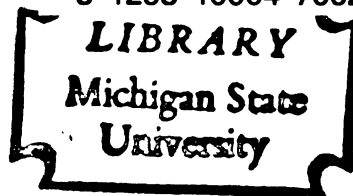




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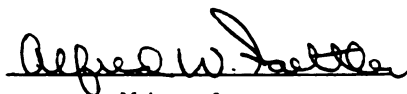
Interaction Between Ozone and Xanthomonas phaseoli on
Navy (Pea) Bean Cultivars 'Seafarer' and 'NEP-2'

presented by

Brian Olson

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INTERACTION BETWEEN OZONE AND *XANTHOMONAS PHASEOLI* ON
NAVY (PEA) BEAN CULTIVARS 'SEAFARER' AND 'NEP-2'

By

Brian Olson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1979

ABSTRACT

INTERACTION BETWEEN OZONE AND *XANTHOMONAS PHASEOLI* ON NAVY (PEA) BEAN CULTIVARS 'SEAFARER' AND 'NEP-2'

By

Brian Olson

Frequently in Michigan both ozone and common bacterial blight (*Xanthomonas phaseoli*, *Xp*) damage occur on dry Navy beans. The research concerns interactions between ozone and *Xp* (rifampin resistant mutant, Ra) on *Phaseolus vulgaris* cultivars 'Seafarer' (ozone-sensitive) and 'NEP-2' (ozone-tolerant). Primary leaves of ten-day-old plants were inoculated with Ra bacteria and sometimes treated with an antioxidant N-(20(2-oxo-1-imidozolidinyl)ethyl)-N-phenylurea (EDU) before an eight hour fumigation with ozone (470-544 $\mu\text{g}/\text{m}^3$). A small sometimes significant synergistic interaction between ozone and blight occurred on both cultivars. Ozone injury on both cultivars was significantly reduced when sprayed with EDU. Field experiments were inoculated with Ra bacteria sprayed with EDU. No significant synergistic interaction occurred between ozone and blight damage on

Brian Olson

either cultivar. 'Seafarer' plants sprayed with EDU were significantly protected from ozone-injury compared to non-sprayed plants, while 'NEP-2' plants were not. Significant differences of total yield only occurred on 'Seafarer' plants inoculated with Ra.

To a good friend

George S. Lee

ACKNOWLEDGMENTS

I would like to thank Dr. Saettler for his guidance and encouragement throughout the research and preparation of this thesis.

I would also like to thank my committee members Dr. Hooker and Dr. Smucker for their suggestions throughout my research and evaluation of this manuscript. I would like to thank, Dave Weller for his valuable technical assistance and Dr. Jones for his evaluation of this manuscript.

I especially thank my wife, Val, for her understanding and patience.

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INTRODUCTION

Michigan annually produces approximately 90 percent of the dry Navy (pea) beans in the United States (30). Common and fuscous bacterial blights caused by *Xanthomonas phaseoli* E.F. Smith Dowson (*Xp*) and *Xanthomonas phaseoli* var. *fuscans* (Burkh.) (*Xpf*), respectively, are major disease problems of Michigan Navy (pea) beans (26). Both diseases are seed borne and occasionally cause significant bean yield reductions (1). In recent years, ozone injury has been observed with increasing frequency in Michigan dry bean fields (15).

Both ozone injury and blight damage are frequently observed in the same bean field. Previous researchers have demonstrated a cross protection phenomenon between ozone and obligate plant pathogens (16, 21). In this study we investigated the possible interaction between ozone injury and bean common blight (*Xp*) damage in Navy bean (*Phaseolus vulgaris* L.) cultivars 'Seafarer' and 'NEP-2'.

LITERATURE REVEIW

Common Blight

Xp was first described by Beach in 1892 (2). In 1924, Burkholder reported *Xpf*, as having identical symptoms as *Xp*, but the *Xpf* bacteria were a different color (5).

Xp and *Xpf* bacteria both have a single polar flagellum and are gram negative, straight rods, obligately aerobic and produce non-diffusible yellow pigments. Both bacteria produce hydrogen sulfide, proteolize milk, hydrolize gelatin, starch and Tween 80, and produce an alkaline reaction with phenol red dextrose agar. Results of biochemical tests for *Xp* and *Xpf* are indistinguishable from those of *Xanthomonas campestris*: *Xp* and *Xpf* are considered *X. campestris* nomenspecies in Bergy's Manual 8th Ed. (4). The only difference between *Xp* and *Xpf* is a brown diffusable pigment produced by *Xpf* in certain culture media (5).

Xp and *Xpf* produce identical symptoms on leaves, stems, pods and seed. Leaf symptoms are most frequently observed, and begin with leaf cells becoming

plasmolyzed causing a water-soaked appearance on the abaxial surface. The water soaked tissue becomes chlorotic and characteristically bright yellow. The chlorotic leaf tissue becomes necrotic and often lesions coalesce, forming large portions of diseased tissue. Heavily infected leaves may prematurely begin senescence (31). Stem and pod infections also begin with water-soaked lesions. The stem lesions become sunken and turn reddish brown in color. Lesions on mature dry pods turn dark brown.

Common and fuscous blights have traditionally been considered late season diseases on dry beans. The first observable symptoms normally occur in late July and early August just after blossom. Recently Weller (27) has detected common and fuscous blight symptoms and the causal bacterial throughout the growing season. Symptoms and bacterial populations begin in the early seedling stage. Weller noted that leaves with blight symptoms were usually located under younger symptomless leaves, causing the entire plant to appear healthy. At blossom, leaves in the outer canopy showed symptoms.

Xp and *Xpf* are seed borne diseases. Seed internally-infected with *Xp* or *Xpf*, are usually yellow or show a darkened hilum (27) and are considered the primary source of inoculum. Bacteria on infected

seedlings are spread as secondary inoculum by blowing or splashing rain.

Weller monitored bacterial populations on leaves, stems, roots, pods and seed with *Xp* and *Xpf* isolates resistant to 50 ppm rifampin (27). Bacterial populations were determined by homogenizing plant material in .01 M phosphate buffer (pH 7.2) and plating the homogenate on nutrient agar media containing rifampin and cycloheximide.

Ozone

Man has known about ozone, the molecule composed of three oxygen atoms since the 1800's. In the late 1860's R.C. Kedzie, chemistry professor at Michigan Agricultural College (M.S.U.), researched ozone detection and suggested the possible health hazard of ozone (18).

Awareness of air pollutants and ozone began in the early 1950's. The modern world had become dependent on the combustion engine and large industries were located in most cities. At this time people in cities such as Los Angeles became aware of smog and its danger to human health.

In 1950 Middleton, Kendrick and Schwain attributed injury on many herbaceous plants in the Los Angeles area to smog or air pollution (25). Haagen-Smit *et al.*, in

1952 experimentally demonstrated air pollution damage on spinach, beets, endive, oats and alfalfa (8). At the present time the Environmental Protection Agency (EPA) determines smog levels by measuring ozone concentration.

In nature ozone is found in both the upper and lower atmospheres. The ozone responsible for plant damage is found in the lower atmosphere, and is formed by the photolytic reaction of NO_2 from gasoline combustion engines with O_2 . Ozone is a strong oxidizing agent and is short-lived. Because ozone is formed with ultraviolet light and is a strong oxidizing agent, ozone levels are generally diurnal, being high in the day and low at night. Ozone also is formed during electrical discharges, such as thunderstorm lightning bolts, but such ozone does not significantly increase ozone levels. For research purposes ozone is usually produced by passing air over a high intensity ultraviolet lamp.

Ozone is continually formed in our lower atmosphere, but is a threat, only when conditions allow ozone formation to exceed ozone decomposition. These conditions include, clear skies and a thermal inversion causing nitrous oxides to be trapped in the lower atmosphere. In Michigan and other temperate regions

this may occur several times a year during the summer months. Ozone episodes are not restricted to metropolitan areas. Drifting air masses can cause damage to agricultural crops in rural areas (9).

Ozone often causes a necrotic stippling or flecking of leaves on affected plants. Bean leaves damaged by ozone are termed bronzed due to the presence of small pin-size necrotic regions on the adaxial leaf surface. The necrotic regions are composed of dead palisade cells; similar symptoms may also occur on bean pods.

In laboratory experiments researchers have exposed plants to high levels of ozone without observable damage (11). Subsequent studies and reviews have shown that ozone is transported into the plant through the stomata and that plants are not injured when the stomata are closed (11). Several important factors regulate stomatal function.

Juhren, Hull, and Went reported light intensities of 3.2 to 4.3 Klx were necessary to obtain traces of oxidant (air pollution) injury on speargrass (*Poa annua* L.) (17). Oxidant injury at 9.7 to 12.9 Klx and 32 to 43 Klx were similar but significantly greater than oxidant damage at 3.2 to 4.3 Klx. Thus sufficient light intensity is necessary to open stomata for ozone injury.

High levels of relative humidity are necessary for maximum stomatal opening. Some researchers have recorded increases in air pollution injury on plants when the relative humidity was increased (11).

In closed chamber fumigation studies, sufficient air must pass through the chamber to prevent carbon dioxide buildup. High CO₂ levels stimulate stomatal closure. Heck and Dunning reported, that the ozone sensitivity of pinto bean and tobacco plants decreased when CO₂ levels were increased (12).

The same authors also demonstrated that soil conditions affect ozone sensitivity (12). Plants growing in clay-loam mixture were least sensitive to ozone, while plants growing in vermiculite and a peat-perlite mixture were the most susceptible to ozone injury. Lack of adequate soil moisture decreases plant's sensitivity to ozone. Inadequate water supply causes stomatal closure, and prevents ozone penetration into the leaves.

Plant age is an important parameter in fumigation studies. Heck and Dunning reported that fully expanded mature pinto bean primary leaves were most sensitive to ozone (12). In other cases, field grown beans are most sensitive to ozone after the blossom stage of plant development (9).

Air pollution studies under field conditions involve some difficulties. Experiments using open-top chambers are limited to space and therefore are inadequate for large scale yield studies. Antioxidant chemicals may also be used to determine ozone injury affects on yield. Several compounds including benomyl have shown protection against ozone damage (13). In this research we have used N-(20(2-oxo-1-imidazolidinyl)-ethyl)-N-phenylurea (EDU), a protective compound recorded as effective against ozone injury on bean plants (6).

