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Effects of elevated ozone on physiological, anatomical and ultrastructural characteristics of four common urban tree species in China

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ABSTRACT

Fast urbanization has led to ozone (O_3) being the main pollutant in summer in most of China. To assess future ground-level O_3 effects on the service of urban greening species and clarify the underlying mechanism of O_3 damage, four common urban greening species, *Ailanthus altissima* (AA), *Fraxinus chinensis* (FC), *Platanus orientalis* (PO) and *Robinia pseudoacacia* (RP) were exposed to non-filtered air (NF) and to elevated O_3 (E-O_3) in open-top chambers. E-O_3 induced visible injury in all species as well as microscopic alterations such as collapse of the palisade parenchyma cells, callose accumulation, or chloroplast and mitochondrial accelerated senescence. E-O_3 significantly reduced light-saturated CO_2 assimilation (A_{sat}), the maximum activity of Rubisco (Vc_{max}), the maximum electron transport rate (J_{max}), and fluorescence parameters such as the quantum yield of noncyclic electron transport (ϕ_{PSII}), and the quenching of photochemical efficiency of PSII (*qP*). It also increased total antioxidant capacity, phenolics and ascorbate contents. No significant interaction between O_3 and species was found in photosynthetic performance and antioxidant systems, suggesting that the four species selected were sensitive to O_3. Of all four species, AA was the most sensitive species due to a combination of earlier injury onset, anatomical features, lower antioxidant responses and higher stomatal conductance. The sensitivity of tree species to O_3 is a factor to be considered for urban greening. Ozone may affect important urban forest ecosystem services by reducing CO_2 assimilation.

Capsule
Ground-level ozone negatively affects common greening tree species in China.

1. Introduction

Tropospheric ozone (O_3) levels are of great concern as this pollutant affects human health, ecosystem services and food security besides being a greenhouse gas (The Royal Society, 2008; IPCC, 2013). For sensitive plants, high O_3 concentration is known to induce visible injury, impair photosynthesis, produce reductions in growth and yield, and alter plant interactions with pests and diseases (Krupa et al., 2000).

Abbreviations: *A*, photosynthetic rate; AA, *Ailanthus altissima*; AOT40, accumulated hourly O_3 concentration over a threshold of 40 ppb during daytime; Asa, ascorbate; A_{sat}, light-saturated photosynthesis; BVOC, biogenic volatile organic compounds; C_a, ambient CO_2 concentration; Car, carotenoid; Chl, chlorophyll; C_i, intercellular CO_2 concentration; F_v'/F_m', actual photochemical efficiency of PSII in the saturated light; FC, *Fraxinus chinensis*; ϕ_{PSII}, the quantum yield of noncyclic electron transport; g_s, stomatal conductance; J_{max}, the maximum rate of electron transport; LM, light microscopy; LMA, leaf mass per area; L_s, stomatal limitation to photosynthesis; O_3, ozone; OTC, open-top chambers; PBS, phosphate buffered saline solution; PO, *Platanus orientalis*; RP, *Robinia pseudoacacia*; TEM, transmission electron microscopy; *qP*, quenching of photochemical efficiency of PSII; Vc_{max}, the maximum carboxylation efficiency; WUE, water use efficiency.

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Projected changes of the annual daily mean maximum eight-hour (DM8H) surface O_3 concentrations are expected to be in the range of 2–8 ppb, −3 to 8 ppb, and −7 to 9 ppb for the 2020s, the 2050s, and the 2090s in summertime for the whole East Asia (Lee et al., 2015). Ozone concentrations in China are rising at a higher rate than in other countries because O_3 precursors (mainly NO_2) have steadily increased at annual growth rate of 5% caused by its fast industrialization and urbanization (Wang and Mauzerall, 2004; Feng et al., 2015a). In Changping site, downwind of Beijing city, the monthly average of peak O_3 concentrations reaches 100 ppb in July, while the AOT40 (accumulated hourly O_3 concentration over a threshold of 40 ppb during daytime) from June to August is 29 ppm h in 2014 (Feng et al., 2015b; Yuan et al., 2015). The yearly average of daily peak O_3 concentration reaches 60 ppb at some of 35 monitoring stations in Beijing during May 2014 to April 2015 (Chen et al., 2015), and short-term projected emissions suggest that O_3 concentration will further increase (Yamaji et al., 2008). Therefore, O_3 concentrations in Beijing frequently exceed the threshold value of 40 ppb and AOT40 critical level of 5 ppm h, which have been established in Europe to protect sensitive tree species against O_3 (LRTAP, 2010).

Cities are characterized by higher levels of pollutant emissions, energy consumption and higher temperatures (heat island effect) than surrounding areas. One of the multiple environmental benefits of vegetation is the improvement in air quality (Nowak et al., 2014). In the central part of Beijing, the removal of pollutants by trees was quantified to be 1261.4 tons in 2002, mostly particles (61%), with O_3 accounting for 20% (Yang et al., 2005). However, biogenic volatile organic compounds (BVOCs) emitted from vegetation such as isoprene and monoterpenes are precursors of O_3 (The Royal Society, 2008), so the contribution of urban trees to O_3 formation can even offset their removal capacity (Yang et al., 2005). Therefore, it is important to select plants with low BVOC emission rates, high pollutant removal capacity and also tolerant to air pollutants when planting trees in cities. The present paper focuses on the latter aspect.

