Differential responses of peach (*Prunus persica*) seedlings to elevated ozone are related with leaf mass per area, antioxidant enzymes activity rather than stomatal conductance[☆]

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ABSTRACT

To evaluate the ozone (O₃) sensitivity among peach tree (*Prunus persica*) cultivars widely planted in Beijing region and explore the possible eco-physiological response mechanisms, thirteen cultivars of peach seedlings were exposed to either charcoal-filtered air or elevated O₃ (E-O₃, non-filtered ambient air plus 60 ppb) for one growing season in open-top chambers. Leaf structure, stomatal structure, gas exchange and chlorophyll *a* fluorescence, photosynthetic pigments, antioxidant defense system and lipid peroxidation were measured in three replicated chambers. Results showed that E-O₃ significantly reduced abaxial epidermis thickness, but no effects on the thicknesses of adaxial epidermis, palisade parenchyma and spongy parenchyma. Stomatal area, density and conductance were not significantly affected by E-O₃. E-O₃ significantly accelerated leaf senescence, as indicated by increased lipid peroxidation and more declines in light-saturated photosynthetic rate and pigments contents. The reduced ascorbate content (ASC) was decreased but antioxidant enzyme activity (CAT, APX and SOD) and total antioxidant capacity (TAC) were significantly increased by E-O₃ among cultivars. The cultivars with visible symptoms also had more reductions in net photosynthetic rate than those without visible symptoms. Ozone sensitivity among cultivars was strongly linked to leaf mass per area (LMA), antioxidant enzymes activity e.g. SOD, APX rather than stomatal parameters (stomatal area, density and conductance) and ASC. Results could provide a theoretical basis for selecting and breeding the ozone-resistant cultivars of peach trees grown in high O₃-polluted regions.

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1. Introduction

Ground-level ozone (O₃) is considered as one of the most phytotoxic air pollutant due to its significant damage to the plants and ecosystem services worldwide (Paoletti et al., 2010). With the fast industrialization and urbanization in the last three decades, the ground-level ozone (O₃) is increasing at a rate of approximately 0.5–2% per year over the midlatitudes of the Northern Hemisphere

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(IPCC, 2013). In many regions of China, high levels of O₃ have been observed (Wang et al., 2010, 2012; Cooper et al., 2014) and are expected to increase further as NO_x precursors are increasing (Feng et al., 2015). The highest hourly ozone value was observed as 316 ppb at Jinan, Shandong Province (Yin et al., 2004). At Miyun, located at the Northeast edge of Beijing, the 95th percentile of daily O₃ concentration ranged from 100 ppb to 162 ppb during April to September based on *in situ* observations (Cooper et al., 2014). Yuan et al. (2015) also reported that the daily mean ambient O₃ concentration was 71.3 ppb, and the maximum hourly peak concentration was 173.6 ppb in the northwest areas of Beijing during June to September 2014. Numerous experimental studies showed that ambient O₃ concentrations with an average of 40 ppb have significantly decreased the yield of crops (rice, wheat, soybean and

potato) and total biomass of trees by 10% and 7% compared with low ozone concentrations air, respectively (Feng and Kobayashi, 2009; Wittig et al., 2009). Furthermore, high O₃ concentrations also caused many physiological and biochemical changes in plants, for instance, decreased photosynthesis, stomatal closure, chlorophyll degradation, metabolic disorder, accelerated senescence and weaken defense against pests and diseases with or without visible foliar injury (Feng et al., 2014; Hoshika et al., 2015; Li et al., 2016; Matyssek et al., 2010; Zhang et al., 2012).

Peach tree (*Prunus persica*) is originated from China and widely cultivated in China. The harvested area and production in China were over 726,068 ha and 12,423,700 tonnes in 2014 (FAOstat, 2014). Peach peculiarities of flavor and aroma result from a balance of sugars, organic acids, phenolic compounds, carotenoids and volatile compounds, making it an appreciated fruit of great commercial importance (Gil et al., 2002). Although there are many studies about the negative impact of O₃ on woody species (Calatayud et al., 2011; Feng et al., 2011; Haberer et al., 2007; Kontunen-Soppela et al., 2010), very little is known about effect of O₃ on orchard trees, especially on the peach trees to date. Mccool and Musselman (1990) and Badiani et al. (1993, 1996) found that elevated O₃ restrained the growth of peach to some magnitudes in America and Europe, respectively, and current O₃ level was over the critical level of peach growth. However, neither of them explored the mechanism on the difference in response to O₃ among cultivars. May–September is the peak of O₃ concentration in Northern China every year, and the period is also the crucial growth season of peach. Due to high overlapping between them, the growth of peach tree may be affected by high O₃ concentration. Furthermore, the presence of O₃ visible symptoms in *Prunus persica* f. *duplex* was observed in field surveys around Beijing (Feng et al., 2014). However, whether O₃ affects growth, physiological and biochemical characteristics of peach tree in China has not been explored to date.

There is a large variation in the sensitivity to O₃ between genotypes or species. Generally, evergreen genotypes/species are more tolerant to O₃ than deciduous genotypes/species (Calatayud et al., 2010; Zhang et al., 2012), and needle-leaf vegetation is more tolerant than broadleaved plants (LRTAP, 2010). The internal mechanism on the O₃ sensitivity has been controversial. Some studies found the O₃ sensitivity was associated with high stomatal conductance (g_s) (Paoletti, 2006; Wittig et al., 2007), but others argued that it depended on antioxidant levels rather than g_s (Feng et al., 2016; Nali et al., 2004). Li et al. (2016) compared 29 deciduous and evergreen woody species with different sensitivity to O₃ and found that both leaf morphology and antioxidant levels were related to O₃ sensitivity. Furthermore, many studies have confirmed that the O₃-induced responses of antioxidant metabolism varied by species or cultivars in the detoxification of reactive oxygen species (ROS), depending on the concentration of O₃ and the duration of the fumigation, e.g. acute vs. chronic ozone exposure (Betzberger et al., 2010; Burkey et al., 2003; Feng et al., 2011). Generally, acute exposure to relatively high O₃ concentrations often caused leaf lesions due to programmed cell death and induced an increase in antioxidant metabolism. In the chronic exposure to relatively low O₃ concentrations, there is still some dispute about the antioxidant response to O₃. Some studies demonstrated that total antioxidant capacity was increased after chronic exposure to elevated O₃ (E-O₃) in soybean plants (Gillespie et al., 2011), whereas others found that antioxidant pools and key enzymes in the apoplast and leaf tissues of wheat were declined by E-O₃ in an O₃-FACE (Feng et al., 2010; Wang et al., 2014a,b). Peach tree consists of nectarines, flat peach, white peach and yellow peach with different leaf traits. Whether there is different response to O₃ among cultivars of peach tree is not so clear. Furthermore, it is not clear if factors contributing to O₃ sensitivity among cultivars or species also play an important role in

different response to E-O₃ among cultivars of the peach tree.

In this study, 13 cultivars of peach seedlings including six nectarines and seven white peaches were exposed to relatively high concentration under local climate conditions. The objectives were (1) to elucidate the eco-physiological response to O₃ among cultivars and find the sensitive and tolerant cultivars of peach seedlings and (2) to explore the intrinsic mechanisms on the differences in O₃ sensitivity among cultivars of peach seedlings.

