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Ethylenediurea (EDU) as a protectant of plants against O₃

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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 (O₃), 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 O₃. EDU is the most widely-used substance in O₃ research, in order to moderate O₃ effects on plant growth. We present a synoptic table with recent literature on EDU applications to plants as a protectant against O₃. This table summarizes important information on these publications, and we hope to be useful 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 (O₃) 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 O₃-induced negative effects, through complex responses, when exposed to O₃ 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 O₃ 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), O₃-sensitive species should be protected.

During the last six decades, plenty of substances have been evaluated as to their efficacy to protect plants against O₃ 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 O₃, 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 (C₄H₁₀N₄O₂) 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 O₃ in research: it is a research tool per se, which can be used for bio-monitoring purposes, O₃

research in remote areas, studying the O₃ 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 O₃ 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 O₃-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 O₃ (i.e. open field experiments, open top chamber experiments - OTC, close chambers, etc.), iii) the O₃ concentration used in the research, iv) the duration of exposure of plants to O₃, v) the applied concentration of EDU, vi) details on the EDU applications (before or after exposure to O₃, 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 repeatedly and at what frequency), x) if any EDU-caused phytotoxicity was reported (+) or not (-), xi) whether EDU finally protected the plants against O₃ (+) 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 O₃ 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=O₃ enriched air, P = purafil, NF+ = non filtered air + a concentration of O₃, CF+=carbon-filtered air + a concentration of O₃, FA=filtered air, FA+=filtered air + a concentration of O₃, OTC = open-top chamber, CSTR = continuously stirred-tank reactor (also known as vat- or backmix reactor), and FACE=Free-Air O₃ Enrichment

A/A	Species	Exposure	Ozone Concentration	Exposure Duration	EDU Concentration	Application	Stage	Method	Repetition	Phytotoxicity	Protection	REF
	Crop Plants											
1	<i>Beta vulgaris</i> 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	<i>Brassica rapa</i> 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 <i>et al.</i> 1995
3	<i>Conyza bonariensis</i> 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 <i>et al.</i> 1994
4	<i>Daucus carota</i> 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	<i>Echinacea purpurea</i> 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 (μ l l ⁻¹); b) 0, 200, 400 and 600 ppm	After the fourth day following the exposure		Spraying	7 days	-	+	Szantoi <i>et al.</i> 2007
6	<i>Glycine max</i> L.	Open-field	Cumulative dose 81.2-101.4 μ l 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 <i>et al.</i> 1990
		OTCs								-	+	Scheepers <i>et al.</i> 2010
7	<i>Lycopersicon esculentum</i> 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

8	<i>Nicotiana tabacum</i> 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 $80 \times \text{nl l}^{-1}$ 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}))	24 h prior to exposure		Spraying	No	-	+
9	<i>Oryza sativa</i> L.	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	-	+	Bissessar and Palmer, 1984
			frequently exceeded 40 nl l^{-1} , occurred less often for 50 nl l^{-1} , and were less frequent above 60 nl l^{-1}	\approx 5 months	0, 150, 300, 450 mg l^{-1}	2 months after sowing	Spraying	7 days	-	-+	Wang et al. 2007	
10	<i>Phaseolus vulgaris</i> 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.)			Spraying	7 days	-	-+	Elagoz and Manning, 2005
			7 h mean of the 5 experiments: 45-49 nl l^{-1}	45-49 nl 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 trifoliolate leaf was expanding	Drench	after 14-17 days	-	+	-
		Closed chambers	0.45 $\mu\text{l l}^{-1}$	4 hours	50 mg EDU in 100 ml aqueous per pot	24 h prior to exposure		Drench	No	-	+	Lee et al. 1981
			0.30 $\mu\text{l l}^{-1}$	3 hours	100 ml of 0.5 mg ml^{-1} EDU	48 h prior to exposure	When the first trifoliolate leaf was fully expanded	Drench	No	-	-	+
		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 (\pm 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)		Drench	a) after 12, 24, 38 days b) after 14 days	+	-+	Kostka-Rick and Manning, 1993c

