

The combined application of ethylenediurea and arbuscular mycorrhizal fungi alleviates ozone damage to *Medicago sativa* L.

Running title: Combined application of EDU and AM fungi alleviates O₃ phytotoxicity to alfalfa

Rongbin Yin ^{1,2}, Zhipeng Hao ¹, Xiangyang Yuan ^{1,3}, Xin Zhang ¹, Siyu Gun ⁴, Xuemei Hu ^{1,2}, Lifan Wang ⁵ and Baodong Chen ^{1,2,*}

¹ State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

² University of Chinese Academy of Sciences, Beijing 100049, China

³ School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, China

⁴ Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China

⁵ College of Landscape Architecture and Forestry, Qingdao Agricultural University, Qingdao 266109, China

*Corresponding author, Baodong Chen, Tel: 0086-10-62849068; Fax: 0086-10-62923549; E-mail: bdchen@rcees.ac.cn. Postal address: No. 18, Shuangqing Road, Haidian District, Beijing 100085, China.

This supplementary material contains 1 method and 2 tables.

Methods S1. Calculation method of mycorrhizal colonization rate and arbuscule abundance:

To determine the mycorrhizal colonization rate (M%) and arbuscule abundance (A%), MYCOCALC software was employed (Trouvelot et al., 1986). The software operated based on the following formulas for computation:

$$M\% = \frac{95 \times n_5 + 70 \times n_4 + 30 \times n_3 + 5 \times n_2 + n_1}{\text{Total number of root segments}} \times 100\% \quad (1)$$

$$A\% = \frac{100 \times mA_3 + 50 \times mA_2 + 10 \times mA_1}{10000} \times M \times 100\% \quad (2)$$

In the formula 2, mA_3 , mA_2 , and mA_1 are respectively calculated based on formula 3, formula 4, and formula 5.

$$mA_3 = \frac{100 \times (95 \times n_5 \times A_3 + 70 \times n_4 \times A_3 + 30 \times n_3 \times A_3 + 5 \times n_2 \times A_3 + n_1 \times A_3)}{\text{Number of colonized root segments} \times m} \quad (3)$$

$$mA_2 = \frac{100 \times (95 \times n_5 \times A_2 + 70 \times n_4 \times A_2 + 30 \times n_3 \times A_2 + 5 \times n_2 \times A_2 + n_1 \times A_2)}{\text{Number of colonized root segments} \times m} \quad (4)$$

$$mA_1 = \frac{100 \times (95 \times n_5 \times A_1 + 70 \times n_4 \times A_1 + 30 \times n_3 \times A_1 + 5 \times n_2 \times A_1 + n_1 \times A_1)}{\text{Number of colonized root segments} \times m} \quad (5)$$

The variable 'm' in formulas 3, 4, and 5 is calculated according to the following formula:

$$m = \frac{M \times \text{Total number of root segments}}{\text{Number of colonized root segments}} \times 100\% \quad (6)$$

In the above formulas, n_1 - n_5 respectively represent the number of root segments showing mycorrhizal colonization levels of 0, <1%, <10%, <50%, <90%, and >90%; while A_1 - A_3 represent the number of root segments with few, moderate, and abundant arbuscules, respectively.

Table S1 Mycorrhizal colonization rate (M) and arbuscule abundance (A) under different experimental treatment. AO₃: ambient air O₃, EO₃: elevated O₃ with 40 nmol·mol⁻¹ O₃ enrichment; E0: spray water, E150: spray 150 mg L⁻¹ EDU aqueous solution, E300: spray 300 mg L⁻¹ EDU aqueous solution. A split-plot ANOVA was performed on the data, with significant effects highlighted in bold ($P < 0.05$). Data are presented as means \pm standard error (SE) (n = 4).

	<i>O</i> ₃	<i>E</i>	M%	A%
		E0	32.89±5.85	18.35±4.73
	AO ₃	E150	38.94±8.44	22.37±7.09
		E300	47.83±6.25	27.88±5.94
		E0	31.26±4.32	18.42±3.95
	EO ₃	E150	32.68±5.11	17.14±3.02
		E300	29.17±5.41	16.54±3.15
	<i>O</i> ₃		0.244	0.395
ANOVA	<i>E</i>		0.372	0.556
	<i>O</i> ₃ × <i>E</i>		0.178	0.276

Table S2 ANOVA outputs of ozone (O_3), EDU (E), AM inoculation (I) and their interactions on biomass, root/shoot ratio, photosynthetic parameters, specific leaf weight, area of the third leaf, stomatal parameters, shoot and root elemental concentrations, and shoot antioxidant enzyme activities, with significant effects highlighted in bold ($P < 0.05$).

Variables	O_3	E	I	$O_3 \times E$	$O_3 \times I$	$E \times I$	$O_3 \times E \times I$
Shoot biomass	0.020	0.483	<0.001	0.615	0.140	0.417	0.866
Root biomass	0.032	0.605	<0.001	0.570	0.102	0.736	0.593
Total biomass	0.022	0.506	<0.001	0.523	0.082	0.552	0.697
Root/shoot ratio	0.164	0.931	0.549	0.989	0.396	0.560	0.751
Net photosynthetic rate	0.572	0.555	0.001	0.144	0.417	0.948	0.593
Stomatal conductance	0.390	0.955	0.009	0.194	0.805	0.989	0.779
PhiPS2	0.030	0.282	<0.001	0.992	0.835	0.773	0.568
Area of the third leaf	0.266	0.388	<0.001	0.958	0.664	0.288	0.134
Specific leaf weight	0.349	0.122	0.019	0.505	0.571	0.212	0.252
Stomatal density	0.280	0.994	0.043	0.399	0.046	0.923	0.875
Stomatal aperture	0.500	0.904	0.063	0.847	0.485	0.799	0.999
MDA	0.010	<0.001	0.371	0.020	0.212	0.398	0.465
Shoot C concentration	0.807	0.199	<0.001	0.716	0.770	0.439	0.245
Shoot N concentration	0.757	0.048	0.462	0.408	0.219	0.859	0.265
Shoot P concentration	0.437	0.637	<0.001	0.075	0.788	0.275	0.039
Root C concentration	0.766	0.889	<0.001	0.754	0.571	0.157	0.284
Root N concentration	0.611	0.164	<0.001	0.281	0.220	0.734	0.177
Root P concentration	0.849	0.051	<0.001	0.298	0.264	0.578	0.166
SOD	0.042	0.016	0.020	0.659	0.437	0.641	0.067
POD	<0.001	0.033	0.432	0.110	0.125	0.008	0.084
APX	0.852	0.179	0.018	0.726	0.537	0.255	0.769
CAT	0.639	0.259	<0.001	0.251	0.267	0.849	0.304