2. Materials and methods

2.1. Experiment site and fumigation treatment

The experiment was conducted in field open-top chambers (OTCs) located at Tangjiapu town (40°45'N, 115°97'E), Yanqing district, northwest of Beijing, China, ~60 km from Beijing city. There were two treatments inside six OTCs: one with charcoal-filtered air (CF) in which ~70% of the O₃ in the ambient air was filtered, another with elevated O₃ (E-O₃, non-filtered ambient air plus extra 60 ppb O₃ during fumigation hours). Every treatment had three chambers replicates. O₃ fumigation started from 26 June to 30 September with a daily maximum of 10 h (from 08:00 to 18:00) except rainy days. During the experimental period covering non-fumigated days, the averaged O₃ concentration in CF and E-O₃ was 28.1 and 87.0 ppb, respectively, and AOT40 (accumulation of hourly O₃ concentration over 40 ppb) was 2.48 and 50.8 ppm h, respectively (Fig. 1). Ozone was generated from pure oxygen by an electrical discharge O₃ generator (HY003, Chuangcheng Co. Jinan, China). A UV absorption O₃ analyzer (Model 49i-Thermo, Thermo Scientific, Massachusetts, USA) was used to continuously monitor O₃ concentrations inside the OTCs. For each of the 13 cultivars, three replicates were grown in each OTC. Possible confounding influences of within OTC differences in growth conditions were minimized by changing plant positions within each OTC weekly.

2.2. Plant materials

Thirteen cultivars of peach seedlings including six nectarines (LJY, 76, RG27, RG29, WC and ZC) and seven white peaches (CJ, DDT, GH2, JY, LH9, LHC and W24) were randomly selected in this study, representing a wide range of peach tree cultivated in surrounding areas of Beijing. One-year-old seedlings of the selected cultivars were individually planted into 30 L plastic pots filled with local loamy soil. The soil was excavated from farmland at 0–10 cm depth, sieved out by a 0.3 mm pore mesh and then mixed for homogeneity carefully. All seedlings were manually irrigated at 1–2 days interval

Fig. 1. Variations in 10 h (8:00–18:00) mean O₃ concentrations and AOT40 from June 26 to September 30 in charcoal-filtered air (CF) and elevated O₃ (E-O₃) treatments.

to keep soil moisture uniform and close to field capacity.

2.3. Visible O₃ injury

Leaves of each seedling were observed every other day until symptoms first appeared and checked twice every week thereafter. The date of first foliar injury onset was defined when plants in three replicated chambers showed the same foliar symptoms. The onset date of visible foliar injury and the injury performance were recorded. The AOT40 at the initial visible injury onset (AOT40_{injury}) was calculated following to the approach described by Zhang et al. (2012) and Li et al. (2016). Notably, we did not quantify the severity of visible injury because it needs much experience and professional techniques.

2.4. Leaf structure

For leaf anatomical traits, six leaves per plant were sampled according to the method of Li et al. (2016). In brief, leaf samples were fixed with 4% mixed fixative of paraformaldehyde and glutaraldehyde for 24 h at 4 °C and washed three times for 15 min with 0.1 M phosphate-buffered saline (pH 7.2). For microscopy examinations, samples were cut with a freezing microtome (CM 1325; Leica, Wetzlar, Germany) and sections were stained with safranin-fast green. The thickness of adaxial epidermis, abaxial epidermis, palisade and spongy parenchyma of the sections was photographed using an Olympus CX 41 fluorescence microscope equipped with an Olympus DP 26 camera and measured with 'Olympus cellSens Standard' Software.

2.5. Stomata structure

The abaxial epidermis was peeled from the leaves using Nail Polish Smear methods based on Liu et al. (2005). In brief, the appropriate plant leaves were selected and coated with nail polish in the abaxial epidermis. The wiping layer was torn using tweezers when the coating layer was slightly connected with the leaf blade after nearly 5 min. The width of the stomata was measured under a research microscope with the help of a precalibrated ocular micrometer. Stomata were counted at random in 20 visual sections on the abaxial epidermis, and final tallies were used to compute their densities. Lengths and widths were measured randomly from 20 stomata on the same specimens using Image J software. Stomata area was counted as length × width.

2.6. Gas exchange, chlorophyll *a* fluorescence and photosynthetic pigments

Gas exchange and chlorophyll *a* fluorescence were measured with a portable leaf photosynthesis system (Licor-6400, LI-COR Inc., Lincoln, NE, USA) fitted with a 6400-40 leaf chamber fluorometer (LCF). Two fully expanded upper leaves from one plant were randomly selected in each chamber. The system controlled saturating PPFD at 1200 μmol m⁻² s⁻¹, block temperature at 30 °C, the CO₂ concentration of air entering the leaf at 400 μmol mol⁻¹ and the relative humidity at 50–60%. The measurements were conducted during 09:00–12:00 h in sunny days. In order to minimize possible confounding influences of environmental fluctuations, all measurements were randomized between O₃ treatments and among cultivars. The values from light-saturated photosynthesis rate (*A*_{sat}), stomatal conductance (*g*_s), intercellular CO₂ concentration (*C*_i), transpiration rate (*Tr*), the actual photochemical efficiency of PSII in the saturated light (*F*_v/*F*_m'), effective quantum yield of PSII photochemistry (*Phi*PSII) and quenching of photochemical efficiency of PSII (*qP*) were downloaded from the instruments. Water

use efficiency (*WUE*) was calculated as the ratio of *A*_{sat} and *Tr*.

Two leaf discs per plant from one plant in each chamber were extracted with 2 ml 95% ethanol in the dark for at least 72 h at 4 °C. The absorbance of pigment extracts was measured at 664, 649 and 470 nm. Concentrations of photosynthetic pigments including chlorophyll *a*, *b* and carotenoids were calculated according to the specific absorption coefficients provided by Lichtenthaler (1987).

2.7. Antioxidants

Three leaves of the third or fourth one from the top per plant were sampled between 12:00 and 14:00 h and quickly wrapped by tinfoil, frozen in liquid nitrogen immediately and stored at –80 °C until analysis. Frozen leaf tissues were ground into power in liquid N₂ with a mortar and a pestle for the biochemical assay. Malondialdehyde (MDA) content was assessed for estimation of lipid peroxidation by 2-thiobarbituric acid-reactive metabolite (TBA) according to the method of Heath and Packer (1968) and calculated by the equation of C_{MDA} (mmol L⁻¹) = 6.45 × (OD₅₃₂ – OD₆₀₀) – 0.56 × OD₄₅₀ in order to rule out the disturbances from nonspecific (OD₆₀₀) and sugar (OD₄₅₀) absorbance. Total antioxidant capacity (TAC) determinations were conducted following the ferric reducing antioxidant power (FRAP) assay, measuring the combined antioxidant effect of the nonenzymatic defenses, offering a putative index of the ability to resist oxidative damage (Benzie and Strain, 1996), expressed as Fe³⁺ equivalents (mmol Fe²⁺ g⁻¹ FM). Reduced ascorbic acid (ASC) contents were measured by monitoring the changes of absorbance at 265 nm (extinction coefficient of 14 mM⁻¹ cm⁻¹) after addition of ascorbate oxidase (Luwe and Heber, 1995).

For antioxidant enzymes measurement: Samples (~0.1 g fresh leaves) were ground in liquid nitrogen and extracted with 2 ml of 50 mM sodium phosphate buffer (pH 7.0, containing 1% vinyl pyrrolidone). The samples were centrifuged at 13,000 r min⁻¹, 4 °C for 20 min and the supernatant was collected. All extracting experiments were carried out in the ice bath. The superoxide dismutase (SOD) activity was determined by estimating its ability of inhibiting the photochemical reduction of nitro blue tetrazolium (NBT) and the absorbance was read at 560 nm. The amount of enzyme that inhibited the 50% NBT reduction was defined as one unit of SOD activity. The ascorbate peroxidase (APX) activity was assayed by monitoring the change of the absorbance at 290 nm after the enzyme extract was mixed a 50 mM potassium phosphate buffer (pH 7.0) containing 30 mM ascorbate and 10 mM H₂O₂. The enzyme activity was determined by monitoring the change in optical density (OD) at 290 nm and one unit of APX activity was defined as the decrease of 0.01 ΔOD per minute. The hydrogen peroxidase (CAT) activity was determined according to the description by Mckee et al. (1997). The change of absorbance at 240 nm was monitored after the enzyme extract was mixed with a 30 mM KH₂PO₄/K₂HPO₄ (pH 7.0) containing 10 mM H₂O₂. The unit of CAT and POD activity was defined as the decrease and increase of 0.01 ΔOD per minute at 240 nm and 470 nm, respectively. All measurements were conducted using spectrophotometric methods.

