PHOTOSYNTHETIC RESPONSE OF YELLOW-POPLAR SEEDLINGS TO THE ANTIOXIDANT CHEMICAL ETHYLENEDIUREA[1]

By Bruce R. Roberts

Abstract. An antioxidant chemical, ethylenediurea (EDU), was applied by soil drench or by stem injection to 2-yr-old containerized seedlings of yellow-poplar (*Liriodendron tulipifera*) growing in the greenhouse. Net photosynthesis (Pn) was measured before EDU treatment and again 2, 4, and 7 days after treatment. The same seedlings were then fumigated with 0, 0.35 or 0.95 ppm ozone (O_3) for 3 hr and Pn remeasured after 2 days. No significant change in Pn was observed for those seedlings treated with EDU (either soil drench or stem injection) when compared to the controls. Following O_3 fumigation, the decline in Pn for EDU-treated seedlings was appreciably less than that for seedlings without EDU. Stomatal conductance measurements taken on foliage from EDU-treated and untreated plants, both before and after O_3 fumigation, suggest that EDU may affect Pn indirectly through its influence on stomatal opening.

The antioxidant chemical N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea (ethylenediurea, EDU) has been shown to be an effective material for reducing the sensitivity of many agricultural and horticultural crops to air pollution injury caused by O_3(5,6,7,8,9,15,18). Although the mechanism of action of ethylenediurea is not clearly understood, reports in the literature suggest that the chemical may alter stomatal resistance (13) and/or membrane permeability (10,11). There is also evidence to indicate that EDU might function biochemically by increasing the concentration of enzymes known to scavenge toxic-free radicals generated in the presence of O_3 (14). Whatever its mode of action, it is important to know whether or not applications of EDU have a significant effect on physiological activity and subsequent growth and development of treated plants. This study was undertaken to investigate the influence of EDU treatment on net photosynthesis (Pn) of yellow-poplar seedlings both before and after fumigation with O_3.

Materials and Methods

Starting in mid-May, 50 two-yr-old seedlings of yellow-poplar were planted in 2-L plastic containers filled with an artificial potting media (Terra Lite 500). The seedlings were placed in the greenhouse under natural photoperiods for 8 wks prior to initiation of the study. During this time the plants were watered thoroughly twice each week and fertilized biweekly with 500 ml of a commerical fertilizer solution containing 200 ppm each of nitrogen, phosphoric acid and soluble potash as 20.0N-8.6P-16.6K. Temperature and relative humidity conditions in the greenhouse were 26±5 C and 72±12%, respectively. Toward the end of July, 36 seedlings of uniform size were selected and 12 were randomly assigned to each of the following treatments: 1) stem injection with 5 ml EDU at a concentration of 500 ppm; 2) soil drench with 250 ml EDU, also at a concentration of 500 ppm; 3) no EDU treatment. These applications of EDU had been used successfully in an earlier investigation on the effectiveness of the chemical in modifying the sensitivity of woody plants to O_3 fumigation (16).

Following the assignment of EDU treatments, but prior to application of the chemical (day 0), Pn was measured on a single leaf from each seedling by a transient measuring technique (Li-Cor 6000 Portable Photosynthesis System). Photosynthetically active radiation, 410-428 $\mu E\ s^{-1} m^{-2}$, was derived from a high pressure sodium lamp filtered through 6 cm of water. Pn was calculated as CO_2 uptake per unit leaf area based on average leaf area determinations made at day 0 and again at the end of the experiment. The leaf used for Pn determinations had a plastochron index value of 5, and leaf area was recorded from a photocopy of the leaf surface measured with an area meter (Hayashi Denko AAM-5). After the measurement at day 0, Pn was remeasured on the same leaf at day 2, 4, and 7. Following the 7

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day measurement, 4 seedlings in each treatment (EDU injection; EDU drench; no EDU) were fumigated for 3 hr in a continuously stirred tank reactor system (12) with one of the following O_3 concentrations: 0, 0.35 or 0.95 ppm (0, 693 or 1881 $\mu g\ m^{-3}$). After O_3 fumigation the seedlings were returned to the greenhouse for 2 days prior to remeasuring Pn on the same leaf as previously described. At this time, the degree of O_3 injury for each seedling was rated subjectively on a scale ranging from 0-10. Seedlings exhibiting no visible foliar injury were rated 0. For those plants showing chlorotic areas between the veins, a rating of 0.5 was scored. Small interveinal necrotic spots rated at 1.0. For ratings of 2 to 10, 20% to 100% of the sensitive leaves, respectively, exhibited bifacial necrosis.

