Prevention of Ozone Injury to Plants by a New Protectant Chemical

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ABSTRACT

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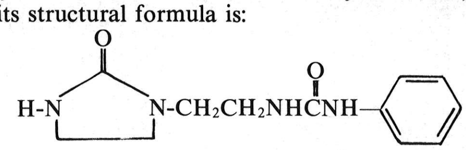
The new chemical N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N′-phenylurea (abbreviated EDU) protects plants against ozone injury. It was effective by foliage and root applications. Dose-response measurements on Pinto beans treated by foliage spray showed that the ozone dose required to cause 50% leaf area injury (ED_{50}) increased linearly with the quantity of this protectant applied. A 500-μg/ml foliage spray to runoff increased the ozone resistance (ED_{50}) of Pinto bean about 30-fold relative to unprotected controls.

Additional key words: air pollution.

Ozone (O_3) occurs widely as an air pollutant in the United States and other industrialized countries (1, 4, 5, 6, 16, 20). It forms in the atmosphere by action of sunlight on other air pollutants, mainly hydrocarbons and nitrogen oxides. It spreads with air currents for many miles and days from the points of origin of the primary pollutants (4, 28). Ozone causes leaf injury on and reduces growth of many plant species at concentrations that presently occur in various agricultural areas (7, 8, 9, 18, 22, 24, 25). The magnitude of economic damage to crops has not been measured definitely because of the difficulties in obtaining O_3-free checks (2, 17). Numerous chemical treatments previously have been reported to suppress O_3 injury on plants with varying degrees of effectiveness (3, 12, 13, 14, 15, 16, 21, 26), but none so far has achieved general use.

In this report, we describe a new chemical that increases the O_3 resistance of several plant species when treated with foliage sprays, root applications, or both. The chemical is N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (abbrevated EDU for ethylenediurea) (27), and its structural formula is:



It is a research chemical not commercially available and not registered for use in crop production. This compound is useful as a survey tool to determine the location and magnitude of crop losses due to O_3. Economics and crop residues may represent serious obstacles to its use as a commercial crop protectant in O_3 problem areas. The present report is confined to laboratory studies on Pinto bean.

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MATERIALS AND METHODS

Preparation of N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (27).—This procedure is provided here because the compound is not commercially available. To a solution of 294 g (2.28 moles) of 2-(2-oxo-1-imidazolidinyl)ethylamine [Aldrich Chemical Company, Cedar Knolls, NJ 07927, or prepared from diethylenetriamine and urea (10)] in 2.5 liters of chloroform, was added 294 ml (2.69 moles) of phenyl isocyanate (lachrymator, use efficient fume hood) over a period of 1.5 hr with external cooling to maintain an internal temperature of 25 C. After being stirred overnight at 25 C, the mixture was cooled in ice, filtered, and the solid product was washed with hexane. Crystallization from 2 liters of ethyl alcohol gave 413 g of EDU white crystals with a melting point of 167-170 C (Table 1).

Plant material.—Pinto beans (*Phaseolus vulgaris*, Pinto 111) grown in vermiculite in 10-cm diameter plastic pots were watered automatically twice daily with 100 ml of modified Hoagland's solution and kept in a growth room illuminated with mixed fluorescent and incandescent lamps (wattage ratio 4:1) with an intensity of 24,000 lux at leaf level for 16 hr/day. The growth room was operated at 24 C and 75% relative humidity (RH) during the light and at 18 C and 85% RH during the dark. Plants to be treated with chemical applied to the roots were grown in sand and watered manually with 100 ml of modified Hoagland's solution once daily. Plants were exposed to O_3 13 days after planting when primary leaves were fully expanded and first trifoliolates were approximately 40 millimeters in width.

Plants were treated by foliage spray applied to both the upper and lower surfaces of the primary leaves to run-off. The EDU was dissolved in water containing 3.6% glycerol and 0.1% of Tergitol Nonionic 15-S-12 surfactant (Union Carbide Corporation, 270 Park Avenue, New York, NY 10017). Approximately 6 ml of solution containing 20-500 μg/ml EDU was applied to each plant. Other surfactants that were equally effective were Tween-20 (ICI United States, Inc., Wilmington, DE 19897) and Triton X-100 (Rohm and Haas, Philadelphia, PA 19105). For root treatment, 4 mg of EDU dissolved in 20 ml of water without surfactant was applied to the surface of the sand.