Ozone and Pathogen Interactions

Heagle and Manning have separately reviewed the interaction of air pollutants and pathogens, primarily fungal pathogens (11, 24). Generally the reviews noted, "ozone-injured plants appear to be more susceptible to invasion by facultative parasitic and facultative saprophytic fungi. Obligate parasitism by fungi appears to be retarded by ozone and ozone-injured host tissues" (24). More recently researchers have studied the interactions between ozone and bacterial and viral plant pathogens.

Brennen and Leone demonstrated protection against ozone damage on tobacco plants (*Nicotiana sylvestris*) inoculated with Tobacco Mosaic Virus six to 12 days

prior to a three to six hour ozone fumigation ($588 \mu\text{g}/\text{m}^3$) (3). One day after ozone fumigation, non-inoculated plants were ozone damaged and TMV-inoculated plants were not. Davis and Smith noted protection against ozone damage on pinto beans (*Phaseolus vulgaris* L. Pinto) inoculated with Bean Common Mosaic Virus six to 12 days prior to ozone fumigation (7). Both researchers observed less protection against ozone-injury when the time between viral inoculation and ozone fumigation was decreased.

The first ozone-bacterial pathogen study demonstrated little of no interaction or cross protection. Kerr and Reinert inoculated red kidney beans (*Phaseolus vulgaris* L.) with *Pseudomonas phaseolicola* (halo bright) and one week later exposed the plants to ozone ($1176 \mu\text{g}/\text{m}^3$) for one hour (20). Ozone fleck symptoms were observed on all leaf areas except areas exhibiting typical necrotic and chlorotic halo bright symptoms.

An interaction between ozone and bacterial leaf-spot of alfalfa (*Xanthomonas alfalfae*) was observed by Howell and Graham (16). Three alfalfa (*Medicago sativa*) cultivars were used, one resistant to ozone and two ozone-sensitive. Plants were split in two groups, one group was inoculated with *X. alfalfae*

24 hrs before ozone fumigation ($346 \mu\text{g}/\text{m}^3$ for four hrs) and the other group was inoculated 24 hrs after fumigation. Plants inoculated before fumigation had less severe ozone injury than non-inoculated plants and plants inoculated after fumigation. Plants inoculated after fumigation developed less bacterial leafspot injury than non-fumigated plants and plants inoculated before fumigation.

Laurence and Wood observed symptom differences on soybean plants (*Glycine max*) fumigated with ozone and inoculated with *Pseudomonas glycinea* (halo bright) (21). Plants were fumigated with ozone 21 days after planting with primary leaves almost fully expanded. Sets of plants were inoculated with *P. glycinea* between two days before and 16 days after ozone fumigation. Plants were fumigated with $400 \mu\text{g}/\text{m}^3$ ozone for four hours producing light to moderate ozone damage on non-inoculated plants. Bacterial symptoms were only less severe on plants inoculated one day before and two days after fumigation than on non-fumigated plants. The authors suggested that reduced bacterial injury may have been due to the production of bacteriostatic or bactericidal compounds in the plant caused by ozone-injury. Ozone injury on soybean was not affected by bacterial inoculations before and after ozone fumigation.

Laurence and Wood also observed an interaction between ozone and *Xanthomonas fragariae* on wild strawberry (22). Reduced bacterial symptoms were recorded in all experiments when plants were exposed to ozone ($392 \mu\text{g}/\text{m}^3$) for three hours before and after bacterial inoculations.

Several workers have reported that soybean and bean plants injured by ozone produce phytoalexin-type compounds, which might account for differences in disease severity of other pathogens (19, 25). One theory suggests, the pathogen or ozone stimulates the production of a general protective type compound which then protects the plant from subsequent biotic or abiotic attack. Howell observed protection against ozone damage when alfalfa plants were first inoculated with *Xanthomonas alfalfae*. He also reported protection against *X. alfalfae* damage when plants were exposed to ozone (16). This protection phenomenon is termed cross protection. Saettler and Rubin (unpublished data) reported the accumulation of coumestrol, a phytoalexin-type compound, in navy bean leaves damaged by ozone. Recognizing that coumestrol may be bactericidal or bacteriostatic and that common blight (*Xp*) and ozone-injury occur on Michigan navy beans we decided to study the interaction of these two diseases on navy beans.

In this research we studied the possible interaction of ozone and common bacterial blight (*Xp*) on two navy bean (*Phaseolus vulgaris* L.) cultivars, ozone susceptible 'Seafarer' and ozone resistant 'NEP-2', grown in the greenhouse and field.

MATERIALS AND METHODS

Greenhouse and Field Bean Plants

Ozone susceptible 'Seafarer' and ozone tolerant 'NEP-2' navy bean cultivars were used in all studies. 'Seafarer' is a commercial variety extensively grown in Michigan and 'NEP-2' is a white bean developed through seed mutation of the black bean cultivar 'San Fernando'.

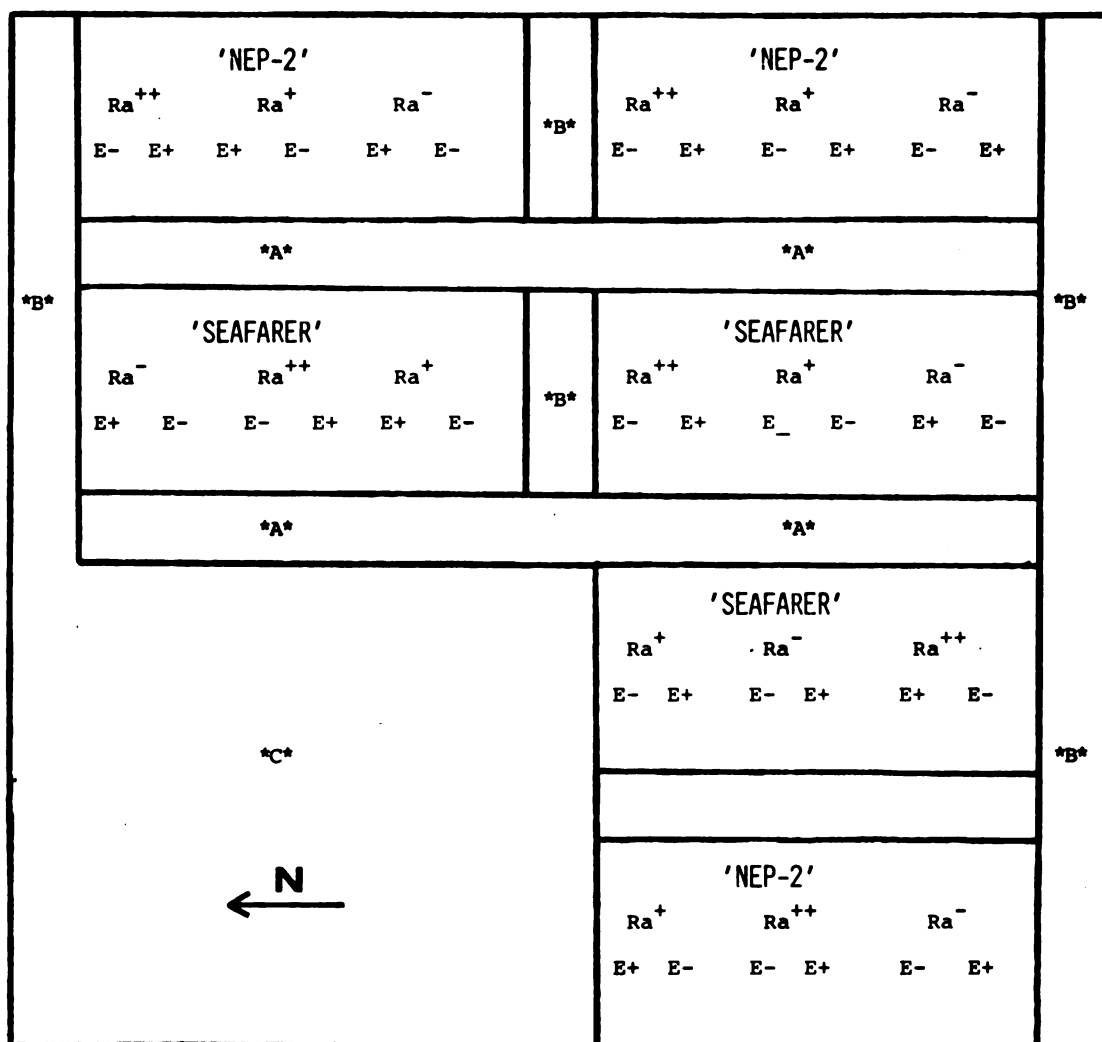
In greenhouse studies seed were germinated in moist vermiculite for two days in the dark. One hundred and twenty germinated seedlings of uniform size were transplanted at 1.5 cm depth in individual 7.5 cm diameter sterile clay pots containing a soil mixture of equal parts (volume) of sterilized peat, vermiculite and sterilized sandy loam soil. Eight days after germination 72 uniform plants were chosen for the pertaining experiment. The plants were fumigated with ozone 11 to 13 days after germination. Plants were watered alternately with deionized water and modified Hoaglands solution (14). The plants were grown in a greenhouse cooled with an evaporative cooler and entering ambient air was drawn through charcoal filters.

Ozone levels in the greenhouse when monitored ranged from zero to $78 \mu\text{g}/\text{m}^3$, while outdoor ambient ozone levels ranged from 78 to $196 \mu\text{g}/\text{m}^3$. Greenhouse temperatures ranged from 22 to 37 C and the relative humidity ranged from 60 to 90%. No supplemental lighting was used, because the experiments were performed from 6/1/78 to 8/26/78.

In field studies land was cultivated and treated with herbicides and fertilizers using conventional practices. Seed were planted on 6/15/78 and the plants were harvested 9/15/78 and 9/17/78 (Fig. 1). Each individual plot consisted of three rows, each 5.4 meters in length.