Results and Discussion

The results of this study show that there was no significant change in Pn for yellow-poplar seedlings treated with EDU (either injection or drench) over the 7 day period following application of the chemical (Table 1). These data suggest that EDU treatment *per se* has no appreciable effect on Pn, and are in general agreement with the results reported by Bennett et al (2) for soil-applied EDU on bean seedlings. While EDU had no significant influence on Pn, seedlings without EDU showed a substantial decrease in Pn rate between day 4 and day 7 (Table 1). The reason for this decline is not clear, but may be related to the large increase in leaf area noted for those plants without EDU (data not shown). While leaf area increased on average of 26% for seedlings treated with EDU, the corresponding increase for untreated plants was substantially greater (41%).

Measurements of leaf stomatal conductance, taken simutaneously with determination of Pn, showed that stomatal opening in all treatments increased gradually over the first 4 days (Table 2). However, between day 4 and day 7 there was a significant decline in stomatal conductance in the EDU treated plants. These data correspond with the trends noted for Pn in Table 1, and suggest that CO_2 assimilation in this study was probably influenced more by stomatal opening than by the direct effect of EDU on the photosynthetic mechanism *per se*. As a general observation, stomatal conductance of EDU-treated plants (either injection or drench) tended to be somewhat greater than corresponding plants without EDU, although the values are only significant for the EDU injection treatment on day 4 (Table 2).

Table 1. The effect of EDU treatment on net photosynthesis of 2-yr-old containerized seedlings of yellow-poplar for a period of 7 days following treatment.

Time	*Photosynthesis*[z] *($mgCO_2m^{-2}s^{-1}$)*		
	EDU Injection[y]	*EDU Drench*[x]	*No EDU*
0	0.313^{ab}	0.345^{ab}	0.314^{ab}
2	0.374^{ab}	0.287^{b}	0.374^{ab}
4	0.363^{ab}	0.333^{ab}	0.407^{a}
7	0.294^{b}	0.320^{ab}	0.217^{c}

[z]Photosynthesis measured on a single leaf from each seedling (plastochron index=5). Each value represents the mean of 5 consecutive measurements on each of 12 seedlings. Mean separation among all treatment-time combinations by Duncan's new multiple range test, 5% level.
[y]5 ml EDU (500 ppm) per seedling.
[x]250 ml EDU (500 ppm) per container.

Table 2. The effect of EDU treatment on stomatal conductance of 2-yr-old containerized seedlings of yellow-poplar for a period of 7 days following treatment.

Time (days)	*Stomatal conductance*[z] *($cm\ s^{-1}$)*		
	EDU Injection[y]	*EDU Drench*[x]	*No EDU*
0	0.197^{abc}	0.247^{bc}	0.191^{bc}
2	0.314^{c}	0.248^{bc}	0.268^{bc}
4	0.677^{d}	0.335^{c}	0.323^{c}
7	0.169^{ab}	0.137^{ab}	0.957^{a}

z, y, x See footnotes, Table 1.

The data on Pn of EDU-treated seedlings following fumigation with O_3 are found in Fig. 1. Two days after fumigation, Pn of those seedlings without EDU showed a reduction of 32%. These results were not unexpected, and illustrate the potential harmful effect of O_3 on physiological activity of fumigated plants (1,4,17). For seedlings treated with EDU drench, Pn decreased 20% at 0.35 ppm O_3 and an additional 7% at 0.95 ppm O_3. Thus, EDU treatment afforded some degree of protection from O_3 in terms of moderating the extend of Pn decline. With the EDU injection treatment Pn actually increased at O_3 concentrations of 0.35 and 0.95 ppm when compared to the values for unfumigated seedllings (Fig. 1). These data may be somewhat misleading and difficult to interpret because of the confounding influence of wounding during the injection process, although the metabolic after-effects of wounding are normally quite short-lived (3).

