# Ethylenediurea (EDU) as a protectant of plants against $O_3$

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**Abstract**

Ethylenediurea (EDU) is an anti-ozonant substance that is recognized as a versatile research tool, and recently attracts increasing interest. As many wild plant species are forced into complex responses by tropospheric ozone ($O_3$), these responses are crucial for the functioning of ecosystems and consequently for the biosphere; thus, countermeasures are required. A plethora of substances have been evaluated as to their effectiveness in protecting plants against $O_3$. EDU is the most widely-used substance in $O_3$ research, in order to moderate $O_3$ effects on plant growth. We present a synoptic table with recent literature on EDU applications to plants as a protectant against $O_3$. This table summarizes important information on these publications, and we hope to be usefull to researchers intended to employ EDU in their research with wild plants, but also to researchers working with air pollution control and other scientists.

*Key words*: Bio-monitoring tool, Anti-ozonant, Ethylene-di-urea (EDU), Plant protection, Tropospheric ozone

## Introduction

Ground-surface ozone ($O_3$) is a greenhouse gas (Krupa and Manning 1988, Chameides *et al*. 1994, Paoletti and Manning 2007) which has long been documented to affect flora (Dugger *et al*. 1966, Saitanis *et al*. 2001, Bermejo *et al*. 2003, Koike *et al*. 2013, Agathokleous *et al*. 2015a). Several cultivated plants, forest trees, and other wild plant species experience $O_3$-induced negative effects, through complex responses, when exposed to $O_3$ concentrations over a species-specific threshold (Dugger *et al*. 1966, Saitanis *et al*. 2001, Bermejo *et al*. 2003, Saitanis *et al*. 2004, Fiscus *et al*. 2005, Hayes *et al*. 2006, Feng *et al*. 2008, Saitanis 2008, Ainsworth *et al*. 2012, Zhang *et al*. 2012, Koike *et al*. 2013, Saitanis *et al*. 2014, Agathokleous *et al*. 2015a, Feng *et al*. 2015). Such effects could be critical for human feeding needs, plant communities, ecosystems function, and thus for the entire biosphere. Given that, nowadays, the $O_3$ concentrations occur at elevated levels and they still continue rising (Chameides *et al*. 1994, Akimoto 2003, Yamaji *et al*. 2008, Kalabokas *et al*. 2013, Akritidis *et al*. 2014, Kleanthous *et al*. 2014, Saitanis *et al*. 2015b), $O_3$-sensitive species should be protected.

During the last six decades, plenty of substances have been evaluated as to their efficacy to protect plants against $O_3$ deleterious effects (e.g. Freebairn *et al*. 1960, Manning *et al*. 1973a, Manning *et al*. 1973b, Francini *et al*. 2011, Agathokleous *et al*. 2014, Saitanis *et al*. 2015a). Tested substances include vitamins, such as ascorbic acid (Freebairn *et al*. 1960), agrochemicals, such as azoxystrobin, benomyl, penconazole, hexaconazole, trifloxystrobin (Manning *et al*. 1973a, Manning *et al*. 1973b, Saitanis *et al*. 2015a), the antitranspirant Di-1-*p*-menthene (Francini *et al*. 2011, Agathokleous *et al*. 2014), and several others. Agrochemicals, such as fungicides and pesticides, would have the big advantage to be applied for both purposes, to protect plants against fungi and pests but also against $O_3$, reducing thus the financial cost in practice (in the framework of integrated plant protection). However, the efficacy of the tested substances is inadequate, except that of ethylenediurea (EDU) (Carnahan *et al*. 1978, Paoletti *et al*. 2009, Feng *et al*. 2010, Manning *et al*. 2011, Agathokleous *et al*. 2015b).

