Ozone effects on photosynthesis of ornamental species suitable for urban green spaces of China

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ABSTRACT

Urban green spaces (UGS) offer several ecosystemical benefits to the urban environment. However, these advantages may be weakened by alterations of plants photophysiological mechanisms due to increasing tropospheric ozone (O₃) concentrations, a serious problem for China. To evaluate their utilization in UGS, we selected three widely-used urban plant species (smoke tree, *Cotinus coggygria* Scop.; marigold, *Tagetes erecta* Linn.; rose, *Rosa chinensis* Jacq.) to investigate their biometric and photophysiological responses to (i) ambient air (AA), (ii) AA + 60 ppb O₃ (AA + 60), and (iii) AA + 120 ppb O₃ (AA + 120) (9 h d⁻¹, from 8:30 am to 5:30 pm). Considering visible injury and biomass production, smoke tree and marigold seem to be O₃-sensitive, whereas rose should be considered more tolerant. The exposure to the pollutant gas reduced photosynthetic efficiency in all seedlings. However, different features were shown throughout our study by the three species here monitored. In smoke tree, stomatal limitations seemed to be its principal weakness. In marigold, the reduction of the photosynthetic performance was mainly ascribable to impairments of both light and dark reactions of photosynthesis. Here, stomatal closure maybe not the cause to limit the photosynthetic rate, but a down-regulated response. Unexpectedly, CO₂ assimilation increased in roses exposed to AA + 60 and did not change in those treated with AA + 120 after one month from the beginning of the exposure (FBE). This seemed due to a better efficacy of these plants in dark reactions of photosynthesis. This feature was confirmed also a month later. In conclusion, the results of this study indicate that non-invasive methods such as gas exchange and chlorophyll fluorescence for monitoring photosynthetic performance of urban plants can be useful to give guidelines to manage UGS in the “climate change era”. Generally, in severe O₃-polluted areas as those of several cities of China, the plants with high-efficient biochemical processes driving a well photosynthetic performance (such as rose) might be a recommended choice.

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Abbreviations: A_{sat}, light-saturated photosynthesis; C_i, intercellular CO₂ concentration; %D, fraction of absorbed light thermally dissipated in PSII antennae; FBE, from the beginning of the exposure; F_v/F_m, actual photochemical efficiency of PSII in the saturated light; g_s, stomatal conductance; J_{max}, light-saturated rate of electron transport; Ls, stomatal limitation to photosynthesis; M9, the daily 9-h mean O₃ concentration; NO_x, nitrogen oxides; OTC, open-top chamber; %P, fraction of absorbed light utilized in PSII photochemistry; Φ_{PSII}, light-adapted apparent quantum efficiency of PSII; PPFD, photosynthetic photon flux density; PSII, photosystem II; qP, photochemical quenching; RH, relative humidity; RuBP, 1,5-diphosphate ribulose; TPU, triose phosphate utilization; UGS, urban green spaces; V_{max}, maximum rate of Rubisco-limited carboxylation; VOCs, volatile organic compounds; %X, fraction of light absorbed by *PSI* neither used in photochemistry nor dissipated in the PSII antenna.

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1. Introduction

Urban green spaces (UGS) offer several ecosystemical benefits to the urban environment, not only in relation to their aesthetic and social values but also for their effects on air quality (Pellegrini, 2014). Plants can beautify cities, improve eco-environment, promote living in harmony between human and nature, and contribute to public health, aesthetic enjoyment and physical and psychological well-being (Jo, 2002; Chen and Jim, 2008), suggesting their irreplaceable roles during urbanization. However, these advantages provided by UGS may be weakened by alterations of plants photophysiological mechanisms due to increasing tropospheric ozone (O₃) concentrations. Currently, O₃ has been proved to be one of the most toxic gaseous substances that significantly impacts plant life (Cotrozzi et al., 2016; Yi et al., 2016).

The fast economic development of China is producing serious air pollution problems. Large quantities of nitrogen oxides (NO_x) and volatile organic compounds (VOCs) are emitted from massive fossil fuel combustion of industrial activities and vehicular traffic. This phenomenon contributes to increasing ambient O_3 concentrations, especially in the regions with dense population, such as Beijing, Shanghai, and Guangzhou areas (Li et al., 2013; Zheng et al., 2014). Taking Beijing as an example, the daily mean (24-h) and hourly peak O_3 concentrations at urban and exurban regions were 46 and 67 ppb, and 181 and 209 ppb, respectively, during May–September 2010 (Feng et al., 2014) (for O_3 , 1 ppb = $1.96 \mu\text{g m}^{-3}$, at 25°C and 101.325 kPa). This confirms the well-known phenomenon of long range transport of O_3 precursors from urban to exurban areas, often leading to higher levels of the pollutant in regions far from cities or industrial zones (Lorenzini et al., 1995). Evenmore, the daily mean (24-h) O_3 concentration in May–September 2014 reached 71.3 ppb in exurban regions (Yuan et al., 2015). Thus, O_3 pollution has not been effectively controlled in recent years, and UGS are still living under high O_3 concentrations, expected to induce serious damages to plants.

Analysis and monitoring of plant responses to urban conditions, defined as “urban plant physiology” (Calfapietra et al., 2015) or “urban plant pathology” (Lorenzini and Nali, 2015), has been identified as a critical component of research programs. It represents an important opportunity to gain immediately an insight in relation to the physiological responses and the mechanisms (type and extent) of plant acclimation/tolerance. Current knowledge of plant photophysiology in the urban environment is mainly based on two non-destructive methodological approaches: (i) gas exchange analysis, and (ii) chlorophyll *a* fluorescence measurement.

