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Ethylenediurea (EDU) pretreatment alleviated the adverse effects of elevated O_3 on *Populus alba* "*Berolinensis*" in an urban area

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ABSTRACT

Ethylenediurea (EDU) has been used as a chemical protectant against ozone (O_3). However, its protective effect and physiological mechanisms are still uncertain. The present study aimed to investigate the changes of foliar visible injury, physiological characteristics and emission rates of volatile organic compounds (VOCs) in one-year-old *Populus alba* "*Berolinensis*" saplings pretreated with EDU and exposed to elevated O_3 (EO, 120 μg/m^3). The results showed that foliar visible injury symptoms under EO were significantly alleviated in plants with EDU application ($p < 0.05$). Under EO, net photosynthetic rate, the maximum photochemical efficiency of PSII and the photochemical efficiency of PSII of plants pretreated with 300 and 600 mg/L EDU were similar to unexposed controls and significantly higher compared to EO-stressed plants without EDU pretreatment, respectively. Malondialdehyde content was highest in EO without EDU and decreased significantly by 14.9% and 21.3% with 300 and 600 mg/L EDU pretreatment, respectively. EDU pretreatment alone increased superoxide dismutase activity by 10-fold in unexposed plants with further increases of 88.4% and 37.5% in EO plants pretreated with 300 and 600 mg/L EDU pretreatment, respectively ($p < 0.05$). Abscisic acid content declined under EO relative to unexposed controls with the effect partially reversed by EDU pretreatments. Similarly, VOCs emission rate declined under EO relative to unexposed plants with a recovery of emission rate observed with 300 and 600 mg/L EDU pretreatment. These findings provided significant evidence that EDU exerted a beneficial effect and protection on the tested plants against O_3 stress.

Introduction

Tropospheric O_3 is regarded as one of the major air pollutants in Asia including China (Gong et al., 2018), formed from photochemical reactions of VOCs and NOx due to the increased consumption of fossil fuels (Paoletti et al., 2010). It is estimated that ground-level O_3 concentrations will continue to increase between 0.5% and 2.0% per year in the Northern Hemisphere during the next several decades, reaching average global surface concentrations of 40–60 μg/m^3 by 2060 (Vingarzan, 2004). In recent decades, many cities and regions of China have experienced high ozone episodes and increasing trends of

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the ground-level O_3 concentration including Shenyang city in this study (Xu et al., 2015; Liu et al., 2019). Generally, elevated O_3 may exert an adverse impact or stress on tree growth, inhibit photosynthesis, induce visible foliar injury and accelerate leaf senescence (Wittig et al., 2007; Fares et al., 2013; Marco et al., 2017). Fortunately, plants can mitigate the abiotic stress or even repair the damage of O_3 by promoting the antioxidant ability and adjusting secondary metabolites, including VOCs (Lu et al., 2009; He et al., 2009; Loreto and Velikova, 2001). In addition, the adverse impact of O_3 on plants can be alleviated by application of some exogenous chemical substances (Runeckles and Resh, 1975; Saitanis et al., 2015; Chen et al., 2018; Zhang et al., 2018). For many years, ethylenediurea (EDU) has been used as one of main chemical protectants against ozone stress (Paoletti, 2009; Manning et al., 2011; Tiwari, 2017, Agathokleous et al., 2018) and to reveal the different responses among the cultivars to ozone toxicity, as indicated for crops (Yuan et al., 2015; Feng et al., 2018; Singh et al., 2018) and trees including some poplars (Carriero et al., 2015; Agathokleous et al., 2016).

