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Modification of Ozone Sensitivity in Seedlings by Ethylenediurea: Soil Application vs. Stem Injection

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Abstract. **An anti-oxidant chemical, EDU, was applied in the greenhouse as a soil drench or by stem injection to 2-year-old containerized seedlings 7 days prior to fumigation with 0.35 or 0.95 ppm ozone (O_3) for 3 hr. EDU treatment reduced the appearance of O_3-induced symptoms (surface bleaching, bifacial necrosis, and chlorosis) on the foliage of red maple, *Acer rubrum* L.; honeylocust, *Gleditsia triacanthos* L.; sweetgum, *Liquidambar styraciflua* L.; and pin oak, *Quercus palustris* Muenchh. Stem injection of EDU was significantly more effective than soil drench at the same concentration, although both treatments afforded some protection at the low O_3 level (0.35 ppm). About 50 times more EDU was required for comparable levels of O_3 protection using soil application as opposed to stem injection. Honeylocust showed the greatest physiological response to EDU as evidenced by changes in root, stem, and leaf dry weight of stem-injected seedlings. All 4 species showed some sensitivity to O_3 at 0.95 ppm in the absence of EDU. Chemical name used: N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea (EDU).**

Air pollution remains an important environmental stress factor influencing plant growth and development. This is particularly true in urbanized areas where ambient levels of atmospheric contaminants are frequently quite high. Of all the vegetation types found in the urban landscape, trees represent the dominant plant material in terms of size and persistence. Consequently, trees often are exposed to recurring levels of air pollution stress.

Ozone (O_3), produced from photochemical activity induced by sunlight on nitrogen oxides and reactive hydrocarbons, generally is considered the air pollutant most injurious to plants (7). Damage from this pollutant can be especially severe during the summer months when concentrations are sufficiently high to injure sensitive vegetation over extensive areas of the United States. Many compounds have been suggested as potential chemicals to protect plants from O_3 injury, including fungicides, vitamins, antitranspirants, and stomatal regulators (10). Although no specific chemical has gained extensive use in agriculture as a protectant from O_3 injury, EDU (supplied by E.I. duPont deNemours and Co., Wilmington, DE 19898), an antioxidant, has been effective in reducing foliar O_3 damage in young tree seedlings (4, 9).

In previous studies with woody plants, EDU was applied as a foliar spray or via soil application. In both instances, undesirable ecological side effects can occur as a result of chemical contact with nontarget organisms. Chemical injection, on the other hand, offers some obvious advantages, such as avoidance of drift, precise dosage control, and improved applicator safety (2, 11). In this investigation, we compared the relative effectiveness of EDU applied either as a soil drench or via stem injection for controlling O_3 injury in young, containerized tree seedlings.

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Materials and Methods

Plant material. Two-year-old seedlings of red maple, honeylocust, sweetgum, and pin oak were used in this study. Sixty seedlings of each species were potted in a mixture of 2 soil:1 peat:1 sand (by volume) in 15-cm diameter plastic containers and grown in the greenhouse under natural photoperiods for 3 months (April–June) before experimentation. At the start of the study in mid-June, 45 uniformly-sized seedlings of each species were selected and assigned randomly to one of 9 EDU-O_3 treatments. Each treatment consisted of 5 seedlings as described in Table 1.

Table 1. The effect of EDU treatment on O_3 injury to 2-year-old containerized seedlings following O_3 fumigation.

		Injury rating[z]		
Species	Treatment	0 ppm	0.35 ppm	0.95 ppm
Red Maple	Injection[y]	0.0 a	0.0 a	0.9 ab
	Soil drench[x]	0.0 a	0.2 ab	3.2 bc
	No EDU	0.0 a	1.8 ab	6.6 c
Honeylocust	Injection	1.3 ab	0.4 a	1.3 ab
	Soil drench	0.2 ab	0.2 a	5.8 bc
	No EDU	0.1 ab	5.0 bc	9.1 c
Sweetgum	Injection	0.0 a	0.0 a	0.1 ab
	Soil drench	0.0 a	1.0 ab	0.8 ab
	No EDU	0.0 a	0.0 a	1.2 b
Pin Oak	Injection	0.0 a	0.1 a	0.1 a
	Soil drench	0.0 a	1.0 ab	5.2 b
	No EDU	0.0 a	2.4 ab	4.4 b

[z]Foliar injury rating from 0–10, 144 hr after exposure to O_3 for 3 hr. Each value represents tthe mean of 5 seedlings. Data were subjected to arcsin transformation prior to statistical analysis. Mean separation among treatments for each species by Duncan's new multiple range test, 5% level.
[y]5 ml EDU (500 ppm) per seedling injected 7 days before O_3 exposure.
[x]250 ml EDU (500 ppm) per container applied 7 days before O_3 exposure.

