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Supplementary Information

Physiological and yield responses of spring wheat cultivars under realistic and acute levels of ozone

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Citation: Chaudhary, N.; Bonfil, D.J.; Tas, E. Physiological and Yield Responses of Spring Wheat Cultivars under Realistic and Acute levels of Ozone. *Atmosphere* **2021**, *12*, 1392. https://doi.org/10.3390/atmos12111392

Academic Editors: Alfredo Rocha and Ana Isabel Miranda

Received: 25 August 2021
Accepted: 20 October 2021
Published: 24 October 2021

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S.1 Preliminary experiment

S.1.1 Objective

Assessing the effect of elevated ozone (O_3) direct exposure at leaf-level on the physiological parameters of commercially available wheat cultivars. Wheat cultivars were exposed to the elevated O_3 in a greenhouse, while keeping other meteorological factors under control.

S.1.2 Experimental setup

Ozone exposure-response measurements were conducted in the greenhouse, located at the Faculty of Agriculture of the Hebrew University (Rehovot, Israel). Experiments were performed during 2014 and 2015 growing seasons. Four different commercial wheat (*T. aestivum*) cultivars: Beit Hashita, Omer, Zahir, and Yuval were investigated, under exposure to O_3. This is the first time to investigate these commercially available cultivars that were bred under semi-arid conditions in Israel. Seeds of each cultivar were sown in six pots of 8-liter capacity, filled with commercially available plant potting mix. Temperature was set to 25/15 °C day/night and relative humidity to 40-50 %/70-80 % day/night. These meteorological conditions inside the greenhouse were maintained to represent typical semi-arid conditions, during the wheat growing season in Israel. Relative humidity (RH) and temperature (T) in the greenhouse were continuously measured using RH and T sensor (Campbell-HC2). Measurements were performed by connecting the commercial portable photosynthesis system LI-6400 XT (LICOR, Lincoln, Nebraska, USA) to an O_3 generator (T.E.I model 146) and a zero-air generator (Figure S1). This system allowed using the LI-6400 instrument to quantify physiological parameters of plants exposed to O_3 level to 65 ppb. The flow rate was set at 500 μmol m^{-2} s^{-1} and a CO_2 concentration of 400 ppm was maintained. Photosynthetically active radiation (PAR) of 1000 μmol m^{-2} s^{-1} was set for internal light source of the leaf chamber. Physiological parameters measurements including stomatal conductance (*gs*), net assimilation rate (*Ps*), and transpiration rate (*Trans*) were carried out on the flag leaf between booting and heading phenological stages.

S.1.3 Ozone exposure and physiological response measurements

First the flag-leaves were allowed to adapt to stable conditions in the laboratory for 60 minutes, where they were exposed to low O_3 concentrations (~30 ppbv). Following this acclimation flag leave physiological performance was tested for *gs*, *Ps*, and *Trans*, as control. Subsequently, elevated O_3 of 65 ppb was introduced into the chamber for an additional 1 hour, and physiological parameters were measured in 4 min time intervals. Physiological measurements were performed under stable conditions, both under 30 ppbv and during the exposure to 65 ppbv. For each cultivar measurements were averaged over 12 repetitions. Figure S.1 presents differences in measured physiological parameters between each time point during exposure to elevated O_3 (65 ppbv) and the corresponding control measurement (30 ppbv). Significant changes were observed in *gs*, *Ps*, and *Trans* for all tested cultivars. Results showed reduction in *gs*, *Ps*, and *Trans* for all tested cultivars except Zahir.