Photosynthesis is the foundation of material cycle and energy conversion of plant, and is the primary carbon source for biomass formation. It is influenced by multiple step processes involving (i) carbon dioxide (CO_2) diffusion from atmosphere to leaf through stomata, (ii) light energy utilization and conversion (light reactions), and (iii) ribulose-1,5 biphosphate carboxylase/oxygenase (RuBisCo) carboxylation (dark reactions). Inhibition of any of these steps may affect the photosynthetic performance. Several experimental studies showed that O_3 stress induced decline in photosynthesis in parallel with decrease in stomatal conductance, while the intercellular CO_2 concentration remained constant or even increased, suggesting that stomatal closure may be only a downward-regulation response to decreased photosynthesis rather than the cause (Watanabe et al., 2014). Furthermore, several authors documented that the direct effect of O_3 on light and dark phases of photosynthesis was the main cause of its decline (e.g. Power and Ashmore, 2002). The detrimental effects of O_3 on potential photosystem II (PSII) photochemical efficiency (F_v/F_m), actual photochemical efficiency of PSII in the saturated light (F_v'/F_m'), light-adapted apparent quantum efficiency of PSII (Φ_{PSII}) and the percentage of open photosynthetic reaction centers (qP) have been widely reported (e.g. Feng et al., 2011b; Pellegrini, 2014; Zhang et al., 2014), suggesting that photochemistry was depressed and the production of NADPH and ATP for CO_2 reduction may be decreased. A reduction of maximum rate of RuBisCo-limited carboxylation (V_{cmax}) has also been considered a main factor being responsible for impairment of photosynthesis (Matyssek et al., 1991; Zheng et al., 2002; Morgan et al., 2004; Fiscus et al., 2005; Pellegrini et al., 2011). These are interesting findings to select and breed O_3 -tolerant species, but so far the photosynthetic mechanisms, including stomatal change, and light and dark reaction responses have not been explored enough.

Biomass production is to some extent the comprehensive reflection of photosynthetic capacity. Studies over the past several decades indicated that chronic O_3 exposure significantly decreased the production of forests (Proietti et al., 2016), crops (Avnery

et al., 2011; Ghude et al., 2014; Chuwah et al., 2015) and grasslands (Gilliland et al., 2016). However, there are some exceptions where O_3 exposure promoted biomass accumulation under particular environmental conditions (e.g. Prozhnerina et al., 2003). That photophysiological mechanisms lead to reverse biomass production responses to O_3 stress among different plant species has rarely been systematically explored.

Smoke tree (*Cotinus coggygria* Scop.), marigold (*Tagetes erecta* Linn.) and rose (*Rosa chinensis* Jacq.) have been widely used in urban landscaping (e.g. smoke tree contributed to 71% of Red Leaves, the most spectacular natural scenery in Fragrant Hills Park, an imperial garden in the northwestern part of Beijing), introduced in residential areas, parks, squares or on sides of roads. They are representative greening and ornamental plant species in cities. Before experiment, we consulted previous literatures to understand how these three species adapt to rising O_3 concentration through regulating photosynthetic mechanism. However, to the best of our knowledge, no study has been performed on this crucial topic. Only few reports on the photosynthetic efficiency responses of these species to other abiotic stresses were achieved (drought and high temperatures; van Iersel and Seymour, 2002; Li et al., 2011; Riaz et al., 2013) and, by the way, only the final photosynthetic rate was exhibited. Thus, we conducted this experiment to investigate the O_3 sensitivity (based on biomass production) of these three plant species, and to explore their crucial photophysiological mechanisms resulting in different biomass responses to elevated O_3 by analyzing stomatal factor along with photochemical and biochemical processes of photosynthesis in order to evaluate their utilization in UGS.

2. Materials and methods

2.1. Plant material and ozone exposure

Experiments were conducted at Zhangtou village ($40^\circ 12' \text{N}$, $116^\circ 8' \text{E}$), Changping District, Beijing, China, where in 2013 and 2014 (the experimental period) the average annual temperature and precipitation were 12.1°C and 542 mm (concentrated from June to August), respectively (Chen et al., 2016).

Seedlings were purchased from a local farmer (smoke trees were one-year old, whereas rose and marigold were just emerged in the current year when relative experiments were conducted), and individually planted into plastic pots (17 cm in height and 22 cm in diameter) filled with native soil. About 10 days later, when plants were adapted to pots conditions, 30 seedlings of smoke tree and 60 of rose and marigold, each, with similar height (102, 24 and 7 cm for smoke tree, rose and marigold, respectively) and stem diameter (12.5, 8 and 4 mm, respectively) were selected and equally distributed to three octagonal open top chambers (OTCs; 2.8 m in height and 4.0 m in diameter), made of aluminum alloy frame covered with stalinite; (Zheng et al., 2011). Three treatments were applied (one for each OTC): (i) ambient air (AA), (ii) AA with the addition of 60 ppb O_3 (AA+60), and (iii) AA with the addition of 120 ppb O_3 (AA+120) (for O_3 , 1 ppb = $1.96 \mu\text{g m}^{-3}$, at 25°C and 101.325 kPa). O_3 was generated from pure oxygen by an O_3 generator (HY003, Chuangcheng Co., Jinan, China) using a high voltage discharge method (Zheng et al., 2013), and then mixed with AA to achieve the target concentration. Depending on the growth season of each species, smoke tree, rose and marigold seedlings were exposed to O_3 (9 h d^{-1} , from 8:30 am to 5:30 pm) from 23 June to 19 October 2013 (118 days), 23 June to 29 October 2013 (128 days), and 5 August to 28 September 2014 (54 days), respectively (with exception of rainy and windy days). During exposure period, the average temperature, relative humidity (RH) and precipitation were 24.3°C , 77.3% and 365.2 mm for smoke tree and rose, respectively, and