Solution preparation. EDU was prepared by dissolving the chemical in warm water to a concentration of 500 ppm. For seedlings receiving stem injection, 5 ml quantities of EDU were pipetted into serum vial caps affixed to the main stem using the technique described by Gregory (5). The chemical was introduced by wounding the stem with a sharp scalpel and allowing the solution to be taken up in the transpiration stream (12). In soil applications of EDU, 250 ml of the chemical were poured on the soil around each plant. Plastic saucers were placed under each container to act as a reservoir for any excess solution. Excess material was always reabsorbed within 3–4 hr. All EDU solutions were prepared and used the same day.

Ozone fumigations. Five plants from each EDU treatment were fumigated with O_3 in concentrations of 0, 0.35 or 0.95 ppm (0, 693, 1881 $\mu g \cdot m^{-3}$) for 3 hr in a continuously stirred tank reactor system (6). All fumigations were initiated 7 days after chemical treatment and included seedlings which had received no EDU. The exposure system, located in a shaded quonset greenhouse, was maintained at 29°C with 35% to 85% RH and natural daylight supplemented with cool-white fluorescent lamps for a light intensity of 203 $\mu mol\ s^{-1}\ m^{-2}$. All fumigations were completed in one day between 0900 and 1515 HR.

After exposure to O_3 the seedlings were transferred back to the greenhouse and evaluated for air pollution injury after 48 and 144 hr. The degree of O_3 injury to the most sensitive leaves of each seedling was rated subjectively on a scale ranging from 0 to 10. Plants exhibiting no visible foliar injury were rated 0. For those plants showing chlorotic areas between the veins, a rating of 0.5 was scored. Interveinal necrotic specks rated a 1.0. For ratings of 2.0 to 10, 20% to 100% of the sensitive leaves, respectively, exhibited bifacial necrosis. Ten days after fumigation the plants were harvested and measurements of height and dry weight of all leaves, stems, and roots were determined for each seedling.

Results and Discussion

Ozone sensitivity. All 4 species showed some sensitivity to O_3 fumigation in the absence of EDU, especially at 0.95 ppm (Table 1). Honeylocust was the most sensitive to O_3 injury, red maple and pin oak intermediate in their response, and sweetgum the most tolerant to this pollutant. Sensitivity to O_3 at 0.35 ppm showed trends similar to those reported for the high concentration of pollutant, except the degree of foliar injury was not as severe (Table 1). Even at the low O_3 concentration, however, individual leaflets of *Gleditsia* gradually abscised during the 6 days following fumigation. This response was particularly evident on young, fully expanded foliage, but there was little impact on immature foliage of the same species.

The observations reported in this study are similar to those reported by Cathey and Heggestad (4) for honeylocust and red maple, particularly at the high O_3 concentrations. The oak species we used (*Quercus palustris*) was different from that used by Cathey and Heggestad (*Quercus alba*), so comparison is difficult. Whereas they found white oak to be very sensitive to O_3, we found pin oak to be intermediate in sensitivity. Both oak species have been reported to be sensitive to O_3 by Jensen et al. (8), which merely points out the discrepancies that can result from differences in methods of diagnosis, genetic makeup, plant vigor, and modifying influences in the environment. Sweetgum was not included in the experiments reported by Cathey and Heggestad, but we found this species to be intermediate in sensitivity to O_3 injury in the absence of EDU, which is in general agreement with the tolerance level reported by Jensen et al. (8).

Our observations on leaf ontogeny as a factor in the susceptibility of *Gleditsia* foliage to O_3 injury are in general agreement with the results reported by the other researchers working with this pollutant (13, 14, 15).

Response to EDU

Protection from O_3 injury. EDU treatment afforded at least some degree of protection from O_3 injury for all species tested (Table 1). In general, injection was more effective than soil application, despite that fact that lesser amounts of EDU were used (5 ml vs. 250 ml). For red maple there was no significant change in foliar injury of EDU-injected seedlings as O_3 exposure increased from 0 to 0.95 ppm. When EDU was applied as a soil drench, however, maple seedlings fumigated at 0.95 ppm showed significantly more foliar damage than similar plants receiving no O_3 treatment. Thus, with this particular species, soil applications of EDU provided some protection at 0.35 ppm O_3 but not at 0.95 ppm, whereas stem injection of EDU provided protection at both O_3 concentrations. Although the data reported in Table 1 were determined after 144 hr, similar trends also were observed after only 48 hr.