Populus alba "*Berolinensis*" is a hybrid poplar of *P.alba* × *P. berolinensis* hybridized by Heilongjiang Institute of Protection Forest of China in the 1980s (Wang et al., 2008). This hybrid poplar is a fast-growing species with high drought or cold tolerance (Huang et al., 2017), and widely planted and afforested in the cities of Northeast China (Xiao et al., 2016; Wang et al., 2017; Xu et al., 2019). It plays a very important role in the ecological protection of shelterbelt construction in China by microclimate regulation, air pollution reduction such as dust retention and absorption of harmful gases (Hu et al., 2014; Wang et al., 2018b; Wu et al., 2018, 2019). In past decades, many studies have been carried out on the effect of EDU on plants including poplar species in the field under ambient O_3 conditions, and the results of these studies are also often contradictory in terms of the protective effect of EDU application on the O_3-stressed plants (Hoshika et al., 2013; Tiwari, 2017; Feng et al., 2018). Actually, plant responses to EDU under O_3 exposure may be much stronger in O_3-sensitive genotypes than in O_3-tolerant ones (Manning et al., 2011). By now, little information is known about the effect of EDU on the hybrid O_3-sensitive poplar of Northeast China exposed to ambient and elevated O_3. Therefore, the objective in this study is to explore the physiological characteristics of a native hybrid poplar with EDU pretreatments by foliar application under elevated O_3 concentration simulated by open top chambers (OTCs) in an urban area. We here hypothesize that (1) EDU alleviates the foliar visible injury induced by elevated O_3 and exert a protective impact on photosynthesis in leaves *P. alba* "*Berolinensis*." (2) EDU mitigates the oxidative stress of elevated O_3 by altering the levels of key enzymes and metabolites that function in antioxidant metabolism.

1. Materials and methods

1.1. Experimental design and treatments

This experiment was conducted at Shenyang Arboretum, Chinese Academy Science, located in the populated central area of Shenyang city (41°46′N, 123°26′E) in the northeast of China. This urban area is in a region belonging to the temperate continental monsoon climate. Average annual precipitation is 755 mm and average annual temperature is 7.4°C (Xu et al., 2017). One-year-old cutting saplings of *P. alba* "*Berolinensis*" (60 cm in average height and 0.8 cm in average diameter) were obtained from a local nursery in Shenyang city, and were transplanted into 25 cm diameter and 20 cm depth pots filled with 2 kg of soil composed of sand, peat, and clay (2:3:1, *V*:*V*:*V*). Organic carbon and pH of the soil were 35.8 g/kg and 6.6, respectively. One sapling was planted in each pot. All the saplings were cultured for 60 days in a growth chamber with temperatures of 25/22°C day/night, 60%–80% relative humidity (RH), and a 12-hr photoperiod under 500 μmol/(m^2·sec) of photosynthetically active radiation (PAR). At the end of a 60-day period, the pots grown plants with or without EDU pretreatment were divided into the two identical sets, respectively. One set was exposed to elevated O_3 environment in OTCs and the second maintained as an ambient air control.

EDU pretreatment and O_3 fumigation were performed in six open top chambers (OTCs). Three of them were used for ambient air (AA, about 40 μg/m^3 O_3) and another three for elevated O_3 (EO, 120 ± 10 μg/m^3, Xu et al., 2015). The OTCs were 4 m in diameter, 3 m in height and equipped with a 45° sloping frustum. The O_3 concentration in the OTCs was monitored by an automatic controller (SDM-CD16AC, Beijing, China) connected to an O_3 analyzer (S-900, Aeroqual, New Zealand). All the data were stored using a data logger (CR800, Campbell Scientific Inc., Logan, UT, USA). Target O_3 concentration was generated from pure medical oxygen using the high-voltage discharge method (XH-2000, Xinghang Industry & Trade Co. Ltd., Shenyang, China).

The original source of EDU was from Prof. W.J. Manning, University of Massachusetts. Two EDU concentrations and one control treatment (distilled water) were applied by foliar pretreatment. EDU and distilled water pretreatments were initiated before O_3 fumigation, and applied twice as a foliar spray in the OTCs. All the leaves of each plant were sprayed by watering until they were visibly saturated. Six treatments were set: ambient air with distilled water, 300 and 600 mg/L EDU, elevated O_3 concentration with distilled water, 300 and 600 mg/L EDU. O_3 fumigation lasted 14 days from July 20 to August 3 in 2016.

The plants were fumigated with EO for 9 hr daily in the daytime (8:00–17:00). During the experiment, the recorded average day time concentration of O_3 in the EO-OTCs was 115.8 μg/m^3. Six healthy saplings were used for the measurements of gas exchange and physiological parameters. In addition, three healthy plants (three pots) of each treatment were used for the measurement of VOC emission rate.