Exposure to ozone.—Plants were exposed to O_3 in air in a 90 × 140-cm chamber of Lucite® acrylic resin 90 cm high. The floor was a 3-cm-thick aluminum honeycomb with vertical cells to permit free flow of air. The top of the chamber had twelve 2.5-cm-diameter holes uniformly spaced to create nearly laminar air flow from bottom to top. The chamber was mounted on a 35-cm-high stainless steel plenum divided by a horizontal baffle. The lower compartment had an air intake at one end and a water atomizer at the other. The atomizer was a spray nozzle supplied with distilled water at controlled head pressure and with compressed air through a pressure regulator. The upper compartment just below the aluminum honeycomb floor served as a mixing chamber. Air was supplied by a squirrel cage fan that took ambient air through a charcoal filter and electric heater. Ozone was fed into the air stream just before it entered the bottom plenum compartment from a supply line equipped with needle valve and flow meter to aid in adjusting the O_3 concentration within the chamber to about 1,600 $\mu g/m^3$ (0.8 ppm). Ozone was generated by a Welsbach T-408 Ozonator (Welsbach Corporation, 3340 Stokely Street, Philadelphia, PA 19129). The excess was split out of the feed line and vented through a bed of charcoal. A Mast ozone meter (Mast Development Company, 2212 East 12th Street, Davenport, IA 52803) continuously sampled the air near the top of the chamber. The meter was calibrated for each fumigation by the phosphate-buffered iodide colorimetric procedure (23), and the reported concentrations are thus corrected. The chamber was illuminated (24,000 lux) by a battery of high-intensity fluorescent lamps mounted just above the top with supplemental incandescent lighting to match the light quality of the growth room. The chamber was operated at about one air change per minute (approximately 1,000 liters/min) corresponding to a linear velocity of 1 meter/min. Temperature was held at 24 C and humidity at 75%. Humidity was measured periodically on a Bendix Psychron (Bendix Environmental Science Division, Baltimore, MD 21204), and the heat input and air pressure or water head on the atomizer were adjusted as needed.

Plants were moved from the growth room to the exposure chamber 30 min before introduction of O_3 began, and they were rated for foliar O_3 injury 48 hr after exposure. The top surfaces of the two primary leaves were assessed for the percentage of the surface showing injury. White necrotic areas and red or bronzed areas were equally considered to be damaged. The two leaves were rated separately to the nearest 10% and averaged for each plant.

Dose-response.—A number (typically 12) of identically treated plants were placed in the chamber (24-plant capacity) and exposed to O_3 in air at 1,600 $\mu g/m^3$ (0.8 ppm). The plants were removed one at a time to give exposures ranging, for example, from 15 to 150 min, to assure that the series would cover a leaf-area injury range of about 10-90%. Ideally, plants with the median exposure would show 50% foliar injury. Two such groups of plants (12 each) having different treatments could be exposed in the chamber simultaneously thus providing side-by-side comparisons from which many random variables troublesome in sequential exposures were eliminated. Ozone exposure dose was defined as the product of exposure time in minutes multiplied by O_3 concentration in ppm giving dose units of ppm-O_3-minutes. The O_3 exposure dose required to cause injury to 50% of the leaf area, defined ED_{50}, was determined graphically by plotting the percent-injury-ratings for 12 plants (average of two leaves per plant, one plant per

TABLE 1. Properties of N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea

Molecular extinction coefficient in water at 238 nm	14,760
Solubility at 25 C in:	
Water	0.75%
Ethanol	1.9 %
Methanol	6.4 %
Methylene chloride	0.3 %
LD_{50} > 14,000 mg/kg (rat, oral)[a]	

[a]A. M. Kaplan and W. C. Kraus, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours & Co., Inc., Newark, DE 19898.