Ethylenediurea ($C_4H_{10}N_4O_2$) is an antiozonant, described as N-[-2-(2-oxo-1-imidazolidinyl) ethyl]-N'-phenylurea], (Wat 1975). It has been included in numerous studies with usually very encouraging results (Paoletti *et al*. 2009, Feng *et al*. 2010, Manning *et al*. 2011, Oksanen *et al*. 2013, Pandey *et al*. 2014, Agathokleous *et al*. 2015b). Although the EDU research had declined for a period due to mis-interpretations and technical problems (Manning *et al*. 2011), the interest has risen again and seems to be increasing (Agathokleous *et al*. 2015b). EDU is something more than just a substance used to protect plants against $O_3$ in research: it is a research tool per se, which can be used for bio-monitoring purposes, $O_3$

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research in remote areas, studying the $O_3$ effects on plants, etc. (Paoletti *et al*. 2009, Manning *et al*. 2011, Oksanen *et al*. 2013, Agathokleous *et al*. 2015b). Nevertheless, its mechanism of action against $O_3$ deleterious effects has not been explained yet (Paoletti *et al*. 2009, Manning *et al*. 2011, Agathokleous *et al*. 2015b) and more multi-aspects research is required.

Considering the growing importance given to EDU as a research tool, we report here a summary table of the features and the overall conclusion of research studies dealing with the $O_3$-EDU-plant interaction, which have been reported in relevant scientific publications over the years. More specifically, the summary table includes the following information: i) the names of the studied plant species, ii) the method of exposure to $O_3$ (i.e. open field experiments, open top chamber experiments - OTC, close chambers, etc.), iii) the $O_3$ concentration used in the research, iv) the duration of exposure of plants to $O_3$, v) the applied concentration of EDU, vi) details on the EDU applications (before or after exposure to $O_3$, etc.), vii) the stage of growth of plants when EDU was applied, viii) the method of applications (via foliage (spray), via root (drench) or injection, etc.), ix) information of the repetition of the EDU application (i.e. if the EDU was applied only once or repentantly and at what frequency), x) if any EDU-caused phytotoxicity was reported (+) or not (-), xi) whether EDU finally protected the plants against $O_3$ (+) or not (-), and xii) the names of authors and the year of publication. All these publications are extensively discussed in a review article published by Agathokleous *et al*. (2015b).

This table can be used as research material in order to help researchers who are interested in plant protection against ozone.

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Table 1. Plant species that have been examined for their response to $O_3$ under EDU treatments. Symbols + and - state presence or not, respectively. When they are placed together, it means that some parameters were present and some others were not. Drench stands for soil drench and spraying for foliar spraying. Phytotoxicity means EDU-caused phytotoxicity, and repetition means repetition of EDU treatments over time. DAS, DAG, DAE stands for, days after seeding, days after germination, and days after emergence, respectively. AA=ambient air, CF=charcoal-filtered air, NF=non-filtered air, 2xAA=twice ambient (AA), EA=$O_3$ enriched air, P = purafil, NF+ = non filtered air + a concentration of $O_3$, CF+=carbon-filtered air + a concentration of $O_3$, FA= filtered air, FA+=filtered air + a concentration of $O_3$, OTC = open-top chamber, CSTR = continuously stirred-tank reactor (also known as vat- or backmix reactor), and FACE=Free-Air $O_3$ Enrichment