1.2. Measurements of foliar visible injury and physiological parameters

The visible injury induced by O_3 fumigation was evaluated by the percentage of leaves with visible injury symptoms relative to total leaves per plant. A fully expanded, intact, healthy, and sun-exposed leaves at the same position (the third leaf from upper parts) were selected from one plant in

each pot to measure gas exchange parameters including net photosynthetic rate (Pn), stomatal conductance (*gs*), intercellular CO_2 concentration (Ci), and transpiration (Tr) by using a portable photosynthesis system (Li-COR 6400, Li-COR, Lincoln, Nebraska, USA). The leaves were exposed to actinic light (1000 μmol/m^2·sec) in leaf cuvette, and maintained for about 20 min until CO_2 assimilation reached a steady state. The temperature, CO_2 concentration and relative humidity were respectively set at 25°C, 400 μmol/mol and 60% in the leaf cuvette. Water use efficiency (WUE) was calculated by Pn divided by Tr (Fischer and Turner, 1978). Chlorophyll fluorescence was measured between 10:00 and 11:30 at ambient temperature in OTCs using a FMS-2 pulse modulated fluorometer (Hansatech, UK). The environmental conditions the tested leaves in plants were similar to gas exchange measurement. The maximum photochemical efficiency of PSII (Fv/Fm) was recorded in dark-adapted (30 min) samples with leaf clips. Steady-state fluorescence yield (Fs) was also recorded. Subsequently, a saturating actinic light pulse of 8000 μmol/m^2·sec for 0.7 sec was used to produce maximum fluorescence yield (Fm') by temporarily inhibiting PSII photochemistry. Photochemical efficiency of PSII (ΦPSII) was calculated (ΦSII = (Fm'–Fs)/Fm') (Maxwell and Johnson, 2000).

The fully expanded and healthy leaves (the third–fifth leaf from upper parts of plant) were harvested for determinations of malondialdehyde (MDA) content, superoxide dismutase (SOD) activity and abscisic acid (ABA) content. MDA content was measured according to the thiobarbituric acid (TBA) reaction method. Leaf tissues (0.2 g) were ground under liquid nitrogen and then homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7.8). After centrifugation at 4°C and 13,000×*g* for 15 min, the supernatant (1 mL) was vortexed with 4 mL of 20% TCA and 0.5% (W/V) TBA, and then heated for 30 min at 95°C. Absorbance was measured at 600, 532 and 450 nm (Heath and Packer, 1968). For superoxide dismutase (SOD; EC 1.15.1.1) activity, 0.5 g fresh leaf was homogenized with 10 mL cold phosphate buffer (100 mM, pH 7.8) containing 0.1 mM EDTA. SOD activity was determined in the supernatant by inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Fridovich, 1974). For measurement of ABA content, 1 g fresh leaves was ground with 5 mL 80% methanol on ice and then centrifuged at 4000 r/mim for 20 min at 4°C. The supernatant was transferred to a C18 column for pigment removal. ABA content in leaves was measured using ELISA kits (Zhuocai Biotechnology Co., Ltd., Shanghai, China).

1.3. Evaluation of VOC emission rate

After two weeks of gas fumigation, VOCs samples were collected by covering the whole above-ground saplings with steel rectangular cylinder (length, width, height was 30, 30 and 80 cm, respectively) surrounded by a transparent polyethylene bag. The VOCs samples were collected with the glass adsorbent tubes (11.5 cm long and 0.4 cm internal diameter) filled with Tenax-TA, Carboxen 1000 and Carbosieve SIII (Supelco Inc., PA, USA) connected to the cylinder by Teflon tube. Sample collection was conducted using a constant-flow type pump with a flow rate of 100 mL/min and the sampling time of 10 min. The air samples were kept at 4°C until analysis. VOCs were separated and detected by a gas chromatograph with flame ionization detection (FID). Analysis of VOCs was carried out using a thermal desorption sample injection system (ACEM 9300, USA) connected by a thermal transfer line to a gas chromatograph (14B, Shimadzu, Japan) with FID.

After sampling, all the leaves enclosed in the bags were removed from the whole plant, and placed in a drying oven at 60°C for 48 hr. The dry weights were used for normalization of VOCs emission rate to unit leaf mass. For the details of the experimental procedures and similar methods can be found in our previous studies (Li et al., 2009; Xu et al., 2012). The emission rate of VOCs was calculated using the following equation

$$ER = \frac{M \cdot V}{22.4 \cdot \Delta t \cdot W}(c_2 - c_1) \times 10^{-3}$$

where, *ER* is the emission rate of VOCs (μg/(g·hr)), mainly consisting of isoprene), c_1 is the isoprene concentration (μg/m^3) over the saplings before the sampling cylinder was closed, c_2 is the isoprene concentration inside the rectangular cylinder after the sampling cylinder closed, *V* is the effective volume of the rectangular cylinder, Δ*t* is time of closing cylinder for the sample collection, *W* is dry weight of all the leaves in the closed cylinder (g), and *M* is the molecular weight of isoprene.

