N-[2-(2-Oxo-1-Imidazolidinyl) Ethyl]-N'-Phenylurea as a Protectant Against Ozone Injury to Laboratory Fumigated Pinto Bean Plants

T. Craig Weidensaul

Head, Laboratory for Environmental Studies, and professor of plant pathology, Ohio Agricultural Research and Development Center, Wooster, 44691.

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ABSTRACT

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The protective capacity of *N*-[2-(2-oxo-1-imidazolidinyl) ethyl]-*N'*-phenylurea against ozone (O_3) injury to pinto bean plants was evaluated. This experimental compound, known as ethylene diurea (EDU), was applied as a foliar spray 1, 3, 7, or 10 days before a 6-hr fumigation with 0, 200, 500, 800, 1,000, or 1,500 μg of O_3 per cubic meter. EDU levels were 0, 500, 1,000, or 5,000 μg/g in water with 0.05 or 0.10% Triton X-100. EDU gave better protection when applications were larger. Treating plants 3 to 7 days before O_3 exposure gave best protection. EDU treatments were most effective in protecting plants exposed to O_3 levels of 800 $\mu g/m^3$ or more. EDU did not protect foliage that had not yet formed when the chemical was applied.

In the last several years more than 60 compounds have been tested as plant protectants against air pollutants. Some have been applied as foliage sprays (1–3,6,9,10,15,19,21,24) and others as soil drenches (1,2,13,14,17,18,20–22,25,26). The most widely tested antioxidants include benzimidizole (23,24,26), carboxin (7,17,19,22,25), and benomyl (2,7,9,11–15,17–22,25). Phenylurea was used as a protectant 5 yr ago (27); foliar protection was present 24 hr after treatment and lasted 1 wk. The relatively new compound, *N*-[2-(2-oxo-1-imidazolidinyl)ethyl]-*N'*-phenylurea (also known as DPX-4891 and ethylene diurea or EDU) has been tested in the laboratory (1,16) and field (2,7), but it is too early to make conclusions about the field performance of the compound.

Controlled fumigations have traditionally been used to test the efficacy of various protectant chemicals (1,4–6,8,12–14,16,18–21, 23,24). Little is known about the effects of protective chemicals on plant yields in the absence of pollutants (*unpublished data*). Objectives of this study were to (i) determine an effective EDU concentration to protect foliage, (ii) establish the most desirable time for application, and (iii) determine if, through systemic distribution, foliar-applied EDU could protect foliage that developed after application.

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MATERIALS AND METHODS

Pinto beans (*Phaseolus vulgaris* L.) were used to assess the effectiveness of EDU as a foliar protectant. Five bean seeds were planted per 0.95-L pot containing a 2:1:1 mixture of silt loam soil, peat, and vermiculite, respectively. After germination, bean plants were thinned to two per pot. Each possible treatment combination was replicated four times. Plants were grown in a carbon-filtered greenhouse with 16-hr daylength supplemented by 8,600 lux of fluorescent light, watered each day to near field capacity, and fertilized weekly with commercial 20-20-20 fertilizer. Greenhouse air filtration prevented ozone levels from exceeding 20 $\mu g/m^3$ (0.01 ppm). EDU levels of 0, 500, 1,000, and 5,000 μg/g were combined with the wetting agent, Triton X-100 (Sigma Chemical Co., St. Louis, MO) at 0.05 and 0.10% and atomized to runoff on upper surfaces of bean leaves 1, 3, 7, or 10 days before fumigation. The 10-day application was made when primary leaves were fully developed. Trifoliate leaves were not present when the 7- and 10-day EDU treatments were made.

All plants were fumigated simultaneously with one of the following ozone (O_3) levels: none, 200 $\mu g/m^3$ (0.10 ppm), 500 $\mu g/m^3$ (0.25 ppm), 800 $\mu g/m^3$ (0.40 ppm), 1,000 $\mu g/m^3$ (0.50 ppm), or 1,500 $\mu g/m^3$ (0.75 ppm) for 6 hr. Ozone was produced by an Orec Model 03V5-0 generator (Ozone Research and Equipment

Corp., Phoenix, AZ). Chamber O_3 levels were monitored serially through constantly conditioned Teflon sample lines and electrically operated solenoid valves directing samples to a Dasibi Model 1003-AH UV ozone meter (Dasibi Environmental Corp., Glendale, CA).