Maintenance of Bacterial Cultures

Xanthomonas phaseoli mutant Ra, resistant to 50 ppm rifampin, was obtained from D.M. Weller (28). Stock cultures were prepared by growing bacteria in liquid buffered-yeast extract (10 g yeast extract per 1000 ml 0.01 M phosphate buffer, pH -7.2) placed on a shaker. After 48 hrs bacteria were transferred to 40% v/v aqueous glycerol and stored at -10 C. To prepare inoculum Ra bacteria were transferred onto yeast extract calcium carbonate agar plates (YCA: 10 g yeast extract, 15 g agar and 1 g CaCO_3 per 1000 ml glass distilled water). After 96 hrs growth, bacteria were transferred



KEY

- Ra^- Non-inoculated
- Ra^+ Inoculated with $Ra\ 10^8$ CFU/ml on 7/6/78.
- Ra^{++} Inoculated with $Ra\ 10^8$ CFU/ml on 7/20/78.
- E^- Sprayed with tap water containing 0.1% v/v Tween 80.
- E^+ Sprayed with EDU (855 g/ml) containing 0.1% v/v Tween 80.
- *A* Unplanted area one meter wide.
- *B* Four border rows of 'Seafarer'.
- *C* Bulk 'Seafarer' planting.
- ** Each individual treatment contains 3 rows and 5 meters in length. The rows are arranged east to west.

Figure 1. Diagram of Field Plot

to fresh YCA plates for 48 hrs.

Inoculum Preparation and Inoculation

Bacteria were rinsed from YCA plates with phosphate buffer. Bacterial concentrations were adjusted to 10^8 colony forming units (CFU)/ml using standard turbidimetric and dilution plate techniques. Plants were inoculated by one of two methods: 1) The abaxial surface of primary leaves were sprayed until runoff with a Devilbiss atomizer operated at 1.4 kg/cm^2 and held 15 to 20 cm from the leaf surface; 2) The abaxial surface of primary leaves were sprayed to a water-soaked appearance with a Devilbiss atomizer operated at 1.4 kg/cm^2 and held 2 to 3 cm from the leaf surface. Several different bacterial concentrations were used.

Inoculum for field experiments was prepared with deionized water instead of phosphate buffer and bacterial concentrations were adjusted to 10^8 CFU/ml. Bacterial suspensions were directed upwards underneath the plants using a knapsack sprayer operated at 1.5 to 2.0 kg/cm^2 delivering 99 ml(inoculum)/lineal meter.

Application of Antioxidant Compound EDU

Aqueous solutions of EDU were prepared to contain 855 $\mu\text{g/ml}$ (active ingredients) EDU and 0.1% v/v Tween 80. In the greenhouse EDU solutions were

prepared in deionized water and were sprayed until runoff on adaxial primary leaf surfaces using a Devilbiss atomizer operated at 1.4 kg/cm^2 and held 15 to 20 cm from the leaf surface. Check plants were sprayed with deionized water containing 0.1% v/v Tween 80. Approximately 0.9 ml of spray solution was applied to each primary leaf.

In the field EDU solutions prepared in tap water were sprayed onto adaxial leaf surfaces with a knapsack sprayer operated at 2.8 to 4.2 kg/cm^2 and delivering 33.46 ml(solution)/lineal meter or 57.25 mg(EDU)/lineal meter. Check plots were sprayed with tap water containing 0.1% v/v Tween 80. All sprays were applied weekly between 1200 and 1300 hours.

Bacterial Populations

Populations of *Xp* Ra bacteria were determined by sampling six primary leaves in the greenhouse studies. Leaf areas were measured with a Li Cor area meter (Model 3000, Lambda Instruments Corp.) using either leaf tracings on paper, giving a $\pm$ five to ten percent error, or by direct leaf measurements. Leaves were then homogenized in a 75 ml of .01 M phosphate buffer pH 7.2 for 2.5 minutes. Homogenates were serially diluted and aliquots plated on rifampin agar media, RAM (50 mg rifampin and 25 mg cycloheximide/1000 ml YCA). Duplicate plates

were prepared for each dilution. After 96 hrs, dilution plates containing 30 — 300 colonies per plate were counted. Final bacterial populations were expressed as the number of CFU/50 cm² leaf tissue.

The presence of Ra bacteria in field studies was confirmed by separately pressing three leaves per plot exhibiting typical blight symptoms onto RAM plates containing 75 µg/ml rifampin and 50 µg/ml cycloheximide. Blighted leaf areas were outlined on the plates; characteristic *Xp* bacterial growth after 96 hrs was considered a positive indication of Ra bacteria.

Ozone Fumigation

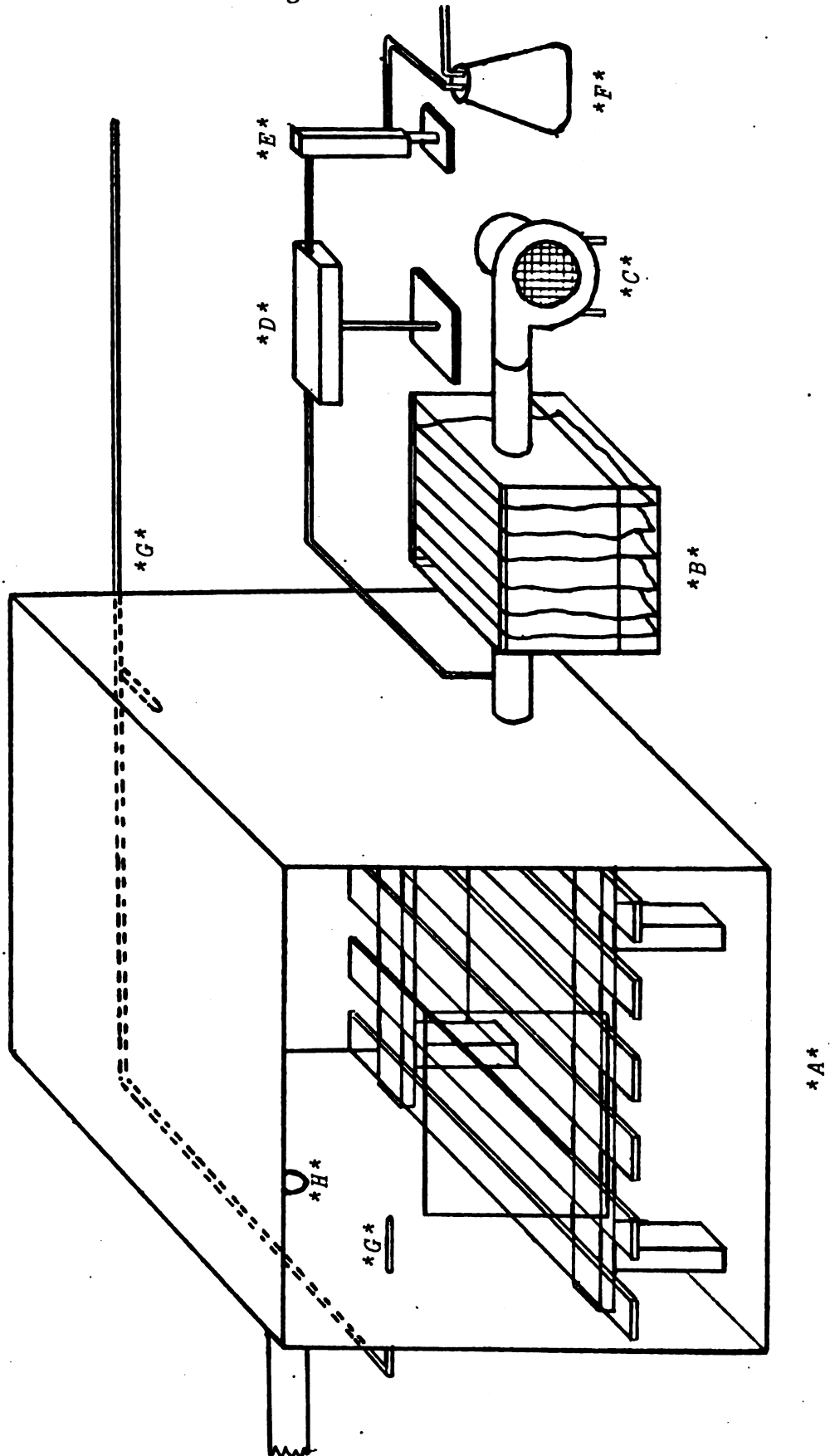
Plants were fumigated with ozone in a 76.2 cm cubical chamber constructed on all sides except the top with .635 cm clear plexiglass lined with aluminum foil on the outside. The chamber top was made of .635 cm glass. Ten 91.44 cm length fluorescent tubes (four 30 watt Cool White and six 30 watt Gro Lux tubes) over the chamber top provided 9.7 Klx at the leaf surface.

Air was forced through the exposure system at 265.0 l/min with a fan (Model 4C443 Dayton Mfg. Co., Chicago, IL) regulated by a rheostat (Cenco). Air initially passed through a 6.35 cm diameter plastic pipe into a 30.5 cm cubical humidifying chamber (Fig. 2) containing 5 — 10 cm of standing distilled water

Figure 2. Ozone Fumigation Chamber

- *A* Exposure chamber
- *B* Humidifying chamber
- *C* Fan
- *D* Ultraviolet lamp (ozone generator)
- *E* Flow meter
- *F* Cotton filter
- *G* Two ozone monitoring inlets
- *H* Exposure chamber exhaust

Figure 2



and six sheets of cheese-cloth hung top to bottom perpendicular to the air flow. The air exited the humidifying chamber through a 6.35 cm diameter plastic pipe and was mixed with ozonated air just prior to entering the exposure chamber.

Ozonated air was generated by passing 6.5 l/min (Lab Crest Flowmeter, Fischer 7 Porter Co., Warminster, PA) of cotton filtered compressed air over an ultraviolet lamp (Model SCT4, Ultraviolet Products, Inc., San Gabriel, CA). Ozone in the exposure chamber was monitored with a Dasibi ozone monitor (Model 1003-AH, Environmental Corp., Glendale, CA).