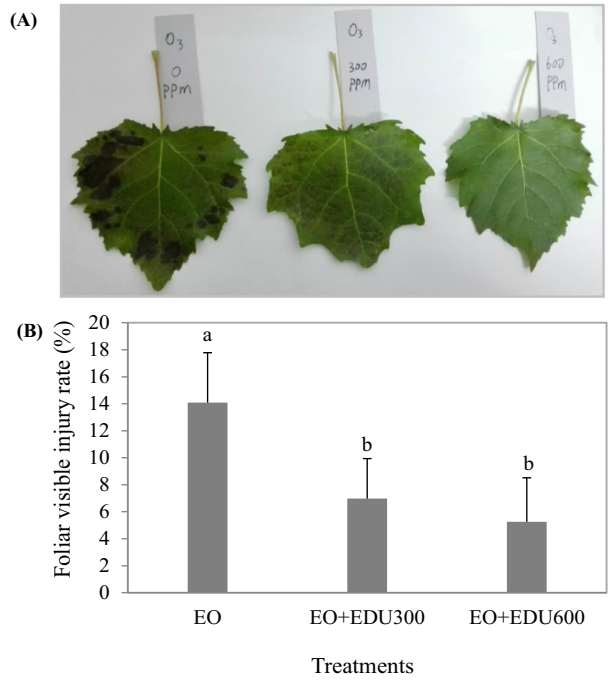

Fig. 1 – Foliar visible symptoms (A) and foliar visible injury rate (B) of *P. alba* "*Berolinensis*" exposed to elevated O_3 (EO). EO + EDU300 – EO with 300 mg/L EDU pretreatment, EO + EDU600 – EO with 600 mg/L EDU pretreatment.

Table 1 – Changes in photosynthetic parameters in leaves of *P. alba* "*Berolinensis*" under AA and EO with pretreatment of different EDU concentrations (0, 300 and 600 mg/L).

Photosynthetic parameters	AA			EO		
	0	300	600	0	300	600
Pn (μmol/(m^2·sec))	10.93a (1.67)	11.00a (2.65)	10.26a (0.67)	5.78b (1.30)	8.68a (2.77)	10.17a (0.75)
gs (mmol/ (m^2·sec))	0.32a (0.08)	0.32a (0.07)	0.35a (0.03)	0.22b (0.03)	0.27b (0.07)	0.24b (0.05)
Ci (μmol/mol)	292.7a (5.1)	292.5a (4.1)	293.5a (1.2)	300.5a (8.7)	284.5a (8.5)	274.2a (12.5)
Tr (μmol/(m^2·sec))	11.9a (1.5)	12.5a (1.7)	12.2a (0.8)	10.6a (1.1)	11.5a (1.8)	10.8a (0.9)
WUE	0.92a (0.07)	0.88a (0.09)	0.84a (0.01)	0.55c (0.09)	0.75b (0.15)	0.94a (0.05)
Fv/Fm	0.78a (0.01)	0.80a (0.04)	0.81a (0.07)	0.65b (0.04)	0.79a (0.02)	0.80a (0.01)
ΦPSII	0.55a (0.07)	0.58a (0.03)	0.60a (0.02)	0.45b (0.04)	0.60a (0.02)	0.62a (0.01)

Data are shown mean and standard deviation (SD) in the parenthesis. AA-ambient air, EO-elevated O_3. Different letters in the same row represented significant difference at 0.05 level among different treatments.

1.4. Statistical analysis

Chambers corresponding to the same treatment were considered statistical replicates. There were three replicate OTCs for AA and EO plot, respectively. One-way ANOVA was used to compare the difference of each parameter in this experiment between AA and EO with and without EDU pretreatment. The significant differences between treatments were evaluated by least significance differences (LSD) at the 95% confidence level by using SPSS statistical software (SPSS 18, Chicago, USA). Difference between the treatments was considered significant at $p < 0.05$. The values presented are the mean of measurements with three replicate plants in each set. Before ANOVA, the normal distribution and homogeneity of the variance of the experimental data (all the variables) were statistically analyzed. Pearson correlation between the physiological parameters was analyzed by two-tailed test.