In Beijing, O_3 concentrations are high enough to induce visible injury in sensitive species and cultivars, including several ornamental trees (Feng et al., 2014). These symptoms are observed in large gardens or urban forests in parks inside the city, or in crop areas or tree plantation surrounding the city rather than in streets where high NO traffic emissions locally scavenge O_3 (due to the titration effect). Ozone effect on plants depends both on the O_3 dose entering the plant through the stomata, which is directly related to water vapor stomatal conductance (g_s), and also on their defense ability to cope with oxidative stress (Matyssek et al., 2007; Paoletti et al., 2008). On the other hand, leaf functional traits are considered to play a role in O_3 sensitivity, e.g. plants with higher leaf mass per area (LMA), or higher thickness or density of mesophyll tissues are usually more tolerant to O_3 (Bussotti, 2008; Zhang et al., 2012).

However, information on the effects of current and predicted future O_3 levels on urban greening species is still very scarce in China. In the present study, we exposed four commonly planted urban greening tree species to an elevated O_3 level which is representative of a future scenario by 2050 on the basis of an annual increase rate of 0.73 ppb/year, observed at Shangdianzi station near Beijing city (Dr. XB Xu, personal communication), and an increase rate of 0.5–2% at a global scale (Vingarzan, 2004). The four species are the tree of heaven (*Ailanthus altissima* (Mill.) Swingle, AA), the Chinese ash (*Fraxinus chinensis* Roxb., FC), the American sycamore (*Platanus orientalis* L., PO) and the black locust (*Robinia pseudoacacia* L., RP). Three of them (AA, FC, RP) are regarded as O_3 sensitive. This study tests the following two hypotheses: (1) O_3 sensitivity differs among investigated species, considering anatomical and ultrastructural changes, photosynthetic performance and antioxidant systems; (2) plants with a higher stomatal conductance, lower antioxidant capacity and thinner leaves are more sensitive to O_3.

2. Materials and methods

2.1. Plant materials

One-year-old seedlings of AA, FC, PO and RP were obtained from a commercial nursery near the experimental site. Bare rooted seedlings were planted in 20 L circular plastic pots on 31 March 2013 and grown at ambient field condition. Pots were filled with native light loamy soil (pH 7.96, organic C 14.7 g/kg; total N 1.64 g/kg, available P 6.59 mg/kg, available K 139.8 mg/kg) randomly selected from a nearby farmland. Plants with similar height and basal diameter were selected. Ten days before O_3 fumigation, they were pre-adapted to open-top chamber (OTC, octagonal base, 12.5 m^2 of growth space with a diameter of 4 m, and 3.0 m in height). All plants were watered at field capacity at 1–3 day intervals to avoid water stress. Solid, slow-release fertilizer (N/P/K = 17/17/17) was applied at a rate of 300 kg ha^{-1} to each plant at July during the experiment.

2.2. O_3 treatments

The experiment was carried out at Changping (40°19′ N, 116°13′ E), Northwest Beijing. The area has a semi-humid continental climate, with a yearly precipitation of 550 mm, and an annual mean temperature of 11.8 °C. Plants were exposed to two O_3 treatments in OTCs for four and a half months (from 1 June to 15 October): non-filtered ambient air (NF, averaged O_3 concentration of 42 ppb from 09:00 to 18:00), and NF supplied with 40 ppb of O_3 (E-O_3, averaged O_3 concentration of 69 ppb from 09:00 to 18:00). The four species and two O_3 treatments were selected for the present study from a wider investigation involving a total of 10 species and six different O_3 regimes in six OTCs. Positional effects were avoided by changing plant positions within each OTC weekly, and by switching them randomly among six OTCs monthly (Feng et al., 2011a). For each O_3 treatment, 4–6 plant replicates were used for each species. Ozone was generated from pure oxygen using an O_3 generator (HY003, Chuangcheng Co., Jinan, China), mixed with ambient air and then piped into OTCs through a PVC tube (11 cm in diameter) using a fan (1.1 kW, 1080 Pa, 19 m^3 min^{-1}, CZR, Fengda, China). The flow rate of pure oxygen was regulated by mass flow controllers so as to achieve the target O_3 concentration at the top of the canopy in the fumigation treatments. An O_3 analyzer (Model 49i-Thermo, USA) was used to continuously monitor O_3 concentrations inside the OTCs via a Teflon solenoid valve switch system connected to a set of Teflon tubes (4 mm in diameter), which collected air from sampling points at approximately 10 cm above the plant canopy in each chamber. The monitors were calibrated by a 49i-PS calibrator (Thermo Scientific, USA) before the experiment and once a month during the experiment. The daily maximum fumigation period was 9 h (from 09:00 to 18:00) through a fan running when there was no rain, fog, mist, or dew, according to the protocols in free air O_3 concentration enrichment system (Feng et al., 2011b). The monthly ambient O_3 concentration (from 09:00 to 18:00) in the open field ranged from 43 ppb (September) to 69 ppb (June), with the highest one hour peak being 153 ppb (at 16 h on 20 September).

2.3. Visible injury

Visible injury was assessed weekly in all plants (4–6 per O_3 treatment). The percentage of injured leaves (for PO, with simple leaves) or leaflets (for the rest of species, with composite leaves)