| A/A | Species | Exposure | Ozone Concentration | Exposure Duration | EDU Concentration | Application | Stage | Method | Repetition | Phytotoxicity | Protection | REF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Crop Plants** | | | | | | | | | | | |
| **1** | *Beta vulgaris* L. | Open-field | Mean 8-h 52-73 nl $l^{-1}$ | ≈ 2 months | 0 or 300 mg $l^{-1}$; up to 30 DAG, 100 mL were given to each plant, thereafter 200 mL were applied. | | 10 DAG | Drench | 10 days | - | + | Tiwari and Agrawal, 2009 |
| **2** | *Brassica rapa* L. | a) Open-field; b) CF and CF+ OTCs | a) The mean 6-h over experimental period was 54.8 nl $l^{-1}$ in the suburban site and 66. nl $l^{-1}$ in the rural; b) CF+: received 80 nl $l^{-1}$ | b) 7 days | 500 mg $l^{-1}$ as a soil drench at 200 ml $pot^{-1}$ | b) 24 h prior to exposure | a) when the first true leaf was fully expanded (1 DAS); b) 19 DAS | Drench | a) 10 days; b) No | - | + | Hassan *et al.* 1995 |
| **3** | *Conyza bonariensis* L. | CSTRs | 0, 0.1, 0.3 and 0.5 µ $l\ l^{-1}$ | 6 hours | 0.3 mg m $l^{-1}$ (w/v) | 1 day before the exposure | The leaves of the primary rosette stage were ≈ two-thirds expanded | Drench | No | - | + | Mersie *et al.* 1994 |
| **4** | *Daucus carota* L. | Open-field | Mean (8-h) of experimental period 36.1 nl $l^{-1}$ | ≈ 3 months | 0, 150, 300 and 450 mg $l^{-1}$; up to 30 DAG, 100 ml EDU was given to each plant, thereafter, 200 ml | | 10 DAG (DAG) | Drench | 10 days | - | + | Tiwari and Agrawal, 2010 |
| **5** | *Echinacea purpurea* L. | OTCs | a) CF or 2xAA; b) CF, AA, 2xNF (12-h AA 29-38 nl $l^{-1}$ during the both periods) | a) 1st year 6 weeks; b) 2nd year 12 weeks (15h/d, everyday) | a) 0, 100, 200 and 300 ppm (µl $l^{-1}$); b) 0, 200, 400 and 600 ppm | After the fourth day following the exposure | | Spraying | 7 days | - | + | Szantoi *et al*. 2007 |
| **6** | *Glycine max* L. | Open-field | Cumulative dose 81.2-101.4 µl $l^{-1}$ h, for the 4 seasons | ≈ 3 to 5 months per year | 0 or 500 mg $l^{-1}$; 4 lit/row | | First application in June, last in September | Drench | 14 days | - | - | Brennan *et al*. 1990 |
| | | OTCs | | | | | | | | - | + | Scheepers *et al*. 2010 |
| **7** | *Lycopersicon esculentum* L. | Open-field | avg hourly 88.41-89.98 nl $l^{-1}$ | 82 days | 400 mg $l^{-1}$ of aqueous solution | | ≈ 1-month-old plants | Drench | 12 days | - | -+ | Varshney and Rout, 1998 |