2. Results

2.1. Foliar visible injury under EO with and without pretreatment of EDU

Significant visible injury symptoms with irregular black dots and patches were observed on the abaxial surface of *P. alba* "*Berolinensis*" leaves after two weeks under EO without EDU pretreatment (Fig. 1A). However, only slight injury symptoms were found under EO plus EDU pretreatments (Fig. 1A). No visible injury symptom emerged under AA. After two weeks of gas fumigation, the foliar visible injury rate was 14.1% under EO without EDU pretreatment (Fig. 1B). Compared to EO without EDU pretreatment, foliar visible injury rate decreased by 50.5% and 62.7% under EO with 300 and 600 mg/L EDU pretreatment, respectively (Fig. 1B).

2.2. Photosynthetic performance under EO with and without pretreatment of EDU

Compared to AA without EDU pretreatment, EO significantly decreased Pn by 47.1% under no-EDU pretreatment ($p < 0.05$) (Table 1). Under EO, Pn with pretreatment of 300 and 600 mg/L EDU was significantly higher by 50.1% and 76.0% than that without EDU pretreatment, respectively. EO significantly decreased *gs* regardless of EDU pretreatment, and no significant difference was observed among EDU pretreatments (Table 1). For Ci and *Tr*, no significant differences were found among treatments. EO significantly decreased WUE in the absence of EDU with partial recovery by treatment with 300 mg/L EDU pretreatment and full recovery of WUE with 600 mg/L pretreatment (Table 1). EO significantly decreased Fv/Fm and ΦPSII under no-EDU pretreatment by 16.7% and 18.1%, respectively (Table 1). Pretreatment of EDU (300 and 600 mg/L) under EO enhanced Fv/Fm and ΦPSII, compared to no-EDU pretreatment.

2.3. Stress physiological characteristics under EO with and without pretreatment of EDU

MDA content in leaves of *P. alba* "*Berolinensis*" showed a maximum increase (by 53.2%) under EO without EDU pretreatment, compared to AA (Fig. 2A). Under EO, MDA content decreased significantly by 14.9% and 21.3% with 300 and 600 mg/L EDU pretreatment, respectively. With increasing of EDU concentration, SOD activity showed an increasing trend regardless of O_3 fumigation (Fig. 2B). Compared to AA, SOD activity significantly increased by 10.4 times, 88.4% and 37.5% under EO with 0, 300 and 600 mg/L EDU pretreatment, respectively ($p < 0.05$). ABA content in leaves of plants exposed to EO with 0, 300 and 600 mg/L EDU pretreatment decreased significantly by 41.3%, 30.8%, and 33.6% (Fig. 2C), respectively ($p < 0.05$). Under EO, ABA content in leaves of plants significantly increased with 300 and 600 mg/L EDU pretreatment relative to the distilled water control ($p < 0.05$), and there was a trend toward EDU stimulation of ABA levels in ambient air as well (Fig. 2C).

2.4. Emission rate of VOC under EO with and without pretreatment of EDU

Under AA, EDU pretreatment did not have a significant effect on VOC emission rate, although there was a trend toward higher emission rates at higher EDU concentrations (Fig. 3). EO significantly decreased VOC emission rate by 64.8% in the absence of EDU pretreatment ($p < 0.05$). Under EO, VOC emission rate was higher by 1.9-fold and 95.5% with 300 and 600 mg/L EDU, respectively, relative to the distilled water pretreatment (Fig. 3). VOC emission rate with 300 mg/L EDU

