

*Supplementary files*

# **Effects of Elevated Temperature and Ozone in *Brassica juncea* L.: Growth, Physiology, and ROS Accumulation**

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**Table 1.** Analyses of variance of the main effects of temperature, O<sub>3</sub>, and sampling date and their interactions on growth parameters, gas exchange characteristics, O<sub>3</sub> flux, carotenoid, ascorbic acid, lipid peroxidation, as well as accumulation of hydroxyl radical, hydrogen peroxide, and superoxide radical. Data were analyzed using three-way or two-way ANOVAs.

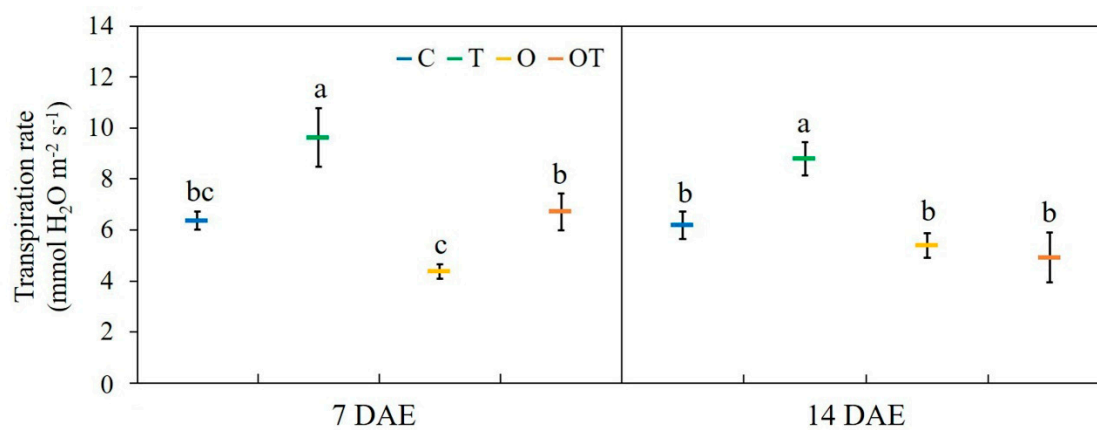
Parameters	Temp.	O <sub>3</sub>	Temp. × O <sub>3</sub>	Date	Temp. × Date	O <sub>3</sub> × Date	Temp. × O <sub>3</sub> × Date
Total fresh weight	<0.001	<0.001	<0.01				
Total dry weight	<0.001	<0.001	0.255				
Shoot dry weight	0.162	<0.001	0.755				
Root dry weight	<0.01	<0.001	0.183				
Shoot:root ratio	<0.01	<0.01	0.125				
Specific leaf area	<0.05	<0.001	0.307				
Photosynthetic rate	<0.001	<0.001	0.051	<0.001	0.655	<0.001	<0.01
Stomatal conductance	<0.001	<0.001	<0.01	<0.01	<0.001	0.064	0.767
Intercellular CO <sub>2</sub>	<0.001	0.601	0.15	<0.001	<0.001	<0.001	0.418
Transpiration rate	<0.001	<0.001	0.053	0.36	0.9	0.903	0.283
Water use efficiency	<0.001	0.616	0.984	<0.01	<0.05	0.148	0.487
Ozone flux	<0.05	<0.001	0.185	<0.05	<0.001	0.322	<0.01
Carotenoid	<0.01	<0.001	0.215	<0.01	0.314	0.542	0.187
Ascorbic acid	<0.001	<0.001	0.765	<0.01	0.372	0.313	0.071
Lipid peroxidation	<0.001	<0.001	0.647	<0.001	0.115	0.858	0.721
Hydroxyl radical	0.135	<0.001	0.788	<0.001	0.985	0.578	0.817
Hydrogen peroxide	<0.001	<0.001	0.344	<0.001	0.475	0.784	0.189
Superoxide radical	<0.01	<0.001	0.215	<0.01	0.314	0.542	0.187

**Table S2.** Comparison of 7 and 14 DAE (days after exposure) differences of each parameter under different ambient and elevated temperature and O<sub>3</sub> treatments, respectively. Data are summarized as means ± SE (n = 5) and were analyzed using the independence t-test. Statistical significance: \*, p ≤ 0.05; \*\*, p ≤ 0.01; \*\*\*, p ≤ 0.001. C: optimal temperatures and ambient O<sub>3</sub>; T: elevated temperature and ambient O<sub>3</sub>; O: optimal temperatures and elevated O<sub>3</sub>; OT: elevated temperature and elevated O<sub>3</sub>; DAE: Days after exposure.

