Response of White bean to Bacterial Blight, Ozone, and Antioxidant Protection in the Field

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ABSTRACT

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An interaction between ozone and bacterial blight incited by *Xanthomonas phaseoli* on white beans (*Phaseolus vulgaris* 'Sanilac') was investigated in the field by reducing ozone injury with the antioxidant EDU (N-2-(2-oxo-1-imidazolidinyl) ethyl-N-phenylurea). Plants were inoculated with *X. phaseoli* at time of flowering and weekly sprays of EDU at 1 kg/ha were begun at the same time. Potentially phytotoxic concentration of ozone in excess of 8 pphm were recorded 11 times during the summer of 1977. Infection with *X. phaseoli* reduced symptoms of foliar ozone injury 19% on plants not treated with antioxidant but *X. phaseoli* did not protect against ozone injury on treated plants. Ozone injury did not protect against blight infection. Plants protected with EDU averaged 38% less ozone injury and became defoliated 3 wk later than control plants. Yields of EDU-protected plants increased 24% relative to unprotected controls. Weight of bean seeds was inversely correlated with per cent leaflet abscission, suggesting that increases in yield of EDU-protected white bean plants may have been due primarily to reduced defoliation rather than to reduce foliar injury.

Additional key words: air pollution.

Interactions among plants, plant pathogens, and ozone have been studies extensively in recent years (7, 19). Infections of plant viruses reduce the susceptibility of plant hosts to ozone injury (1,2,4,23), although an exception to this pattern has been reported recently (26). Reduced ozone injury has been observed on plants infected with wheat stem rust fungus (10) and *Botrytis* (17). Ozone increased the incidence and severity of *Botrytis* infections on potato (21), geranium (20), and onions (32); however, ozone reduced leaf infection and decreased hyphal growth of wheat stem rust (9) and crown rust of oats (6), and reduced infectivity of powdery mildew on barley (11). Plants infected with bacterial pathogens and exposed to ozone show increased resistance to ozone injury and/or increased resistance to bacterial infection (13,14). Inoculation with a hypersensitive-response-inducing pseudomonad 24 hr before exposure to ozone reduced ozone symptom expression in soybean foliage, but inoculation 4 hr before exposure to ozone increased foliar injury symptoms (25). Alfalfa showed significantly reduced bacterial leafspot injury after exposure to ozone, and bacterial infection also reduced ozone injury to alfalfa leaves (12).

With few exceptions (1,32) these interaction studies were conducted under controlled laboratory conditions. Similar experiments in the field present the problem of finding a suitable ozone-free area to use as a control. Open-top chambers (8,18) equipped with charcoal-filtration systems have been used in the field to study effects of ambient oxidants on yield and crop quality (16,28) and on ozone-*Botrytis* interactions (33). But differences in ozone response between field-grown plants and those grown in an open-top chamber have been observed (28,33) and perhaps are caused by reduced light intensity and higher humidity in the chambers. The development of foliar sprays that offer protection against ozone injury (15,22,24) suggested an alternate method for protecting test plants in the field. A compound known as EDU [N-(2-oxo-1-imidazolidinyl) ethyl-N′-phenylurea] appears to be the most effective specific antioxidant for beans (3) and onions (32) developed to date. The chemical characteristics of this compound have been described (3).

White bean (*Phaseolus vulgaris* L 'Sanilac') is a widely-grown crop in southern Ontario, and the cultivar is highly susceptible to ozone injury in the field (31). *Xanthomonas phaseoli* (E.F. Sm.) Dows. is a wide-spread pathogen on white beans in Ontario, and it is the primary agent of epidemic outbreaks of bacterial blight in southern Ontario (27,29,30).

The purpose of this study was to investigate the interaction between bacterial blight, ozone, and foliar injury and yield of white beans, and to assess the effectiveness of EDU in reducing ozone injury on white beans in the field.

MATERIALS AND METHODS

This field trial was conducted in Stouffville, Ontario, about 40 km NE of Toronto. A calibrated Bendix Model 800 chemiluminescence-type ozone analyzer (Bendix Co., Ronceverte, WV 24970) maintained by the Air Quality and Meterology Section, Ministry of the Environment, was used to monitor ambient ozone concentrations during the experiment. The instrument was located about 100 m from the bean plots.

Seeds of white bean were sown 28 May 1977 in plots measuring 2 × 1 m in three rows 2 m long spaced 45 cm apart. The plots were spaced 1 m apart and prepared and planted according to standard commercial practices. Four treatments were replicated four times in a randomized Latin square design. The treatments were: (i) inoculation with *Xanthomonas phaseoli*; (ii) spraying with EDU; (iii) inoculation and spraying with EDU; and (iv) control.