24.2 °C, 77.0% and 36.1 mm for marigold. To attenuate positional effects, pots were re-positioned within the OTCs every 7 days, and re-distributed among OTCs every 10–15 days (as described by Feng et al., 2011a). O₃ concentration into each OTC was continually measured and automatically stored by an O₃ analyzer (Model 49i, Thermo Scientific, Franklin, MA, USA). The percentage of actual to target increment was from –3.9% to +17.4% during O₃ exposure, which basically achieved the target concentration. Temperature and RH within two OTCs during O₃ exposure were measured every 5 min using a weather station (Campbell Scientific, North Logan, Utah, USA; Fig. S1 in appendix): the average temperatures for smoke tree, rose and marigold within the two chambers were 24.2 and 24.3 °C, 22.9 and 23.0 °C, and 23.9 and 24.2 °C; the averages for RH were 77.0 and 77.3%, 76.0 and 76.2%, 78.1 and 77.0%. This data confirms that there were similar micro-meteorological conditions among OTCs. The plants were irrigated enough with running water after 5:30 pm every day (after O₃ fumigation); and visible foliar injury was checked on each plant, in order to detect its time of onset.

2.2. Ecophysiological analyses

For each species, five plants were randomly collected from each OTC, and one fully expanded leaf for each plant was selected and marked for ecophysiological analyses, which were taken on two measuring times: on 23 July (30 days from the beginning of exposure, FBE) and 23 August (60 days FBE) 2013 for both smoke tree and rose, and on 22 August (17 days FBE) and 6 September 2014 (32 days FBE) for marigold. For smoke tree and rose, the AOT40 (Accumulated O₃ exposures Over a Threshold of 40 ppb, 08.00 a.m.–08.00 p.m.; sensu de Leew and van Zantvoort, 1997) at the first and second measuring times were 4.0 and 6.6, 15.0 and 24.7 and 24.1 and 41.4 ppm h in AA, AA + 60 and AA + 120 OTCs, respectively. For marigold, they were 5.8 and 9.7, 15.4 and 26.5, 24.6 and 43.0 ppm h.

Gas exchange and chlorophyll *a* fluorescence measurements were conducted using a LI-6400 photosynthesis system with a leaf chamber fluorometer (LI-COR, Lincoln, NE, USA). The automatic program of the LI-6400 photosynthesis system was used to generate the response of CO₂ assimilation rate (*A*) to photosynthetic photon flux density (PPFD), according to Gomes et al. (2006) with some minor modifications: measurements were taken following a gradient of PPFD (1500, 1200, 900, 600, 250, 150, 75, 0 μmol photons m^{–2} s^{–1}) under a constant CO₂ concentration of 400 ppm and a block temperature of 25 °C. *A*/PPFD curves were fitted by the “Photosynthesis Work-Bench” software and were used to obtain light-saturated photosynthesis (*A*_{sat}), and corresponding stomatal conductance (*g*_s) and intercellular CO₂ concentration (*C*_i) at the PPFD of 1200 μmol m^{–2} s^{–1}.

The automatic program in the LI-6400 photosynthesis system was also used to generate *A*/*C*_i curves, according to Feng et al. (2011b) with some minor modifications: measurements were taken by changing the CO₂ concentration (400, 300, 20, 10, 50, 400, 575, 800, 1000, 1200, 1500 ppm) under a constant PPFD of 1200 μmol m^{–2} s^{–1} and a block temperature of 25 °C. Then, *V*_{max}, light-saturated rate of electron transport (*J*_{max}) and triose phosphate utilization (TPU) were calculated from the *A*/*C*_i curves generated from the “Photosynthesis Work-Bench” software. Actual photochemical efficiency of PSII in the saturated light (*F*_v'/*F*_m', *qP* and Φ_{PSII}) were extracted from the measurements at 400 ppm of CO₂. The distribution of light energy absorbed by PSII was calculated according to Demmig-Adams et al. (1996): fraction of light absorbed by PSII antenna that is thermally-dissipated (%D) = 1 – (*F*_v'/*F*_m') × 100, fraction of light absorbed by PSII antenna that is used in photochemistry (%P) = (*F*_v'/*F*_m') × *qP* × 100, and

fraction of light absorbed by PSII antenna not used in photochemistry nor dissipated in the antenna (%X) = (*F*_v'/*F*_m') × (1 – *qP*) × 100.

2.3. Biomass analysis

At the end of the experiment, five plants for each species from each OTC were randomly harvested, and their dry weights were determined after oven-drying at 75 °C until constant weight.

2.4. Statistics

Measurements were carried out on five (*n* = 5) replicates for each treatment and species. We note that the lack of treatments replication may raise concerns about pseudo-replication (Hurlbert, 1984). However, we believe the benefit of using more treatments outweighs this limitation, as published by Hewitt et al. (2014) and others. The normality of data was preliminarily tested by the Shapiro-Wilk *W* test. Ecophysiological data were analyzed using one-way repeated measures analysis of variance (ANOVA), whereas biomass ones were analyzed by one-way ANOVA. Comparisons among means were determined by Fisher LSD post-test. Statistical analyses were performed using SPSS 16.0 computer package.