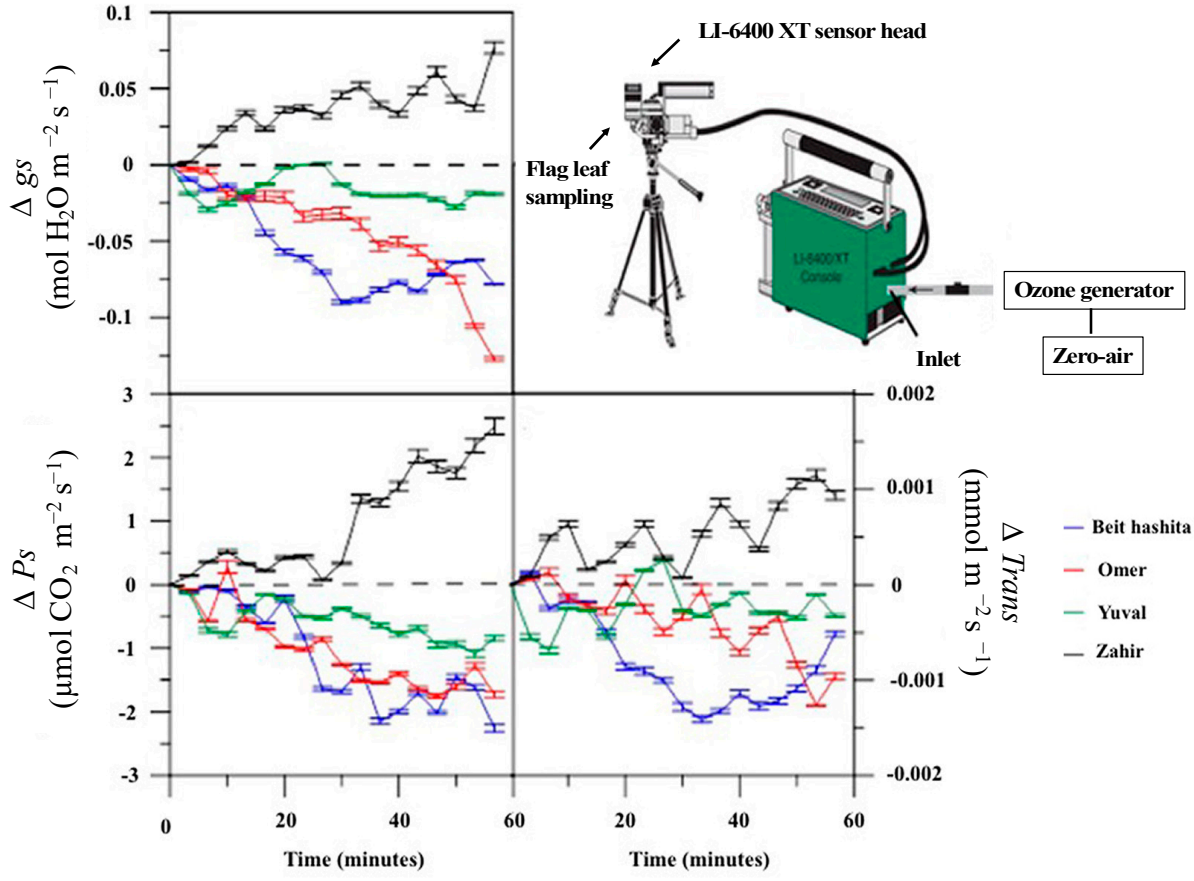

Figure S1. Differences in stomatal conductance (*gs*), net assimilation rate (*Ps*), and transpiration rate (*Trans*) of four wheat cultivars due to direct exposure of the flag leaf to O_3. *gs*, *Ps*, *Trans* were calculated as the difference between their values following an exposure to O_3 at 65ppbv vs. O_3 at 30 ppbv (Sect. S.1.2).