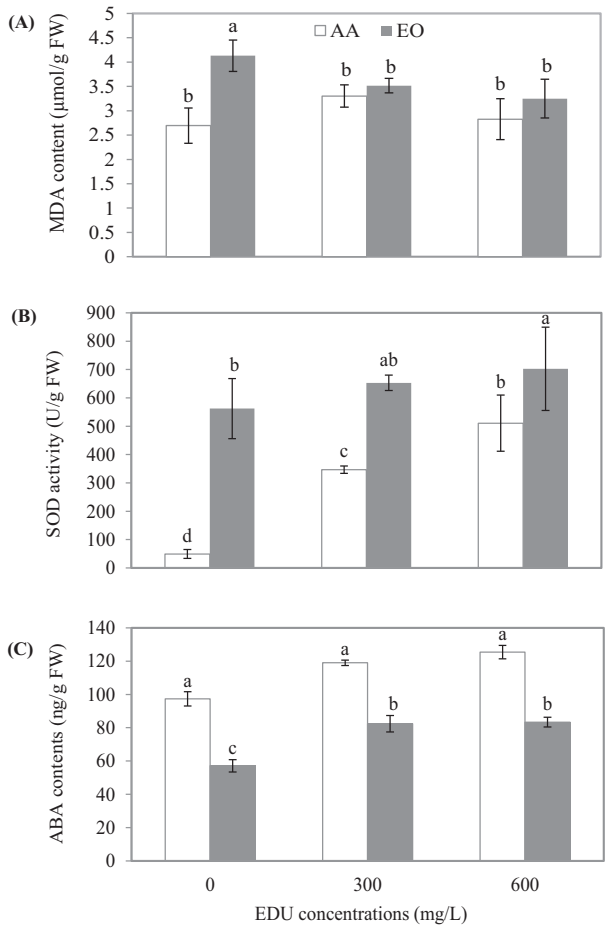

Fig. 2 – MDA content (A), SOD activity (B) and ABA content (C) in leaves of *P. alba* "*Berolinensis*" under ambient air (AA) and elevated O_3 (EO).

pretreatment showed a highest value among EDU pretreatments under EO, and was similar to rates observed under AA conditions.

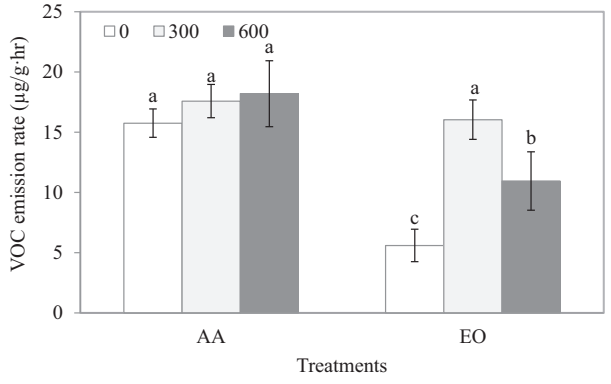

Fig. 3 – VOC emission rate of *P. alba* "*Berolinensis*" under ambient air (AA) and elevated O_3 (EO) with pretreatment of EDU (0 mg/L, distilled water), (300 mg/L) and (600 mg/L), respectively.

2.5. Associations between gas exchange, physiological parameters and VOCs emission

The correlation coefficients between all the physiological parameters were shown in Table 2. Pn showed an extremely significant positive correlation with *g*s, *T*r and WUE ($p < 0.01$), respectively. Similarly, *g*s showed an extremely significant positive correlation with *T*r (0.904), ABA content (0,579) and VOCs emission rate (0.627), respectively (Table 2). MDA content did not show significant correlation with most of physiological parameters except for Fv/Fm (−0.683). ABA content had significant positive correlation with Pn, *g*s, WUE, Fv/Fm, and ΦPSII, respectively (Table 2). VOCs emission rate showed a highly significant positive correlation with *g*s (0.627), Fv/Fm (0.614), ΦPSII (0.633), and ABA content (0.840).

3. Discussion

Over the past decades, O_3 effects on foliar visible injury have been studied on poplar trees across the world (Marzuoli et al., 2009; Feng et al., 2019). In present study, we found that elevated O_3 significantly induced foliar visible injury of a hybrid poplar planted widely in the cities of China, which is consistent with other studies (Strohm et al., 1999). However, the foliar visible injury induced by elevated O_3 (120 μg/m^3) was significantly reduced by pretreatment with increasing concentrations of EDU. Similar results in other plants have been reported by Szantoi et al. (2007, 2009). They observed that more severe foliar injury occurred on O_3-exposed plants (80 μg/m^3) without EDU treatment than those with EDU treatment. In this study, we found that foliar application of EDU (600 mg/L) was most effective in preventing visible O_3 injury of *P. alba* "*Berolinensis*," in agreement with other reports (Bortier et al., 2001; Szantoi et al., 2009).