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| **8** | *Nicotiana tabacum* L. | Open-field | from app. 40 to almost 60 nl $l^{-1}$ | 23 days | 0, 150, 300, 600 mg $l^{-1}$ a.i. | | | Drench | 10 days | - | + | Bytnerowicz *et al.* 1993 |
| | | Plexiglas chambers | CF or CF+: maintained at 80‹ nl $l^{-1}$ during exposures (7h/d) | 5 or 6 days | 0 or 300 mg $l^{-1}$ (also U (70 mg $l^{-1}$), or PU (159 mg $l^{-1}$l) | 24 h prior to exposure | | Spraying | No | - | + | Godzik and Manning, 1998 |
| | | Open-field | during day light hours of June, July and August averaged 29, 35 and 22 nl $l^{-1}$respectively | 12 weeks on field | 50 WP at rate 1 kg ha-1 a.i. + 0.1% Tween-20 | | 4-6 leaf | Spraying | 8-10 days | - | + | Bisessar and Palmer, 1984 |
| **9** | *Oryza sativa* L. | Open-field | frequently exceeded 40 nl $l^{-1}$, occurred less often for 50 nl $l^{-1}$, and were less frequent above 60 nl $l^{-1}$ | ≈ 5 months | 0, 150, 300, 450 mg $l^{-1}$ | | 2 months after sowing | Spraying | 7 days | - | -+ | Wang *et al.* 2007 |
| **10** | *Phaseolus vulgaris* L. | Open-field | In 1st year, avg hourly 41-59 nl $l^{-1}$ for a total of 303 h; in 2nd year, for 355 h. | 2.5-3 months | 300 mg $l^{-1}$ (a.i.) | | Full expansion of primary leaves | Spraying | 7 days | - | -+ | Elagoz and Manning, 2005 |
| | | Open-field | 7 h mean of the 5 experiments: 45-49 n $l^{-1}$ | | 0 or 100 mg $l^{-1}$ at 200 ml/plant (dose of 20 mg per plant or 0.71 g $m^{-2}$) | 10-19 DAS | When the first trifoliate leaf was expanding | Drench | after 14-17 days | + | - | Kostka-Rick and Manning, 1993a |
| | | Closed chambers | 0.45 µl $l^{-1}$ | 4 hours | 50 mg EDU in 100 ml aqueous per pot | 24 h prior to exposure | 3-week-old plants | Drench | No | - | + | Lee *et al* . 1981 |
| | | Closed chambers | 0.30 µl $l^{-1}$ | 3 hours | 100 ml of 0.5 mg $ml^{-1}$ EDU | 48 h prior to exposure | When the first trifoliate leaf was fully expanded | Drench | No | - | + | Lee *et al* . 1997 |
| | | a) NF glasshouse; b) CF or O3 greenhouse | b) CF or 60-75 nl $l^{-1}$ (7h/d) | b) 13 days | a) 0, 300, 400, 500, 600, and 800 mg $l^{-1}$ ; b) 0, 100, 200, 300, and 400 mg $l^{-1}$ ; 200 ml (± 4 ml) of the solution was applied to each pot. | a) 6 days after emergence; b) 12 days after emergence (3 days prior to exposure) | a) when primarily leaf expanded; b) 12 days after emergence | Drench | a) after 12, 24, 38 days b) after 14 days | + | -+ | Kostka-Rick and Manning, 1993c |

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| | | CSTRs | 0, 0.1, 0.3 and 0.5 µ l $l^{-1}$ | 6 hours | 0.3 mg $ml^{-1}$ (w/v) | 1 day before the exposure | When the second set of trifoliate leaves was at ≈ 80% expansion. | Drench | No | - | + | Mersie *et al.* 1994 |
| | | Plexiglas chambers | 1.6 mg $m^{-3}$ (0.8 µl $l^{-1}$ at 20 °C) | a) 2h for primary leaves; b) 4h (6h/d) for trifoliate leaves on older plants | 50 ml of 0.4% and 0.6% (w/v) | 24h before exposure | At least primary leaves fully expanded | Drench | No | - | -+ | Chanway and Runeckles, 1984 |
| | | Closed chambers | 6h mean of 48 nmol $mole^{-1}$, 6h/d, 6d/w | plants were fumigated 29 times | 0, I50 and 300 mg $l^{-1}$ at a rate of 200 ml/pot (total concentration of 60-120mg/plant/3 l soil). | | The primary leaves of the bean plants were developed and the first trifoliate leaf was expanding (11DAE) days after emergence | Drench | 10 or 20 days | - | + | Astorino *et al.* 1995 |
| | | OTCs | NF, CF, CF+AA, CF+2xAA | ≈ 50 days | 200 ml of a 150 mg $l^{-1}$ solution | 1st application 2 weeks after the OTCs treatments | When the primary leaves were fully expanded. | Drench | 14 days | - | + | Brunschon-Harti *et al* . 1995 |
| | | Growth chambers | 0.3 µl $l^{-1}$ | 3 hours | a) 0, 30, 50, or 150 µg m $l^{-1}$; b) 0, 5, 15, 20, 25, 30µg $ml^{-1}$ | plants remained 2 days in EDU-enriched solution and then placed in EDU-free nutrient solution | When the second set of trifoliate leaves reached ≈ 80% expansion | hydroponically (dissolved in nutrient solution) | No | - | + | Gatta *et al* . 1997 |
| | | Closed chambers | 100, 250, 400, 500 or 750 nl $l^{-1}$ | 6 hours | 0, 500, 1000, 5000 mg $l^{-1}$ + Triton X-100 at 0.05 and 0.10 % | 1, 3 ,7 or 10 days prior to fumigation | When primary leaves were fully developed for the 10 days prior to fumigation, but primary leaves were not present for the 1, 3, 7 days prior to fumigation | Spraying | No | - | + | Weidensaul, 1980 |
| | | Growth chambers | 80 nl $l^{-1}$ | a) 12.5 to 797 min b) 150 or 300 min | a) 0 to 500 mg $l^{-1}$ + 3.6% glycerol + 0.1% Tergitol Nonionic 15-S-12 surfactant; b) 0 or 4 mg per 20 ml water | | 13 days after planting when primary leaves were fully expanded and first trifoliolates were ≈ 40 millimeters in width. | a)Spraying; b)Drench | No | - | + | Carnahan *et al* . 1978 |

