and *Alternaria* spp., especially *A. alternata* were isolated more frequently from *Botrytis* lesions than from the leaf surface, indicating actual invasion of the already parasitized onion tissue.

Yield effects.—Prior to *Botrytis squamosa* inoculation, the weight of onion bulbs was 28% greater in the F chamber than in the UF chamber (Table 2). In the final harvest following *Botrytis* inoculation, only a 21.6% increase in bulb weight over the UF treatment was found. Although onions in the field yielded 9.3% more than in the UF chamber, the difference was not statistically significant at $P = 0.05$.

TABLE 1. Leaf assessment indices for percent necrosis and number of lesions caused by *Botrytis* spp. on onion foliage in open-top chamber treatments at Bradford, Ontario, 1975[x,y]

Time of assessment	Chamber treatment	Necrosis (%)	Number of lesions caused by *Botrytis* spp.
Before inoculation with *Botrytis squamosa*	Charcoal filtered (F)	0.32 a	0.11 a
	Unfiltered (UF)	1.84 b	0.24 a
	Check (NC)	1.49 b	2.95 d
After inoculation with *Botrytis squamosa*	Charcoal filtered (F)	0.50 a	0.83 b
	Unfiltered (UF)	2.46 c	1.97 c
	Check (NC)	... HARVESTED ...	

[x]Tested at $P = 0.05$, treatments followed by the same letter are not significantly different, mean separation by columns.

[y]Leaf assessment indices are means of percent necrosis and number of lesions caused by *Botrytis* sp. from all respective assessments throughout the 1975 season on a per leaf basis.

DISCUSSION

Responses to ozone in open-top chambers.—Differences in symptom expression and yield between the UF and F chambers were attributed to reduced ozone levels. No other significant differences in environmental conditions between the chambers were observed. The negligible numbers of *Botrytis* lesions in either chamber before inoculation also eliminated the pathogen as a factor in the observed symptomology and yield effects. Thus, before inoculation with *Botrytis squamosa*, the almost six-fold reduction in leaf necrosis and corresponding 28% increase in bulb size in the F air were due to diminished ozone air pollution. These results are similar to open-top chamber studies conducted with other crops at other locations (13).

It is arguable that, because ozone levels in the F chamber were only reduced by 35 to 65%, an adequate picture of ozone effects was not portrayed. Bennett et al. (2) cogently assert that low ozone levels, since they reflect normal ambient concentrations to which plants have adapted, would better serve as controls rather than atmospheres completely filtered of this pollutant. Hence, ozone reductions of the scale presented in this paper are representative of characteristic 0 to 5 pphm background concentrations in Ontario and thus fairly approximate remote ambient conditions.

Plants grown under low light intensities tend to be more sensitive to ozone (9), and this was reflected in the UF chamber where plants had more injury than those in the field. The light intensity in the chambers was reduced by 10 to 35% by the walls and covering mesh on the top. These lower light intensities were no doubt responsible for the increase in the amount of foliage, the longer leaves and delay of onion maturity in the chambers. Because of differences in growth habit and in susceptibility to ozone of chamber-grown plants, caution is warranted in making comparisons between chamber and field-grown plants.

Leaf necrosis and *Botrytis* lesions observed on the five youngest leaves underestimated the actual extent of injury sustained by the whole plant. Some of these leaves were not fully expanded and, hence, were not maximally susceptible to the pollutant or pathogen. Nevertheless, these leaves gave the most accurate index of newly occurring ozone and *Botrytis* injury. Older leaves are more subject to senescence, mechanical injury, lodging, and invasion by saprophytic organisms, making it difficult to distinguish ozone and *Botrytis* injury.

TABLE 2. The effects of open-top chamber treatments, ozone injury, and incidence of *Botrytis* sp. on onion yield at Bradford, Ontario, 1975

	Yield[x] (metric tons/ha)	
Chamber treatment	Before inoculation with *Botrytis*	After inoculation with *Botrytis*
Charcoal-filtered (F)	38.3 a[y]	66.7 a[z]
Unfiltered (UF)	30.0 b	54.8 b
Check (NC)	...	60.0 ab

[x]Mean separation by column.
[y]Significant at $P = 0.01$.
[z]Significant at $P = 0.05$.