A culture of *X. phaseoli* was obtained from V. R. Wallen, Canada Department of Agriculture, Ottawa, Canada. The culture was maintained on plates of yeast extract-dextrose-calcium carbonate agar. Inoculum was obtained from a 2-day-old culture grown on Difco nutrient agar at 27 C. Plants were sprayed at time of flowering with a suspension containing about 5×10^7 cells/ml delivered via a pressure sprayer at nozzle pressures that caused

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water soaking of the leaf tissue. Plants were sprayed with water prior to inoculation in order to provide a favorable microclimate for bacterial infection (27).

The EDU (DPX-4891, 50WP, [0.5 lb AI/lb]) was obtained from E. L. Jenner, E. I. DuPont de Nemours & Co., Wilmington, DE 19898. Plants were sprayed to runoff with EDU at a rate of 1.1 kg/ha active ingredient + 0.1% Tween-20 (ICI Inc., Wilmington, DE 19897) with a knapsack sprayer. Application of EDU began 5 July 1977 at the start of flowering and the plants were sprayed at 8-day intervals until 17 Aug 1977, a total of seven applications.

Plants were rated for disease reaction and ozone symptoms from July 5 (when ozone symptoms were clearly visible) until 15 Aug when severe defoliation began on plots not sprayed with EDU. Three plants were chosen at random from center rows of each plot and percent leaf necrosis induced by ozone and/or bacterial blight was assessed in increments of 10% on five middle leaves of each plant. Leaves that exhibited ozone damage or blight symptoms were collected and examined for fungal or bacterial pathogens. Leaves were surface-sterilized in 0.5% sodium hypochlorite for 2 min, then washed in distilled water. Leaf sections were incubated at 27 C in petri plates containing Difco agar and 2% malt extract.

The plots were harvested on 2 Sept 1977. Yields were determined from the weight of shelled beans from the middle rows of each plot.

In vitro studies of EDU toxicity. A bacterial suspension containing 5×10^7 cells/ml was diluted from 10^{-3} to 10^{-6} and 0.1 ml of the dilution was spread on the surface of nine nutrient agar plates containing 0, 0.3, 0.5, and 1.0 g/L EDU plus 0.06% Tween-20. Isolates from plates were tested for pathogenicity by injecting aqueous suspensions of colonies into primary leaf nodes of 2-wk-old bean plants in the field.

RESULTS

Ambient ozone concentrations. Ozone concentrations during the daylight hours (0600-2000 hours) of June, July, and August, 1977, averaged 42, 42, and 28 μliters/m^3, respectively. Hourly average concentrations exceeded 80 μl/m^3 twice in June for a total of 11 hr, seven times in July for 20 hr, and twice in August for 3 hr above 80 μl/m^3. Eight excursions above 80 μl/m^3 occurred between 27 June and 18 July, when the beans were in the flowering stage when field-grown white beans become most susceptible to foliar ozone injury (5).

Foliar injury. Upper surface flecking and bronzing symptoms induced by ozone were first observed on mature leaves during the week of 5 July, about 40 days after emergence. Lesions covered about 15% of leaf surface at this time. Foliar injury symptoms and premature abscission increased in intensity on older bean leaves as the season progressed, and 90% of leaflets on control plants had abscissed by 22 August. In contrast, foliar ozone symptoms were significantly less severe on plants infected with *X. phaseoli* or sprayed with EDU (Table 1). Bacterial blight infection decreased foliar ozone injury by an average of 19% in plots unprotected by the antioxidant, but on plots sprayed with EDU, ozone injury increased slightly in bacteria-infected plants. The antioxidant itself reduced foliar ozone injury on white beans by 38%, and only 13 to 20% of leaflets had abscissed on sprayed plants by 22 August (Table 1).

Symptoms of bacterial blight developed on inoculated plants about 10 days after the 5 July inoculation. Bacterial blight lesions were located predominantly on the margins and near the base of the leaflets. By 15 August lesions covered about 15 to 17% of leaf surface of infected plants and no symptoms of blight were found on uninoculated plants (Table 1). The percent leaf area infected was not significantly different between plants treated or not treated with EDU, but defoliation was significantly less on EDU-treated plants. No pod infections were observed.

Effects on yield. Yields of plants infected with bacterial blight and those of control plants were not significantly different. However, plants sprayed with the antioxidant EDU had significantly greater yields than unsprayed plants, whether inoculated with bacterial blight or not (Table 2). Yields of uninfected plants sprayed with the antioxidant averaged 24% greater than unsprayed plants. Infected plants protected with EDU averaged 16% greater yield than unsprayed plants. Correlation of yield with percent defoliation was significant ($r = 0.97$; $p > 0.05$), but correlations of yield with amount of foliar ozone injury or the total of ozone and bacterial injury were not significant.