11	<i>Pisum sativum</i> 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	<i>Raphanus sativus</i> 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 $\text{ml} (\pm 2 \text{ ml})$ of the solution was applied to each pot, containing $\approx 350 \text{ ml}$ 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	<i>Sesamum indicum</i> 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	<i>Solanum tuberosum</i> L.	Open-field	the cumulative dose was 45-65 $\mu\text{l l}^{-1}$ 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

	CSTRs	0.10 µl l ⁻¹ or CF (5h/d)	≈ 2 weeks	a) 0, 50, 150, and 250 mg l ⁻¹ a.i. in tap H ₂ O, 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 ₂ O, 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	<i>Trifolium repens</i> 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 trifoliolate leaf	Drench	14 days		Fumagalli <i>et al.</i> , 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 <i>et al.</i> , 2010b	
16	<i>Trifolium subterraneum</i> 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 l ⁻¹ aqueous solution	on the same day transferred to the fields	Drench	14 days		Tonnejck 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 trifoliolate leaves were fully expanded	Drench	14 days		Tonnejck and van Dijk, 2002	
17	<i>Triticum aestivum</i> 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 <i>et al.</i> , 2009	

	a) Open-field; b) CF and NF OTCs	mean concentration 27.7–59.1 ng 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 ng 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 ng l^{-1} , occurred less often for 50 ng l^{-1} , and were less frequent above 60 ng l^{-1}	≈ 5 months	0, 150, 300, 450 mg l^{-1}	≈ 3.5 months after sowing	Spraying	7 days	-	-+	Wang et al. 2007
18	<i>Vigna mungo</i> L.	41.3–59.9 ng l^{-1} (12-h) during the growth period	3 months	400 mg l^{-1}	10 DAG	Drench	10 days	-	+	Singh et al. 2010a
19	<i>Vigna radiata</i> L.	64–69 ng l^{-1}	80 days	0 or 500 mg l^{-1}	1 week after emergence	Drench	7 days	-	+	Agrawal et al. 2005
20	Wild Plants <i>Rudbeckia laciniata</i> L.	CF, NF, 2xAA; Mean 12-h and 24-h in the ambient air were 33–26 ng 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

21	<i>Fagus sylvatica</i> 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	<i>Fraxinus americana</i> 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 <i>et al.</i> 1987
23	<i>Fraxinus excelsior</i> L.	Open-field	AOT40 for the growing season was 32.49 µl l ⁻¹ h	6 months	450 mg l ⁻¹	first in April	Injection	21 days	+	Paoletti <i>et al.</i> 2007
		Open-field	AOT40 over the period was 32.5 µl l ⁻¹ h	5 months	450 mg l ⁻¹	first in April	Injection	21 days	+	Paoletti <i>et al.</i> 2008
24	<i>Fraxinus pennsylvanica</i> 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 <i>et al.</i> 1987
25	<i>Liriodendron tulipifera</i> 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	Drench	No	-	Cannon <i>et al.</i> 1993
26	<i>Pinus taeda</i> 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	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 <i>et al.</i> 2003

27	<i>Populus × euramericana</i>	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) \approx 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	<i>Populus deltoides × maximowiczii</i>	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) \approx 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	<i>Populus maximowiczii × berolinensis</i>	Open-field	mean AOT40 of the seasons was $24.6 \pm 0.5 \mu\text{l l}^{-1} \text{h}$	6 months per growing season	1 (1st season) to 2 L of water (2nd and 3rd seasons) of 0 or 450 $\text{mg l}^{-1} \text{tree}^{-1}$	Cuttings planted the last autumn		Drip	7 days	-	+	Hoshika et al. 2013
30	<i>Populus nigra</i> L.	Open-field (but pots)	AOT40 (4 months) reached 6170 $\text{nl l}^{-1} \text{h}$	1 growing season	0 or 5 mg plant^{-1}	When the plants were \approx 50 cm tall		Injection	14 days	-	+	Bortier et al. 2001
31	<i>Prunus serotina</i> 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	\approx 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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