Stomatal conductance measurements taken 2 days after fumigation with O_3 show an increase in stomatal opening for EDU-injected seedlings at both O_3 levels used in this study (Fig. 2). These data correspond with the trends for Pn of EDU-injected seedlings noted earlier (Fig. 1), and show that increased CO_2 exchange is probably related to enhanced stomatal opening. However, it is still not possible from these results to distinguish between the effects of EDU on stomatal behavior and those associated with the wounding phenomenon. Yellow-poplar seedlings receiving no EDU treatment exhibited an unusual stomatal response to O_3 fumigation (Fig. 2). At 0.35 ppm O_3, stomatal conductance declined 55% as might be expected, but at 0.95 ppm O_3 conductance was substantially greater than in the absence of the pollutant. These data may be explained on the basis that at high pollutant concentrations guard cell integrity and photosynthetic capacity are functionally damaged. Thus, even though stomatal conductance increases (Fig. 2), CO_2 assimilation remains depressed (Fig. 1).

The degree of foliar injury for seedlings fumigated with O_3 is recorded in Table 3. As previously reported (16), stem injection of EDU seems to be an effective means of reducing the sensitivity of many woody plants to O_3 damage. In this study, stem injection of EDU reduced foliar in-

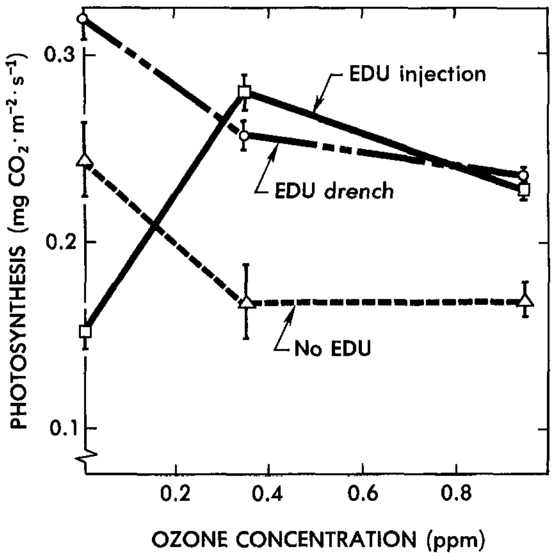

Fig. 1. Net photosynthesis of 2-yr-old containerized yellow-poplar seedlings 2 days after fumigation with O_3. Each value represents the mean of 5 consecutive measurements made on individual leaves (plastochron index = 5) from each of 4 seedlings. Injection treatments made with 5 ml EDU (500 ppm) per seedling; drench treatments with 250 ml (500 ppm) per container. Ozone fumigations were for 3 hr at concentrations of 0, 0.35 and 0.95 ppm (0, 693 and 1881 $\mu g\ m^{-3}$).

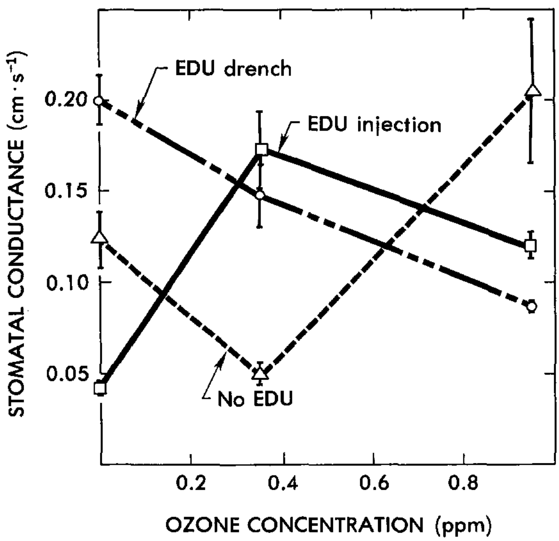

Fig. 2. Stomatal conductivity of 2-yr-old containerized yellow-poplar seedlings 2 days after fumigation with O_3. Refer to legend for Fig. 1.