2.8. Statistical analysis

The log-transformed *g*_s and CAT and other all variables in original data passed Shapiro-Wilk and Levene's tests for normality and homogeneity of variance, respectively. Then, they were subjected to analysis of variance (ANOVA) with mixed linear model to test the effects of O₃, cultivars and their interaction using JMP software (SAS Institute, Cary, NC, USA), in which each OTC represented the statistical unit (*n* = 3). Turkey's Honestly Significant Difference (HSD) test was applied to identify significant differences. The

relationships between cultivar-specific leaf traits (stomata structure, antioxidant defense system and leaf structure) in CF plants and corresponding response variables (AOT40_injury, relative change defined as reduction in A_{sat} by E-O₃ relative to CF treatment) were assessed by ordinary least square regression using SPSS 20 (SPSS 20, Chicago, IL, USA), in which each cultivar was represented by one data point. $P \leq 0.05$ was considered as statistically significant.

3. Results

3.1. Visible injury

No visible injury was observed in any of the plants under CF conditions. However, plants exposed to E-O₃ showed typical visible symptoms including reddish stipple, brown spots, and chlorotic and necrotic patches on their leaves (Table 1). At end of the experiment, only three cultivars including 2 nectarines (WC and RG29) and 1 white peach (DDT) seedlings did not exhibit visible O₃ injury. The most sensitive cultivars were GH2, JY, LH9 and LHC, which displayed visible symptoms on the upper surface of leaves at AOT40 values below 20 ppm h. The cultivars with highest AOT40_injury values (>30 ppm h) were RG27, 76 and ZC (three nectarines) (Table 1). Nearly all of the white peach cultivars except DDT showed more or less visible symptoms when plants were exposed to AOT40 of 50.8 ppm h. The injuries induced by E-O₃ became more severe over time and the leaf senescence was accelerated by E-O₃ in some cultivars.

Among the tested cultivars, three distinct groups with respect to O₃ sensitivity could be identified: sensitive cultivars (GH2, JY, LHC and LH9), intermediate cultivars (CJ, W24, LJY, RG27, 76 and ZC) and resistant cultivars (RG29, DDT and WC) based on symptom onset timing.

3.2. Leaf structure

E-O₃ significantly reduced abaxial epidermis thickness by 3.06%, but no effects on the thicknesses of adaxial epidermis, palisade parenchyma and spongy parenchyma (Table 2). Cultivars differed significantly on the thicknesses of abaxial epidermis, palisade parenchyma and spongy parenchyma. A significant interaction between O₃ and cultivars was found in all four variables ($P < 0.05$) as indicated by the increase in some cultivars like W24, DDT and WC, but the decrease in other cultivars like LHC and RG27 compared

with CF. Furthermore, E-O₃ also significantly reduced leaf mass per area (LMA) by 8.49% across 13 cultivars although there was no significant interaction between O₃ and cultivars (Table 2).

3.3. Stomata structure

Compared with CF plants, E-O₃ significantly decreased stomatal area and density of 13 cultivars by 26.0% and 13.5%, respectively ($P < 0.001$). The declines in stomatal area were significant in cultivars of GH2 and LHC, although no significant interaction between O₃ and cultivars was found (see Table 3).

3.4. Photosynthesis parameters and water use efficiency

From Table 4, E-O₃ significantly reduced A_{sat} by 39.9% across all cultivars, especially for white peach GH2 (−46.1%), JY (−52.4%) and LH9 (−66.3%) showing the O₃ symptoms earlier, but the interaction between O₃ and cultivars was close to significant ($P = 0.08$). E-O₃ significantly increased C_i by 10.6%, reduced WUE by 29.4%, but effect on g_s was close to significant ($P = 0.087$).

3.5. Chlorophyll a fluorescence

E-O₃ significantly reduced F_v/F_m' , Φ_{PSII} and qP by 10.8%, 30.3% and 22.3% across all cultivars, respectively, as compared to those grown in CF chambers (Table 5). Although no significant interaction between O₃ and cultivars was found, E-O₃ reduced F_v/F_m' among 13 cultivars in a large variation from 5.0% to 23.3%, Φ_{PSII} from 5.3 to 46.3% and qP from 3.05 to 36.2%.

3.6. Photosynthetic pigment content

Compared to CF, E-O₃ significantly reduced chlorophyll *a*, *b*, *a+b* and carotenoid contents by 21.3%, 20.9%, 19.4% and 19.7% across 13 cultivars, respectively. However, no interaction between O₃ and cultivars was found for all of these photosynthetic pigment variables (Table 6).

3.7. Antioxidant system and lipid peroxidation

E-O₃ significantly increased the activities of SOD and CAT by 16.2% and 59.3%, respectively, but not on APX across 13 cultivars (Table 7). However, no significant interaction between O₃ and cultivars was observed. Moreover, there was no significant

Table 1

AOT40 at the initial visible O₃ symptom onset and the description of the visible symptoms in 10 cultivars of peach seedlings exposed to elevated ozone (E-O₃).

Cultivars	White peach/ nectarines	Symptom onset date (days after fumigation)	AOT40 (ppm.h)	Description of symptoms
GH2	white peach	15	13.6	Necrotic spots appeared at the edges of adaxial surface of fully expanded leaves. For serious-injured leaves, stippling became brown. Leaf senescence was accelerated
JY	white peach	19	15.4	Yellow reddish interveinal stippling on upper surface and accelerated leaf senescence
LH9	white peach	25	17.1	Brown stippling on the upper surface of the leaf, finally coalescing to form large brown patches
LHC	white peach	29	19.7	Light brown interveinal stippling and also finally become dark purple stippling
W24	white peach	34	22.3	Brown stippling on the upper surface of the leaf, finally coalescing to form large brown patches. Stippling more abundant in older leaves
CJ	white peach	39	25.9	Chlorosis firstly appeared in fully developed leaves and then leaves turned yellow with pale white stipple widespread on the surface of leaves
LJY	nectarine	48	29.4	Necrotic spots appeared at the edges of adaxial surface of fully expanded leaves. In very affected leaves, stippling becoming brown
RG27	nectarine	51	30.6	Light brown interveinal stippling and also finally become dark purple stippling
76	nectarine	60	35.5	Leaf yellowing associated with accelerated senescence
ZC	nectarine	66	44.1	No visible symptoms occurred in developed leaves, but the expanding leaves showed curing
RG29	nectarine	—	—	—
DDT	white peach	—	—	—
WC	nectarine	—	—	—

Table 2
Adaxial epidermis, abaxial epidermis, palisade parenchyma, spongy parenchyma and leaf mass per area (LMA) of 13 cultivars of peach seedlings growing in charcoal-filtered air (CF) and elevated [O₃] (E-O₃). Data are shown as Mean ± SE (n = 3); different letters indicate statistical differences (Turkey's test, P < 0.05).