Thirty-six plants were randomly placed in the chamber on six 0.635 cm x 6.35 cm x 71.12 cm glass plates elevated 22.86 cm from the chamber floor. Temperature was maintained at $22\text{ C} \pm 2\text{ C}$ and relative humidity was maintained at $70\% \pm 10\%$. The plants were placed in the chamber at 0800 hours and a eight hour ozone fumigation with 470 to 549 $\mu\text{g}/\text{m}^3$ was initiated at 0900 hours.

Ozone and Blight Symptom Ratings and Yield Determinations

Ozone and bacterial blight symptoms were recorded on each individual plant in the greenhouse experiments. Ozone injury was recorded as the percentage of damaged

primary leaf tissue and was recorded at the following times: 1) two days after ozone fumigation; 2) the day of bacterial sampling; 3) sometime between fumigation and sampling. Blight symptoms were recorded on a scale of 0 to 100 (0 to 15, no symptoms to light water-soaking; 15 to 35, moderate to heavy water-soaking; 35 to 65, light to severe chlorosis; 65 to 85, light to moderate necrosis; 85 to 100, severe to complete necrosis). Blight symptoms were always recorded at the time of bacterial population sampling and sometimes between ozone fumigation and bacterial sampling.

Ozone injury and blight symptoms were individually recorded in the field as percent of damaged leaf tissue. Symptoms were recorded every five days from 7/10/78 to 8/9/78 and every two days from 8/9/78 to 9/8/78.

For each plot a three meter length of the middle row was harvested for yield data. The plants were dried for two weeks in the greenhouse before weight determinations were measured on total plants, seed plus pods and seed. Pod blight symptoms were recorded as the number of lesions per pod and 100 pods were examined per plot.

Statistical Tests. In this research we analyzed all of the data using the Northwestern University's Statistical Package for the Social Sciences (SPSS) program on Michigan State University's Control Data

6500 computer. Ozone and bacterial symptom data was always transformed with the following equation before analysis, $(180/3.14) \times \{\arcsin [\sqrt{\text{value}}]\}$. All of the experiments were analyzed using the multi-variable analysis of variance subprogram (MANOVA). In each case one independent variable was used and a suitable statistical design was formulated. The experiment designed to analyze the affect of EDU on Ra colony forming units was analyzed using SPSS's Student-Newman-Keuls test.

RESULTS

Greenhouse Experiments

Effects of inoculum concentration and ozone fumigation on blight and ozone injury and bacterial population of 'Seafarer' plants. 'Seafarer' plants were inoculated until runoff with 10^4 , 10^6 and 10^8 Ra CFU/ml one day before ozone fumigation. This experiment was designed to determine the effects of ozone injury on different bacterial populations and the effect of different inoculum concentrations on the severity of ozone injury. Bacterial symptoms were recorded ten days after ozone fumigation just prior to sampling for bacterial populations. Ozone injury was recorded two and ten days after fumigation. Ra bacterial symptoms and populations were not significantly different between ozone fumigated and non-fumigated plants (Table 1). The severity of ozone injury was not significantly different between plants inoculated with Ra bacteria and those plants that were non-inoculated (Table 1).

TABLE 1. Effects of Ra Inoculum Concentration and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'Seafarer' Plants.

Experiment Number ^a	Inoculum Concentration CFU/ml ^b	Ozone Fumigation ^c	Ozone Injury Symptoms ^d		Blight Symptoms ^e 10 day	Log Bacterial Populations Ra CFU/50 cm ² leaf area ^f
			2 day	10 day		
One	10 ⁴	-	0.0	0.0	0.0	5.817
	10 ⁶	+	15.0	14.0	0.0	6.596
	10 ⁶	-	0.0	0.0	0.8	8.459
	10 ⁸	+	8.8	7.0	0.6	8.416
	10 ⁸	-	0.0	0.0	3.2	9.818
	10 ⁸	+	9.7	7.0	2.7	9.821
	Check	-	0.0	0.0	0.0	
Two	10 ⁴	-	0.0	0.0	0.0	—
	10 ⁶	+	38.6	38.6	0.0	2.302
	10 ⁶	-	0.0	0.0	5.0	7.899
	10 ⁸	+	30.5	36.6	2.2	7.859
	10 ⁸	-	0.0	0.0	2.1	9.639
	10 ⁸	+	37.3	44.3	2.1	9.686
	Check	-	0.0	0.0	0.0	
Three	10 ⁴	-	0.0	0.0	0.0	5.229
	10 ⁶	+	2.3	3.0	0.0	4.975
	10 ⁶	-	0.0	0.0	0.0	7.117
	10 ⁸	+	6.5	4.0	0.0	6.896
	10 ⁸	-	0.0	0.0	1.6	8.476
	10 ⁸	+	5.5	5.6	1.6	8.518
	Check	-	0.0	0.0	0.0	
Mean	10 ⁴	-	0.0	0.0	0.0	3.682
	10 ⁶	+	18.7	18.5	0.0	4.624
	10 ⁶	-	0.0	0.0	1.9	7.825
	10 ⁸	+	15.3	15.9	0.9	7.724
	10 ⁸	-	0.0	0.0	2.3	9.311
	10 ⁸	+	17.5	18.9	2.2	9.342
	Check	-	0.0	0.0	0.0	
	10 ⁴	-	11.5	12.5	0.0	
	10 ⁶	+				
	10 ⁶	-				
	10 ⁸	+				
	10 ⁸	-				
	10 ⁸	+				
	Check	-				

Levels of Significance

	Ozone Symptoms ^g
	2 day 10 day
Experiments	.00001*
Inoculum Concentration	.10662 .00001*
	Blight Symptoms ^g
	10 day
Experiments	.00001*
Inoculum Concentration	.00009*
Ozone Fumigation	.20135
Inoculum Concentration by Ozone Fumigation	.34774
	Log of Bacterial Populations
Experiments	.00002*
Inoculum Concentration	.00001*
Ozone Fumigation	.41676
Inoculum Concentration by Ozone Fumigation	.43080

^a Values for each of the three experiments and their means.

^b Bacterial concentrations were sprayed until runoff on the abaxial primary leaf surface 1 day before ozone fumigation.

^c Plants were ozone fumigated (+) and not fumigated (-) 10 days after seed germination.

^d Ozone symptoms were recorded 2 and 10 days after ozone fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue.

^e Blight symptoms were recorded 10 days after fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis).

^f Bacterial populations were sampled 10 days after fumigation.

^g Ozone and blight symptom values were transformed $(180/3.14) \times \{\arcsin[\sqrt{\text{value}}]\}$ and analyzed.

* Significant at the five percent level.

Effects of inoculum concentration and ozone fumigation on blight and ozone injury and bacterial populations of 'NEP-2' plants. The same experimental procedure as above was performed on the cultivar 'NEP-2' with the following exceptions. Ozone injury was recorded at two, six and nine days after ozone fumigation and bacterial symptoms were recorded six and nine days after fumigation. Ra bacterial populations were sampled nine days after ozone fumigation. There were no significant differences between the three replicate experiments with respect to bacterial symptoms and populations (Table 2). Ozone fumigation had no significant effect on bacterial symptoms or populations (Table 2). Nine days after fumigation ozone injury was significantly more severe on Ra bacteria inoculated plants than non-inoculated plants (Table 2).

Effect of Ra bacteria, inoculation time and ozone fumigation on ozone blight symptoms and bacterial populations of 'Seafarer' plants. Primary leaves of 'Seafarer' plants were inoculated (10^6 Ra CFU/ml) to a water-soaked appearance, four and two days prior to fumigation with ozone. The experiment was designed to determine the effect of inoculation time on ozone injury and the effect of ozone fumigation on Ra bacteria populations and symptoms. Ozone injury was recorded two, six and ten days after ozone fumigation.

TABLE 2. Effects of Inoculum Concentrations and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'NEP-2' Plants.

Experiment Number ^a	Inoculum Concentration CFU/ml ^b	Ozone Fumigation ^c	Ozone Injury Symptoms ^d			Blight Symptoms ^e		Log Bacterial Populations ^f CFU/50 cm ² leaf area
			2 day	6 day	9 day	6 day	9 day	
One	10 ⁴	-	.0	.0	.0	.0	.0	5.225
		+	2.0	3.1	3.9	.0	.0	5.679
	10 ⁶	-	.0	.0	.0	.0	.0	7.444
		+	4.1	3.9	4.3	.0	.0	7.698
	10 ⁸	-	.0	.0	.0	.0	2.9	8.907
		+	1.4	3.3	3.3	.0	3.7	9.128
Two	Check	-	.0	.0	.0	.0	.0	
		+	2.6	3.3	3.6	.0	.0	
	10 ⁴	-	.0	.0	.0	.0	.0	4.395
		+	3.8	4.3	4.5	.0	.0	5.870
	10 ⁶	-	.0	.0	.0	.0	.0	7.877
		+	7.6	7.6	7.2	.0	.0	7.899
Three	10 ⁸	-	.0	.0	.0	1.0	1.4	8.989
		+	7.4	6.2	8.4	.5	6.7	8.993
	Check	-	.0	.0	.0	.0	.0	
		+	4.6	3.9	3.5	.0	.0	
	10 ⁴	-	.0	.0	.0	.0	.0	5.507
		+	3.6	4.5	5.4	.0	.0	4.180
Mean	10 ⁶	-	.0	.0	.0	.0	.0	7.749
		+	4.1	7.0	8.9	.0	.0	7.252
	10 ⁸	-	.0	.0	.0	1.0	7.6	9.229
		+	4.6	7.6	7.3	1.0	5.7	9.221
	Check	-	.0	.0	.0	.0	.0	
		+	3.9	4.1	4.9	.0	.0	
Mean	10 ⁴	-	.0	.0	.0	.0	.0	5.042
		+	3.1	3.9	4.5	.0	.0	5.576
	10 ⁶	-	.0	.0	.0	.0	.0	7.690
		+	5.3	6.2	6.8	.0	.0	7.616
	10 ⁸	-	.0	.0	.0	.7	3.9	9.042
		+	4.5	5.7	6.3	.5	5.3	9.114
Check		-	.0	.0	.0	.0	.0	
		+	3.8	3.7	4.0	.0	.0	

Levels of Significance

Experiments	Ozone Symptoms ^g		
	2 day	6 day	9 day
Inoculum Concentration	.00143*	.01688*	.02177*
	.23616	.05691	.00620*
Experiments	Blight Symptoms ^g		
	9 day		
Ozone Fumigation	.05456		
	.14051		
Experiments	Log of Bacterial Populations		
Inoculum Concentration	.99374		
	.00001*		
Ozone Fumigation	.20747		
	.18652		

^a Values presented are for each of the three experiments and their means.