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| **11** | *Pisum sativum* L. | Closed chambers or greenhouse | a) 0 or 250 nl $l^{-1}$; b) 0, 200, 220, 250 or 280 nl $l^{-1}$ | a) 4 hours; b) 3 hours | 25 ml of 0 or 150 mg $l^{-1}$ per cell | 24 h prior to exposure | When the plants consisted of four leaves (20 DAS) | Drench | No | - | + | Zilinskas *et al*. 1990 |
| **12** | *Raphanus sativus* L. | a) Open-field; b) CF and CF+ OTCs | a) The mean 6-h over the experimental period was 54.8 nl $l^{-1}$ in the suburban site and 66.9 nl $l^{-1}$ in the rural; b) CF+: received 80 nl $l^{-1}$ | b) 7 days | 500 mg $l^{-1}$ as a soil drench at 200 ml $pot^{-1}$ | b) 24 h prior to exposure | a) when the first true leaf was fully expanded (1 DAS); b) 19 DAS | Drench | a) 10 days; b) No | - | + | Hassan *et al*. 1995 |
| | | Open-field | 7-h mean and AOT40 during the experimental period was 36 nl $l^{-1}$ and 1286 nl $l^{-1}$ h, respectively | 5 weeks | 0 or 0.2 g EDU in 1 lit of distilled water (100 ml/tray) | 11 days after emergence | When the first true leaves were 1-2 cm | Drench | after 14 days | - | + | Pleijel *et al*. 1999 |
| | | Greenhouse | a) NF; b) CF or 60-75 nl $l^{-1}$ (7h/d, 6d/w) | b) 13 days | a) 0, 300, 400, 500, 600, and 800 mg $l^{-1}$ ; b) 0, 100, 200, 300, and 400 mg $l^{-1}$ ; 100 ml (± 2 ml) of the solution was applied to each pot, containing ≈ 350 ml of substrate. | b) 4 days prior to exposure | When cotyledons of the radish plants were fully developed and the first pair of true leaves was expanding | Drench | a) after 11 days; b) No | + | -+ | Kostka-Rick and Manning, 1993b |
| | | Open-field | not exceeded moderate levels (7h mean lower than 50 nl $l^{-1}$ ) | | 100 ml $plant^{-1}$; 100 mg $l^{-1}$ (10 mg $plant^{-1}$) | 10-14 DAS | When the cotyledons were fully developed and the first pair of leaves started to expand | Drench | No | - | -+ | Kostka-Rick *et al*. 1993 |
| **13** | *Sesamum indicum* L. | Open-field | Seasonal mean 10 hr was 91 nl $l^{-1}$ | ≈ 3 months | 0, 125, 250, 375, 500 mg $l^{-1}$ | | ≈ 14-days-old plants | Drench | 7 days | - | + | Wahid *et al*. 2012 |
| **14** | *Solanum tuberosum* L. | Open-field | the cumulative dose was 45-65 µl $l^{-1}$ h during the first 3 years and the last, but the fourth year was ≈ 2 times higher | | 6.7 kg ai/ha (500 mg $l^{-1}$) | | | Drench | 21 days | - | + | Clarke *et al*. 1990 |