3. Results

3.1. Visible injury and biomass production

In smoke tree, O₃ visible injury was shown only under AA + 120 treatment, at the AOT40 of 13.5 ppm h, in form of brown stains in interveinal areas of the upper surface of older leaves. Then, they spread into patches, finally leading to withered leaves (Fig. 1A). Differently, in marigold plants it emerged in both AA + 60 and AA + 120 samples, at the AOT40 of 8.6 and 6.4 ppm h, respectively, in form of leaf yellowing chlorosis covering a large portion of the interveinal areas of leaves (Fig. 1B). No symptoms were detected in rose.

Concerning total biomass production, seedlings of smoke tree were affected only by AA + 120 (–32%), whereas marigold ones were altered even by AA + 60 (–29%) and even more by AA + 120 (–40%). Conversely, AA + 60 treatment induced biomass production of rose (+16%), while AA + 120 did not act on this feature (Table 1). Taking into account each organ, root biomass production of smoke tree was reduced by 33 and 53% under AA + 60 and AA + 120 treatments, respectively, while leaf and stem biomass productions were only affected by AA + 120 treatment (–54 and –33%, respectively). All parts of marigold were suppressed, with reduction of 13, 25, 35 and 50% in leaf, stem, flower and root under AA + 60 treatment, respectively, and 19, 40, 45 and 67% under AA + 120 one. No organ of rose showed changes under elevated O₃, although the total biomass increased in plants exposed to AA + 60 treatment.

3.2. Dynamics of gas exchanges

According to the one-way repeated measures ANOVA, the interaction between O₃ and time was significant for all the gas exchange parameters, with the exception of *V*_{max} in smoke tree plants (Figs. 2 and 3). Here, *A*_{sat} decreased already after one month FBE due to AA + 60 exposure (–22%, in comparison to AA) and even more to AA + 120 one (–56%). After two months FBE, AA plants showed lower values than on the previous measurement (–23%), while decreases due to O₃-addition were similar between AA + 60 and AA + 120 treatments (–17 and –22%, respectively) (Fig. 2A). Differently, only after 30 days FBE *g*_s was lower in AA + 60 plants (–29%) and even more in AA + 120 ones (–52%) than in those exposed to AA, although AA plants showed a decrease of *g*_s between the measuring times (–34%) (Fig. 2B). Increased O₃ induced changes of *C*_i only after 60 days FBE, when its addition to AA led to a similar increase

Fig. 1. O₃ symptoms on leaves of *Cotinus coggygia* (A) and *Tagetes erecta* (B).

of this parameter regardless of the concentration (+15 and +18% in AA + 60 and AA + 120 plants in comparison to AA ones, respectively) (Fig. 2C). Differently, A_{sat} of marigold plants was depressed by AA + 60 exposure and even more by AA + 120 one after 17 and 32 days FBE (–34 and –67%, firstly; –16 and –42%, secondly in comparison to AA, respectively) and similar responses were shown in terms of g_s (–27 and –50%; –32 and –67%). However, AA plants showed a decrease of A_{sat} (–23%) and an increase of g_s (+30%) throughout the exposure (Fig. 2D, E). Only on the first measuring time, C_i was affected by O₃-addition increasing similarly in AA + 60 and AA + 120 plants (+12 and +15%, respectively), although AA induced a raise of this parameter between 17 and 32 days FBE (+9%) (Fig. 2F). In roses, A_{sat} showed different responses between times of analysis: firstly, it increased only in AA + 60 plants (+16%); secondly, it decreased similarly in both AA + 60 and AA + 120 ones (–30 and –43%, respectively). Also in this species, A_{sat} decreased in AA plants throughout the exposure (–33%) (Fig. 2G). After 30 days FBE, only AA + 120 treatment caused a drop of g_s (–35%, in comparison to AA),

whereas the following analysis showed a similar decrease of this parameter among plants exposed to O₃-addition (–52 and –58% in AA + 60 and AA + 120, respectively), and also AA plants showed lower values then the previous measuring time (–19%) (Fig. 2H). Finally, C_i was only affected by increased O₃ concentrations after two months FBE decreasing under AA + 60 exposure (–16%, in comparison to AA) and increasing under AA + 120 one (+9%) (Fig. 2I).

In comparison to AA, O₃-addition induced a drop of V_{cmax} of smoke tree only at the second time of analyses, although it is not possible to evaluate the differences among samples since the interaction O₃ × time was not significant, as previously reported (Fig. 3A). Similarly, J_{max} and TPU were affected by O₃-addition only after 60 days FBE (Fig. 3B and C): the former parameter decreased due to AA + 60 (–26%) and even more to AA + 120 (–51%), while the second one dropped similarly among treatments (–31% and –34% in AA + 60 and AA + 120 plants, respectively). In marigold plants, V_{cmax} , J_{max} , and TPU showed the same responses at both measuring times (Fig. 3D–F): firstly, they decreased due to AA + 60 (–26%,

Table 1
Biomass production of *Cotinus coggygia*, *Tagetes erecta* and *Rosa chinensis* plants exposed to ambient air (AA), AA with the addition of 60 ppb O₃ (AA + 60) and AA with the addition of 120 ppb O₃ (AA + 120) (9 h d^{–1}, from 8:30 a.m. to 5:30 p.m.; 118, 47 and 128 days, respectively). Data are shown as mean ± standard deviation (n = 5), –: no record. Following one-way ANOVA, for each species different letters indicate significant differences ($P \leq 0.05$). *** $P \leq 0.001$, * $P \leq 0.05$. Abbreviation: DW, dry weight.