Table 3. The effect of EDU treatment on foliar injury of 2-yr-old containerized seedlings of yellow-poplar following O_3 fumigation.

Ozone	*Injury rating*[z]		
Concentration (ppm)	*EDU Injection*[y]	*EDU Drench*[x]	*No EDU*
0	0.025[a]	0.045[a]	0.105[a]
0.35	0.043[a]	0.150[ab]	0.138[ab]
0.95	0.338[b]	0.463[c]	0.500[c]

[z]Foliar injury rating from 0-10, 48 hr. after exposure to O_3 for 3 hr. Each value represents the mean of 4 seedlings. Data were subjected to arscin transformation prior to statistical analysis. Mean separation among treatments for each O_3 concentration by Duncan's new multiple range test, 5% level.
[y]5 ml EDU (500 ppm) per seedling.
[x]250 ml EDU (500 ppm) per container.

jury 69% at 0.35 ppm O_3 and 32% at 0.95 ppm O_3 when compared to seedlings receiving no EDU. Based on the degree of damage, it is reasonable to anticipate that Pn would decrease as foliar injury increases. There was a significant increase in foliar injury for both EDU treatments as the fumigation level increased from 0.35 to 0.95 ppm O_3 (Table 3). Correspondingly, a decrease in Pn was noted for these same treatments (Fig. 1). However, for seedlings without EDU, as foliar injury increased from 14% to 50%, Pn remained unchanged. These results are unexpected and may have resulted from the fact that folilar injury ratings were recorded for entire seedlings, while Pn measurements were made on a single leaf from each seedling. Thus, there may not be the strong correlation between foliar injury and Pn which normally would be expected in a study of this type.

The results of this investigation show that EDU treatment, while affording protection from O_3-induced foliar injury in yellow-poplar seedlings, does not adversely affect Pn. These data would suggest that the chemical could probably be used safely for protecting high value urban trees from air pollution injury without the risk of altering normal physiological activity. This is an important consideration for arborists who may contemplate use of EDU in the urban landscape.

Literature Cited

1. Barnes, R. L. 1972. *Effects of chronic exposure to ozone on photosynthesis and respiration of pines.* Environ. Pollut. 3:133-138.
2. Bennett, J. H., E. H. Lee and H. E. Heggestad. 1978. Apparent photosynthesis and leaf stomatal diffusion in EDU treated ozone-sensitive bean plants. **In** Proc. 5th Annu. Plant Growth Regulator Working Group, Blacksburg, VA.
3. Birdwell, R. G. S. 1979. Plant Physiology, 2[d]ed. MacMillian Publ. Co., Inc., New York, NY, 726 pp.
4. Carlson, R. W. 1979. *Reduction in the photosynthetic rate of Acer, Quercus and Fraxinus species caused by sulfur dioxide and ozone.* Environ. Pollut. 18: 159-170.
5. Carnahan, J. E., E. L. Jenner and E. K. Wat. 1978. *Prevention of ozone injury to plants by a new chemical protectant.* Photopathology 68:1225-1229.
6. Cathey, H. M. and H. E. Heggestad. 1982. *Ozone and sulfur dioxide sensitivity of petunia: Modification by ethylenediurea.* J. Amer. Soc. Hort. Sci. 107:1028-1035.
7. Cathey, H. M. and H. E. Heggestad. 1982. *Ozone sensitivity of herbaceous plants: modification by ethylenediurea.* J. Amer. Soc. Hort. Sci. 107:1035-1042.
8. Cathey, H. M. and H. E. Heggestad. 1982. *Ozone sensitivity of woody plants: modification by ethylenediurea.* J. Amer. Soc. Hort. Sci. 107:1042-1045.
9. Clark, B., M. Henninger and E. Brennan, 1978. *The effect of two antioxidants on foliar injury and tuber production in 'Norchip' potato plants exposed to ambient oxidants.* Plant Dis. Reptr. 62:715-717.
10. Evans, L. S. and I. P. Ting. 1973. *Ozone induced membrane permeability changes.* Amer. J. Bot. 60:155-162.
11. Heath, R. L. and P. E. Frederick. 1979. *Ozone alteration of membrane permeability in Chlorella. I. Permeability of potassium ions as measured by rubidium tracer.* Plant Physiol. 64:455-459.
12. Heck, W.W., R. B. Philbeck and J. A. Dunning. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants. USDA Publ. ARS-5-181.
13. Koziol, M. J. and F. R. Whatley. 1984. Gaseous Air Pollutants and Plant Metabolism. Butterworths Scientific. Cambridge, England. 466 pp.
14. Lee, E. H., J. H. Bennett and H. E. Heggestad. 1981. *Retardation of senescence in red clover leaf discs by a new antiozonant, N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N'-phenylurea.* Plant Physiol. 67:347-350.
15. Legassicke, B. C. and D. P. Ormrod. 1981. *Suppression of ozone injury on tomatoes by ethylenediurea in controlled environments and in the field.* HortScience. 16:183-184.
16. Roberts, B. R., K. F. Jensen and H. M. Cathey. 1985. *Modification of ozone sensitivity in seedlings by ethylenediurea: soil application vs stem injection.* J. Amer. Soc. Hort. Sci. 11:178-180.
17. Tingey, D. T. and G. E. Taylor, Jr. 1982. Variation in plant response to ozone: a conceptual model of physiological events, p. 113-138. **In** M. H. Unsworth and D. P. Ormrod (eds). Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworths Scien-