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| | | CSTRs | 0.10 µ1 $l^{-1}$ or CF (5h/d) | ≈ 2 weeks | a) 0, 50, 150, and 250 mg $l^{-1}$ a.i. in tap H20, equivalent to doses of 0, 15, 45, and 75 mg a.i. $l^{-1}$ soil volume, respectively; b) 0 or 50 mg $l^{-1}$ a.i. in tap $H_20$, equivalent to doses of 0 or 15 mg a.i. $l^{-1}$ soil volume, | 1 day before the exposure | 20 days after planting | Drench | No | -+ | + | Eckardt and Pell, 1996 |
| **15** | *Trifolium repens* L. | Open-field | AOT40 from day of emergence to the final harvest were 15463 nl $l^{-1}$ h for experiment 1 and 12098 nl $l^{-1}$ h for experiment 2. | 3 months | 0 or 100 mL of a 150 mg $l^{-1}$ solution | | From the emergence of the first trifoliate leaf | Drench | 14 days | - | + | Fumagalli *et al*. 1997 |
| | | Open-field | 30.3-46.6 nl $l^{-1}$ (12-h) during the growth period | ≈ 2 months | 0, 150 and 300 mg/L | | 10 DAG | Drench | 10 days | - | + | Singh *et al*. 2010b |
| **16** | *Trifolium subterraneum* L. | Open-field | 7-h daily mean 51-58 nl $l^{-1}$ and 21-26 nl $l^{-1}$ among sites of 1st year and 2nd year, respectively | 4 and 8 weeks the 1st and 2nd year, respectively | 100 ml per pot of a 150 $mg^{-1}$ aqueous solution | on the same day transferred to the fields | When the first trifoliate leaves were fully expanded | Drench | 14 days | - | -+ | Tonneijck and van Dijk, 1997 |
| | | Open-field | average AOT40 1600nl $l^{-1}$ h for all the seasons, plots and experiments | 3 growing seasons | 100 ml per pot of a 150 mg $l^{-1}$ aqueous solution | | When the first trifoliate leaves were fully expanded | Drench | 14 days | - | -+ | Tonneijck and van Dijk, 2002 |
| **17** | *Triticum aestivum* L. | Open-field | 34.2-54.2 nl $l^{-1}$ (8-h) during the growth period | ≈ 4 months | 0 and 400 mg $l^{-1}$ (100 ml $plant^{-1}$) | | 10 DAG | Drench | 12 days | - | + | Singh *et al*. 2009 |

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| | | a) Open-field; b) CF and NF OTCs | mean concentration 27.7-59.1 nl $l^{-1}$ | | a) 0, 200, 300, 400 and 500 mg $l^{-1}$; b) 0 and 400 mg $l^{-1}$ | a) Initially up to 40 DAG, 100 ml $plant^{-1}$ EDU solution was given, thereafter, 200 ml $plant^{-1}$ EDU was applied as soil drench up to 110 DAG. EDU was applied up to 50 DAG for vegetative phase and after 50 DAG for reproductive phase. However, flag leaf was treated with EDU twice a day as foliar spray after it was fully expanded. | At different stages of plant development i.e. vegetative phase (10–50 DAG), reproductive phase (60–110 DAG) as soil drench and foliar spray on flag leaf (60–110 DAG) | Drench but Spraying on flag leaf | 10 days | - | + | Singh and Agrawal, 2010 |
| | | Open-field | were low during December and January, but thereafter it often exceeded 40 nl $l^{-1}$ levels for several hours during February and March. | ≈ 4 months | 0, 150, 300 and 450 mg $l^{-1}$; up to 40 DAG, 100 ml EDU was given to each plant, thereafter, 200 ml EDU | | 10 DAG (DAG) | Drench | 10 days | - | + | Tiwari *et al* . 2005 |
| | | Open-field | frequently exceeded 40 nl $l^{-1}$, occurred less often for 50 nl $l^{-1}$, and were less frequent above 60 nl $l^{-1}$ | ≈ 5 months | 0, 150, 300, 450 mg $l^{-1}$ | | ≈ 3.5 months after sowing | Spraying | 7 days | - | -+ | Wang *et al.* 2007 |
| **18** | *Vigna mungo* L. | Open-field | 41.3-59.9 nl $l^{-1}$ (12-h) during the growth period | 3 months | 400 mg $l^{-1}$ | | 10 DAG | Drench | 10 days | - | + | Singh *et al* . 2010a |
| **19** | *Vigna radiata* L. | Open-field | 64-69 nl $l^{-1}$ | 80 days | 0 or 500 mg $l^{-1}$ | | 1 week after emergence | Drench | 7 days | - | + | Agrawal *et al* . 2005 |
| | **Wild Plants** | | | | | | | | | | | |
| **20** | *Rudbeckia laciniata* L. | OTCs | CF, NF, 2xAA; Mean 12-h and 24-h in the ambient air were 33–26 nl $l^{-1}$, over the 12-wk period | 12 weeks | 0, 200, 400 or 600 mg $l^{-1}$ | After the fourth day following the exposure | | Spraying | 7 days | - | -+ | Szantoi *et al* . 2009 |

