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Determinants of stomatal sluggishness in ozone-exposed deciduous tree species

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HIGHLIGHTS

- Our knowledge of ozone (O_3) effects on dynamic stomatal response is still limited.
- Determinants of O_3-induced stomatal sluggishness were examined in deciduous tree species in open-top chambers.
- Ozone exposure slowed closing of stomata after leaf cutting.
- Stomatal sluggishness was well explained by stomatal O_3 flux per net photosynthesis.
- Stomatal sluggishness depended both on ozone flux and on the capacity for detoxification or repair.

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ABSTRACT

Our knowledge of ozone effects on dynamic stomatal response is still limited, especially in Asian tree species. We thus examined ozone effects on steady-state leaf gas exchange and stomatal dynamics in three common tree species of China (*Ailanthus altissima*, *Fraxinus chinensis* and *Platanus orientalis*). Seedlings were grown and were exposed to three levels of ozone in open-top chambers (42, 69, 100 nmol mol^{-1} daylight average, from 09:00 to 18:00). At steady-state, ozone exposure induced an uncoupling of photosynthesis and stomatal conductance, as the former decreased while the latter did not. Dynamic stomatal response was investigated by cutting the leaf petiole after a steady-state stomatal conductance was reached. Ozone exposure increased stomatal sluggishness, i.e., slowed stomatal response after leaf cutting, in the following order of sensitivity, *F. chinensis* > *A. altissima* > *P. orientalis*. A restriction of stomatal ozone flux reduced the ozone-induced sluggishness in *P. orientalis*. The ozone-induced impairment of stomatal control was better explained by stomatal ozone flux per net photosynthesis rather than by stomatal ozone flux only. This suggests that ozone injury to stomatal control depends both on the amount of ozone entering a leaf and on the capacity for biochemical detoxification or repair. Leaf mass per area and the density of stomata did not affect stomatal sluggishness.

1. Introduction

Tropospheric ozone (O_3) is recognized as a significant phytotoxic air pollutant and greenhouse gas (Bytnerowicz et al., 2007; Serengil et al., 2011). Ozone concentrations have been increasing in the northern hemisphere since the pre-industrial age (Akimoto, 2003; Vingarzan, 2004). Especially in East Asian countries, further increases in O_3 concentrations are predicted throughout this century because of rapid economic growth (Ohara et al., 2007; Yamaji et al., 2008).

The phytotoxic nature of O_3 may cause adverse effects on physiological and biochemical processes in tree species (Karnosky et al., 2003; Matyssek and Sandermann, 2003; Ashmore, 2005; Paoletti, 2007). There is still little information on effects of O_3 on native plant species of Asia (Royal Society, 2008). Many studies on European and North American species reported that O_3 may reduce carbon assimilation, and limit the growth of trees (e.g., Wittig et al., 2007, 2009). On the other hand, the effect of O_3 on stomatal conductance is not straightforward (Mansfield, 1998; Paoletti and Grulke, 2005). Ozone has been reported to induce stomatal closure (Wittig et al., 2007). However, slower or less efficient stomatal control may occur, especially a weaker ability to close stomata, referred to as "O_3-induced stomatal sluggishness" (Paoletti, 2005; Mills et al., 2009; Paoletti et al., 2009; Paoletti and Grulke, 2010; Hoshika et al., 2012a, 2013a,b; Dumont et al., 2013). Sluggishness may occur because O_3 reduces stomatal sensitivity to abscisic acid (ABA) (Mills et al., 2009). This loss of stomatal response to ABA is related to O_3-induced ethylene emission (Wilkinson and Davies, 2010). Although our knowledge of the mechanism is still

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limited, such a reduced stomatal control may impair an efficient water use for plants (Sun et al., 2012).

Stomatal O_3 flux is a crucial factor for the assessment of O_3 effects, because stomata are the principal interface for entry of O_3 into a leaf (Omasa et al., 2002; Karlsson et al., 2007; Mills et al., 2010). Stomatal response to environmental stimuli may be related to leaf anatomical traits such as stomatal density (e.g., Drake et al., 2013). Small stomata and their general association with high density of stomata provide the capacity for rapid response in the stomatal conductance of a leaf (Aasamaa et al., 2002; Hetherington and Woodward, 2003; Drake et al., 2013). This implies that fast response of stomata in leaves with high stomatal density and/or lower stomatal conductance during O_3 exposure may result in less diffusion of O_3 into a leaf, and may lead to a decrease in O_3-induced injury (Pääkkönen et al., 1995).

Foliar O_3 injury may also depend on the available resource for repair or biochemical O_3 detoxification of a leaf (e.g., Tausz et al., 2007; Paoletti et al., 2008). Wieser et al. (2002) suggested that antioxidative capacity increased with increasing leaf mass per area (LMA). Inherent LMA may thus be roughly related to sensitivity to O_3 stress (Bussotti, 2008; Zhang et al., 2012). Also Musselman and Minnick (2000) suggested that plant tolerance to O_3 stress may depend on its photosynthetic capacity because detoxification and repair require energy (Noctor and Foyer, 1998). The sensitivity to foliar O_3 injury may thus be explained by the ratio of stomatal O_3 flux to net photosynthesis (Fredericksen et al., 1996; Kolb and Matyssek, 2001), indicating a balance between O_3 exposure of mesophyll cells and availability of photosynthates for repair or detoxification.

