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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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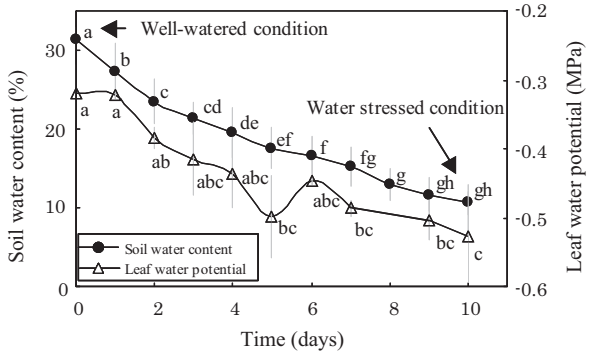

Fig. 1. Changes in soil water content (±S.D., $n = 18$) (%)and predawn leaf water potential (±S.D., $n = 2–3$) after irrigation until field capacity. Different letters indicate significant differences among mean values (Tukey HSD test, $P < 0.05$).

of 500 μmol 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 μmol 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 μmol 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 μmol 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).

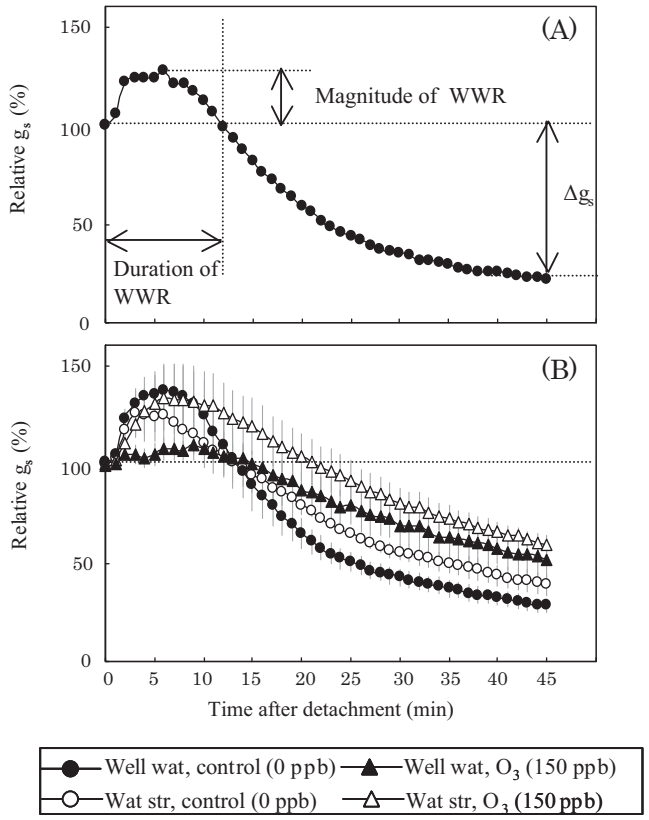

Fig. 2. Time courses of stomatal conductance (g_s) after severing a leaf at time 0. Graph A shows an example of calculation of WWR magnitude and duration, and of the degree of g_s decrease (Δg_s). At first, g_s showed a transient increase called 'wrong-way response' (WWR) and a subsequent decrease with 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.

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

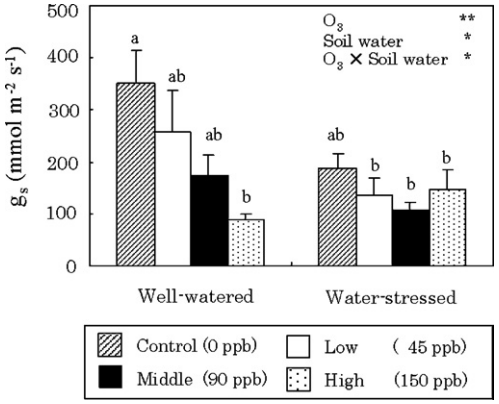

Fig. 3. Effect of 1-h exposure to four levels of O_3 (control: 0 ppb; low: 45 ppb; middle: 90 ppb; high: 150 ppb) and soil water availability (well-watered 30% water content; water-stressed: 6–15% water content) on steady-state stomatal conductance (g_s). Data are means (N = 5–8) ± SE. * denotes significance at 5% levels; ** denotes significance at the 1% levels; n.s. indicates no significance. Different letters indicate significant differences among bars (Tukey HSD test, P < 0.05).