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| **21** | *Fagus sylvatica* L. | Semi-OTCs | target 30 nl $l^{-1}$ day and 15 nl $l^{-1}$ night / except during three 14-day episodes, 3 OTCs received ozone at target concentrations of 80 nl $l^{-1}$ day and 30 nl $l^{-1}$ night. | 5 months background ozone | 1st and 2nd episodes: 0.5 ml of a 500 mg $l^{-1}$ ; 3rd episode: 0.25 ml of 1000 mg $l^{-1}$ - 6r 0 | 2 days before each episode | | Injection | 2 times | - | - | Ainsworth and Ashmore, 1992 |
| **22** | *Fraxinus americana* L. | Open-field | cumulative dose was 49.5 µl $l^{-1}$ h | ≈ 4 months | 250 ml of 500mg $l^{-1}$ aqueous solution | | 2- year-old seedlings | Drench | 10 days | - | - | Elliott *et al* . 1987 |
| **23** | *Fraxinus excelsior* L. | Open-field | AOT40 for the growing season was 32.49 µl $l^{-1}$ h | 6 months | 450 mg $l^{-1}$ | first in April | Adult trees | Injection | 21 days | - | + | Paoletti *et al* . 2007 |
| | | Open-field | AOT40 over the period was 32.5 µl $l^{-1}$ h | 5 months | 450 mg $l^{-1}$ | first in April | Adult trees | Injection | 21 days | - | + | Paoletti *et al* . 2008 |
| **24** | *Fraxinus pennsylvanica* L. | Open-field | cumulative dose was 49.5 and 85.6 µl $l^{-1}$ h for the 1st and 2nd season, respectively | ≈ 4 months | 250 ml of 500 mg $l^{-1}$ aqueous solution | | 2 and 3-year-old seedlings | Drench | 10 days | - | - | Elliott *et al* . 1987 |
| **25** | *Liriodendron tulipifera* L. | CSTRs | CF, CF+0.07 µl $l^{-1}$ and CF+0.15 µl $l^{-1}$ (6h/d, 7d/w) | 12 weeks | 1000 mg $l^{-1}$ (250 ml to each plant) | 8 days before fumigation | One-year-old containerized seedlings | Drench | No | - | - | Cannon *et al.* 1993 |
| **26** | *Pinus taeda* L. | OTCs | CF, NF, 1.5xAA, 2.0xAA, and 2.5xAA; mean cumulative 12-h was 91.4 µl $l^{-1}$ h | ≈ 7 months | 0, 150, or 300 mg $l^{-1}$ + Tween 20 (≈ 0.02 ml $l^{-1}$ ) | 2 days before exposure | ≈ 6-month-old seedlings | Spraying | 14 days | - | + | Kuehler and Flagler, 1999 |
| | | Open-field | Most concentrations were in the range of >40–70 nl $l^{-1}$. Peak one hour concentrations were 113, 102, and 118 nl $l^{-1}$ for the 1st, 2nd and 3rd year, respectively | 3 growing seasons | 0, 150, 300 or 450 mg $l^{-1}$ + 0.02 ml $l^{-1}$ Tween 20 | spraying | | Spraying | 14 days | - | -+ | Manning *et al* . 2003 |