In this study, we examined O_3 effects on steady-state leaf gas exchange and dynamic stomatal response under severe water stress imposed by cutting a leaf in three tree species that are common in China. The objective of the study was to test whether the degree of O_3-induced injury to stomatal control was related to stomatal density, LMA, stomatal O_3 flux or the ratio of O_3 flux to net photosynthesis.

2. Materials and methods

2.1. Plant materials

We used one-year-old seedlings of *Ailanthus altissima* (Mill.) Swingle, *Fraxinus chinensis* Roxb. and *Platanus orientalis* L. Before bud burst, bare rooted seedlings were planted in 20 L circular plastic pots on 31 March, 2013 and grown at ambient field condition (outdoors). Pots were filled with native light loamy soil. Seedlings with similar height and basal diameter were selected for this study and pre-adapted to open-top chamber conditions for 10 days before O_3 fumigation. All plants were watered at field capacity at 1–3 day intervals to avoid water stress.

2.2. O_3 treatments

The experiment was carried out in three open-top chambers (OTC, octagonal base, 12.5 m^2 of growth space and 3.0 m of height) in Changping (40°19′ N, 116°13′ E), Northwest Beijing with warm temperate and semi-humid continental climate. The annual mean temperature is 11.8 °C, and total precipitation is 550 mm. All seedlings were exposed to the following treatments for three months with a daily maximum of 9 h (from 09:00 to 18:00) when there was no rain, fog, mist or dew: nonfiltered ambient air (NF, averaged O_3 concentration of 42 nmol mol^{-1} from 09:00 to 18:00), NF supplied with 40 nmol mol^{-1} of O_3 (NF + 40, averaged O_3 concentration of 69 nmol mol^{-1} from 09:00 to 18:00), and NF supplied with 80 nmol mol^{-1} of O_3 (NF + 80, averaged O_3 concentration of 100 nmol mol^{-1} from 09:00 to 18:00). Ozone was generated from pure oxygen by an O_3 generator (HY003, Chuangcheng Co., Jinan, China). The concentrations of O_3 in the OTCs were continuously monitored at approximately 10 cm above the plant canopy using a UV absorption O_3 analyzer (Model 49*i*-Thermo, USA). The monitors were calibrated by a 49iPS calibrator (Thermo Scientific, USA) before the experiment and once per month during the experiment. The chambers were turned off in the evening and the door was opened to allow for dew formation. Four or five potted plants per each tree species were set in each OTC. There was no replication of O_3 treatments. In order to eliminate the positional and chamber effects (Potvin and Tardif, 1988), the plant positions were changed every week within each OTC, and all seedlings were switched between chambers every month, according to Feng et al. (2008, 2011).

2.3. Measurements of leaf traits and gas exchange

Measurements of leaf gas exchange were carried out with a portable infra-red gas-analyzer (Model 6400, Li-Cor Instruments, Lincoln, NE, USA), at controlled value of leaf temperature (30 °C), leaf-to-air vapor pressure deficit (1.5 kPa), saturating light (1500 μmol m^{-2} s^{-1} of PPFD, photosynthetic photon flux density) and ambient CO_2 concentration (400 μmol mol^{-1}) from 8 to 14 August, 2013. Four or five plants per each O_3 treatment were used. A fully expanded sun leaf (leaf order: 4th to 6th in a shoot) was selected as a target. When stomatal conductance reached the equilibrium under constant light at 1500 μmol m^{-2} s^{-1}, the methodology described by Paoletti (2005) was applied to assess dynamic variations of stomatal conductance after cutting the leaf petiole (Fig. 1). Data were logged at 1 min intervals for 30 min after cutting the petiole. Two phases of stomatal response were observed (Fig. 1). At first, stomatal conductance showed an increase called as the transient "wrong-way response" (WWR) (Powles et al., 2006). This transient increase is due to a difference in turgor pressure between guard cell and epidermal cells. Subsequently, stomatal conductance decreased with increasing leaf water stress. In the present study, the magnitude of WWR and time for 50% decrease of stomatal conductance were recorded. All gas exchange measurements were performed from 9:00 to 12:00 to avoid the midday depression of stomatal conductance (cf. Zhang and Gao, 1999).

After measurement of leaf gas exchange, the same leaves were analyzed for determining the leaf mass per unit area (LMA). Three leaf disks (12 mm diameter) per measured leaf were punched out and dried in an oven at 70 °C for 1 week and then weighed. LMA was calculated as the ratio of dry mass to area of the leaf disks. The stomatal density was determined by the SUMP method (Koike et al., 1998), which involves making a replica of the abaxial leaf surface using a celluloid sheet (Universal Micro-printing, SUMP, Tokyo, Japan). Stomata were counted at 5–7 locations, randomly chosen from interveinal fields, of a total area of 0.4 mm^2, under a light microscope.

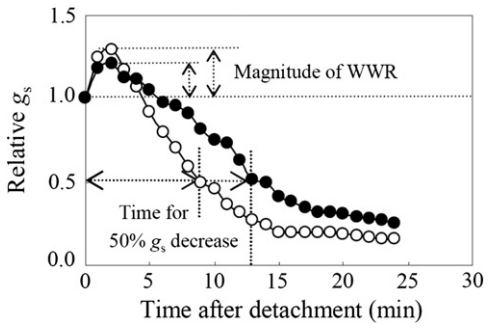

Fig. 1. Example of time course of stomatal conductance (g_s) after severing a *F. chinensis* leaf at time zero (open circle: NF, closed circle: NF + 80), with calculation of wrong way response (WWR) magnitude, and time for 50% decrease in stomatal conductance with increasing leaf water stress.