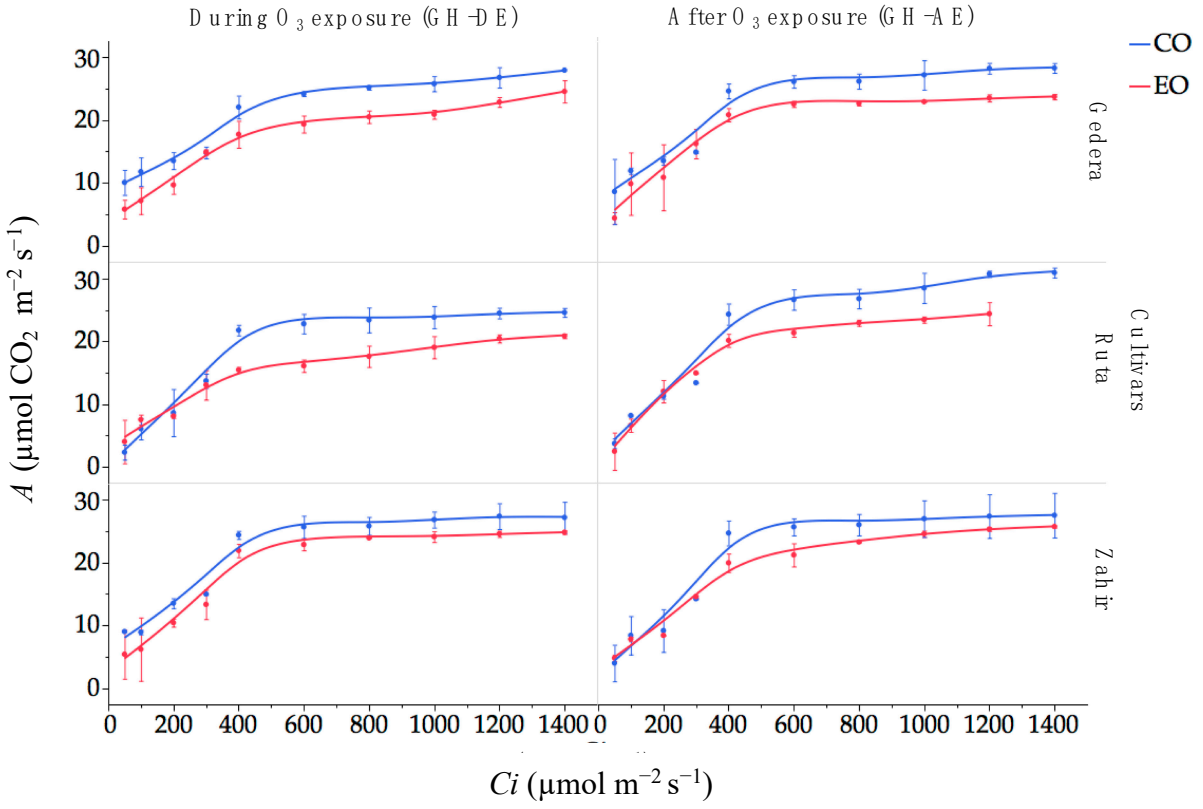

Figure S2. Greenhouse (GH) experiment: CO_2 response curve, *A*/*Ci*, during O_3 exposure (GH-DE) and after O_3 exposure measurements (GH-AE) of all cultivars during the exposure to O_3 (ozone) and corresponding control (control). Values are Mean (n = 3). See more details on the greenhouse experiment in Sects. 2.7 and 3.3.1 in the main text.