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| **27** | *Populus* × *euramericana* | a) Greenhouse chambers; b) Open field | a) mean concentration 84.9 nl $l^{-1}$ , 8 h/d; b) 7 hour daily mean concentrations were 56 nl $l^{-1}$ and 59 nl $l^{-1}$ for the 1st and 2nd growing season respectively | a) 10 days; b) 2 growing seasons | a) 0.5 ml distilled water, 0.5 ml of 250 mg $l^{-1}$ EDU or 1.0 ml of 1000 mg $l^{-1}$ EDU; b) 1500 mg $l^{-1}$ or 3000 mg $l^{-1}$ in 1st season and 500 mg $l^{-1}$ in 2nd season | a) 1 day before the exposure; 2) ≈ 3 months after the cuttings plantation | a) 80 day old (40-60 cm); b) X | Injection | a) No; b) 2 or 3 weeks | - | -+ | Ainsworth *et al.* 1996 |
| **28** | *Populus deltoides* × *maximowiczii* | a) Greenhouse chambers; b) Open field | a) mean concentration 84.9 nl $l^{-1}$ , 8 h/d; b) 7 hour daily mean concentrations were 56 nl $l^{-1}$ and 59 nl $l^{-1}$ for the 1st and 2nd growing season respectively | a) 10 days; b) 2 growing seasons | a) 0.5 ml distilled water, 0.5 ml of 250 mg $l^{-1}$ EDU or 1.0 ml of 1000 mg $l^{-1}$ EDU; b) 1500 mg $l^{-1}$ or 3000 mg $l^{-1}$ in 1st season and 500 mg $l^{-1}$ in 2nd season | a) 1 day before the exposure; 2) ≈ 3 months after the Cuttings plantation | a) 80 day old (40-60 cm); b) X | Injection | a) No; b) 2 or 3 weeks | - | -+ | Ainsworth *et al.* 1996 |
| **29** | *Populus maximoviczii x berolinensis* | Open-field | mean AOT40 of the seasons was 24.6 ± 0.5 µl $l^{-1}$ h | 6 months per growing season | 1 (1st season) to 2 L of water (2nd and 3rd seasons) of 0 or 450 mg $l^{-1}$ $tree^{-1}$ | | Cuttings planted the last autumn | Drip | 7 days | - | + | Hoshika *et al* . 2013 |
| **30** | *Populus nigra* L. | Open-field (but pots) | AOT40 (4 months) reached 6170 nl $l^{-1}$.h | 1 growing season | 0 or 5 mg $plant^{-1}$ | | When the plants were ≈ 50 cm tall | Injection | 14 days | - | + | Bortier *et al.* 2001 |
| **31** | *Prunus serotina* L. | Open-field | daily maximum hours > 40 nl $l^{-1}$ were 172, 146, 181 and 167 for the 1st, 2nd, 3rd and 4th year respectively | 4 growing seasons | a) 1000 mg $l^{-1}$ EDU; b) 1000 mg $l^{-1}$ EDU + 0.06% Ortho X-77 or + 0.05% Tween-20; 40-50, 70-80, 100-110, 130-140 ml/tree in 1st, 2nd, 3rd and 3th year respectively | 1st application mid June to mid July | | Spraying | ≈10 days | - | + | Long and Davis, 1991 |

Note: Because there is no gas treatment in the open-field experiment, it is difficult to identify the EDU effect as a protection against $O_3$. However, we assumed that EDU-induced positive change is protective effect of EDU against $O_3$ in the open-field experiments, because the experiments were conducted under relatively high concentration of $O_3$ and the EDU is well known to have protective effect against $O_3$."

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