Cultivars	adaxial epidermis thickness (μm)		abaxial epidermis thickness (μm)		Palisade parenchyma thickness (μm)		Spongy parenchyma thickness (μm)		LMA (g m ⁻² DM)	
	CF	E-O ₃	CF	E-O ₃	CF	E-O ₃	CF	E-O ₃	CF	E-O ₃
GH2	9.29 ± 0.65	9.76 ± 0.41	7.46 ± 0.20a-d	7.25 ± 0.34cd	33.7 ± 1.95ab	34.2 ± 2.40ab	37.9 ± 0.63cde	38.8 ± 1.30cde	103 ± 6.23a	89.1 ± 0.78a
JY	9.89 ± 0.16	9.27 ± 0.50	7.41 ± 0.29a-d	7.39 ± 0.13a-d	34.8 ± 1.08ab	37.2 ± 0.79ab	40.0 ± 0.52a-e	45.6 ± 0.98abc	86.6 ± 4.10a	91.7 ± 1.19a
LH9	9.02 ± 0.24	10.2 ± 0.10	7.52 ± 0.31a-d	7.68 ± 0.21a-d	35.8 ± 1.19ab	36.8 ± 2.32ab	41.9 ± 0.27a-e	40.3 ± 1.92a-e	91.9 ± 6.70a	104 ± 2.63a
LHC	9.58 ± 0.17	9.48 ± 0.14	7.46 ± 0.09a-d	7.32 ± 0.04bcd	42.4 ± 4.05a	35.4 ± 0.86ab	44 ± 1.65a-d	39 ± 1.60b-e	109 ± 3.38a	84.4 ± 2.97a
W24	10.1 ± 0.43	10.3 ± 0.33	7.59 ± 0.18a-d	8.38 ± 0.26a	33.3 ± 2.99ab	39.4 ± 2.28ab	38.3 ± 0.75cde	46.7 ± 0.97ab	94.9 ± 3.64a	108 ± 4.52a
CJ	9.94 ± 0.24	9.89 ± 0.11	7.55 ± 0.13a-d	8.35 ± 0.11ab	36.0 ± 0.91ab	32.3 ± 0.65ab	41.1 ± 0.35a-e	39 ± 1.46b-e	109 ± 1.85a	95.7 ± 3.56a
LJY	9.64 ± 0.17	9.86 ± 0.13	7.68 ± 0.14a-d	7.65 ± 0.21a-d	30.3 ± 0.62b	35.0 ± 1.08ab	37.3 ± 1.40de	38.4 ± 0.74cde	101 ± 5.37a	84.1 ± 3.83a
RG27	9.65 ± 0.01	9.00 ± 0.18	7.43 ± 0.04a-d	7.16 ± 0.11cd	33.9 ± 1.40ab	32.5 ± 0.82ab	39.8 ± 1.47a-e	38.3 ± 1.83cde	112 ± 1.95a	90.5 ± 2.91a
76	9.59 ± 0.21	9.15 ± 0.26	7.75 ± 0.09a-d	7.89 ± 0.14a-d	36.4 ± 1.64ab	31.9 ± 0.11ab	47.6 ± 2.26a	40.1 ± 1.02a-e	110 ± 2.83a	95.3 ± 1.58a
ZC	10.2 ± 0.31	9.41 ± 0.15	7.64 ± 0.09a-d	7.54 ± 0.16a-d	29.4 ± 0.38b	34.1 ± 0.26ab	35.5 ± 2.29e	39.5 ± 0.60b-e	117 ± 5.03a	106 ± 4.51a
DDT	9.62 ± 0.28	9.57 ± 0.15	7.35 ± 0.04a-d	8.12 ± 0.29abc	35.5 ± 0.81ab	38.2 ± 3.44ab	41.4 ± 0.17a-e	42.9 ± 1.79a-e	106 ± 2.90a	92.9 ± 3.22a
RG29	9.19 ± 0.30	9.92 ± 0.09	7.07 ± 0.06d	7.56 ± 0.08a-d	33.9 ± 0.53ab	32.4 ± 0.49ab	38.8 ± 1.70cde	42.3 ± 2.11a-e	109 ± 4.31a	101 ± 4.04a
WC	9.61 ± 0.23	9.25 ± 0.04	7.35 ± 0.33a-d	7.94 ± 0.26a-d	38.4 ± 2.04ab	41.3 ± 4.70a	40.6 ± 2.21a-e	40.7 ± 1.31a-e	104 ± 3.92a	95.5 ± 1.28a
ANOVA										
O ₃	0.812		0.003		0.483		0.317		0.010	
Cultivars	0.125		0.003		0.002		0.0003		0.570	
O ₃ × Cultivars	0.022		0.025		0.034		<0.0001		0.455	

Table 3
Stomatal area and density of 13 cultivars of peach seedlings growing in charcoal-filtered air (CF) and elevated [O₃] (E-O₃). Data are shown as Mean ± SE (n = 3); different letters indicate statistical differences (Turkey's test, P < 0.05).

Cultivars	Stomatal area (μm ²)		Stomatal density (mm ⁻²)	
	CF	E-O ₃	CF	E-O ₃
GH2	1209 ± 106ab	651 ± 90.6c	303 ± 10.1ab	248 ± 12.0ab
JY	1023 ± 119abc	749 ± 42.4abc	285 ± 21.8ab	323 ± 8.80a
LH9	916 ± 191abc	778 ± 103abc	278 ± 40.9ab	222 ± 31.1b
LHC	1273 ± 199a	727 ± 73.3bc	268 ± 7.30ab	249 ± 16.0ab
W24	887 ± 87.3abc	867 ± 67.5abc	288 ± 14.2ab	243 ± 15.9ab
CJ	1120 ± 71.7abc	813 ± 62.1abc	297 ± 6.00ab	258 ± 15.9ab
LJY	951 ± 62.2abc	887 ± 105abc	325 ± 20.2a	237 ± 18.3ab
RG27	1280 ± 192a	894 ± 94.3abc	280 ± 20.8ab	272 ± 10.9ab
76	1022 ± 131abc	684 ± 41.1bc	288 ± 16.4ab	246 ± 7.30ab
ZC	1128 ± 72.2abc	855 ± 59.3abc	290 ± 5.80ab	249 ± 14.7ab
DDT	966 ± 29.5abc	711 ± 26.2bc	303 ± 23.5ab	257 ± 8.80ab
RG29	1082 ± 54.9abc	821 ± 107abc	298 ± 30.0ab	228 ± 15.9ab
WC	903 ± 31.2abc	747 ± 18.3abc	288 ± 24.2b	251 ± 5.00ab
ANOVA				
O ₃	<0.0001		<0.0001	
Cultivars	0.299		0.452	
O ₃ × Cultivars	0.224		0.191	

difference among cultivars in the activities of APX and SOD across two O₃ treatments, but the very close significant difference in CAT activity was found (Table 7).

E-O₃ significantly decreased ASC content by 24.0% across 13 cultivars (Table 8). However, a significant interaction between O₃ and cultivars was found. The reduction of ASC by E-O₃ in ten cultivars ranged from 7.03% to 66.5% except LH9 (+49.7%), ZC (+26.7%) and DDT (+8.0%). On the other hand, E-O₃ significantly increased TAC content by 23.5% across 13 cultivars despite no significant interaction. E-O₃ significantly increased MDA contents by 30.9% across 13 cultivars, but no interaction between O₃ and cultivars was found although the largest increase in GH2 cultivar was 92.0% (Table 8).

3.8. Correlations between variables

As shown in Table 9, LMA in CF plants was positively correlated with AOT40_injury and negatively correlated with reductions in A_{sat} induced by E-O₃. However, neither stomatal parameters (SA, SD and g_s) nor antioxidants (TAC, ASC and A-TAC, A-ASC) were found to

be significantly correlated with these two response parameters. For antioxidant enzymes, SOD was negatively correlated with reductions in A_{sat}. A-SOD was positively correlated with AOT40_injury, and negatively correlated with reductions in A_{sat}. APX was positively correlated with reductions in A_{sat} but A-APX was positively correlated with AOT40_injury. There were no significant correlations between the two response parameters and CAT or A-CAT. g_s/LMA was negatively correlated with reductions in A_{sat}.