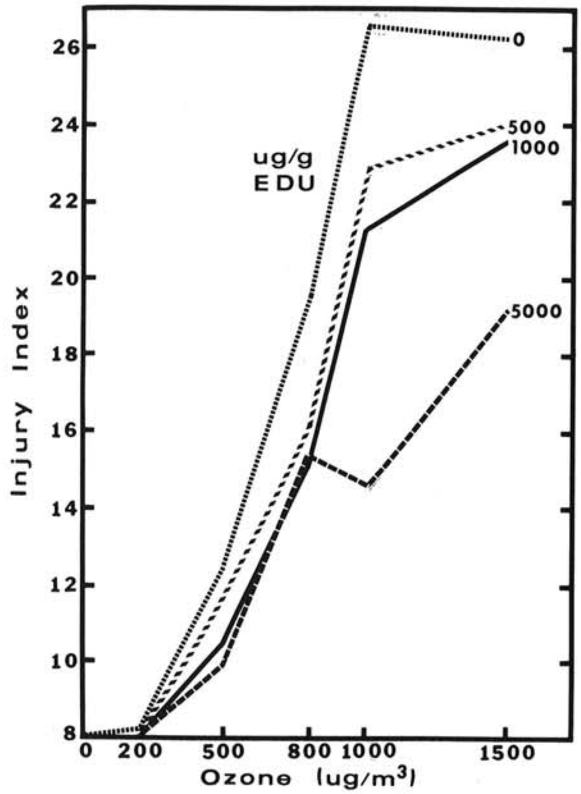

Fig. 1. Interaction of ozone and ethylene diurea (EDU) levels on ozone-induced foliage injury on pinto bean. Ozone fumigations lasted 6 hr with 0, 200, 500, 800, 1,000, and 1,500 $\mu g/m^3$. Injury ranged from 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis). Eight leaflets per plant were rated: index value range is 8–64. The interaction was significant at $P = 0.0001$.

Temperature (25.5 C) and humidity (75% RH) were measured by dry-bulb copper-constantan thermocouples and wet-bulb depression calculations, respectively. Twelve Vitalite 60-W fluorescent tubes (Durotest Corp., North Bergen, NJ) with a sunlight spectrum were used above each Teflon fumigation chamber (101.6-cm cube) to provide 7,700 lux at plant height. Contaminants were removed from ambient air by brominated charcoal filters. Filtered air was drawn into each chamber from a duct running beneath the inline chambers. Ozone was introduced into the clean-air line through electrically operated micrometer needle valves (Hoke electromechanical valve actuater, Hoke Inc., Cresskill, NJ).

Fumigated plants were returned to the carbon-filtered greenhouse for 3 days before O_3 injury was rated. The rating system was an index of 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis) for each primary leaf and leaflet. Since primary leaves and leaflets of two trifoliate leaves were evaluated, each plant received a rating between 8 and 64.

The systemic distribution and protective ability of EDU was tested by later fumigating foliage that had not formed when EDU was applied. Pinto bean plants were produced in the same fashion as previously described. EDU (0–5,000 $\mu g/g$ was applied when the first trifoliate leaf was fully expanded but before the second trifoliate was well formed. The same rating system was used to quantify injury after 6-hr fumigation at the ozone levels mentioned earlier.

Data were analyzed by analysis of variance, and means were separated by Duncan's new multiple range test at significance levels of $P = 0.01$ and $P = 0.0001$.

RESULTS AND DISCUSSION

Ozone injury increased with successive O_3 exposure levels beyond 200 $\mu g/m^3$. Neither the 200 $\mu g/m^3$ O_3 treatment nor control (filtered air) caused injury. All levels of EDU significantly reduced the severity of O_3-induced injury (Table 1) and, unlike dimethyl sulfoxide (28), effectively prevented injury even when applied 1 day before fumigation. Although significantly better than the control, EDU's effectiveness diminished as the length of time between application and fumigation increased (Table 2). EDU did not protect the second trifoliate leaf against O_3 injury via systemic

TABLE 1. Influence of ozone (O_3) and ethylene diurea (EDU) on leaf injury of pinto bean

Ozone concentration		Leaf injury at EDU levels ($\mu g/g$)							
($\mu g/m^3$)	(ppm)	0		500		1,000		5,000	
0	0.00	8.0 a[y]	(8–9)[z]	8.0 a	(8–8)	8.0 a	(8–8)	8.0 a	(8–9)
200	0.10	8.2 b	(8–10)	8.0 a	(8–9)	8.0 a	(8–8)	8.0 a	(8–9)
500	0.25	12.4 c	(8–20)	11.7 d	(8–26)	10.5 e	(8–19)	9.9 f	(8–19)
800	0.40	19.5 g	(8–39)	16.2 h	(9–36)	15.2 i	(8–29)	15.4 j	(8–33)
1,000	0.50	26.6 k	(11–45)	22.8 l	(11–48)	21.3 m	(8–39)	14.6 n	(9–28)
1,500	0.75	26.3 o	(10–49)	24.0 p	(8–45)	23.6 q	(8–45)	19.2 r	(8–40)

[y]Means followed by the same letter are not different at $P = 0.0001$.
Index from 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis). Eight leaflets were evaluated: index value range possible per plant is 8–64.
[z]Values in parentheses are ranges of injury index totals for eight leaflets within a given O_3/EDU treatment over all times of application before O_3 exposure (1, 3, 7, or 10 days).