^b Bacterial suspensions were sprayed until runoff on the abaxial primary leaf surfaces 1 day before ozone fumigation.

^c Plants were ozone fumigated (+) and non-fumigated (-) 10 days after seed germination.

^d Ozone symptoms were recorded 2, 6, and 9 days after ozone fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue. Values presented are the means from 3 individual ratings.

^e Blight symptoms were recorded 6 and 9 days after ozone fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis). Values presented are the means of 3 individual ratings.

^f Bacterial populations were sampled 9 days after ozone fumigation. Values presented are the means of 3 individual samples each containing 3 plants.

^g Ozone and blight symptom values were transformed $(180/3.14) \times \{\arcsin[\sqrt{\text{value}}]\}$ and analyzed.

* Significant at the five percent level.

Ra bacterial populations were sampled ten days after ozone fumigation. Two and six days after ozone fumigation, ozone injury was significantly more severe on bacterial inoculated plants than non-inoculated plants. Ozone injury was not affected by the different inoculation times. Blight symptoms and Ra bacterial populations were not significantly different between ozone-fumigated and non-fumigated plants (Table 3).

Effect of Ra bacteria, inoculation time and ozone fumigation on ozone and blight symptoms and bacterial populations of 'NEP-2' plants. This experiment was performed the same as the experiment above except that ozone and blight symptoms were recorded two and six days after ozone fumigation and Ra bacterial populations were sampled six days after fumigation. Six days after fumigation blight symptoms and bacterial populations were significantly more severe and greater, respectively, on ozone-fumigated than non-fumigated plants (Table 4). Ozone injury recorded six days after fumigation was significantly more severe on Ra bacteria inoculated plants than non-inoculated plants. Note, ozone injury was observed in the zone of bacterial inoculation for both 'Sefarer' and 'NEP-2' plants.

TABLE 3. Effect of Ra Bacteria, Inoculation Time and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'Seafarer' Plants.

Experiment Number ^a	Inoculation Time ^b days	Bacteria ^c	Ozone Fumigation ^d	Ozone Injury Symptoms ^e			Blight Symptoms ^f		Bacterial Populations ^g Ra CFU/50 cm ² leaf area
				2 day	6 day	10 day	6 day	10 day	
One	4	-	-	.0	.0	.0	.0	.0	
	4	-	+	.7	1.3	1.4	.0	.0	
	4	+	-	.0	.0	.0	2.1	99.0	9.739
	4	+	+	5.8	4.9	4.5	2.0	99.0	9.693
	2	-	-	.0	.0	.0	.0	.0	
	2	-	+	1.4	1.3	1.4	.0	.0	
	2	+	-	.0	.0	.0	8.4	22.1	9.414
	2	+	+	1.7	3.7	2.9	9.6	20.0	9.419
Two	4	-	-	.0	.0	.0	.0	.0	
	4	-	+	5.1	4.7	3.1	.0	.0	
	4	+	-	.0	.0	.0	1.3	99.0	9.697
	4	+	+	11.0	6.6	3.4	2.1	99.0	9.730
	2	-	-	.0	.0	.0	.0	.0	
	2	-	+	7.6	5.8	4.3	.0	.0	
	2	+	-	.0	.0	.0	1.6	99.0	9.544
	2	+	+	8.8	10.5	5.0	4.8	99.0	9.672
Three	4	-	-	.0	.0	.0	.0	.0	
	4	-	+	37.5	38.7	39.7	.0	.0	
	4	+	-	.0	.0	.0	99.0	99.0	9.829
	4	+	+	49.0	49.0	46.2	99.0	99.0	9.943
	2	-	-	.0	.0	.0	.0	.0	
	2	-	+	53.3	51.1	52.2	.0	.0	
	2	+	-	.0	.0	.0	18.3	99.0	9.932
	2	+	+	43.4	47.9	54.0	6.2	99.0	10.124
Mean	4	-	-	.0	.0	.0	.0	.0	
	4	-	+	14.4	14.9	14.7	.0	.0	
	4	+	-	.0	.0	.0	34.1	99.0	9.755
	4	+	+	21.9	20.2	18.0	34.4	99.0	9.789
	2	-	-	.0	.0	.0	.0	.0	
	2	-	+	20.8	19.4	19.3	.0	.0	
	2	+	-	.0	.0	.0	9.4	99.0	9.630
	2	+	+	18.0	20.7	20.6	6.9	99.0	9.738

Levels of Significance

	Ozone Symptoms ^h		
	2 day	6 day	10 day
Experiments	.00001*	.00001*	.00001*
Inoculation Time	.78610	.24348	.08712
Bacteria Inoculation	.04908*	.01148*	.06242
Inoculation Time by Bacteria Inoculation	.00633*	.42466	.54782

	Blight Symptoms ^h	
	6 day	10 day
Experiments	.00001*	.00001*
Inoculation Time	.00001*	.00001*
Ozone Fumigation	.90165	.92656
Inoculation Time by Ozone Fumigation	.77589	.92656

	Log of Bacterial Populations
Experiments	.00001*
Inoculation Time	.11844
Ozone Fumigation	.21545
Inoculation Time by Ozone Fumigation	.55978

^a Values presented are for each of the three replicate experiments and their means.

^b Primary leaves were inoculated 4 and 2 days before ozone fumigation.

^c Abaxial leaf surfaces were inoculated until a water-soaked appearance with (-) phosphate buffer or (+) 10⁶ Ra CFU/ml.

^d Plants were (+) ozone fumigated 12 days after fumigation or (-) not fumigated.

^e Ozone symptoms were recorded 2, 6 and 10 days after fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue. Values presented are the means from 3 individual plant ratings.

^f Bacterial blight symptoms were recorded 6 and 10 days after fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis). Values presented are the means of 3 individual ratings.

^g Bacterial populations were sampled 10 day after ozone fumigation. Values presented are the means of 3 individual samples each containing 3 plants.

^h Ozone and blight symptom values were transformed (180/3.4) x {arcsin[sqrt(value)]} and analyzed.

* Significant at the five percent level.

TABLE 4. Effect of Ra Bacteria, Inoculation Time and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'NEP-2' Plants.

Experiment Number ^a	Inoculation Time ^b days	Bacteria ^c	Ozone Fumigation ^d	Ozone Injury Symptoms ^e		Blight Symptoms ^f		Log Bacterial Populations ^g Ra CFU/50 cm ² leaf area
				2 day	6 day	2 day	6 day	
One	4	-	-	0.0	0.0	0.0	0.0	
	4	-	+	7.3	8.9	0.0	0.0	
	4	+	-	0.0	0.0	20.0	80.0	9.561
	4	+	+	7.2	8.0	20.0	80.0	9.755
	2	-	-	0.0	0.0	0.0	0.0	
	2	-	+	10.0	8.9	0.0	0.0	
	2	+	-	0.0	0.0	20.0	21.0	9.490
Two	4	-	-	0.0	0.0	0.0	0.0	
	4	-	+	3.2	3.9	0.0	0.0	
	4	+	-	0.0	0.0	2.0	80.0	9.654
	4	+	+	4.2	4.9	2.0	80.0	9.862
	2	-	-	0.0	0.0	0.0	0.0	
	2	-	+	4.1	3.6	0.0	0.0	
	2	+	-	0.0	0.0	0.0	20.0	9.555
Three	4	-	-	0.0	0.0	0.0	0.0	
	4	-	+	12.7	11.6	0.0	0.0	
	4	+	-	0.0	0.0	20.0	80.0	9.527
	4	+	+	18.1	18.7	20.0	80.0	9.680
	2	-	-	0.0	0.0	0.0	0.0	
	2	-	+	14.4	11.1	0.0	0.0	
	2	+	-	0.0	0.0	5.0	15.6	9.192
Mean	4	-	-	0.0	0.0	0.0	0.0	
	4	-	+	7.7	8.1	0.0	0.0	
	4	+	-	0.0	0.0	14.0	80.0	9.581
	4	+	+	9.8	10.5	14.0	80.0	9.765
	2	-	-	0.0	0.0	0.0	0.0	
	2	-	+	9.5	7.8	0.0	0.0	
	2	+	-	0.0	0.0	8.3	18.9	9.412
	2	+	+	9.2	10.7	8.3	25.8	9.568

Levels of Significance

	Ozone Symptoms ^h	
	2 day	6 day
Experiments	.00001*	.00001*
Inoculation Time	.65364	.77677
Bacteria Inoculation	.58576	.01760*
Inoculation Time by Bacterial Fumigation	.05137	.91306
	Blight Symptoms ^h	
	2 day	6 day
Experiments	.00001*	.00011*
Inoculation Time	.00001*	.00001*
Ozone Fumigation	1.00000	.00570*
Inoculation Time by Ozone Fumigation	1.00000	.00570*
	Bacterial Populations	
Experiments	.00001*	
Inoculation Time	.00005*	
Ozone Fumigation	.00013*	
Inoculation Time by Ozone Fumigation	.70689	

^a Values presented are for each of three replicat experiments and their means.

^b Primary leaves were inoculated 4 and 2 days before ozone fumigation.

^c Abaxial leaf surfaces were inoculated until a water-soaked appearance with (-) phosphate buffer or (+) 10⁶ Ra CFU/ml.

^d Plants were ozone fumigated (+) or non-fumigated (-) 12 days after seed germination.

^e Ozone symptoms were recorded 2 and 6 days after ozone fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue. Values presented are the means from 3 individual plant ratings.

^f Bacterial blight symptoms were recorded 2 and 6 days after ozone fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis). Values presented are the means of 3 individual ratings.