Parameters	C		T		O		OT	
	7 DAE	14 DAE	7 DAE	14 DAE	7 DAE	14 DAE	7 DAE	14 DAE
Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	29.67 ± 0.59	<b>24.96 ± 0.66**</b>	26.05 ± 0.90	26.01 ± 0.47	23.83 ± 0.72	<b>17.51 ± 1.22*</b>	21.53 ± 1.62	<b>11.76 ± 0.90**</b>
Stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ )	0.67 ± 0.07	0.59 ± 0.07	1.62 ± 0.23	<b>0.88 ± 0.09*</b>	0.33 ± 0.59	<b>0.50 ± 0.06*</b>	0.76 ± 0.10	<b>0.36 ± 0.08*</b>
Intercellular CO <sub>2</sub> ( $\text{mmol mol}^{-1}$ )	265.93 ± 11.15	287.70 ± 7.33	319.26 ± 3.52	<b>295.31 ± 3.25**</b>	231.74 ± 9.25	<b>302.69 ± 2.22***</b>	307.07 ± 4.21	316.58 ± 7.60
Transpiration rate ( $\text{mmol mol}^{-1}$ )	6.37 ± 0.35	6.17 ± 0.53	9.63 ± 1.14	8.78 ± 0.65	4.37 ± 0.28	5.38 ± 0.48	6.72 ± 0.72	4.91 ± 0.98
Water use efficiency ( $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$ )	4.74 ± 0.38	3.79 ± 0.36	2.89 ± 0.38	3.08 ± 0.24	5.53 ± 0.34	<b>3.29 ± 0.14***</b>	3.28 ± 0.21	3.01 ± 0.85
Ozone flux ( $\text{nmol m}^{-2} \text{ s}^{-1}$ )	3.97 ± 0.41	4.30 ± 0.70	9.62 ± 1.38	<b>5.24 ± 0.54*</b>	19.63 ± 1.77	<b>29.82 ± 3.39*</b>	45.33 ± 6.04	<b>21.63 ± 4.55*</b>
Carotenoid ( $\text{mg g}^{-1} \text{ FW}$ )	1.63 ± 0.03	<b>1.85 ± 0.03*</b>	1.47 ± 0.07	1.73 ± 0.13	1.28 ± 0.07	1.59 ± 0.12	1.13 ± 0.10	1.15 ± 0.08
Ascorbic acid ( $\text{mg g}^{-1} \text{ FW}$ )	0.73 ± 0.03	0.71 ± 0.03	0.55 ± 0.02	<b>0.43 ± 0.02*</b>	0.58 ± 0.03	0.53 ± 0.02	0.34 ± 0.01	0.32 ± 0.01
Lipid peroxidation ( $\text{nmol g}^{-1} \text{ FW}$ )	0.07 ± 0.01	<b>0.32 ± 0.03***</b>	0.30 ± 0.02	<b>0.40 ± 0.02*</b>	0.52 ± 0.04	<b>0.72 ± 0.04**</b>	0.76 ± 0.11	0.86 ± 0.07
Hydroxyl radical ( $\text{A}_{540} \text{ g}^{-1} \text{ FW}$ )	0.06 ± 0.009	<b>0.04 ± 0.007*</b>	0.07 ± 0.009	<b>0.04 ± 0.007*</b>	0.09 ± 0.004	<b>0.06 ± 0.004*</b>	0.10 ± 0.005	0.07 ± 0.01
Hydrogen peroxide ( $\mu\text{mol g}^{-1} \text{ FW}$ )	0.42 ± 0.07	<b>0.87 ± 0.07**</b>	0.78 ± 0.08	<b>1.33 ± 0.20*</b>	1.08 ± 0.10	<b>1.78 ± 0.12*</b>	1.50 ± 0.08	<b>1.89 ± 0.05*</b>
Superoxide radical	0.09 ± 0.01	0.10 ± 0.04	0.16 ± 0.04	0.14 ± 0.02	0.28 ± 0.02	0.27 ± 0.04	0.25 ± 0.03	<b>0.34 ± 0.03*</b>

(A<sub>580</sub> g<sup>-1</sup> FW)

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**Figure S1.** Transpiration rate of *Brassica juncea* L. under different ambient and elevated temperature and O<sub>3</sub> treatments at 7 DAE and 14 DAE. Data are plotted as means ± SE (n = 5). Different letters signify significant differences among treatments at p < 0.05 according to Tukey's HSD test.



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