Plant Species	Ozone Treatments	Biomass Production (g DW plant ^{–1})				
		Leaf	Stem	Flower	Root	Total
<i>Cotinus coggygia</i>	AA	56 ± 12 b	122 ± 9 b	–	88 ± 10 b	265 ± 8 c
	AA + 60	60 ± 11 b	109 ± 6 b	–	59 ± 10 a	227 ± 21 b
	AA + 120	26 ± 7 a	82 ± 13 a	–	41 ± 9 a	149 ± 20 a
	Ozone	*	**	–	**	***
<i>Tagetes erecta</i>	AA	16 ± 1 b	20 ± 3 c	20 ± 2 b	6 ± 1 b	63 ± 4 c
	AA + 60	14 ± 1 a	15 ± 2 b	13 ± 2 a	3 ± 1 a	45 ± 6 b
	AA + 120	13 ± 1 a	12 ± 1 a	11 ± 2 a	2 ± 1 a	38 ± 2 a
	Ozone	**	***	***	***	***
<i>Rosa chinensis</i>	AA	37 ± 6 a	36 ± 3 a	–	21 ± 1 a	94 ± 9 a
	AA + 60	40 ± 7 a	43 ± 4 a	–	26 ± 5 a	109 ± 7 b
	AA + 120	31 ± 2 a	37 ± 10 a	–	24 ± 3 a	92 ± 10 a
	Ozone	ns	ns	–	ns	*

Fig. 2. Trends of light-saturated photosynthesis (A_{sat}) (A, D, G), stomatal conductance (g_s) (B, E, H) and intercellular CO_2 concentration (C_i) (C, F, I) in *Cotinus coggygria*, *Tagetes erecta* and *Rosa chinensis* plants exposed to ambient air (AA, white), AA with the addition of 60 ppb O_3 (AA + 60, grey) and AA with the addition of 120 ppb O_3 (AA + 120, black) (9 h d^{-1} , 8:30 a.m.–5:30 p.m.) from 23 June to October 2013, from 12 August to 28 September 2014 and from 23 June to 29 October 2013, respectively. Bars represent standard deviation of the mean ($n=3$). For each parameter, results of one-way repeated measurements ANOVA are reported, asterisks showing the significance of factors (ozone and time) and their interaction for: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = $P > 0.05$. Different letters indicate significant differences ($P \leq 0.05$).

–21, and –32% in comparison to AA, respectively) and even more to AA + 120 treatment (–60, –59, and –72%), and later only due to AA + 120 one (–44, –54, and –47%). Furthermore, these parameters decreased between times of analysis in AA plants (–36, –31, –25%). Finally, V_{cmax} , J_{max} and TPU of rose plants showed an increase due to AA + 60 after one month FBE (+27, +11 and +25%, respectively), while only J_{max} was negatively affected by AA + 120 (–24%). Differently, these parameters showed three different responses after two months FBE: V_{cmax} decreased only in AA + 120 plants (–17%), J_{max} increased only in AA + 60 (+43%), while TPU was not affected by O_3 -addition. However, they decreased throughout exposure in AA plants (–30, –46 and –46%, respectively).

3.3. Dynamics of chlorophyll a fluorescence

According to the one-way repeated measures ANOVA, the interaction between O_3 and time was not significant for all the chlorophyll a fluorescence parameters in smoke tree plants. Here, only the factor O_3 was significant for F_v/F_m' , Φ_{PSII} and qP since the gas pollutant decreased these parameters, while Φ_{PSII} and qP were significant also in terms of time suggesting that they dropped comparing measuring times (Fig. 4A–C). Marigold plants showed decreases of F_v/F_m' due to O_3 -addition after 17 days FBE (–13% in AA + 60 plants and even more in AA + 120 ones, –31%), whereas only AA + 120 exposure decreased this parameter at the

Fig. 3. Trends of maximum rate of Rubisco-limited carboxylation (V_{cmax}) (A, D, G), light-saturated rate of electron transport (J_{max}) (B, E, H) and triose phosphate utilization (TPU) (C, F, I) in *Cotinus coggygia*, *Tagetes erecta* and *Rosa chinensis* plants exposed to ambient air (AA, white), AA with the addition of 60 ppb O_3 (AA + 60, grey) and AA with the addition of 120 ppb O_3 (AA + 120, black) (9 h d^{-1} , 8:30 am–5:30 pm) from 23 June to October 2013, from 12 August to 28 September 2014 and from 23 June to 29 October 2013, respectively. Bars represent standard deviation of the mean ($n=3$). For each parameter, results of one-way repeated measurements ANOVA are reported, asterisks showing the significance of factors (ozone and time) and their interaction for: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = $P > 0.05$. Different letters indicate significant differences ($P \leq 0.05$).

following measurement (–16%), although also AA plants showed a decrease between times of analysis (–6%) (Fig. 4D). Differently, Φ_{PSII} decreased due to AA + 60 and even more to AA + 120 at both times of measurement (–26 and –55%, –32 and –46% after 30 and 60 days FBE, respectively). Also Φ_{PSII} dropped in AA plants throughout the exposure (–18%) (Fig. 4E). The interaction $O_3 \times \text{time}$ was not significant for qP of marigold and F_v'/F_m' of rose, although O_3 decreased these parameters and their values were lower on the second time of measurement in comparison to the first one (Fig. 4F, G). In rose plants, Φ_{PSII} and qP decreased only due to AA + 120

treatment after one month FBE (–30 and –15%, respectively), whereas they were also affected by AA + 60 (–26 and –10%) and even more by AA + 120 (–60 and –48%) at the following analysis. Also for these parameters, AA induced a decrease throughout time (–12 and –10%, respectively) (Fig. 4H, I).