TABLE 2. Relationship of time of application of ethylene diurea (EDU) to severity of ozone (O_3)-induced leaf injury

Application time before fumigation (days)	Leaf injury at EDU levels ($\mu g/g$)							
	0		500		1,000		5,000	
1	13.6 a[y]	(8–34)[z]	13.0 b	(8–32)	13.6 a	(8–40)	12.1 c	(8–40)
3	16.3 d	(8–40)	12.6 e	(8–34)	15.1 f	(8–35)	11.7 g	(8–32)
7	16.6 h	(8–49)	15.4 i	(8–48)	12.5 j	(8–35)	11.9 k	(8–27)
10	20.8 l	(8–45)	19.5 m	(8–46)	16.5 n	(8–45)	14.5 o	(8–38)

[y]Means followed by the same letter are not different at $P = 0.0001$.
Index from 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis). Eight leaflets were rated: index value range possible per plant is 8–64.
[z]Values in parentheses are ranges of injury index totals for eight leaflets within a given EDU/time-of-application treatment over all O_3 levels (0, 200, 500, 800, 1,000, and 1,500 $\mu g/m^3$).

distribution before its development. There was, however, a significant ($P = 0.01$) interaction between the O_3 level administered and the type of leaf injured. First trifoliate leaves were injured more than primary leaves, which were more severely affected than the second trifoliate leaf. These results suggest that protection of bean foliage against O_3 by EDU applied on foliage can only be accomplished by regular applications on developing foliage. If EDU is systemically distributed within the plant after foliage treatment, its concentration is apparently diluted and not sufficient to protect new foliage.

In a previous study, Triton X-100 (0.05%) used as a surfactant had no effect on O_3-induced injury to pinto bean plants (28). Triton X-100 at 0.05% did not protect plants against O_3 injury in the present study, but 0.1% of the surfactant provided significantly greater protection than the control.

Although all levels of EDU tested reduced the severity of injury, the treatments became more effective at O_3 levels in excess of 800 $\mu g/m^3$ (Fig. 1). At O_3, levels lower than 500 $\mu g/m^3$, EDU gave little protection compared with controls because injury was much less at the lower O_3 exposures. Both the average and upper ends of injury index ranges increased as O_3 concentrations were increased. The index values decreased at a given O_3 exposure as more EDU was used. Table 1 shows the nature of the O_3/EDU interaction. The choice of EDU level depends primarily on the anticipated O_3 level and on the level of injury acceptable to the investigator. No level gave complete protection at O_3 levels above 200 $\mu g/m^3$ for 6 hr, but depending on the crop species, yields may not be significantly reduced with average injury indices of less than 10–15.

A highly significant interaction between time before fumigation and EDU levels indicated that protection with 500 $\mu g/g$ of EDU was only slightly better than the control (Table 2). Maximum protection with EDU applied to foliage at 1,000 and 5,000 $\mu g/g$ was obtained in treatments applied between 3 and 7 days before fumigation, after which plant sensitivity increased sharply (Fig. 2). The interaction effects may be due largely to the fact that plants treated 7 and 10 days before fumigation had injury on untreated foliage (ie, foliage that formed after EDU treatment); hence, there was a sudden increase in injury on the 7- and 10-day-treated plants. Leaves treated 10 days before fumigation were adequately protected, however, as shown by the absence of injury on many leaves (Table 2). EDU gave better protection at 5,000 $\mu g/g$ than at 1,000 $\mu g/g$ applied any time up to 10 days before fumigation. Control plants also became more sensitive after the stage of development at which the 7-day treatment would normally be made (Fig. 2).

A significant interaction between O_3 level and time of EDU treatment showed that EDU effectiveness decreased more rapidly with time of application at higher O_3 levels (Fig. 3). The amount of injury increased rapidly after the 7-day treatment, especially at O_3 levels of 800, 1,000, and 1,500 $\mu g/m^3$. This increase in sensitivity was less pronounced at 500 $\mu g/m^3$. Injury did not occur over time in the absence of O_3 or as a result of exposure to O_3 at 200 $\mu g/m^3$.