4. Discussion

4.1. Effects of E-O₃ among peach cultivars

Besides foliar symptoms, E-O₃ also induced serious effects on the thickness of the abaxial epidermis, but not on palisade parenchyma and spongy parenchyma in leaves of peach seedlings. In addition, leaf senescence was significantly accelerated by E-O₃ as indicated by the significant decrease in chlorophyll contents and increase in MDA contents. Furthermore, E-O₃ induced the change in chlorophyll and MDA contents in white peach cultivars (−28.2% and +42.1%, respectively) higher than those in nectarine cultivars (−8.15% and +19.4%, respectively), suggesting that white peach cultivars are more sensitive to O₃ than nectarine cultivars.

As a gate O₃ entering into plants, stomata react to changing environmental parameters by opening or closing. In this study, no significant differences in stomatal area and density were found among 13 peach cultivars under CF treatments. Stomatal area and density were significantly increased by E-O₃ in different magnitudes (P < 0.001), which are fully different from those in tobacco (Saitanis and Karandinos, 2002) and birch (*Betula pendula* Roth.) (Frey et al., 1996). The different results may be attributed to plant properties and/or O₃ levels.

In this study, E-O₃ induced a large amount of reduction in A_{sat} in most cultivars with an uncoupling of the relationship between A_{sat} and g_s. The reductions in A_{sat} in white peach cultivars like GH2, JY and LH9 were higher than nectarine cultivars like ZC, RG29 and WC, suggesting that white peach cultivars was more severely damaged by E-O₃ than nectarine cultivars. However, the effect of E-O₃ on g_s was not consistent among cultivars as indicated by an increase in some cultivars and a decrease in others (Table 4), suggesting that g_s may not contribute to the significant difference among cultivars in response to E-O₃. Moreover, O₃-induced an increase in C_i,

Table 4

Photosynthesis parameters (light-saturated photosynthesis rate (A_{sat}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and water use efficiency (WUE) of peach seedlings growing in charcoal-filtered air (CF) and elevated $[\text{O}_3]$ (E-O_3). Data are shown as Mean $\pm$ SE ($n = 3$); different letters indicate statistical differences (Turkey's test, $P < 0.05$).

Cultivars	A_{sat} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		C_i ($\mu\text{mol mol}^{-1}$)		WUE ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)	
	CF	E- O_3	CF	E- O_3	CF	E- O_3	CF	E- O_3
GH2	11.5 $\pm$ 1.18abc	6.20 $\pm$ 0.85d-g	0.10 $\pm$ 0.01	0.17 $\pm$ 0.01	226 $\pm$ 19.7a	309 $\pm$ 5.73a	8.83 $\pm$ 0.86a	4.02 $\pm$ 0.18ab
JY	11.7 $\pm$ 1.67ab	5.60 $\pm$ 1.19efg	0.24 $\pm$ 0.04	0.27 $\pm$ 0.07	290 $\pm$ 23.1a	340 $\pm$ 9.08a	5.2 $\pm$ 0.77ab	2.30 $\pm$ 0.21b
LH9	11.9 $\pm$ 0.98ab	4.00 $\pm$ 0.51g	0.11 $\pm$ 0.004	0.12 $\pm$ 0.04	196 $\pm$ 4.29a	304 $\pm$ 17.4a	7.02 $\pm$ 0.18ab	4.05 $\pm$ 0.50ab
LHC	10.2 $\pm$ 0.04a-e	7.40 $\pm$ 0.33b-g	0.31 $\pm$ 0.08	0.24 $\pm$ 0.06	319 $\pm$ 13.1a	312 $\pm$ 18.6a	3.77 $\pm$ 0.31ab	3.60 $\pm$ 0.69ab
W24	11.4 $\pm$ 1.15abc	6.90 $\pm$ 0.57c-g	0.30 $\pm$ 0.04	0.14 $\pm$ 0.01	318 $\pm$ 8.71a	301 $\pm$ 13.1a	3.94 $\pm$ 0.32ab	4.31 $\pm$ 0.19ab
CJ	11.4 $\pm$ 0.59abc	7.50 $\pm$ 1.08b-g	0.23 $\pm$ 0.04	0.19 $\pm$ 0.05	269 $\pm$ 31.1a	290 $\pm$ 23.8a	6.11 $\pm$ 0.86ab	4.57 $\pm$ 0.84ab
LJY	9.8 $\pm$ 0.75a-f	5.30 $\pm$ 0.49fg	0.15 $\pm$ 0.02	0.15 $\pm$ 0.04	271 $\pm$ 16.0a	301 $\pm$ 18.5a	6.37 $\pm$ 0.56ab	3.77 $\pm$ 0.43ab
RG27	11.4 $\pm$ 0.93abc	6.70 $\pm$ 0.51d-g	0.24 $\pm$ 0.04	0.19 $\pm$ 0.03	290 $\pm$ 19.8a	311 $\pm$ 17.6a	5.42 $\pm$ 0.71ab	3.61 $\pm$ 0.48ab
76	13.1 $\pm$ 0.50a	7.60 $\pm$ 0.28b-g	0.31 $\pm$ 0.09	0.21 $\pm$ 0.01	297 $\pm$ 20.7a	315 $\pm$ 0.62a	4.61 $\pm$ 0.29ab	3.69 $\pm$ 0.36ab
ZC	10.8 $\pm$ 0.92a-d	7.40 $\pm$ 0.74b-g	0.29 $\pm$ 0.03	0.27 $\pm$ 0.05	317 $\pm$ 6.61a	330 $\pm$ 10.9a	4.05 $\pm$ 0.50ab	2.83 $\pm$ 0.30b
DDT	9.70 $\pm$ 1.33a-f	5.70 $\pm$ 0.50e-g	0.31 $\pm$ 0.08	0.18 $\pm$ 0.05	262 $\pm$ 48.5a	306 $\pm$ 21.3a	6.19 $\pm$ 1.46ab	4.1 $\pm$ 0.93ab
RG29	9.80 $\pm$ 0.99a-f	7.60 $\pm$ 0.26b-g	0.35 $\pm$ 0.09	0.17 $\pm$ 0.02	310 $\pm$ 17.9a	315 $\pm$ 16.0a	3.97 $\pm$ 0.27ab	3.77 $\pm$ 0.69ab
WC	10.7 $\pm$ 1.07a-d	8.40 $\pm$ 0.32b-g	0.33 $\pm$ 0.09	0.17 $\pm$ 0.03	288 $\pm$ 20.9a	306 $\pm$ 10.1a	4.98 $\pm$ 0.17ab	5.15 $\pm$ 0.64ab
ANOVA								
O_3	<0.0001		0.087		0.026		<0.001	
Cultivars	0.113		0.308		0.609		0.194	
O_3 *Cultivars	0.080		0.977		0.867		0.456	

Table 5

Chlorophyll *a* fluorescence parameters of peach seedlings growing in charcoal-filtered air (CF) and elevated $[\text{O}_3]$ (E-O_3). Data are shown as Mean $\pm$ SE ($n = 3$); different letters indicate statistical differences (Turkey's test, $P < 0.05$).