Also in terms of distribution of light energy, one-way repeated measures ANOVA showed none $O_3 \times \text{time}$ interaction in smoke tree. Here, O_3 induced a reduction of %P and an increase of %D, but only the latter parameter showed highest values at the second analysis in comparison to the first one (Fig. 5A, B). Differ-

Fig. 4. Trends of actual photochemical efficiency of PSII in the saturated light (F_v'/F_m') (A, D, G), light-adapted apparent quantum efficiency of PSII (Φ_{PSII}) (B, E, H) and photochemical quenching (qP) (C, F, I) in *Cotinus coggygria*, *Tagetes erecta* and *Rosa chinensis* plants exposed to ambient air (AA, white), AA with the addition of 60 ppb O_3 (AA+60, grey) and AA with the addition of 120 ppb O_3 (AA+120, black) (9 h d^{-1} , 8:30 a.m.–5:30 p.m.) from 23 June to October 2013, from 12 August to 28 September 2014 and from 23 June to 29 October 2013, respectively. Bars represent standard deviation of the mean ($n=3$). For each parameter, results of one-way repeated measurements ANOVA are reported, asterisks showing the significance of factors (ozone and time) and their interaction for: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns= $P > 0.05$. Different letters indicate significant differences ($P \leq 0.05$).

ently, %X was only affected by O_3 , but a clear response was not shown throughout the exposure (Fig. 5C). In marigold plants, %P decreased after 17 days FBE due to AA+60 treatment (–26%) and even more to AA+120 one (–55%), whereas dropped similarly in both AA+60 and AA+120 plants at the following measurement (–37 and –46%, respectively). Furthermore, AA plants showed a decrease of this parameter between analyses (–18%). Oppositely, %D increased in AA+60 and even more in AA+120 plants (+17 and +29%) after 17 days FBE and raised only in AA+120 ones after 32 days FBE (+10%). Here, %X was affected only by AA+60 at the first

measuring time (+27%), whereas at the second one it increased in AA+60 and even more in AA+120 treatments (+19 and +44%, respectively). In rose plants, %P dropped only due to AA+120 after 30 days FBE (–33%), whereas due to AA+60 and even more to AA+120 after 60 days FBE (–23 and –59%, respectively). Also AA plants showed a decrease of this parameter between measuring times (–16%). Oppositely, %D increased only in AA+120 plants after 30 days FBE (+26%), whereas in AA+60 and even more in AA+120 plants after 60 days FBE (+11 and +23%, respectively). AA plants showed an increase of %D between times of analysis (+11).

Fig. 5. Trends of the fractions of absorbed light utilized in PSII photochemistry (%P) (A, D, G), absorbed light thermally dissipated in PSII antennae (%D) (B, E, H) and light absorbed by PSII neither used in photochemistry nor dissipated in the PSII antenna (%X) (C, F, I) in *Cotinus coggygria*, *Tagetes erecta* and *Rosa chinensis* plants exposed to ambient air (AA, white), AA with the addition of 60 ppb O₃ (AA+60, grey) and AA with the addition of 120 ppb O₃ (AA+120, black) (9 h d⁻¹, 8:30 a.m.–5:30 p.m.) from 23 June to October 2013, from 12 August to 28 September 2014 and from 23 June to 29 October 2013, respectively. Bars represent standard deviation of the mean (n=3). For each parameter, results of one-way repeated measurements ANOVA are reported, asterisks showing the significance of factors (ozone and time) and their interaction for: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = $P > 0.05$. Different letters indicate significant differences ($P \leq 0.05$).

Finally, O₃-addition increased %X only after 60 days FBE: +20% in AA+60 plants and even more in AA+120 ones (+55%).

4. Discussion

Results of the present study show some of the integrated photophysiological mechanisms that may confer O₃-sensitivity/tolerance to smoke tree, marigold and rose seedlings, evaluated to improve their management in UGS responding to climate change.

Plant sensitivity to O₃ is generally identified by the timing or extent of leaf injury (Bermejo et al., 2003; Li et al., 2015). On this basis, smoke tree and even more marigold seem to be O₃-sensitive, whereas rose should be considered tolerant. However, for some species (e.g. Soja et al., 1998), impairments of photosynthesis occurred prior to visible injury formation, which may lead to decrease in biomass production in the absence of visible injury (Pleijel et al., 1999). Therefore, biomass variation reflected by inhibited photosynthesis may be considered as an earlier indicator than visible injury to assess plant tolerance to the pollutant gas,

especially at low O_3 concentrations (Pleijel et al., 1999). Also following this approach, smoke tree and marigold showed an O_3 -sensitivity since their total biomass tended to decrease chasing the concentration gradient of O_3 . In rose plants, differently, biomass increased under AA+60 treatment and was not affected under AA+120 one compared to AA seedlings, showing again a greater O_3 -tolerance of this species. The O_3 -induced biomass increment has been previously reported. Prozhnerina et al. (2003), for instance, recorded a similar increase by 4–18% in young birch genotypes under 65 ppb of O_3 exposure for 8 weeks (10 h d^{-1} and 7 d week^{-1}), mainly attributed to a delay in bud burst. Similar results were also found in $1.5\text{--}1.7 \times$ ambient O_3 -treated birch genotypes with different root-shoot ratio responses (Yamaji et al., 2003).