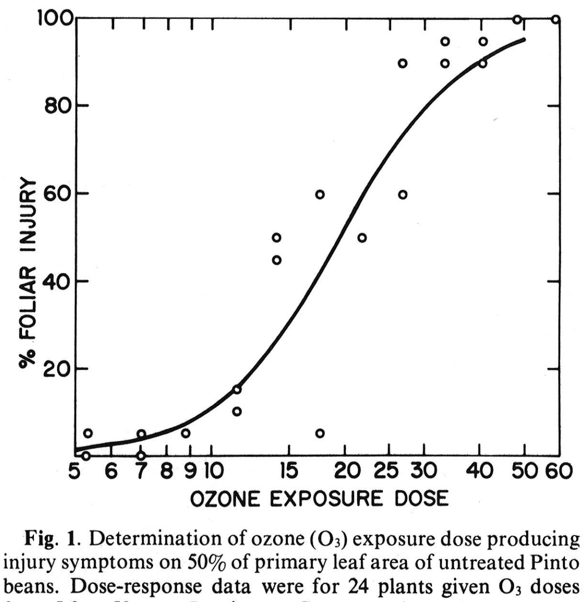

Fig. 1. Determination of ozone (O_3) exposure dose producing injury symptoms on 50% of primary leaf area of untreated Pinto beans. Dose-response data were for 24 plants given O_3 doses from 5.3 to 59 ppm-O_3 minutes. Curve was drawn by computer-fitting the dose-response equation to the 24 data points. This gave ED_{50} = 19.3 ppm O_3-minutes and n = 3.2 (n is an exponent characteristic of system).

TABLE 2. Protection of Pinto bean from ozone injury with foliage treatments[a] of N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (EDU)

Exposure to ozone at 1,600 $\mu g/m^3$ (0.8 ppm) in air		EDU Treatment	Foliar damage
Minutes	Dose[b]	(μg/ml)	(%)[c]
12.5	10	0	5
20	16	0	10
31	25	0	75
50	40	0	100
18	14.4	20	0
24	19	20	0
31	25	20	0
41	33	20	30
55	44	20	30
71	57	20	60
94	75	20	80
24	19	50	0
31	25	50	0
41	33	50	0
55	44	50	10
71	57	50	10
94	75	50	55
74	59	100	10
90	72	100	25
109	87	100	8
135	108	100	30
160	128	100	57
122	98	500	5
245	196	500	5
318	254	500	20
389	311	500	15
582	465	500	10
718	574	500	55
797	637	500	70

[a]Plants grown in vermiculite. The EDU concentration indicated was sprayed on primary leaves at about 6 ml per plant on the day before exposure of plants to O_3. Spray solution contained 0.02% Tergitol Nonionic 15-S-12.

[b]Ozone dose was the product of exposure time in minutes and O_3 concentration in ppm in the air giving exposure dose units of ppm-O_3-minutes.

[c]Each entry represents a single plant.

TABLE 3. Protection of Pinto bean from ozone injury with root treatments[a] of N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (EDU)

Exposure to ozone at 1,600 $\mu g/m^3$ (0.8 ppm) in air			Foliar damage
Minutes	Dose[b]	Treatment	(%)[c]
150	120	None	88
150	120	4 mg EDU	0
300	240	4 mg EDU	8

[a]Plants grown in sand. The EDU in 20 ml water was poured over the sand one day preceding fumigation.

[b]Ozone dose was product of exposure time in minutes and concentration of ozone in air in ppm. Dose units were ppm-O_3-minutes.

[c]Average of two plants.

dose) on a linear ordinate against the corresponding 12 O_3 doses on a logarithmic abscissa as in Fig. 1. Because of individual plant variability the data points showed considerable scatter necessitating curve fitting to locate the ED_{50}. Curve fitting was done by computer employing the MLAB program (11) which gave a least-squares fit of the data to the dose-response function (19) and also computed the ED_{50} O_3 exposure dose for each 12- or 24-plant determination.

$$\text{response} = \frac{\text{dose}^n}{\text{dose}^n + ED_{50}{}^n}$$

which becomes:

$$\frac{\%\ \text{foliar injury}}{100} = \frac{Z^n}{Z^n + ED_{50}{}^n}$$

in which:

Z = ozone exposure dose = $c \times t$.
c = ozone concentration in ppm in air.
t = exposure time in minutes.
n = a constant characteristic of system.

To explore the relationship between degree of O_3 protection conferred by treatments and the concentration of EDU applied, ED_{50} O_3 exposure doses were determined on groups of plants (24 each) that had been treated with EDU foliage sprays in the concentration range 0-500 μg/ml. The O_3 exposure time range for each set of plants was increased with increasing EDU treatments to assure reaching a high level of injury at the maximum exposure time. Exposures ranged from 150 min for untreated plants to 800 min for plants treated with 500 ppm EDU.