A protectant chemical used in the field to evaluate genetically controlled sensitivity of plants to O_3 injury should not in itself alter yields. It has not yet been established conclusively that all plants respond similarly in this regard. EDU is presently being field tested in Ohio under various conditions to determine its influence on yields of horticultural, agronomic, and forest crops. It is hoped that EDU can serve as a satisfactory and less expensive screening tool than field chambers. Results of tests to date indicate that EDU does not affect plant yields by itself.

EDU appears promising as a protectant against O_3 foliar injury, but effectiveness is maximum only when treatments are made at no more than weekly intervals. The degree of protection depends on the inherent sensitivity of the crop, the amount of EDU applied, and the amount of O_3 injury acceptable. Greater foliar protection is obtained with more EDU and when EDU is applied within a few days of O_3 fumigation.

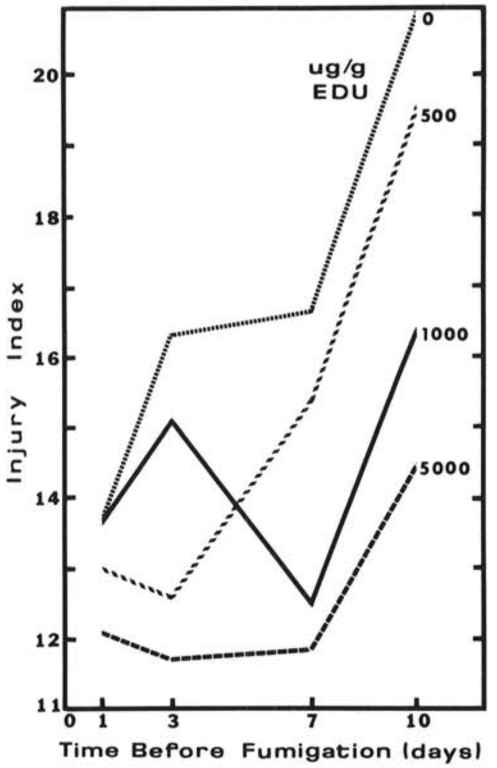

Fig. 2. Relationship of ethylene diurea (EDU) and time of application before fumigation on ozone-induced foliage injury on pinto bean. Ozone fumigations lasted 6 hr with 0, 200, 500, 800, 1,000, and 1,500 $\mu g/m^3$. Injury index ranged from 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis). Eight leaflets per plant were rated: index value range is 8–64. The interaction was significant at $P = 0.0001$.

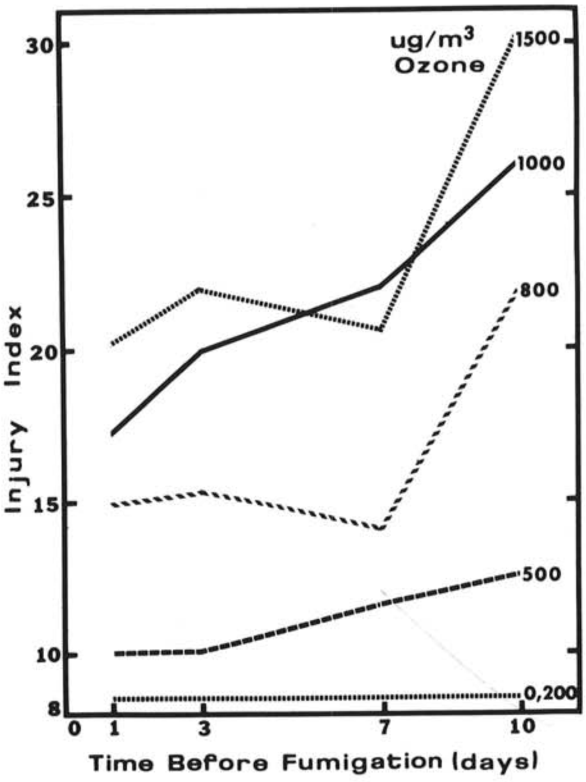

Fig. 3. Interaction of ozone and time of ethylene diurea treatment on ozone-induced foliage injury to pinto bean. Ozone fumigations lasted 6 hr with 0, 200, 500, 800, 1,000, and 1,500 $\mu g/m^3$. Injury index ranged from 1 (no visible injury) to 8 (severe bleaching and bifacial necrosis). Eight leaflets per plant were rated: index value range is 8–64. The interaction was significant at $P = 0.0001$.

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