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Both ozone exposure and soil water stress are able to induce stomatal sluggishness

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ABSTRACT

We tested whether short-term exposure to realistic ozone pollution (≤150 ppb, 1 h) and soil water stress (soil water content ≤15%) slow stomatal dynamics in an ozone-sensitive cultivar of snapbean. Both ozone exposure and water stress caused stomata to be sluggish in the degree of closure after leaf severing, while ozone also delayed the time the closing signal was perceived. Ozone-induced aberrations lasted up to the night and caused incomplete closure of stomata. No synergic effect was observed in the dynamic measurements. In contrast, at steady-state, water stress protected the plants from the negative ozone effects on stomatal conductance. Ambient ozone peaks may thus cause sluggish stomatal response and increase leaf water loss both under well watered and drought conditions.

1. Introduction

Tropospheric ozone (O_3) is an important secondary pollutant resulting from emission of volatile organic compounds and nitrogen oxides, and is also recognized as a significant greenhouse gas (Bytnerowicz et al., 2007; Serengil et al., 2011). Ozone is seriously phytotoxic and causes negative effect on plants (e.g., NIES, 1980, 1984; Omasa et al., 2002; Paoletti, 2007). Stomatal O_3 uptake is crucial for assessing the adverse effect of O_3 on plants (Omasa et al., 1979; UNECE, 2004; Paoletti and Manning, 2007; Grulke et al., 2007a; Cieslik et al., 2009). However, our understanding about stomatal responses to O_3 is still imperfect (Paoletti and Grulke, 2005). Previous studies reported that O_3 generally induces both stomatal closure (Wittig et al., 2007, 2009) and sluggishness of stomatal response to change of environmental factors (Paoletti and Grulke, 2005, 2010). Because plants live in a fluctuating environment, both steady-state stomatal conductance and stomatal dynamics play an important role in regulating leaf gas exchange. Ozone-induced sluggishness of stomata has been reported in response to change in photosynthetic photon flux density (PPFD) (Reich and Lassoie, 1984; Reiling and Davison, 1995; Paoletti, 2005; Grulke et al., 2007a; Paoletti and Grulke, 2010), vapor pressure deficit (VPD) (Grulke et al., 2007b) and severe water stress imposed by severing a leaf (Paoletti, 2005; Paoletti et al., 2009; Mills et al., 2009). Such aberrations may increase nighttime transpiration, as reported for several tree species exposed to daytime O_3 exposure in controlled (Skärby et al., 1987; Matyssek et al., 1995; Wieser and Havranek, 1995; Günthardt-Goerg et al., 1997; Grulke et al., 2007c) and ambient conditions (Grulke et al., 2004). Ozone is known to induce up-regulation of ethylene emission, which has been suggested to be responsible for a reduction in stomatal sensitivity to abscisic acid (ABA) and thus to closure (Wilkinson and Davies, 2010). Several stressors, including water deficit, are known to stimulate ethylene production by plants (Morgan and Drew, 1997).

Climate change is expected to increase O_3 levels and alter precipitation regimes, regionally increasing the probability of drought (Ciais et al., 2005). Drought has the capacity to limit O_3 injury through stomatal closure (Tingey and Hogsett, 1985), while O_3-induced impairment of stomatal response may increase susceptibility to drought (Nali et al., 2004).

Our main objective was to test whether short-term exposure to realistic O_3 pollution and soil water stress, singly and in combination, may slow stomatal dynamics in an ozone-sensitive cultivar of snapbean (*Phaseolus vulgaris*, S156).