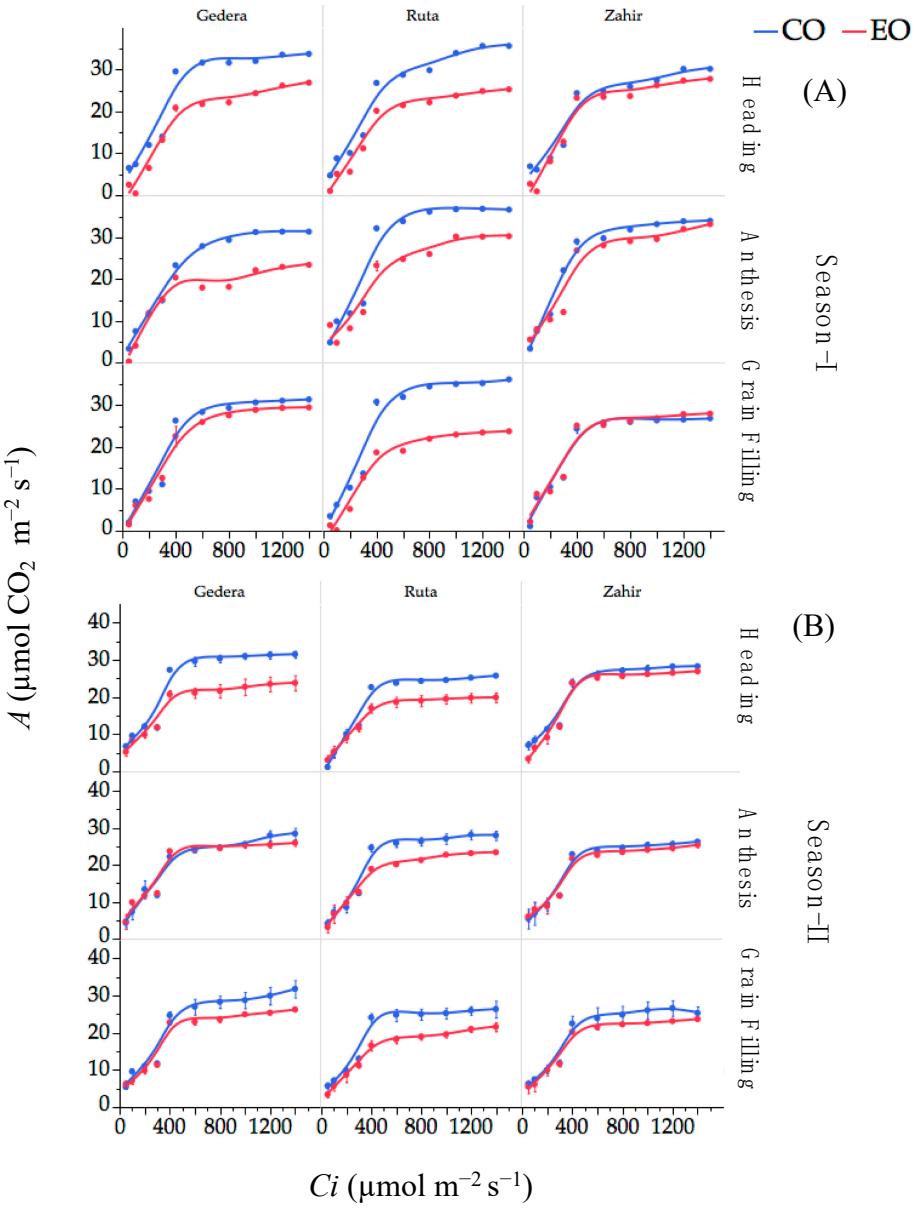

Figure S3. CO_2 response curve during the open top chamber (OTC) experiment: (A) *A*/*Ci* curves of all cultivars in OTC enriched by O_3 (OTC-EO) and corresponding values under control conditions (OTC-CO) during season-I. (B) *A*/*Ci* curves for all the phenological stages during season-II. Values are Mean (n = 4). See more details on the OTC experiment in Sects 2.7 and 3.3.2 in the main text.