^g Bacterial populations were sampled 6 days after ozone fumigation. Values presented are the means of 3 individual samples each containing 3 plants.

^h Ozone and blight symptom values were transformed $(180/3.14) \times \{\arcsin[\sqrt{\text{value}}]\}$ and analyzed.

* Significant at the five percent level.

Effect of EDU, Ra bacteria and ozone fumigation on ozone and blight symptoms and Ra bacterial populations of 'Seafarer' plants. Primary leaves of 'Seafarer' plants were inoculated until runoff with 10^6 Ra CFU/ml and sprayed with EDU, two and one day, respectively, before fumigation with ozone. The experiment was designed to determine the effect of EDU and ozone fumigation on Ra bacterial populations and blight symptoms. The effects of bacterial inoculation on ozone injury was also observed. Ozone injury was recorded two, six and ten days after fumigation and bacterial symptoms were recorded six and ten days after fumigation. Bacterial populations were sampled ten days after fumigation. Ozone symptoms in each replicate experiment were not significantly different (Table 5). Blight symptoms were significantly less severe on ozone fumigated plants than non-fumigated plants. There were no significant differences in Ra bacterial populations between ozone-fumigated and non-fumigated plants. Blight symptoms were also significantly less severe on EDU sprayed plants than check sprayed plants. Ozone injury was not affected by bacteria inoculation.

Effect of EDU, Ra bacteria and ozone fumigation on ozone and blight symptoms and Ra bacterial populations of 'NEP-2' plants. The same experiment described above

was performed on 'NEP-2' with the following exceptions. Ozone injury was recorded two, five and eight days after ozone fumigation and blight symptoms were recorded five and eight days after fumigation. Bacterial populations were sampled eight days after fumigation. Ozone-fumigated plants had significantly higher bacterial populations than non-fumigated plants. Blight symptoms recorded eight days after fumigation were significantly more severe on ozone fumigated plants than non-fumigated plants. There were no significant differences in Ra bacterial populations or blight symptoms between EDU sprayed and check plants (Table 6). Bacterial inoculated plants exhibited significantly greater ozone injury than non-inoculated plants at two, five and eight days after ozone fumigation. 'Seafarer' and 'NEP-2' plants were totally protected from ozone injury when sprayed with EDU (Table 5 and 6). EDU sprayed plants were always a darker green than the non-sprayed plants.

Ozone injury on 'Seafarer' and 'NEP-2'. The cultivar 'NEP-2' is classified as field tolerant to ozone injury. We simultaneously ozone-fumigated ten-day-old 'Seafarer' and 'NEP-2' plants to determine their differences in ozone sensitivity (Table 7). The primary leaves of both cultivars were equally sensitive to ozone fumigation.

TABLE 5. Effect of EDU, Ra Bacteria and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'Seafarer' Plants.

Experiment Number ^a	Bacteria ^b	EDU Sprayed ^c	Ozone Fumigation ^d	Ozone Injury Symptoms			Blight Symptoms ^f		Log Bacterial Populations ^g Ra CFU/50 cm ² leaf area
				2 day	6 day	10 day	6 day	10 day	
One	+	-	-	0.0	0.0	0.0	10.0	10.0	8.728
	+	-	+	11.1	13.4	9.6	10.0	10.0	8.779
	+	+	-	0.0	0.0	0.0	10.0	11.0	8.907
	+	+	+	0.0	0.0	0.0	10.0	10.0	8.860
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	9.8	9.8	7.6	0.0	0.0	
	-	-	-	0.0	0.0	0.0	0.0	0.0	
Two	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	0.0	15.2	8.399
	+	-	+	9.4	6.8	7.4	0.0	3.1	8.640
	+	+	-	0.0	0.0	0.0	0.6	2.7	8.813
	+	+	+	0.0	0.0	0.0	0.0	2.9	8.832
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	11.1	6.7	7.7	0.0	0.0	
Three	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	0.0	0.5	8.176
	+	-	+	15.6	10.2	8.3	0.0	0.8	8.198
	+	+	-	0.0	0.0	0.0	0.0	0.8	8.154
	+	+	+	0.0	0.0	0.0	0.0	0.6	8.157
	-	-	-	0.0	0.0	0.0	0.0	0.0	
Mean	-	-	+	10.3	10.7	8.7	0.0	0.0	
	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	3.3	8.6	8.434
	+	-	+	12.0	10.1	8.4	3.3	4.6	8.539
	+	+	-	0.0	0.0	0.0	3.4	5.0	8.625
	+	+	+	0.0	0.0	0.0	3.3	4.5	8.616
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	10.4	9.0	8.0	0.0	0.0	
	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	

Levels of Significance

	Ozone Symptoms ^h		
	2 day	6 day	10 day
Experiments	.60905	.12139	.86641
Bacteria Inoculations	.49656	.61223	.81097
	Blight Symptoms ^h		
	10 day		
Experiments	.00001*		
EDU Sprayed	.01274*		
Ozone Fumigation	.00769*		
Ozone Fumigation by EDU Sprayed	.03191*		
	Bacterial Populations		
	10 day		
Experiment	.00001*		
EDU Sprayed	.05552		
Ozone Fumigation	.48013		
Ozone Fumigation by EDU Sprayed	.40609		

^a Values presented are for each of the three experiments and their means.

^b Abaxial primary leaf surfaces were sprayed until runoff with (+) 10^6 Ra CFU/ml or (-) phosphate buffer 2 days before ozone fumigation.

^c Adaxial primary leaf surfaces were sprayed with (+) EDU or (-) deionized water.

^d Plants were (+) ozone fumigated and (-) non-fumigated 10 days after seed germination.

^e Ozone symptoms were recorded 2, 6 and 10 days after ozone fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue. Values presented are the means from 3 individual plant ratings.

^f Blight symptoms were recorded 6 and 10 days after ozone fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis). Values presented are the means of 3 individual ratings.

^g Bacterial populations were sampled 10 days after ozone fumigation. Values presented are the means of 3 individual samples each containing 3 plants.

^h Ozone and blight symptom values were transformed $(180/3.14) \times \{\arcsin[\sqrt{\text{value}}]\}$ and analyzed.

* Significant at the five percent level.

TABLE 6. Effect of EDU, Ra Bacteria and Ozone Fumigation on Ozone and Blight Symptoms and Bacterial Populations of 'NEP-2' Plants.

Experiment Number ^a	Bacteria ^b	EDU Sprayed ^c	Ozone Fumigation ^d	Ozone injury Symptoms ^e			Blight Symptoms ^f		Log Bacterial Populations ^g Ra CFU/50 cm ² leaf area
				2 day	5 day	8 day	5 day	8 day	
One	+	-	-	0.0	0.0	0.0	0.0	0.9	8.486
	+	-	+	2.2	2.3	2.2	0.0	1.4	8.454
	+	+	-	0.0	0.0	0.0	0.0	0.6	8.540
	+	+	+	0.0	0.0	0.0	0.0	1.4	8.755
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	1.9	1.9	1.8	0.0	0.0	
	-	+	-	0.0	0.0	0.0	0.0	0.0	
Two	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	0.0	1.0	8.647
	+	-	+	1.0	1.9	2.0	0.0	1.3	8.732
	+	+	-	0.0	0.0	0.0	0.0	1.3	8.819
	+	+	+	0.0	0.0	0.0	0.0	1.6	8.841
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	1.1	1.5	2.2	0.0	0.0	
Three	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	0.0	0.5	8.442
	+	-	+	4.1	5.8	5.9	0.0	2.0	8.793
	+	+	-	0.0	0.0	0.0	0.0	0.2	8.468
	+	+	+	0.0	0.0	0.0	0.0	0.3	8.709
	-	-	-	0.0	0.0	0.0	0.0	0.0	
Mean	-	-	+	1.0	1.7	1.8	0.0	0.0	
	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	
	+	-	-	0.0	0.0	0.0	0.0	0.8	8.525
	+	-	+	2.4	3.3	3.4	0.0	1.6	8.660
	+	+	-	0.0	0.0	0.0	0.0	0.7	8.609
	+	+	+	0.0	0.0	0.0	0.0	1.1	8.768
	-	-	-	0.0	0.0	0.0	0.0	0.0	
	-	-	+	1.3	1.7	2.0	0.0	0.0	
	-	+	-	0.0	0.0	0.0	0.0	0.0	
	-	+	+	0.0	0.0	0.0	0.0	0.0	

Levels of Significance

	Ozone Symptoms ^h		
	2 day	5 day	8 day
Experiments	.05504	.02877*	.02147*
Bacteria Inoculations	.03530*	.00950*	.01781*
	Blight Symptoms ^h		
	8 day		
Experiments	.04106*		
EDU Sprayed	.14035		
Ozone Fumigation	.01953*		
EDU Sprayed by Ozone Fumigation	.48125		
	Bacterial Populations		
Experiments	.00857*		
EDU Sprayed	.07104		
Ozone Fumigation	.00773*		
EDU Sprayed By Ozone Fumigation	.81588		

^a Values presented are for each of the three experiments and their means.

^b Abaxial primary leaf surfaces were sprayed until runoff with 10⁶ Ra CFU/ml (+) or phosphate buffer (-) 2 days before ozone fumigation.

^c Adaxial primary leaf surfaces were sprayed with EDU (+) or deionized water (-).

^d Plants were ozone fumigated (+) and non-fumigated (-) 10 days after seed germination.

^e Ozone symptoms were recorded 2, 5 and 8 days after ozone fumigation. Symptoms were recorded as the percentage of ozone damaged primary leaf tissue. Values presented are the means from 3 individual plant ratings.

^f Blight symptoms were recorded 5 and 8 days after ozone fumigation. Blight symptoms were recorded on a scale of 0-100 (0-15, no symptoms to light water-soaking; 15-35, moderate to heavy water-soaking; 35-65, light to severe chlorosis; 65-85, light to moderate necrosis; 85-100, severe to complete necrosis). Values presented are the means of 3 individual ratings.

^g Bacterial populations were sampled 10 days after ozone fumigation. Values presented are the means of 3 individual samples each containing 3 plants.