Photosynthesis is the physiological foundation of biomass formation, which can be regulated by stomatal and/or non-stomatal factors (Zhang et al., 2014). In our study, O_3 exposure reduced photosynthetic efficiency in all seedlings, as largely reported in previous studies on several species (e.g. Flowers et al., 2007; Pellegrini et al., 2011; Zhang et al., 2011; Cotrozzi et al., 2016). However, different features were shown by the three species here monitored throughout the experiment (to be quite clear, comparisons among species is not the aim of the present study). In smoke tree plants, already at the first measuring time (one month FBE) O_3 -addition induced a reduction of CO_2 assimilation following the concentration gradient of the pollutant gas. Currently, there is debate regarding the principal mechanism that induces decrease in photosynthetic rate, with evidence of direct effects of O_3 on light and dark reactions of photosynthesis (Power and Ashmore, 2002) or through an indirect stomatal closure effect (Noormets et al., 2001). Here, both stomatal and mesophyll limitations were shown. However, the alterations at mesophyll level seemed to be diffusional rather than biochemical since V_{cmax} , J_{max} and TPU did not decrease in comparison to AA plants. This behavior has been observed by other authors in several species (e.g. Sun et al., 2014). The constraint in photoassimilation seemed to be not ascribable also to light reactions of photosynthesis considering that the PSII performance and the distribution of light energy were not largely affected by O_3 and did not follow the behaviors of gas exchange parameters in both times of analysis. The scenario changed at the second measuring time (two months FBE). At this point, the oxidative pressure presumably became stronger since also plants exposed only to AA showed a reduction of A_{sat} in comparison to the previous analysis, confirming an high O_3 -sensitivity of this species. Furthermore, at this time, no more differences were shown between AA+60 and AA+120 in terms of photosynthetic rate, suggesting that all plants exposed to increasing O_3 might have reached a lower bound level. Although there were no differences in g_s among treatments, we speculate that all plants greatly closed stomata since their values were at the same levels shown by AA+120 plants one month before. Thus, the stomatal limitation seems to be the Achilles' heel of this species as previously reported for other species (e.g. *Ginkgo biloba*; He et al., 2007). However, also the mesophyll impairments in photochemistry CO_2 fixation seemed to be more drastic at the second measuring time since the values of C_i were higher in plants exposed to O_3 -addition than those exposed only to AA. Indeed, after 60 days FBE, these plants began to show decreases in Rubisco carboxylation efficiency and regeneration capacity (especially in AA+120 ones), as well as in TPU. However, interestingly, smoke tree was the only species where in AA these parameters did not decrease throughout time, confirming that the stomatal limitation might be its principal weakness.

At the first measuring time also marigold showed a contraction of CO_2 assimilation twinned to both reduction of O_3 uptake and store of CO_2 in substomatal chamber, with A_{sat} and g_s decreasing following the gradient of O_3 concentration, and C_i reaching higher values than AA plants. However, differently to smoke tree, here the

responsibility seemed to be more ascribable to a mesophyll limitation since V_{cmax} , J_{max} and TPU decreased due to O_3 addition, again following the concentration gradient of the pollutant gas. Many Authors (Calatayud et al., 2007; Pina and Moraes, 2010; Pellegrini et al., 2011) similarly found that O_3 -induced differences in photosynthesis are the result of non-stomatal factors, potentially driven by either photosystem oxidation, (i) limiting the energy for RuBP regeneration from the lower pools of Calvin cycle intermediates, (ii) decreasing the efficiency of Rubisco due to direct enzyme oxidation or (iii) reducing CO_2 transport to the enzymes. Furthermore, previous studies reported that the limitation of photosynthesis is correlated with the reduction in the efficiency of energy conversion of PSII as shown by decreased ability of electron transport chain and reduced quinone pool (Thwe et al., 2014). This outcome leads to speculate that the limitation of photoassimilation might be also due to contractions of light reactions of photosynthesis, which could be explained as a chronic photoinhibition or a consequence of the decline in demand for reducing power and energy (NADPH and ATP, Pellegrini, 2014). Moreover, the effects of oxidative stress on photosynthetic process were well represented by data obtained from the analysis of energy distribution. Increasing O_3 limited the photosynthetic process in AA+60 and AA+120 plants (as indicated by %P), distributing the excess of energy into thermal dissipation (%D increased) and in alternative ways in AA+60 ones (%X increased), according to Calatayud et al. (2002). Other divergences with smoke tree emerged at the second measuring time (two months FBE) since A_{sat} , g_s and C_i showed similar behaviors than 15 days before. Although A_{sat} was negatively affected also by AA (in comparison to the previous measurement), confirming that also this species is O_3 -sensitive, differences between O_3 -addition treatments were still present in gas exchange parameters. These responses suggest that AA+60 plants were still able to partially defend themselves from the oxidative pressure. This outcome looked like to be twinned to a capacity of these plants to protect their dark reactions of photosynthesis since V_{cmax} , J_{max} and TPU decreased only under AA+120 treatment. Moreover, an ability of marigold exposed to AA+60 to enact some antioxidant mechanisms seemed to be confirmed by the behavior in PSII performance. Indeed, in these plants the exposure led only to a photoinhibition and not to a chronic photodamage. Certainly, it is known that plants have very efficient enzymatic and non-enzymatic antioxidant mechanisms which work in concert to control the cascades of uncontrolled oxidation and protect cells from oxidative damage by scavenging reactive oxygen species (Gill and Tuteja, 2010). We can speculate that these responses of AA+60 plants were presumably due to their ability to reduce the energy addressed to photosynthesis, dissipating it not as heat but in alternative ways. However, marigold could not carry out similar responses under the highest O_3 concentration. Thus, in this species the reduction of photosynthetic performance and consequently of biomass production seemed to be mainly ascribable to impairments of light and dark reactions of photosynthesis. In this case, stomata closure maybe not the cause to limit photosynthetic rate, but the down-regulated response to decreased carbon assimilation to balance the CO_2 concentration between ambient and the substomatal chamber (Watanabe et al., 2014).