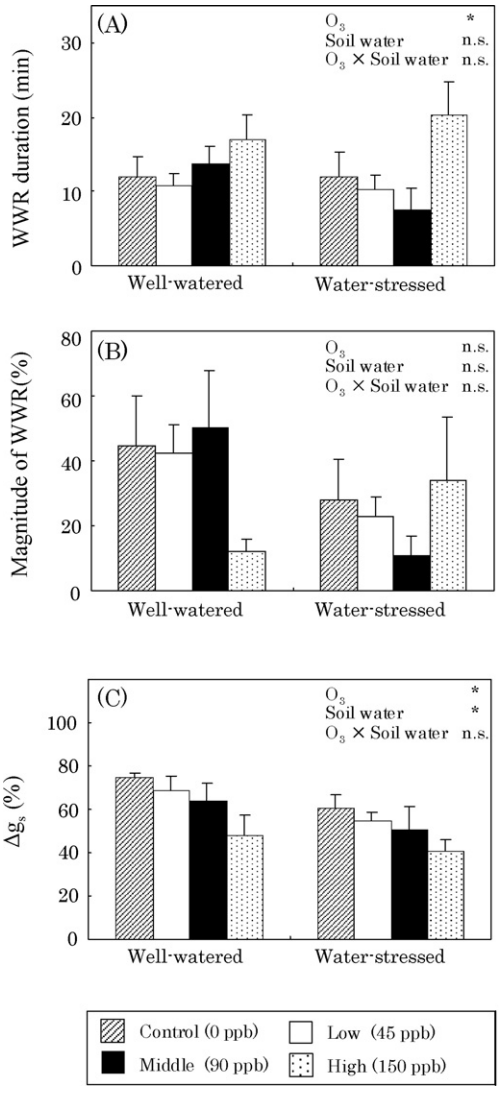

Fig. 4. Effects of 1-h O_3 exposure (Control: 0 ppb; low: 45 ppb; middle: 90 ppb; high: 150 ppb) and soil water availability (well-watered 30% water content; water-stressed: 6–15% water content) on dynamic g_s response after severing a leaf (A: duration of transient increase in g_s after severing; B: magnitude of transient increase in g_s after severing; C: degree of g_s decrease at 45 min after severing). Data are means (N = 3–4) ± SE. * denotes significance at the 5% levels; n.s. indicates no significance.

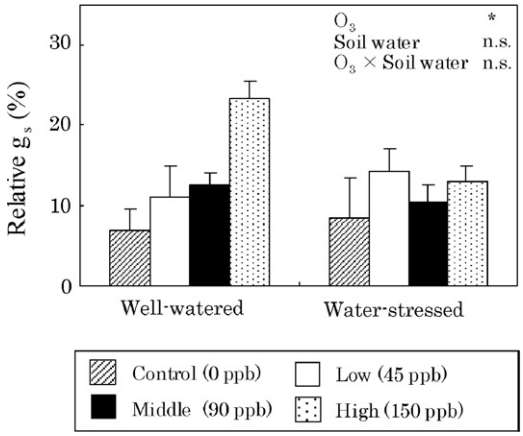

Fig. 5. Effects of 1-h O_3 exposure (Control: 0 ppb; low: 45 ppb; middle: 90 ppb; high: 150 ppb) and soil water availability (well-watered 30% water content; water-stressed: 6–15% water content) on nocturnal steady-state stomatal conductance (g_s) relative to the pre-exposure daytime value. Data are means (N = 2–4) ± SE. * denotes significance at the 5% levels; n.s. indicates no significance.

water-stressed plants, high O_3 and control treatment resulted in 41% and 61% stomatal closure, respectively.

3.2. Nocturnal stomatal conductance

Fig. 5 shows nocturnal steady-state g_s relative to daytime pre-exposure g_s. Ozone exposure caused an increase of nocturnal g_s. A reduced soil water availability did not affect nocturnal g_s. Nocturnal g_s increased in well-watered plants from 7% in the control plants to 23% in the high O_3 treatment.