In vitro studies. At concentrations up to 1.0 g/L, EDU had no effect on number of *X. phaseoli* colonies that grew on nutrient agar. Isolates of colonies grown with 1.0 g/L of EDU in nutrient agar showed no decrease in infectivity when tested on white beans in the field.

DISCUSSION

Infection with *X. phaseoli* significantly reduced ozone injury on field-grown white beans. These results are consistent with the pattern of reduced ozone injury found by other workers who have investigated ozone-bacteria or ozone-virus interactions. However, we found no protective effect of bacterial infection against ozone injury when plants were sprayed with the antioxidant EDU. Instead, there was a small but statistically significant increase in ozone injury on *X. phaseoli*-infected plants in EDU-protected plots compared with uninfected antioxidant-treated plants. Possible mechanisms for the protective effects of bacterial infection in reducing ozone injury have included reduced ozone uptake due to impairment of stomatal function and reduced availability of potential ozone-injury sites (12). These mechanisms are at variance with the observation that EDU-protected plants with 17% of leaf tissues affected by bacterial blight had 5% more foliar ozone injury than plants with no blight infection. The antioxidant had no effect on the amount of leaf tissue affected by blight and no effect on *X. phaseoli* in vitro, suggesting that the lack of protective effect on EDU-treated plants was not the result of a direct EDU-bacteria interaction on the surface of the leaf. The protective effect of *X. phaseoli* infection in reducing ozone injury on white beans not treated with EDU would appear to have little commercial significance, because the total amount of leaf injury on infected plants (53%) was nearly the same as on control plants (57%). Moreover, the reduced foliar injury on bacteria-infected plants did not translate into increased yield; seed yields from the two treatments were not significantly different.

We found no evidence that exposure to ozone protected bean

TABLE 1. Effects of ozone, the antioxidant EDU, and bacterial blight (caused by *Xanthomonas phaseoli*) on foliar injury and defoliation of white beans[x]

Treatment	Leaf necrosis		Defoliation (%)
	Ozone injury (%)	Blight injury (%)	
X. phaseoli	38 a[z]	15 a	93 a
EDU (antioxidant)[y]	19 b	0 b	20 b
X. phaseoli + EDU	24 c	17 a	13 b
Control	57 d	0 b	90 a

[x]Means of four replicate plots, evaluated 15 August and 22 August 1977 Stouffville, Ontario.
[y]Weekly applications of EDU (N-2-(2-oxo-1-imidazolidinyl) ethyl-N-phenylurea) 1 kg/ha active ingredient.
[z]Means in columns followed by the same letter are not significantly different, $P = 0.01$, when tested by one-way analysis of variance.

TABLE 2. Effects of ozone, the antioxidant EDU, and bacterial blight (caused by *Xanthomonas phaseoli*) on yield of white beans[y]

Treatment	Yield (g/1,000 seeds)
Xanthomonas phaseoli	164 a[z]
EDU (anti-oxidant)	197 b
Xanthomonas phaseoli + EDU	191 c
Control	159 a

[y]Means of four replicate plots harvested 2 Sept 1977 at Stouffville, Ontario.
[z]Means followed by the same letters are not significantly different, $P = 0.01$, when tested by one-way analysis of variance.

plants from infection by *X. phaseoli*. The amount of leaf tissue affected by blight was the same in antioxidant-treated and in unprotected plants. In controlled environment studies, Howell and Graham (12) reported that exposure to ozone significantly reduced foliar injury to alfalfa by *X. alfalfa*, and *Pell et al.* (25) found a similar protective effect in soybeans exposed to ozone and subsequently inoculated with a hypersensitive-response-inducing pseudomonad.

The antioxidant EDU very effectively reduced foliar ozone injury and defoliation on field-grown white beans. These effects were reflected in increased yields of EDU-treated plants. Because premature defoliation has been implicated in reducing yields of beans affected by bacterial blight (31) and ozone (5), the increased yields of EDU protected plants may be due more to reduced defoliation of these plants than to reduced amount of injured leaf tissue. Bacteria-infected plants treated with EDU had a total of 41% leaf injury from blight and ozone; uninfected plants treated with EDU had 19% leaf injury caused by ozone alone. Although the blight-infected plants had a slightly reduced yield relative to uninfected plants, this reduction was not statistically significant. Similarly, percent defoliation was not significantly different between the two treatments. In their study on ozone effects on onion, Wukasch and Hofstra (32) found that EDU increased yield of field-grown onions by 38%. They suggested that this increase was due to the protective effect of EDU in reducing ozone injury and subsequent *Botrytis* spp. infection of the onion foliage. In our study, reduced leaflet abscission appeared to be of greater importance than reduced foliar injury in increasing yield of white beans.

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