A third different response to O_3 throughout the exposure was shown by rose plants. After one month FBE, unexpectedly, CO_2 assimilation rate increased in plants exposed to AA+60 and did not change in the AA+120 ones, confirming the behavior in biomass production. This phenomenon confirmed something already indicated by the other species: photosynthesis behavior of the first period was more closely related to the final biomass productions since initially leaves were in better conditions facilitating carbohydrate accumulation, while later their health went bad (i.e. due to lipid peroxidation or chlorophyll degradation); (Feng et al., 2011b). This phenomenon was partially supported by trends of g_s , C_i , V_{cmax} ,

$J_{\max}$ and TPU. In AA+60 plants, neither stomatal nor mesophyll limitations were shown. Particularly, the increment in A_{sat} of AA+60 plants seemed ascribable to a better efficacy of dark reactions of photosynthesis (V_{cmax} , $J_{\max}$ and TPU increased). Probably, it could be due to an antioxidant capacity of these plants, allowing them to cope with O_3 stress. Indeed, also PSII performance and the distribution of light energy were not affected by the exposure. Also in AA+120 seedlings, although the light constraints at stomatal and mesophyll levels, in Rubisco regeneration capacity and in light reactions of photosynthesis, A_{sat} , V_{cmax} and TPU were not affected by the high concentration of the pollutant gas. Still more than 25% of captured light energy was utilized in PSII photochemistry, which cannot limit the reducing power and energy for biochemical processes (Dubinsky, 1980; Niyogi, 1999). Thus, the higher O_3 -tolerance of rose was again certified, similarly to other several species (Prozherina et al., 2003; Yamaji et al., 2003). A different situation took place after 60 days FBE. At this point, there was a ruin of CO_2 assimilation already in plants exposed to only AA (in comparison to the previous analysis), and a further decrease happened due to O_3 -addition (similarly among treatments). These falls could be ascribable to stomatal limitations since g_s showed the same trend of A_{sat} , while the mesophyll were shown only in plants exposed to AA+120. Anyway, also at this time point, dark reactions of photosynthesis seemed to be the strong point of this species, considering the ability of AA+60 plants to preserve these exercises as demonstrated by unchanged values of V_{cmax} and TPU, as well as higher ability in Rubisco regeneration. Furthermore, in AA+120 plants the C_i increment seemed to be mainly due to a diffusional issue, although also a biochemical one could have occurred since a slight decrease in V_{cmax} was observed ($J_{\max}$ and TPU were not affected). However, reductions of A_{sat} might also be ascribable to impairments of efficiency of PSII photochemistry (reduced F_v'/F_m' and Φ_{PSII}) and a reduced capacity for reoxidizing Q_A during actinic illumination (reduced qP , Pellegrini et al., 2015). This outcome was also confirmed by decreased %P. Despite A_{sat} decreased similarly among plant exposed to O_3 -addition, the oxidative pressure of light reactions of photosynthesis was less in AA+60 seedlings, as confirmed also by the distribution of light energy. Thus, an ability of roses to cope with O_3 was shown also after two months of exposure.

5. Conclusions

Considering biomass production, smoke tree and marigold seem to be O_3 -sensitive, whereas rose should be considered more tolerant. In our study, the exposure to the pollutant gas reduced photosynthetic efficiency in all species. However, different features were shown throughout the experiment by the three species here monitored: regarding smoke tree, stomatal limitations of photosynthesis seemed to be its principal weakness; differently, in marigold, the reduction of photosynthetic performance and consequently biomass production, was mainly ascribable to impairments of both light and dark reactions of photosynthesis (here, stomatal closure maybe not the cause to limit the photosynthetic rate, but a down-regulated response); finally, rose showed a better efficacy in dark reactions of photosynthesis, probably due to an antioxidant capacity able to preserve the photosynthetic biochemical processes, which could be the reason of its less sensitivity to the pollutant.

In conclusion, the results of this study indicate that non-invasive methods as gas exchange and chlorophyll fluorescence for monitoring photosynthetic performance of urban plants can be useful to give guidelines to ameliorate the management in UGS in the “climate change era”. Generally, in severe O_3 -polluted areas as those of several cities of China, the plants with high-efficient biochemical processes driving a well photosynthetic performance (such as

rose) might be a recommended choice. Further studies involving more urban plants are needed since each species, as merged by this study, has its own photophysiological features to cope with O_3 stress.

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