Cultivars	F_v'/F_m'		Φ_{PS2}		qP	
	CF	E- O_3	CF	E- O_3	CF	E- O_3
GH2	0.53 $\pm$ 0.03a	0.42 $\pm$ 0.01a	0.13 $\pm$ 0.01a-d	0.11 $\pm$ 0.01a-d	0.24 $\pm$ 0.004a	0.259 $\pm$ 0.014a
JY	0.52 $\pm$ 0.02a	0.42 $\pm$ 0.04a	0.15 $\pm$ 0.01abc	0.08 $\pm$ 0.01d	0.29 $\pm$ 0.02a	0.191 $\pm$ 0.018a
LH9	0.47 $\pm$ 0.02a	0.43 $\pm$ 0.05a	0.15 $\pm$ 0.01a-d	0.09 $\pm$ 0.01bcd	0.32 $\pm$ 0.03a	0.21 $\pm$ 0.01a
LHC	0.50 $\pm$ 0.04a	0.47 $\pm$ 0.03a	0.14 $\pm$ 0.003a-d	0.08 $\pm$ 0.01bcd	0.30 $\pm$ 0.03a	0.18 $\pm$ 0.01a
W24	0.55 $\pm$ 0.04a	0.51 $\pm$ 0.02a	0.16 $\pm$ 0.01a	0.11 $\pm$ 0.01a-d	0.31 $\pm$ 0.04a	0.22 $\pm$ 0.02a
CJ	0.59 $\pm$ 0.03a	0.56 $\pm$ 0.04a	0.12 $\pm$ 0.004a-d	0.12 $\pm$ 0.002a-d	0.21 $\pm$ 0.02a	0.21 $\pm$ 0.02a
LJY	0.45 $\pm$ 0.04a	0.38 $\pm$ 0.03a	0.13 $\pm$ 0.006a-d	0.11 $\pm$ 0.01a-d	0.29 $\pm$ 0.03a	0.28 $\pm$ 0.02a
RG27	0.53 $\pm$ 0.03a	0.45 $\pm$ 0.03a	0.15 $\pm$ 0.004a-d	0.10 $\pm$ 0.01a-d	0.28 $\pm$ 0.02a	0.24 $\pm$ 0.03a
76	0.49 $\pm$ 0.04a	0.46 $\pm$ 0.01a	0.13 $\pm$ 0.01a-d	0.08 $\pm$ 0.002bcd	0.27 $\pm$ 0.03a	0.18 $\pm$ 0.01a
ZC	0.46 $\pm$ 0.06a	0.48 $\pm$ 0.02a	0.14 $\pm$ 0.01a-d	0.10 $\pm$ 0.002a-d	0.31 $\pm$ 0.03a	0.22 $\pm$ 0.01a
DDT	0.47 $\pm$ 0.06a	0.36 $\pm$ 0.02a	0.13 $\pm$ 0.02a-d	0.08 $\pm$ 0.01cd	0.27 $\pm$ 0.01a	0.23 $\pm$ 0.02a
RG29	0.55 $\pm$ 0.03a	0.49 $\pm$ 0.04a	0.15 $\pm$ 0.004ab	0.08 $\pm$ 0.004bcd	0.27 $\pm$ 0.01a	0.17 $\pm$ 0.02a
WC	0.54 $\pm$ 0.02a	0.52 $\pm$ 0.02a	0.16 $\pm$ 0.003a	0.13 $\pm$ 0.01a-d	0.29 $\pm$ 0.01a	0.24 $\pm$ 0.01a
ANOVA						
O_3	0.016		<0.0001		<0.0001	
Cultivars	0.257		0.105		0.601	
O_3 *Cultivars	0.995		0.283		0.606	

Table 6

Photosynthetic pigment contents of peach seedlings growing in charcoal-filtered air (CF) and elevated $[\text{O}_3]$ (E-O_3). Data are shown as Mean $\pm$ SE ($n = 3$); different letters indicate statistical differences (Turkey's test, $P < 0.05$).

Cultivars	Chlorophyll <i>a</i> content ($\times 10 \text{ mg m}^{-2}$)		Chlorophyll <i>b</i> content ($\times 10 \text{ mg m}^{-2}$)		Carotenoids content ($\times 10 \text{ mg m}^{-2}$)		Chlorophyll <i>a+b</i> content ($\times 10 \text{ mg m}^{-2}$)	
	CF	E- O_3	CF	E- O_3	CF	E- O_3	CF	E- O_3
GH2	22.5 $\pm$ 6.43ab	15.5 $\pm$ 1.56ab	7.78 $\pm$ 4.03	6.01 $\pm$ 0.76	8.6 $\pm$ 0.77bc	6.10 $\pm$ 0.50bc	30.3 $\pm$ 10.4ab	21.5 $\pm$ 2.32ab
JY	18.3 $\pm$ 2.49ab	6.30 $\pm$ 2.78b	8.84 $\pm$ 0.52	1.79 $\pm$ 1.36	8.97 $\pm$ 1.65bc	4.26 $\pm$ 0.33c	19.0 $\pm$ 10.5ab	8.13 $\pm$ 4.14b
LH9	18.7 $\pm$ 2.11ab	19.3 $\pm$ 3.64ab	8.29 $\pm$ 1.20	7.57 $\pm$ 1.26	6.95 $\pm$ 0.98bc	7.50 $\pm$ 1.47bc	27.0 $\pm$ 3.23ab	26.9 $\pm$ 4.90ab
LHC	20.0 $\pm$ 3.11ab	13.2 $\pm$ 6.97ab	7.41 $\pm$ 2.06	3.75 $\pm$ 3.33	8.01 $\pm$ 0.71bc	5.84 $\pm$ 1.58bc	27.4 $\pm$ 5.07ab	17.0 $\pm$ 10.2ab
W24	24.5 $\pm$ 3.33ab	17.3 $\pm$ 5.17ab	7.48 $\pm$ 4.08	6.56 $\pm$ 2.13	9.85 $\pm$ 0.65b	6.94 $\pm$ 1.46bc	31.9 $\pm$ 7.22ab	23.8 $\pm$ 7.30ab
CJ	27.8 $\pm$ 3.73a	17.8 $\pm$ 1.39ab	12.3 $\pm$ 1.20	7.77 $\pm$ 1.28	19.0 $\pm$ 1.18a	18.1 $\pm$ 1.19a	40.1 $\pm$ 4.92a	25.6 $\pm$ 2.65ab
LJY	16.6 $\pm$ 3.01ab	11.6 $\pm$ 2.25ab	4.83 $\pm$ 3.13	4.02 $\pm$ 1.08	7.05 $\pm$ 0.35bc	4.03 $\pm$ 0.50c	21.4 $\pm$ 6.12ab	15.6 $\pm$ 3.30ab
RG27	25.9 $\pm$ 2.66a	15.8 $\pm$ 1.80ab	8.43 $\pm$ 3.32	5.66 $\pm$ 0.93	9.83 $\pm$ 0.69b	6.39 $\pm$ 1.01bc	34.4 $\pm$ 5.73ab	21.5 $\pm$ 2.72ab
76	21.4 $\pm$ 0.53ab	18.7 $\pm$ 1.25ab	6.34 $\pm$ 2.67	7.24 $\pm$ 0.65	8.52 $\pm$ 1.14bc	5.87 $\pm$ 0.25bc	27.8 $\pm$ 2.78ab	26.0 $\pm$ 1.90ab
ZC	15.8 $\pm$ 2.10ab	22.5 $\pm$ 4.95ab	5.07 $\pm$ 2.77	8.81 $\pm$ 2.26	7.39 $\pm$ 0.32bc	7.18 $\pm$ 1.41bc	20.8 $\pm$ 4.86ab	31.3 $\pm$ 7.20ab
DDT	23.6 $\pm$ 2.88ab	19.6 $\pm$ 4.02ab	9.84 $\pm$ 0.92	7.64 $\pm$ 1.95	8.16 $\pm$ 0.78bc	6.77 $\pm$ 0.62bc	33.4 $\pm$ 3.80ab	27.3 $\pm$ 5.97ab
RG29	21.7 $\pm$ 2.03ab	21.4 $\pm$ 0.73ab	8.95 $\pm$ 0.90	8.72 $\pm$ 0.12	8.10 $\pm$ 0.33bc	9.19 $\pm$ 0.55bc	30.7 $\pm$ 2.90ab	30.2 $\pm$ 0.78ab
WC	18.9 $\pm$ 2.28ab	17.9 $\pm$ 4.21ab	8.44 $\pm$ 0.54	6.76 $\pm$ 1.82	7.46 $\pm$ 0.24bc	6.45 $\pm$ 1.26bc	27.4 $\pm$ 2.82ab	24.7 $\pm$ 6.03ab
ANOVA								
O_3	0.001		0.053		<0.001		0.015	
Cultivars	0.113		0.368		<0.001		0.099	
O_3 *Cultivars	0.318		0.717		0.144		0.735	

suggesting that the reduction in A_{sat} by O_3 largely attributed to non-stomatal factors, i.e. impaired photosynthetic activity including reduced Rubisco activity, ribulose-1, 5-bisphosphate (RuBP) regeneration, decrease in chlorophyll *a* fluorescence or pigments (Akhtar et al., 2010; Feng et al., 2016).