2. Materials and methods

Seeds of the ozone sensitive cultivar S156 of snapbean developed at the Raleigh USDA-ARS (Burkey and Eason, 2002; Flowers et al., 2007; Booker et al., 2009), were planted in 17-cm (1.7–1) pots, filled with sand:peat:soil = 1:1:1 (v:v:v). Seed were planted over several days so that same-age (4-week old) plants were used in the experiment. All plants were grown in a room with controlled environmental conditions (air temperature of 20 °C, PPFD

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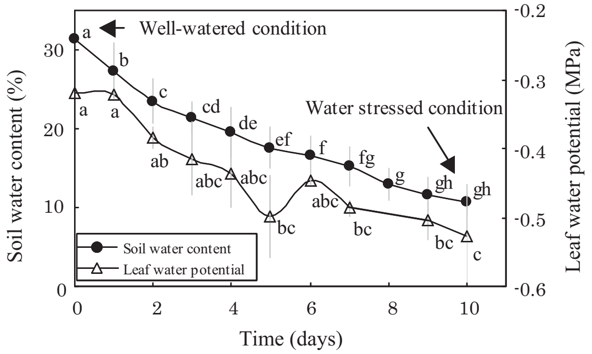

Fig. 1. Changes in soil water content (±S.D., $n = 18$) (%)and predawn leaf water potential (±S.D., $n = 2–3$) after irrigation until field capacity. Different letters indicate significant differences among mean values (Tukey HSD test, $P < 0.05$).

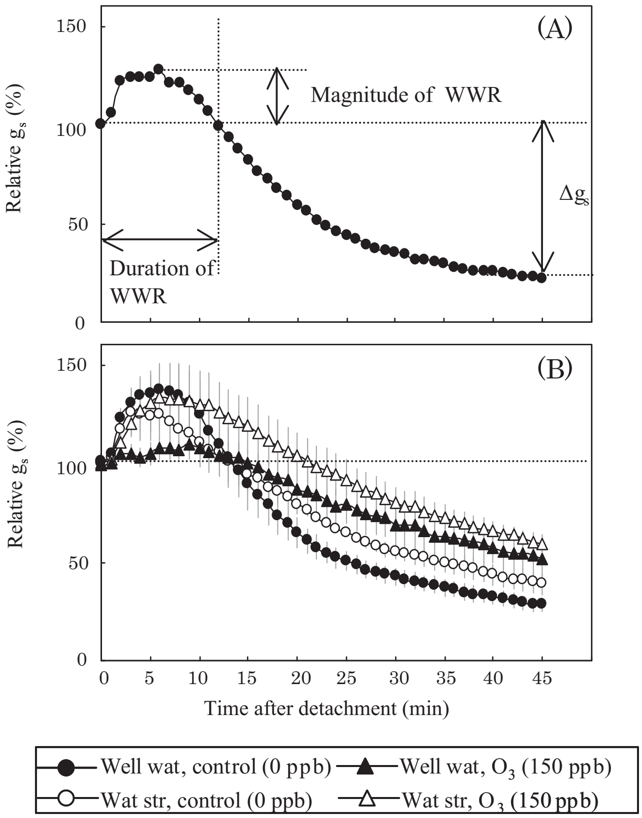

Fig. 2. Time courses of stomatal conductance (g_s) after severing a leaf at time 0. Graph A shows an example of calculation of WWR magnitude and duration, and of the degree of g_s decrease (Δg_s). At first, g_s showed a transient increase called 'wrong-way response' (WWR) and a subsequent decrease with increasing leaf water stress. Graph B shows the time course of average g_s (±SE) under well-watered condition (soil water content > 30%) and water-stressed condition (soil water content = 6–15%) in control (0 ppb O_3) and ozone-exposed (150 ppb O_3) leaves.

of 500 $\mu mol\ m^{-2}\ s^{-1}$, and relative humidity of 55%). Well-watered plants were supplied with water every day and in particular immediately before the ozone exposure, while water-stressed plants received no irrigation for 10 days before O_3 fumigation. Soil water content was more than 30% in well-watered plants and 6–15% in water-stressed plants, corresponding to a predawn leaf water potential of −0.32 MPa and −0.53 MPa, respectively (Fig. 1). Water content was measured in 18 pots with an ECH2O EC-5 soil moisture sensor (Decagon Devices, Pullman WA, USA) at root level. Leaf water potential was measured at dawn in a selection of 2–3 plants per day by means of an SKPM 1400 chamber pressure (Skye, Powys, UK).