^h Ozone and blight symptom values were transformed $(180/3.14) \times \{\arcsin[\sqrt{\text{value}}]\}$ and analyzed.

* Significant at the five percent level.

TABLE 7. Ozone Injury on 'Seafarer' and 'NEP-2' Plants in the Greenhouse.

Experiment	Percent Leaf Tissue Damaged with Ozone Injury	
	'Seafarer' ¹	'NEP-2' ¹
1	30	32
2	33	28
3	14	8
4	10	10
5	8	7
Mean	19	17

¹Each value presented is the mean of 15 individual plant ratings. The plants were fumigated with ozone (470-549 $\mu\text{g}/\text{m}^3$) for eight hours. All the non-fumigated check plants had no ozone injury. The analysis of variance levels of significance of ozone injury between cultivars for all five experiments was .80055.

TABLE 8. Cultured Ra Bacteria Affected by EDU.¹

Experiment	Colony Forming Units					
	EDU ($\mu\text{g}/\text{ml}$)					
	<u>0.0</u>	<u>3.4</u>	<u>7.9</u>	<u>28</u>	<u>111</u>	<u>433</u>
1	183a	127b	130b	121b	113b	122b
2	245a	231ab	210bc	200c	182c	188c

¹Each value presented is the CFU mean from five plates. Values without the same letters are significantly different at the 0.05 level.

Ra cultured bacteria affected by EDU. An experiment was designed to determine the effect of EDU on *Ra* bacterial growth (Table 8). RAM plants containing EDU were prepared by separately sterilizing the RAM and EDU stock solution (886 $\mu\text{g/ml}$) and combining the two solutions to make five different EDU dilutions (443, 111, 28, 7.9 and 3.4 $\mu\text{g/ml}$). *Ra* bacteria were plated on the RAM-EDU plates and after 96 hours there was a significant 33% reduction of CFU on plates containing 443 $\mu\text{g/ml}$ EDU in one out of two experiments performed.

Field Experiment

The field experiment was designed as a split plot experiment, with three blocks each containing plots of 'Seafarer' and 'NEP-2' plants. Each cultivar plot in each block was split into non-inoculated and *Ra* inoculated (Time 1, 7/6/78; Time 2, 7/20/78) subplots. Each subplot was split into EDU sprayed and non-sprayed plots. The experiment was designed to determine the effect of EDU (plants protected against ozone injury) on blight symptoms and the effect of *Ra* bacteria on ozone injury.

Blight symptoms were not significantly different in either cultivar between EDU sprayed plants (protected against ozone injury) and non-sprayed plants (Tables 9 and 11). In nine out of ten cases EDU significantly

TABLE 9. Blight Symptoms on 'Seafarer' Plants in the Field.¹

Date	Check ²		Ra Inoculated 7/6/78 ³		Ra Inoculated 7/20/78 ³	
	Check ⁴	EDU ⁵	Check ⁴	EDU ⁵	Check ⁴	EDU ⁵
7/10	0	0	0	0	0	0
7/15	0	0	0	0	0	0
7/20	0	0	1	0	0	0
7/25	0	0	1	1	0	0
7/30	0	0	1	1	0	0
8/04	0	0	0	0	6	7
8/09	0	0	2	2	13	12
8/11	1	1	2	2	10	10
8/13	0	0	2	1	11	11
8/15	1	1	3	3	16	12
8/17	0	2	4	2	15	13
8/19	1	3	4	2	18	15
8/21	1	3	2	1	15	10
8/23	7	7	7	8	35	30
8/25	5	5	7	7	23	30
8/27	5	7	8	7	17	23
8/29	7	3	7	7	13	18
8/31	1	2	4	13	10	18
9/02	8	13	15	22	8	22
9/06	19	5	17	17	25	12
9/08	7	18	8	13	10	15

¹ Bacterial symptoms were recorded as the percentage of leaf tissue damaged² Check were not inoculated with Ra bacteria.³ Plants were inoculated with 10⁸ Ra CFU/ml.⁴ Check plants were sprayed weekly with tap water containing 0.1% v/v Tween 80.⁵ Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

Levels of Significance

<u>Date</u>	<u>Ra Bacteria</u>	<u>EDU</u>	<u>Ra Bacteria by EDU</u>	<u>Block</u>
8/19	.00017*	.58295	.58633	.23044
8/21	.01332*	.62333	.62843	.04341*
8/23	.00126*	.77312	.84604	.18841
8/25	.00337*	.61446	.76857	.23870
8/27	.00247*	.36905	.49817	.37948
8/29	.02632*	.85253	.52330	.18901
8/31	.10891	.17434	.72125	.93807
9/02	.03654*	.00195*	.24665	.01354*
9/06	.77090	.26036	.70897	.04202*
9/08	.81871	.01488*	.46947	.24319

*Significant at the five percent level.

TABLE 10. Ozone Symptoms on 'Seafarer' Plants in the Field.¹

Date	Check ² 4	Check ² EDU ⁵	Ra Inoculated Check ⁴	7/6/78 ³ EDU ⁵	Ra Inoculated Check ⁴	7/20/78 ³ EDU ⁵
7/10	0	0	0	0	0	0
7/15	0	0	0	0	0	0
7/20	0	0	0	0	0	0
7/25	0	0	0	0	0	0
7/30	0	0	0	0	0	0
8/04	0	0	0	0	0	0
8/09	0	0	0	0	0	0
8/11	0	0	0	0	0	0
8/13	0	0	1	0	1	0
8/15	0	0	0	0	2	0
8/17	1	0	2	0	2	0
8/19	1	0	4	0	5	0
8/21	1	0	4	0	3	0
8/23	6	0	13	0	17	0
8/25	27	3	50	2	47	0
8/27	33	5	50	2	33	7
8/29	27	7	33	1	35	6
8/31	35	1	75	5	79	20
9/02	23	17	43	13	33	13
9/06	12	2	50	7	30	17
9/08	22	14	30	23	17	10

¹Ozone symptoms were recorded as the percentage of damaged leaf tissue.²Check plants were not inoculated with Ra bacteria.³Plants were inoculated with 10^8 CFU/ml.⁴Check plants were sprayed weekly with tap water containing 0.1% v/v Tween 80.⁵Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

Levels of Significance

<u>Date</u>	<u>Ra Bacteria</u>	<u>EDU</u>	<u>Ra Bacteria by EDU</u>	<u>Block</u>
8/19	.25897	.00519*	.25897	.96080
8/21	.17832	.00045*	.17832	.72997
8/23	.10080	.00010*	.10080	.51755
8/25	.31390	.00004*	.18870	.82589
8/27	.72626	.00105*	.45508	.33586
8/29	.93263	.00897*	.79994	.29102
8/31	.01791*	.00003*	.19000	.43981
9/02	.31565	.00125*	.12946	.20565
9/06	.12315	.01907*	.21296	.22158
9/08	.32507	.32502	.99368	.00200*

*Significant at the five percent level.

reduced ozone injury on 'Seafarer' plants (Table 10). EDU did not significantly affect the total plant weight, pod and seed weight, or seed weight of 'Seafarer' plots (Table 13). Ra bacteria inoculations did significantly affect the total plant weight, pod and seed weight and seed weight of 'Seafarer'. In all three weight categories the greatest weight was the bacterial inoculation on 7/6/78 and the least was bacterial inoculation on 7/20/78. Neither EDU nor Ra bacterial inoculation affected the number of blight lesions per 100 pods (Table 14).

In one out of ten cases EDU significantly reduced ozone injury on 'NEP-2' plants (Table 12). Neither EDU nor Ra inoculation significantly affected, total plant weight, pod and seed weight, seed weight and blight lesions per 100 pods of 'NEP-2' plants (Tables 13 and 14).

TABLE 11. Bacterial Blight Symptoms on 'NEP-2' Plants in the Field.¹

Date	Check ² Check ⁴	Check ² EDU ⁵	Ra Inoculated Check ⁴	7/6/78 ³ EDU ⁵	Ra Inoculated Check ⁴	7/20/78 ³ EDU ⁵
7/10	0	0	0	0	0	0
7/15	0	0	0	0	0	0
7/20	0	0	0	0	0	0
7/25	0	0	1	1	0	0
7/30	1	2	2	3	0	1
8/04	3	3	3	5	8	7
8/09	3	5	2	5	10	4
8/11	1	3	3	2	12	6
8/13	2	3	3	4	10	6
8/15	3	7	4	3	8	8
8/17	3	4	3	5	7	8
8/19	7	10	7	9	15	12
8/21	5	7	9	6	13	13
8/23	13	23	8	7	27	22
8/25	23	30	18	17	28	37
8/27	13	15	17	12	37	33
8/29	10	20	7	12	25	32
8/31	20	24	10	10	13	22
9/02	12	13	9	7	13	13
9/06	20	52	27	19	30	60
9/08	15	14	10	10	7	18

¹Bacterial symptoms were recorded as the percentage of damaged leaf tissue.²Check plants were not inoculated with bacteria (Ra).³Plants were inoculated with 10^8 CFU/ml.⁴Check plants were sprayed weekly with tap water containing 0.1% v/v Tween 80.⁵Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

Levels of Significance

<u>Date</u>	<u>Ra Bacteria</u>	<u>EDU</u>	<u>Ra Bacteria by EDU</u>	<u>Block</u>
8/19	.18673	.89604	.55901	.01181*
8/21	.09727	.89965	.67615	.73513
8/23	.12275	.82635	.58089	.31832
8/25	.31988	.57744	.85377	.01238*
8/27	.03444*	.73300	.90573	.62698
8/29	.04415*	.22180	.91137	.80236
8/31	.33803	.53762	.87219	.01256*
9/02	.21450	.96715	.85246	.02865*
9/06	.16711	.07545	.17584	.00918*
9/08	.52579	.29279	.36009	.00333*

* Significant at the five percent level.