Furthermore, the observed declines in F_v/F_m' , $PhiPSII$ and qP (Table 5) suggest that E- O_3 increased light energy dissipation of the antenna pigment and caused reduction in the efficiency of excitation energy captured by the open PSII reaction centers (Zhang et al., 2014). The relatively higher decrease in $PhiPSII$ in white peach cultivars like *JY*, *LH9* and *LHC* indicates that white peach seedlings have a weaker capacity for carbon metabolism and/or a low utilization of ATP and NADPH compared with the nectarine cultivars (Subrahmanyam and Rathore, 2000).

Although E- O_3 reduced the ASC content, with exceptions of *LH9*, *ZC* and *DDT*, the activities of CAT, APX and SOD were significantly increased by E- O_3 compared to CF. It may explain why the TAC content was increased by E- O_3 (Tables 7 and 8). Our results suggest that E- O_3 produced damage to the antioxidant system of the peach seedlings and increased the unbalance between antioxidants and antioxidative enzymes. The damaged activating detoxification and repair mechanisms induced by E- O_3 may finally have negative effects on peach productivity.

4.2. Possible causes for the different sensitivities to O_3 among cultivars

It has been reported that the O_3 sensitivity of plants is linked to O_3 flux controlled by stomata (Dizengremel et al., 2009; Paoletti, 2006) and antioxidant capacity determined by antioxidant pools and enzymes (Feng et al., 2010; Li et al., 2016; Wang et al., 2015). In this study, two O_3 response variables were used: AOT40_injury and the changes in A_{sat} . AOT40_injury has been frequently considered as an indicator of O_3 sensitivity (Calatayud et al., 2007; Li et al., 2016; Zhang et al., 2012). The O_3 -induced reduction in A_{sat} is a functional response variable related to plant primary production that has been commonly used to assess the O_3 sensitivity among species (Li et al., 2016). Moreover, we found that the AOT40_injury was almost closely significantly linked to negative effects of O_3 on photosynthesis ($P = 0.091$).

In the current experiment, we try to find the relationship between patterns of response and leaf properties associated with O_3 sensitivity among cultivars in one species. We found that the O_3

sensitivity among cultivars was strongly linked to LMA (Table 9). Bussotti (2008) found that O_3 sensitivity among different tree species was associated with LMA. This conclusion was further validated in recent studies including a larger number of woody species (Li et al., 2016; Zhang et al., 2012). Larger LMA may be attributed to a larger density of mesophyll tissues and/or leaf thickness (Wieser et al., 2002). In contrast, lower LMA contributes to higher O_3 sensitivity due to less apoplastic fraction in the leaves (Li et al., 2016; Niinemets, 1999). The tested cultivars consisted of nectarines and white peaches, and there were significant differences in the abaxial epidermis, palisade parenchyma and spongy parenchyma thicknesses among these 13 cultivars (Table 2). Thus, it can be inferred that LMA not only may be one key parameter to determine plant sensitivity to O_3 among different woody species, but also among different cultivars with different leaf traits for the same species.

Besides the structural leaf traits i.e. LMA, the biochemical parameters, such as activities of SOD, APX and A-SOD, A-APX also played a role in the O_3 sensitivity among peach cultivars, as indicated by the significant correlation with AOT40_injury, and the reduction in A_{sat} (Table 9). The activities of SOD and APX on a mass basis represent inherent physiological and biochemical features among cultivars. The different activities of SOD and APX among peach cultivars determined their different tolerance to O_3 . Feng et al. (2016) also found similar results among different winter wheat cultivars investigated in an O_3 -FACE. In addition, the activities of A-SOD and A-APX on a leaf-area basis were also significantly related to O_3 sensitivity among cultivars, suggesting that effective O_3 uptake contributes to the O_3 sensitivity among cultivars when considering both stomatal O_3 uptake and leaf detoxification (Wieser et al., 2002; Wrzaczek et al., 2013).

In general, stomatal O_3 uptake mainly depends on the O_3 concentration at the leaf surface and stomata conductance. Therefore, stomatal properties may also play an important role in determining O_3 sensitivity among species or cultivars. However, in our study, neither stomatal structure (stomatal area and density) nor g_s were significant correlation with AOT40_injury and with reduction in A_{sat} , although g_s was measured many times. It suggests that both stomatal structure and g_s does not contribute to the different sensitivity to O_3 among selected cultivars. Similar findings were reported in the tree species and wheat cultivars (Feng et al., 2016; Li et al., 2016; Zhang et al., 2012). Interestingly, there were significant negative correlations between g_s /LMA, i.e. the g_s for leaf ozone

Table 7
Activities of hydrogen peroxidase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) of peach seedlings growing in charcoal-filtered air (CF) and elevated [O_3] (E- O_3). Data are shown as Mean $\pm$ SE ($n = 3$); different letters indicate statistical differences (Turkey's test, $P < 0.05$).

Cultivars	CAT ($U\ g^{-1}\ FW$)		APX ($U\ g^{-1}\ FW$)		SOD ($U\ g^{-1}\ FW$)	
	CF	E- O_3	CF	E- O_3	CF	E- O_3
GH2	136 $\pm$ 14.9a	228 $\pm$ 111a	85.8 $\pm$ 34.1	201 $\pm$ 50.4	288 $\pm$ 73.9a	436 $\pm$ 97.7a
JY	68.5 $\pm$ 15.5a	77.4 $\pm$ 12.9a	180 $\pm$ 9.17	173 $\pm$ 99.8	318 $\pm$ 25.4a	326 $\pm$ 50.6a
LH9	49.5 $\pm$ 13.3a	94.9 $\pm$ 3.67a	255 $\pm$ 102	241 $\pm$ 19.4	270 $\pm$ 32.8a	370 $\pm$ 117a
LHC	27.1 $\pm$ 1.83a	97.8 $\pm$ 18.5a	86.6 $\pm$ 29.6	144 $\pm$ 60.8	324 $\pm$ 11.3a	413 $\pm$ 90.0a
W24	51.4 $\pm$ 9.84a	200 $\pm$ 90.0a	156 $\pm$ 21.9	228 $\pm$ 50.8	314 $\pm$ 55.4a	294 $\pm$ 70.6a
CJ	72.9 $\pm$ 21.1a	66.5 $\pm$ 34.0a	187 $\pm$ 19.9	88.2 $\pm$ 23.1	355 $\pm$ 40.6a	311 $\pm$ 23.0a
LJY	99.0 $\pm$ 52.2a	167 $\pm$ 18.0a	157 $\pm$ 80.2	215 $\pm$ 75.6	286 $\pm$ 54.0a	385 $\pm$ 75.0a
RG27	48.5 $\pm$ 20.7a	65.9 $\pm$ 21.6a	139 $\pm$ 25.9	207 $\pm$ 90.2	312 $\pm$ 39.5a	491 $\pm$ 112a
76	108 $\pm$ 27.5a	112 $\pm$ 23.0a	220 $\pm$ 49.1	169 $\pm$ 35.9	312 $\pm$ 14.0a	400 $\pm$ 81.4a
ZC	49.1 $\pm$ 19.8a	188 $\pm$ 19.6a	186 $\pm$ 42.9	280 $\pm$ 104	307 $\pm$ 25.0a	209 $\pm$ 62.8a
DDT	81.6 $\pm$ 38.0a	73.6 $\pm$ 14.1a	105 $\pm$ 35.1	154 $\pm$ 45.6	223 $\pm$ 12.9a	361 $\pm$ 69.6a
RG29	64.3 $\pm$ 39.2a	54.6 $\pm$ 11.0a	89.9 $\pm$ 22.5	110 $\pm$ 23.5	300 $\pm$ 44.5a	402 $\pm$ 76.2a
WC	148 $\pm$ 81.6a	175 $\pm$ 52.4a	223 $\pm$ 32.5	139 $\pm$ 40.0	414 $\pm$ 30.6a	276 $\pm$ 78.4a
ANOVA						
O_3	<0.001		0.340		0.044	
Cultivars	0.052		0.252		0.788	
O_3 *Cultivars	0.321		0.659		0.290	

