Ethylenediurea Induces Hormesis in Plants

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Abstract
Tropospheric ozone levels are elevated throughout the northern hemisphere. The potential threat of ozone to vegetation urges for studying plant protection methods. The chemical ethylenediurea (EDU) is the most extensively utilized substance for protecting plants against ozone damage in research projects. This commentary provides collective evidence showing hormetic responses of plants to EDU and suggests that EDU may act as a conditioning agent against elevated ozone exposures. This article also suggests testing different substances in a hormetic framework for protecting plants against ozone damage. The concept of hormesis provides a significant perspective for reducing the economic cost for plant protection.

Keywords
antiozonant, biphasic, ethylenediurea, homeostasis disruption, hormesis, ozone, plant protection

Background
Tropospheric ozone (O₃) levels noted important increases after the industrial revolution. Beginning in the early 1950s, when it was realized that air pollution affects plants in the United States, O₃ effects on vegetation have been widely studied in the northern hemisphere. Thus, adverse effects of elevated O₃ levels on vegetation are well-documented nowadays.

Early after it was observed that air pollution causes negative effects on vegetation, trials began for testing potential protectants of vegetation against air pollution. With the progressive elevation of O₃ levels throughout the northern hemisphere and the accumulated evidence on O₃ impacts in vegetation, more studies investigated potential phytoprotectants against O₃ damage. Although a plethora of substances has been screened as to the efficacy to protect plants against O₃ damage, only the chemical compound ethylenediurea (EDU) has been extensively studied due to the well-documented protection it offers to plants.¹ However, despite the importance of dose–response studies before setting large-scale experiments dealing with mechanisms, only a few studies derived EDU dose responses, whereas the number of studies investigated EDU mode of action in relatively O₃ clean air remains limited. This is of particular importance since EDU is also used as a research tool for studying O₃ effects on vegetation under open-field conditions, without using any exposure facilities, by attributing differences between EDU treatments to O₃.

Is Hormesis the Key to the EDU Mode of Action in Protecting Plants Against O₃ Damage?
A recent study suggests that EDU may protect plants against O₃-induced damage through hormesis,² thus calling for more detailed reexamination of the published literature. Indeed, from the first years of active EDU research, stimulation of total fresh weight of tobacco (Nicotiana tabacum L.) callus was found.² Furthermore, robust dose–response studies revealed evidence for EDU-induced hormesis (0-800 mg L⁻¹) in radish (Raphanus sativus L.) plants (root dry weight and cotyledon fresh weight) grown in nonfiltered air with O₃ ≈25 ppb; the maximum stimulation occurred at 300 mg EDU L⁻¹.² In a further experiment with carrot (Daucus carota L.) grown under...
≈36 ppb mean ambient O₃ during the experimental period, EDU (0-450 mg L⁻¹) induced hormetic-like effects (Figure 1) in a variety of growth, production, and nutrition end points at 60 and 90 days after germination; the maximum stimulation occurred at 150 mg EDU·L⁻¹.¹ Studies with radish,³ carrot,⁴ and other plant species¹ suggest that EDU protects plants against O₃ when applied at concentrations within the low-dose zone.

Photosynthetic pigments are utilized as simple and effective biomarkers of plant stress. A review of the literature suggests that photosynthetic pigments were studied in 25% of the published studies with EDU. However, the vast majority of these studies were conducted under open-field conditions with no control O₃ treatment, or with commonly high O₃ levels, and/or with third potential confounding environmental factors, making it thus difficult to elucidate the EDU per se mode of action in plants. Only a few studies investigated EDU mode of action in plants, independently of O₃.⁵-⁸ Two spray applications of EDU did not affect growth of tobacco seedlings in O₃ clean air.⁶ Interestingly, EDU at 10 or 100 mg L⁻¹ did not affect total carotenoids content (TCar), but at 500 mg L⁻¹, TCar was ≈190% greater, compared with 0 mg EDU·L⁻¹, at relatively O₃ clean air. Plants treated with EDU at 500 mg L⁻¹ had nearly 130% higher total chlorophyll content (TChl) than plants treated with EDU at 0 mg L⁻¹; however, the difference was nonsignificant. As a result, TChl/TCar ratio in plants treated with 500 mg EDU·L⁻¹ was 66% that of control plants.⁶ In a similar experiment, pinto bean (Phaseolus vulgaris L.) plants treated once with EDU concentrations up to 250 mg L⁻¹ had nonsignificantly different TChl content, albeit slightly higher, than control plants at O₃ clean air.⁷ However, it appeared to be higher when measured 5 and 8 days after EDU treatment, but not when measured at 12 and 15 days after EDU treatment,⁷ as EDU may not persist in the apoplast for so long.¹ In a different study,⁸ TCar, TChl, and TChl/TCar ratio were not changed by EDU, but chlorophyll a content to chlorophyll b content ratio was increased by a single soil application of 500 mg EDU·L⁻¹ in snap bean (P vulgaris) under O₃ clean air. Previous studies⁵-⁸ along with additional experiments (E. Agathokleous, unpublished data, 2015), conducted under relatively O₃ clean air, provide indications of direct effects of EDU on the photosynthetic pigments. If this is the case, greater content of photosynthetic pigments in EDU-treated than EDU-untreated plants often found under field conditions with no high O₃ levels may be more upon EDU direct effects rather than O₃-induced chlorophyll degradation.

Conclusions and Future Opportunities

This commentary provides collective evidence showing clear hormetic responses of plants to EDU. It also reaffirms that the EDU-conferring protection against O₃ damage occurs in the low-dose region (left of no-observed-adverse-effect-level) and suggests that EDU may act as a conditioning agent against a higher pressure by elevated O₃ concentrations. This commentary also challenges the air pollution research community to consider testing different substances in a hormetic framework for protecting plants against O₃ damage. For instance,

Figure 1. Typical examples of ethylenediurea-induced hormetic dose responses from published literature. When needed, dose and response data were estimated from figures of the reviewed articles using image analysis software (Adobe Photoshop CS4 Extended v.11; Adobe Systems Incorporated, City of San José, California).
chemicals and their respective concentrations within the low-dose zone can be selected among hundreds of chemicals well-documented to induce hormesis in plants; these can be found in a series of available review papers and in the Calabrese-Blain hormesis database. The lines of evidence listed herein call for more studies on EDU mode of action in O_3 clean air, such as in closed chambers, and at the absence of any confounding stress in the experimental plants so as to first reveal the EDU mode of action and then apply EDU at field conditions. The concept of hormesis provides a significant perspective for reducing the economic cost for plant protection.

Declaration of Conflicting Interests

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