TABLE 12. Ozone Symptoms on 'NEP-2' Plants in the Field.¹

Date	Check ² 4	EDU ⁵	Ra Inoculated Check ⁴	7/6/78 ³ EDU ⁵	Ra Inoculated Check ⁴	7/20/68 ³ EDU ⁵
7/10	0	0	0	0	0	0
7/15	0	0	0	0	0	0
7/20	0	0	0	0	0	0
7/25	0	0	0	0	0	0
7/30	0	0	0	0	0	0
8/04	0	0	0	0	0	0
8/09	0	0	0	0	0	0
8/11	0	0	0	0	0	0
8/13	0	0	0	0	0	0
8/15	0	0	0	0	0	0
8/17	0	0	0	0	0	0
8/19	0	1	0	0	0	0
8/21	0	0	0	0	0	0
8/23	0	0	0	0	0	0
8/25	0	0	10	0	0	0
8/27	0	0	0	0	0	0
8/29	2	0	0	0	0	0
8/31	2	0	2	0	2	2
9/02	4	7	10	5	5	5
9/06	5	4	4	2	2	4
9/08	4	2	4	1	3	1

¹Ozone symptoms were recorded as the percentage of leaf tissue damaged.²Check plants were not inoculated with Ra bacteria.³Plants were inoculated with 10^8 CFU/ml.⁴Check plants were sprayed weekly with tap water containing 0.1% v/v Tween 80.⁵Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

Levels of Significance

<u>Date</u>	<u>Ra Bacteria</u>	<u>EDU</u>	<u>Ra Bacteria by EDU</u>	<u>Block</u>
8/19	-	-	-	-
8/21	-	-	-	-
8/23	-	-	-	-
8/25	-	-	-	-
8/27	-	-	-	-
8/29	.44649	.43324	.31697	.07317
8/31	.80071	.24033	.55895	.18909
9/02	.43356	.71147	.14749	.00788*
9/06	.61167	.77961	.72607	.00635*
9/08	.59966	.03165*	.79804	.05436

* Significant at the five percent level.

TABLE 13. Plant, Seed, and Pod Weight from Field 'Seafarer' and 'NEP-2' Plants Treated with Ra Bacteria and EDU.¹

Cultivar	Sample	Check ²		Dry Weight g		Ra Inoculated 7/20/78 ³	
		Check ⁴	EDU ⁵	Check ⁴	EDU ⁵	Check ⁴	EDU ⁵
'Seafarer'	Total Plant Weight	490	590	566	652	436	458
	Pod and Seed Weight	323	355	382	388	300	295
	Seed Weight	236	255	285	282	216	214
'NEP-2'	Total Plant Weight	622	592	535	603	558	566
	Pod and Seed Weight	374	353	332	351	326	339
	Seed Weight	267	253	239	251	233	264

Levels of Significance

Cultivar	Sample	Ra Bacteria		Ra Bacteria by EDU		Block
		EDU	Block	EDU	Block	
'Seafarer'	Total Plant	.00593 *	.05144	.57273	.13903	
	Pod and Seed	.02582 *	.63060	.78499	.04277 *	
	Seed	.03829 *	.80987	.85841	.05367	
'NEP-2'	Total Plant	.67889	.73959	.67140	.03796 *	
	Pod and Seed	.69557	.90075	.84934	.04613 *	
	Seed	.84848	.67361	.72852	.02796 *	

¹ Samples are from 3 meter sections harvested from each plot² Check plants were not inoculated with Ra bacteria.³ Plants were inoculated with 10⁸ CFU/ml.⁴ Check plants were sprayed with tap water weekly containing 0.1% v/v Tween 80.⁵ Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

* Significant at the five percent level.

TABLE 14. Effect of Ra Bacteria and EDU on Bacterial Blight Lesions on Pods of 'Seafarer' and 'NEP-2' Plants.¹

Cultivar	Rep.	Check ² Check ⁴	EDU ⁵	Ra Inoculated 7/6/78 ³ Check ⁴	EDU ⁵	Ra Inoculated 7/20/78 ³ Check ⁴	EDU ⁵
'Seafarer'	1	8	12	2	15	11	5
	2	12	2	3	3	10	5
	3	8	5	4	6	5	5
	Mean	9.3	6.3	3	0.8	8.6	5
'NEP-2'	1	12	12	15	4	22	23
	2	12	17	20	17	22	25
	3	14	16	17	7	19	13
	Mean	12.6	15	17.3	9.3	21	20.3

¹Values presented are the number of blight lesions per 100 pods.

²Check plants were not inoculated with Ra bacteria.

³Plants were inoculated with 108 CFU/ml.

⁴Check plants were sprayed weekly with tap water containing 0.1% Tween 80.

⁵Plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% Tween 80.

<u>Cultivar</u>	Levels of Significance			<u>Block</u>
	<u>Ra Bacteria</u>	<u>EDU</u>	<u>Ra Bacteria by EDU</u>	
'Seafarer'	.53539	.74434	.10661	.04725
'NEP-2'	.01045	.25124	.08773	.12880

DISCUSSION

The data presented shows a small trend for a synergistic interaction between ozone and Ra bacteria in Navy (pea) bean plants. The interaction was so small that it was only observed in greenhouse experiments and not the field experiments.

Greenhouse experiments were arranged so that bacterial inoculations always occurred before ozone fumigation to simulate the occurrence of bacterial blight and ozone injury in the field. Blight injury begins in the early seedling stage and ozone injury begins after blossom (9, 27). In seven out of sixteen observations ozone injury was significantly more severe on plants inoculated with bacteria than on plants not inoculated with bacteria. Not all of the ozone injury observations showed significant differences. This inconsistency was probably caused by the inability to detect such small ozone injury differences between inoculated and non-inoculated plants. Bacterial populations were significantly different in two of six experiments and always the bacterial populations were higher in ozone fumigated plants than non-fumigated

plants and in another experiment bacterial symptoms were significantly less severe on ozone fumigated plants than non-fumigated plants. But in the latter case increased bacterial populations did not correlate with increased blight symptoms. This inconsistency was probably do to the experimental error in detecting small differences of blight symptoms and bacterial populations.

In one experiment blight symptoms on non-EDU sprayed plants were significantly higher than EDU sprayed plants. Greater bacterial populations were observed on the EDU sprayed plants but the differences were not significant. EDU (433 $\mu\text{g/ml}$) in rifampin agar media (RAM) did significantly reduce Ra CFU by 33 percent. This EDU concentration is half the concentration at which EDU was sprayed onto the plants. The high EDU concentration in the culture plate probably does not represent the EDU concentration found in the leaf tissue. Probably the EDU concentration in the leaf tissue is much lower than the concentration at which it was applied, therefore the bacterial concentrations would not be affected.

In the field experiment no cross protection or synergistic interactions were observed. The only significant differences in yield data were with 'Seafarer' plants. Plants inoculated at 7/6/78 had the greatest yield and plants inoculated at 7/20/78 had the

lowest yield. There are a few reasons to help explain why check plots were not the highest yielding plots. In observing the blight symptoms data (Table 9) it is apparent that *Xp* bacteria had infected the check plots and some of the bacteria were *Xp* rifampin resistant mutant Ra (Table 15). Additionally, there may have been some naturally *Xp* infected seed which was planted which also may have contributed to the spread of volunteer *Xp* bacteria (Table 15).

In the field experiment ozone injury was significantly reduced by EDU on 'Seafarer' plants but not on 'NEP-2' plants. This suggests that 'Seafarer' is ozone sensitive and 'NEP-2' is ozone tolerant in the field. However 'Seafarer' and 'NEP-2' were equally susceptible to ozone injury and were significantly protected from ozone injury with EDU in the greenhouse experiments. In the field perhaps 'NEP-2' plants are as sensitive to ozone as 'Seafarer' plants, but because 'NEP-2' plants mature later than 'Seafarer' plants, the ozone sensitivity of 'NEP-2' plants does not coincide with the ozone episodes in August. Or maybe, primary leaves are more sensitive to ozone than trifoliolate leaves. When we began these experiments we made the assumption that primary leaves were similar to trifoliolate leaves in their response to ozone and *Xp* bacteria. Weller has demonstrated using the *Xp* rifampin resistant mutant

TABLE 15. Presence of Ra Bacteria on 'Seafarer' and 'NEP-2' Leaves with Blight Symptoms in the Field.¹

Cultivar	Rep.	Check ⁴	Check ² EDU ⁵	Ra Inoculated 7/6/78 ³ Check ⁴	EDU ⁵	Ra Inoculated 7/20/78 ³ Check ⁴	EDU ⁵
'Seafarer'	1	- - -	- - -	+ - +	+ + +	+ + +	+ + -
	2	+ - +	+ - +	- - -	- - -	+ + +	+ + +
	3	- - -	+ + +	+ + +	+ - +	+ + +	+ + +
'NEP-2'	1	- - -	- - -	- - -	- - +	+ + +	+ + +
	2	- - -	- + +	- - -	- + -	+ + +	- + +
	3	- - -	- - -	- - -	+ - -	- - -	+ + +

¹ Leaves exhibiting blight symptoms were pressed onto RAM plates containing 75 µg/ml rifampin and 50 µg/ml cycloheximide. After 96 hours characteristic *Xp* bacterial growth was considered a positive indication of Ra bacteria (+). Leaves were sampled on 8/21/78 and 8/23/78/

² Check plants were not inoculated with Ra bacteria.

³ Plants were inoculated with 10⁸ CFU/ml.

⁴ Check plants were sprayed weekly with tap water containing 0.1% v/v Tween 80.

⁵ Check plants were sprayed weekly with EDU (855 µg/ml) containing 0.1% v/v Tween 80.

(Ra) that primary and trifoliate leaves have similar patterns of blight symptoms and bacterial populations. In the case of 'Seafarer' there was no contradiction of ozone sensitivity between field and greenhouse experiments. To determine the cause of ozone sensitivity differences between the field and greenhouse experiments on 'NEP-2', both young (primary leaves) and old (trifoliate leaves) should be fumigated with ozone under controlled conditions.

Contrary to our initial hypothesis and unlike the other studies concerning the interaction between bacterial plant pathogens and ozone we found no cross protection reaction occurring between *Xp* and ozone injury. Instead we found a small synergistic reaction occurring between *Xp* and ozone injury. If there was a cross protection interaction, coumestrol probably would not be responsible. Wyman and VanEtten showed that coumestrol is neither bacteriostatic nor bacteriocidal against the Xanthomonads (29).

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