Determination of cumulative ozone and fungal effects would have necessitated assessment of the same cohort of five leaves throughout the season.

In addition to underestimating injury to the entire plant, the amount of necrosis on the youngest five leaves did not adequately account for the 28% difference in bulb weight of the F over the UF treatment. The lower leaves not used in the assessment had greater amounts of damage and probably also had a significant impact on bulb growth. The general chlorosis observed in the UF chamber was no doubt a contributory factor as well. Reduced photosynthesis has been observed in plants at ozone levels too low to cause visible injury (24). A combination of these factors, and possibly others, were responsible for the suppression of bulb growth.

The most critical growth stage in terms of effects of leaf area on onion yield is when the plant is reaching maximum vegetative development and initial bulb enlargement (1, 7). This critical stage occurred in chamber treatments coincidentally with high ozone levels in July. New leaves that emerged after the beginning of August exhibited very little ozone damage and this might have compensated to some extent for earlier injury. More new leaves emerged in the chambers after 1 August than in the field due to chamber effects delaying plant maturity.

Responses to Botrytis in open top chambers.—The results indicate that 'Autumn Spice' onions were predisposed to *B. squamosa* infection when previously injured by ozone. After inoculation with the mycelial suspension, many more *B. squamosa* lesions developed in the chamber supplied with ambient air than in the chamber with filtered air (Table 1). Even before inoculation, the number of *Botrytis* lesions throughout the entire growing season was consistently, though not significantly, higher in the UF than in the F Chamber.

Two mechanisms are suggested to explain the greater susceptibility of ozone-injured onions to *Botrytis* sp. Firstly, it is well established that ozone causes membrane disruption and subsequent cell leakage (14). The exosmosis of nutrients from plant organs through the stomates or other pathways has been shown to be important in *Botrytis* spp. prepenetration activities (3). Ozone injury could thus have enhanced germination and penetration into the leaf by *Botrytis*. Secondly, a marked increase in flecking and tipburn occurred in the UF chamber, and more sporulation was observed on these necrotic leaf tips than in the F chamber during periods of high relative humidity. Small (22) noted in greenhouse experiments that leaves, which were necrotic before *B. squamosa* inoculation, produced secondary inoculum spores twice as fast as did healthy leaves. Subsequent experiments showed that necrotic leaves generally gave rise to a greater number of conidiophores than did healthy leaves, and Small suggested that necrotic leaves may have been of greater importance to Botrytis leaf blight epidemiology than previously realized. Perhaps ozone flecking and tipburn permitted greater saprophytic colonization of necrotic onion tissue. Such colonization may promote the build up of formidable amounts of secondary inoculum and thereby increase the incidence of Botrytis blight.

Isolation of fungi from onion tissue.—The *Botrytis* species isolated from lesions on chamber-enclosed onions were similar to those found in earlier studies (7, 11, 19, 20, 21). Growth of *Botrytis allii* and *B. cinerea* from healthy and lesioned tissue, especially in nonsurface-sterilized leaf pieces, indicated a rather superficial association with the onion leaf. On the other hand, *B. squamosa* was found to be present only on lesions. Invasion by secondary fungi such as *Stemphylium botryosum*, *Penicillium* spp., *Alternaria* spp., and others may be partly responsible for the low frequency of recovery of *Botrytis* spp. Alternatively, Segall and Newhall (21) and, more recently, Clark and Lorbeer (3), observed that lesions of *Botrytis* spp. on onion leaves may result from tissue breakdown associated with enzymes or toxins without subsequent invasion by the pathogen. Such a situation was referred to as a unique case of pathogenesis without ensuing parasitism. Lesions thus created would present an ideal infection court for many saprophytes or facultative parasites like those already mentioned. *Stemphylium* spp. especially have been regarded as important onion pathogens in the Bradford muck vegetable growing areas (19).

Further studies are required to understand ozone sensitization of onions to *Botrytis* spp. The open-top chambers provided a means to explore further the insidious nature of ozone injury in terms of symptom expression and yield reduction in bulbing onions. The utility of open-top chambers can also be extended to include air pollutant-plant disease interaction, particularly with appropriate modifications for dew production.

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