tific, London, England.
18. Weidensaul, T. C. 1980. *N-[2-oxo-1-imidazolidinyl) ethyl]-N'-phenylurea as protectant against ozone injury to laboratory fumigated pinto bean plants.* Phytopathology 70:42-45.

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Abstracts

Weatherington, R. 1986. **Your eroding right to fire without cause.** Agrichemical Age 30 (1): 27-29.

For many years an employee not covered by an employment or union contract has been able to quit at will. Since the employee could walk without a reason, employers also had the right to terminate an employee at their will, without reason. This balance has generally existed since the start of the industrial revolution. But, because of an explosion in state laws and court rulings, the dogmatic employment-at-will tradition is dead or dying in most states. The absolute right to hire and fire has basically changed. Laws covering civil rights, age discrimination, and equal employment have been in place for years, but courts are increasingly being brought into the relationship between employers and employees in new ways. Now, in more than two-thirds of the states, owners have lost some type of wrongful discharge lawsuits, and there is no slowdown in sight. Some estimates project that unjust dismissal suits could skyrocket to 300,000 filings a year. All firms should have their attorneys check their company employment practices, and they should review these practices at least once a year so timely adjustments can be made. It is possible for a handbook written today to be out of date a year from now.

Haller, J. M. 1986. **The ideal arborist—one professional opinion.** Arbor Age 6(7): 22-24.

I would like to propose my explanation for the alarming shortage of qualified personnel. The problem seems to be that people the industry needs are exceptional types—types that occur infrequently in nature and that are not mass produced in schools. The ideal arborist should be intelligent, dedicated, sensitive. with an inexhaustible curiosity about all things arboreal and a reverence for all forms of plant life. In addition, he should be physically strong and agile enough to perform all phases of tree work in his own person. It is not enough to stand on the ground and direct all operations by telephone. Such a remote-control operator becomes a kind of glorified bookkeeper. Unfortunately, the educated, studious person is seldom the physically able type, while contrariwise the physically able type is seldom educated and studious. Those who can climb do not study, and those who study do not climb. Nature doesn't produce the requisite ideal type in sufficient numbers. Sadly, the conscious or unconscious goal of every climber is to get out of the tree and into the office. The born arborist is the man who works in trees because he prefers it to any other activity whatsoever, the man who couldn't be persuaded to do anything else, the man who loves his work with all his heart and soul.