Table 8

Reduced ascorbic acid (ASC), total antioxidant capacity (TAC) and malondialdehyde (MDA) contents of peach seedlings growing in charcoal-filtered air and elevated $[O_3]$ (E- O_3). Data are shown as Mean $\pm$ SE (n = 3); different letters indicate statistical differences (Turkey's test, $P < 0.05$).

Cultivars	ASC ($\mu\text{mol g}^{-1}$ FW)		TAC ($\mu\text{mol g}^{-1}$ FW)		MDA (nmol g^{-1} FW)	
	CF	E- O_3	CF	E- O_3	CF	E- O_3
GH2	22.5 $\pm$ 5.58a	7.53 $\pm$ 1.38a	189 $\pm$ 12.6a	284 $\pm$ 40.8a	30.0 $\pm$ 3.19b	57.6 $\pm$ 2.51ab
JY	20.5 $\pm$ 6.16a	9.21 $\pm$ 3.11a	228 $\pm$ 53.4a	254 $\pm$ 81.3a	32.4 $\pm$ 5.39ab	46.8 $\pm$ 15.2ab
LH9	11.3 $\pm$ 1.07a	16.9 $\pm$ 0.89a	168 $\pm$ 21.3a	289 $\pm$ 13.1a	42.3 $\pm$ 8.83ab	66.1 $\pm$ 9.31a
LHC	15.7 $\pm$ 3.81a	9.90 $\pm$ 2.03a	240 $\pm$ 94.8a	235 $\pm$ 4.28a	38.1 $\pm$ 7.41ab	43.5 $\pm$ 6.74ab
W24	13.7 $\pm$ 1.64a	11.7 $\pm$ 1.93a	230 $\pm$ 37.3a	242 $\pm$ 15.8a	43.6 $\pm$ 5.94ab	46.7 $\pm$ 11.1ab
CJ	14.8 $\pm$ 3.57a	8.74 $\pm$ 2.56a	146 $\pm$ 38.5a	367 $\pm$ 65.1a	36.9 $\pm$ 5.33ab	56.3 $\pm$ 8.62ab
LJY	20.0 $\pm$ 3.48a	12.8 $\pm$ 2.40a	264 $\pm$ 5.16a	333 $\pm$ 54.2a	40.2 $\pm$ 0.44ab	59.5 $\pm$ 2.37ab
RG27	18.5 $\pm$ 3.78a	12.6 $\pm$ 3.39a	222 $\pm$ 22.4a	196 $\pm$ 33.7a	43.8 $\pm$ 3.60ab	55.1 $\pm$ 5.83ab
76	13.8 $\pm$ 0.77a	10.7 $\pm$ 0.52a	173 $\pm$ 24.4a	242 $\pm$ 46.4a	40.7 $\pm$ 3.23ab	46.0 $\pm$ 7.79ab
ZC	12.8 $\pm$ 1.20a	16.2 $\pm$ 0.71a	167 $\pm$ 29.4a	170 $\pm$ 12.7a	42.7 $\pm$ 4.55ab	42.2 $\pm$ 6.20ab
DDT	12.8 $\pm$ 1.34a	13.9 $\pm$ 1.67a	190 $\pm$ 28.7a	342 $\pm$ 58.2a	29.5 $\pm$ 2.16b	42.1 $\pm$ 3.06ab
RG29	13.6 $\pm$ 2.92a	12.7 $\pm$ 0.38a	326 $\pm$ 91.6a	216 $\pm$ 44.2a	36.0 $\pm$ 6.36ab	44.2 $\pm$ 2.79ab
WC	16.7 $\pm$ 1.50a	14.3 $\pm$ 2.44a	257 $\pm$ 37.8a	288 $\pm$ 31.7a	39.8 $\pm$ 4.22ab	43.4 $\pm$ 2.54ab
ANOVA						
O_3	0.001		0.006		<0.001	
Cultivars	0.872		0.379		0.306	
O_3 *Cultivars	0.031		0.084		0.541	

Table 9

Pearson coefficient (r) and levels of significance (P value) in the correlations between parameters in CF plants at the end of the experiment and the AOT40 at the initial O_3 symptom onset (AOT40_injury), reduction in A_{sat} , respectively (n = 13). The symbols ** and * indicate significances at $P < 0.01$ and $P < 0.05$, respectively.

	AOT40_injury		Reduction in A_{sat}	
	r	P	r	P
SA	0.060	0.435	-0.237	0.218
SD	0.142	0.347	0.020	0.475
g_s	0.455	0.093	-0.252	0.406
LMA	0.728**	0.009	-0.644*	0.018
g_s /LMA	0.275	0.221	-0.637**	0.010
g_s /A-TAC	0.498	0.072	-0.371	0.106
TAC	-0.219	0.272	-0.446	0.127
ASC	0.459	0.091	0.105	0.367
A-TAC	0.109	0.382	-0.630	0.010
A-ASC	-0.129	0.338	-0.129	0.338
CAT	-0.100	0.784	-0.096	0.377
APX	-0.036	0.461	0.478*	0.049
SOD	0.152	0.338	-0.533*	0.030
A-CAT	0.027	0.470	-0.178	0.281
A-APX	0.549*	0.050	0.175	0.284
A-SOD	0.672*	0.017	-0.742**	0.002

SA: stomatal area, SD: stomatal density, g_s , stomatal conductance, LMA: leaf mass per area, TAC: total antioxidant capacity, ASC: reduced ascorbic acid, A-TAC: area-based TAC, A-ASC: area-based ASC, A-CAT/A-APX/A-SOD: area-based hydrogen peroxidase/ascorbate peroxidase/superoxide dismutase.

uptake expressed per unit leaf mass, and a reduction in A_{sat} ($P < 0.01$). The opposite result suggests that neither the leaf area-based O_3 flux index nor the mass-based ozone flux index accounts for the different O_3 sensitivity among peach cultivars.

5. Conclusions

In this study, the eco-physiological responses to elevated O_3 among 13 cultivars of peach seedlings were investigated in field open-top chambers. The cultivars showing visible foliar symptoms earlier also exhibited larger reductions in A_{sat} . Among the tested cultivars, three distinct groups with respect to O_3 sensitivity could be identified: sensitive cultivars (GH2, JY, LHC and LH9), intermediate cultivars (CJ, W24, LJY, RG27, 76 and ZC) and resistant cultivars (RG29, DDT and WC) based on symptom onset timing. The white peach cultivars were more sensitive to O_3 than nectarine cultivars. LMA and the activities of antioxidant enzymes (SOD, APX and A-

SOD, A-APX) rather than stomatal parameters (stomatal area, density and g_s) or the antioxidant (ASC and A-ASC) contributed significantly to the different O_3 sensitivity among the 13 cultivars.

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