RESULTS

Pinto bean plants grown as described developed visible symptoms of O_3 injury on 100% of the surface area of the two primary leaves following exposure to air containing O_3 at a concentration of 1,600 $\mu g/m^3$ (0.8 ppm) for 150 min. A 25-min exposure at the same concentration produced visible injury on approximately 50% of this area. The trifoliolates were less sensitive and showed no visible injury. Strict adherence to the stated growth conditions was necessary for obtaining reproducible O_3 injury responses. Plants older than 13 days, or grown at higher temperatures, or grown with less abundant watering were all markedly less easily injured by O_3 than the standard 13-day old Pinto bean.

Treatments with EDU, either as foliage sprays (Table 2) or root applications (Table 3), had the effect of reducing the extent of visible foliar injury symptoms that developed following exposure to O_3 in the air. Plants sprayed to run-off with 500 μg/ml EDU usually survived exposure to atmospheric O_3 at a concentration of 1,600 $\mu g/m^3$ (0.8 ppm) for 150 min without visible injury but nontreated plants under the same conditions developed O_3 markings over the entire surface area of their primary leaves. A second such exposure of the treated plants usually produced less than 10% leaf injury. Incorporation of surfactants in the foliar spray solution was necessary for maximum effectiveness on Pinto bean. The protective effect of EDU spray treatments appeared to be present as soon as applied because foliar injury ratings were essentially the same whether O_3 exposures followed the treatments by 30 min or by 24 hr. Root application of 4 mg EDU per plant gave protection comparable to that of the 500-μg/ml foliar spray.

Quantitative measurements on the sensitivity of Pinto bean to O_3 injury and on the protective effects of EDU treatments were made in terms of the O_3 exposure dose that was required to produce visible injury on 50% of the area of the primary leaves (ED_{50}). Standard 13-day-old plants without EDU treatment showed an ED_{50} O_3 exposure dose of 20 ppm-O_3-min (0.8 ppm in air for 25 min). A 24-plant determination of that parameter is presented in Fig. 1. Plants treated with EDU foliar sprays required longer O_3 exposures to reach the 50% injury level, and data defining that relationship are presented in Fig. 2 in which 24 plants were used to determine each data point.

DISCUSSION

EDU is a new chemical with significant ability to protect plants from injury by atmospheric O_3. Measured in terms of the O_3 exposure dose that causes visible injury to 50% of the primary leaf area, a 500-μg/ml EDU foliage spray increased the O_3 resistance of Pinto bean plants 30-fold relative to untreated controls. Thus, in Fig. 2 the ED_{50} O_3 exposure dose was 20 ppm-O_3-min in untreated plants and 600 ppm-O_3-min in plants sprayed with 500 μg/ml EDU. The data (Fig. 2) further showed that the O_3 exposure dose for 50% injury increased in linear proportion to the increasing concentrations of EDU in the foliage spray applied. That relationship implies the existence of an antagonistic interaction in definite proportions between EDU and O_3 within the plant. The nature of this interaction has not been determined and is not readily evident in the chemistry of EDU, which is not an exceptional reducing agent. EDU has utility as a survey tool for measuring O_3 damage to crops through field tests in which the compound is used to suppress yield and quality reductions caused by O_3. Other authors will report those studies (29).

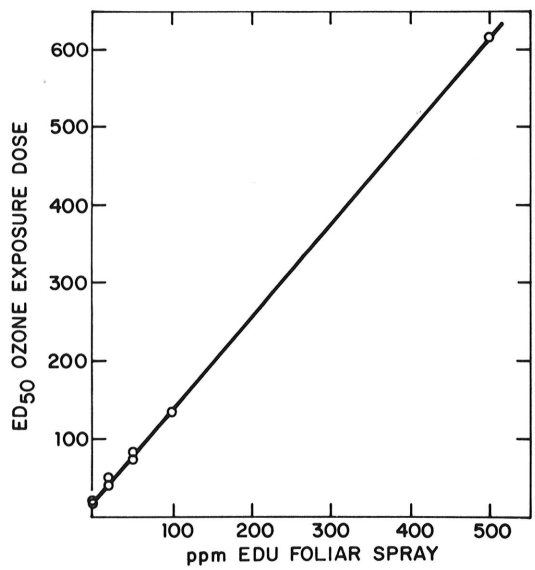

Fig. 2. Ozone exposure dose causing injury to 50% primary leaf area (ED_{50}) as a function of spray concentration of N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (EDU) treatment. All plants were sprayed with a solution of the indicated concentration of EDU and 0.02% Tergitol Nonionic 15-S-12. Each data point represents an ED_{50} determination on 24 plants.

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