4. Discussion

Increasing O_3 exposure under optimal water availability progressively decreased steady-state g_s. Although the mechanisms that regulate stomatal responses to acute and chronic O_3 exposure may differ, the unifying result is a reduction of steady-state g_s, in both crops and trees, under different experimental conditions (e.g., Grulke et al., 2007a; Wittig et al., 2007). Dynamic measurements of g_s in Arabidopsis, in contrast, showed a rapid decrease triggered by acute exposure (Vahisalu et al., 2010) and followed by reopening to overshooting values (Moldau et al., 2011). Soil water stress is usually considered to reduce O_3 injury because it reduces g_s and thus O_3 entering into a leaf (Tingey and Hogsett, 1985). The result of our steady-state measurements supports this conclusion. Simulated water deficit resulted in no effect of O_3 on g_s (Fig. 3). During the dry summer of 2003 in Central Europe, no difference in steady-state g_s was reported for beech trees exposed to ambient and twice-ambient O_3 in free air (Löw et al., 2006). Figures obtained from steady-state observations have been used to model plant responses to O_3 in a changing climate (e.g., Ollinger et al., 2002).

Measurements of dynamic stomatal responses revealed that exposure to increasing O_3 concentrations made stomata sluggish i.e. progressively increased the duration of WWR and reduced the degree of stomatal closure over time (Fig. 4A and C). After leaf severing, duration of WWR and following linear reduction in g_s are related to induction and execution of guard cell osmoregulation, respectively (Powles et al., 2006). Omasa (1990) reported that stomatal response was affected by the O_3-induced effects such as slight increase in permeability of epidermal cell membranes and alteration of the osmotic pressure modulating a balance in turgor between the guard and subsidiary cells. Ozone may also delay stomatal responses by stimulating ethylene production and reducing

stomatal sensitivity to ABA (Wilkinson and Davies, 2010), or temporarily desensitizing the guard cells by blocking the K^+ channels (Vahisalu et al., 2010).

We also demonstrated, for the first time that also a mild soil water stress is able to cause stomatal sluggishness by reducing the degree of stomatal closure, while no effect was recorded on the time the closing signal is perceived. Following Powles et al. (2006), this response suggests that water stress affects execution rather than induction of guard cell osmoregulation. In contrast with steady-state g_s, water deficit did not provide any protection from O_3 effects on stomatal dynamics.

The effect of water stress on stomatal sluggishness, however, lasted shorter than the effect of ozone exposure. The imperfect stomatal closure at night, in fact, with higher nocturnal steady-state g_s after short-term O_3 exposure in well-watered plants, may be considered as a long-lasting effect of the aberrations induced by ozone. Enhanced nocturnal g_s induced by O_3 has been reported for several plant species (Skärby et al., 1987; Matyssek et al., 1995; Wieser and Havranek, 1995; Grulke et al., 2004, 2007c). Ozone-induced incomplete closure of stomata at night may translate into losing control of water efflux at dark (Skärby et al., 1987; Grulke et al., 2007c). Increase in nocturnal g_s may also enhance O_3 uptake at night. Günthardt-Goerg et al. (1997) reported that considerable O_3 uptake at night induced leaf injury in *Betula pendula*, *Populus* × *euramericana*, and *Alnus glutinosa*. Matyssek et al. (1995) reported that birch species exposed to O_3 at night showed great reductions in growth.

Ozone peaks may exceed 0.1 ppm h in suburban and rural areas of California in the United States (Heath et al., 2009), southern Europe (Paoletti, 2006) and East Asia such as Japan (Takigawa et al., 2007). The present study revealed that realistic short-term O_3 exposure ($\leq$150 ppb) induced stomatal sluggishness with or without drought stress. Although the effect is lighter than for ozone, also soil water deficit can induce stomatal sluggishness. Climate change brings about the risk of drought and flooding (Bytnerowicz et al., 2007). Ozone- and drought-induced loss of stomatal function may enhance both leaf water loss and O_3 uptake. Current modeling efforts of O_3 effects on plants have been developed using steady-state parameters (Emberson et al., 2000; Grünhage et al., 2001) and the O_3-induced losing control of dynamic stomatal response was ignored. The results presented here suggest to reconsider the role of O_3 pollution on leaf gas exchange and highlight complex interactions between ozone and drought. Further improvement about our understanding of stomatal response to O_3 and drought will contribute to assess climate change impacts on plant water balance and susceptibility to stress.

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