The fully expanded central leaf of the second trifoliate leaf was selected as a target leaf. After 1-h exposure to 1000 $\mu mol\ m^{-2}\ s^{-1}$ light, steady state leaf gas exchange was measured with a portable infra-red gas-analyzer (CIRAS-2 PP Systems, Herts, UK), equipped with a 2.5 cm^2 leaf cuvette which controlled leaf temperature (20 °C), leaf-to-air vapour pressure deficit (0.9 kPa), saturating light (1800 $\mu mol\ m^{-2}\ s^{-1}$) and CO_2 concentration (365 ppm). Ozone exposure to one of four levels (±SD), i.e. low (48 ± 6.7 ppb), middle (87 ± 9.4 ppb), high (150 ± 10.9 ppb), and control (0 ppb, no O_3), was then carried out for 60 further min by a web fumigation system (Velikova et al., 2005; Pinelli and Tricoli, 2008). Ozone was added by an O_3 generator (Model Heliozon, Milano, Italy) to the fumigating air for the target leaf through a teflon tube. The concentration around the leaf was recorded with an ozone monitor (Mod. 205, 2B Technologies, Boulder CO, USA), and adjusted through mass flow controllers (Mod. GFC171S A alborg). At 30 min after the end of O_3 exposure, steady-state stomatal conductance (g_s) was measured again. When g_s reached equilibrium under constant light at 1800 $\mu mol\ m^{-2}\ s^{-1}$, the methodology described by Paoletti (2005) was applied to assess dynamic variations of g_s after cutting the leaf petiole (Fig. 2). Data were logged at 1 min intervals in the 45 min after severing. In the experiment, two phases of g_s response were observed (Fig. 2). At first, g_s increased for Iwanoff effect. This transient increase called as the transient 'wrong-way response' (WWR) is due to a difference in turgor pressure between guard cell and epidermal cells (Omasa and Maruyama, 1990; Powles et al., 2006). Subsequently, stomatal conductance decreased with increasing leaf water stress. WWR duration and magnitude of WWR and g_s decrease at 45 min (Δg_s) were recorded.

After O_3 exposure and steady-state measurements, 22 plants were placed in the dark for 10 h. Nocturnal steady-state g_s was measured with leaf temperature of 20 °C, leaf-to-air vapour pressure deficit of 0.9 kPa, no exposure to light and CO_2 concentration of 365 ppm.

Data were checked for normal distribution and homogeneity of variance (Levene's test). Percents were arcsine square root transformed prior to analysis. Effects of soil water status and O_3 exposure were tested using two-way analysis of variance (ANOVA). Results were considered significant at $p < 0.05$. Differences among means were tested by Tukey's HSD test. Statistical analysis was performed with STATISTICA software (6.0, StatSoft Inc., Tulsa, OK, USA), according to Statsoft (2001).

3. Results

3.1. Steady-state and dynamic stomatal responses

Ozone exposure induced a decline of steady-state g_s under well-watered conditions (Fig. 3). g_s was 73% in the low O_3 treatment and 25% in the high O_3 treatment compared to the control plants. A reduced soil water availability significantly reduced g_s relative to well-watered plants and resulted in no effect of O_3 on g_s.

After severing a leaf, two phases of g_s response were observed (Fig. 2): a transient increase as WWR and then a linear decrease. Ozone exposure increased WWR duration from 11.9 ± 2.7 min in the control plants to 17.1 ± 3.2 min in the high O_3 treatment, while the effect of water stress was not significant (Fig. 4A). Magnitude of WWR was not affected by both O_3 and soil water availability (Fig. 4B). Ozone reduced the degree of stomatal closure over time (Δg_s) in both soil water conditions (Fig. 4C). Also soil water deficit reduced Δg_s relative to the optimal soil water availability. In well-watered plants, the high O_3 treatment resulted in smaller stomatal closure than in control leaves (48% vs. 75%). In