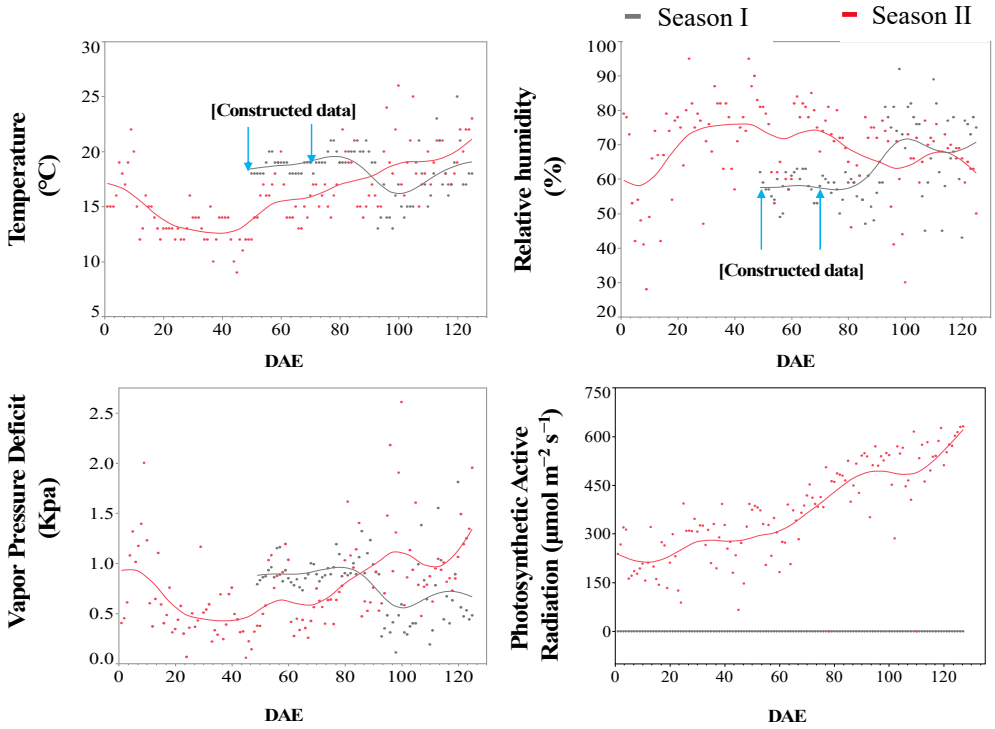

Figure S4. Measured meteorological data during the open top chamber (OTC) experiment: Presented are temperature, relative humidity, vapor pressure deficit and photosynthetic active radiation during the season I and II inside the OTCs. Data includes here from all experimental duration (Dec-Apr) for both seasons; In season I, sensors were installed at 31 January 2017 (49 days after emergence (DAE)). For season-II from 11 December 2017 (DAE day 1). Constructed values shows the modified data for season I data from OTC and ambient regression up to the period of sensors without shelter. In season I, PAR sensor was not installed. See more details on the OTC experiment in Sects. 2.4 and 3.1.2 in the main text.

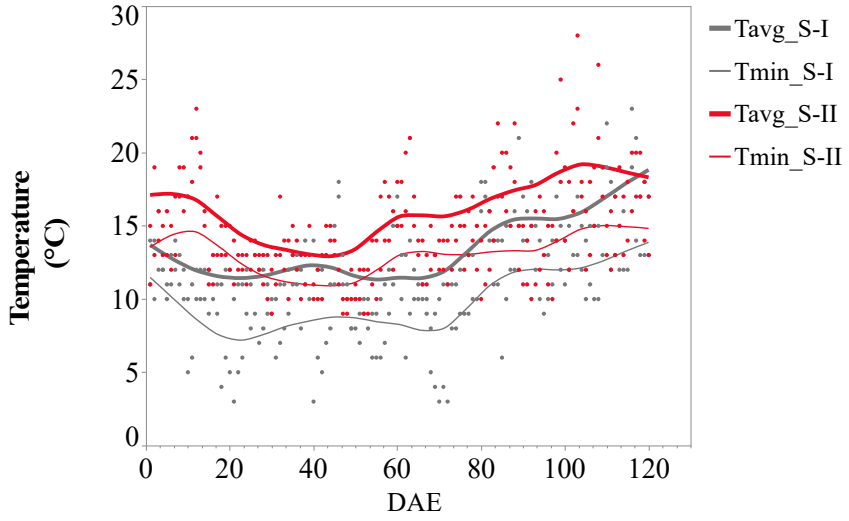

Figure S5. Ambient daily average Temperature (Tavg) and daily minimum temperature (Tmin) from emergence to maturity of plant for OTC experiment during season-I (black) and II (red). See more details on OTC experiment in Sects. 2.4 and 4.1 in the main text.