Comparable responses to O_3 fumigation following EDU treatment were observed for honeylocust and pin oak (Table 1). With both of these species, as with red maple, EDU afforded only limited protection when applied as a soil drench but gave complete protection when introduced via stem injection. For sweetgum seedlings, no significant differences in foliar injury were noted between stem-injected or soil-applied EDU at any fumigation level (0, 0.35, or 0.95 ppm); therefore, chemical effectiveness could not be tested because of the O_3 tolerance level exhibited by this species.

Phytotoxicity. EDU treatment by itself, without O_3 fumigation, did not cause any phytotoxicity except to stem-injected honeylocust seedlings. With these plants, a foliar injury rating of 1.3 was noted 144 hr after EDU treatment (Table 1).

Although Cathey and Haggestad (4) reported slight marginal burning on the foliage of some woody plants following soil application of EDU, their observations were determined at a concentration of 2000 ppm (250 ml), well above the EDU concentration used in this study. Based on the results of the present investigation, it seems that EDU is a relatively safe chemical in terms of its phytotoxic potential for most woody plants.

Growth changes. Since honeylocust was especially sensitive to O_3 fumigation in our study, we investigated the influence of EDU and O_3 treatments on height and dry weight changes in this species. In every instance, the height and dry weight of seedlings injected with EDU was greater than corresponding plants without EDU (Table 2). Although the differences were statistically significant only for leaf dry weight, the trend was consistent for all the growth parameters measured whether or not the seedlings were fumigated with O_3.

Although stem injection of EDU appeared to stimulate height and dry weight of honeylocust seedlings, this did not occur in response to soil-applied EDU (Table 2). In fact, the growth of seedlings treated with soil-applied EDU generally was less than that observed for similar plants receiving no EDU. The most pronounced differences in growth, however, occurred between stem-injected and soil-applied EDU treatments. These differences were particularly significant for leaf and stem dry weight, regardless of the O_3 level to which the seedlings were exposed (Table 2).

Table 2. The effect of EDU treatment and O_3 fumigation on height and dry weight of 2-year-old containerized honeylocust seedlings.

Growth Parameter[z]	Treatment	Fumigation level 0 ppm	0.35 ppm	0.95 ppm
Height (cm)	Injection[y]	34.7 ab	35.0 b	36.3 b
	Soil drench[x]	20.7 a	23.2 ab	29.1 ab
	No EDU	28.3 ab	33.1 ab	29.2 ab
Leaf dry wt. (g)	Injection	0.9 d	1.1 e	1.1 e
	Soil drench	0.4 a	0.4 a	0.6 bc
	No EDU	0.5 ab	0.7 c	0.5 ab
Stem dry wt. (g)	Injection	1.1 bc	1.1 bc	1.3 c
	Soil drench	0.4 a	0.3 a	0.4 a
	No EDU	0.6 ab	0.6 ab	0.5 a
Root dry wt. (g)	Injection	3.1 ab	3.3 b	3.0 ab
	Soil drench	1.4 a	1.4 a	1.0 ab
	No EDU	0.9 ab	2.1 ab	1.7 ab

[z]Growth measured 144 hr after exposure to O_3 for 3 hr. Each value represents the mean of 5 seedlings. Mean separation among treatments for each growth parameter by Duncan's new multiple range test, 5% level.

[y]5 ml (500 ppm) per seedling injected 7 days before O_3 exposure.

[x]250 ml EDU (500 ppm) per container applied 7 days before O_3 exposure.

Whereas EDU-injected plants showed more growth than untreated plants, it is impossible from this study to determine whether this is a cause or effect relationship, i.e., is increased growth the result of EDU protection or is EDU protection the result of increased growth? The mechanism by which EDU influences plant sensitivity to O_3 is not well understood (3), but evidence indicates that the chemical may enhance enzymes capable of scavenging toxic-free radicals produced when O_3 interacts with plant tissue (1).

Conclusions

The results of this study verify the effectiveness of EDU in reducing sensitivity to O_3 injury in certain woody plants. Furthermore, the present investigation shows that stem injection is an efficient means of introducing the chemical into woody plants, both in terms of the degree of protection afforded as well as the quantity of EDU required. For all species fumigated with O_3 at 0.95 ppm, foliar injury was reduced an average of 91% by stem injection of 500 ppm EDU and 26% by soil application of chemical at the same concentration. Thus, the capacity to protect foliage from O_3 injury can be enhanced appreciably by injecting EDU directly into the stem.

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