Gas exchange parameters of photosynthetic apparatus are reconsidered as very important indices when assessing O_3 sensitivity. In this study, elevated O_3 significantly decreased Pn, possibly related to adverse effects on the electron transport rate through reduction in Fv/Fm ratio, suggesting effects on PSII and the ability to reduce the primary acceptor QA (Feng et al., 2011; Rai and Agrawal, 2014). EDU was found to be very efficient in mitigating the negative effects of O_3 on photosynthesis probably due to its high detoxification ability (Zhang et al., 2018). In this study, leaves of *P. alba* "*Berolinensis*" maintained higher levels of Pn under EO plus EDU pretreatment, than that of EO alone, suggesting that the adverse effect of O_3 on photosynthesis was alleviated by EDU treatment. This protection induced alleviating the negative effects on electron transport components Fv/Fm and ΦPSII (Table 1). The finding in this study was in agreement with some studies in other plants (Agrawal and Agrawal, 1999; Zhang et al., 2018). Generally, O_3 decreases Pn and *g*s, increases Ci and inhibits *T*r, which has been reported for an urban shrub in our previous experiment (Xu et al., 2017). Actually, Pn showed a significant positive correlation with *g*s, *T*r, WUE, Fv/Fm (Table 2), respectively. The synergetic changes in these parameters were observed in this study. However, we found that no significant effect was observed in *g*s, Ci, and *T*r among the EDU treatments regardless of O_3 fumigation. The result was

Table 2 – Pearson correlation coefficients between physiological parameters in leaves of *P. alba* "Berolinensis."

	Pn	gs	Ci	Tr	WUE	Fv/Fm	ΦPSII	MDA	SOD	ABA	VOC
Pn	1										
gs	0.779**	1									
Ci	−0.281	0.265	1								
Tr	0.768**	0.904**	0.134	1							
WUE	0.893**	0.483*	−0.507*	0.412	1						
Fv/Fm	0.524*	0.305	−0.368	0.179	0.664**	1					
ΦPSII	0.428	0.264	−0.429	0.246	0.495*	0.649**	1				
MDA	−0.148	−0.056	0.156	0.153	−0.346	−0.683**	−0.255	1			
SOD	−0.323	−0.329	−0.306	−0.242	−0.288	−0.151	0.212	0.319	1		
ABA	0.588*	0.597**	0.016	0.436	0.563*	0.680**	0.544*	−0.421	−0.287	1	
VOC	0.649*	0.627**	−0.135	0.533*	0.579*	0.614**	0.633**	−0.235	−0.256	0.840**	1

* Indicates significance of correlation at 0.05 level.
** Indicates significance of correlation at 0.01 level (two-tailed test).

consistent with many reports that EDU does not affect gas exchange parameters of plants (Hassan et al., 2007; Paoletti et al., 2008; Manning et al., 2011). In addition, plants showed a significant lower WUE under EO than ambient air control ($p < 0.05$), which indicated that O_3 fumigation could inhibit photosynthesis and exacerbate evaporation of water (Gao et al., 2016). In our study, no significant change of Fv/Fm was observed between the two EDU concentrations of pretreatments (300 and 600 mg/L) regardless of O_3 fumigation. A similar study was reported that Fv/Fm did not vary significantly in *Fraxinus excelsior* exposed to 450 mg/L EDU (Contran et al., 2009).

As a strong oxidant, elevated O_3 can cause membrane lipid peroxidation of tissue cells in plants (Xu et al., 2015; Singh et al., 2018). Malondialdehyde (MDA), the product of membrane lipid peroxidation, can accumulate in plants resulting from oxidative stress by elevated O_3 (Xu et al., 2015). The result in this study also confirmed that elevated O_3 significantly increased MDA content, indicating the occurrence of oxidative stress and increase of lipid peroxidation under O_3 fumigation. EDU is known to protect the membranes against lipid peroxidation through increased levels of ROS scavenging enzymes (Tiwari and Agrawal, 2009; Paoletti et al., 2009; Feng et al., 2010). In present study, MDA content under O_3 stress was significantly reduced with EDU pretreatments, which indicated that the reduction in lipid peroxidation in EDU treated plants confirmed the anti-oxidative property of EDU. However, no significant difference in MDA content in this study was found under ambient air among EDU pretreatments, which implied that the protective effect by EDU was specific for elevated O_3 stress and not effective in alleviating the lipid peroxidation associated with general cellular metabolism under ambient O_3 level (Singh et al., 2018).

In addition, the antioxidant enzyme SOD activity in this study increased significantly in EDU-treated plants relative to non EDU-treated controls, which suggested that EDU protection involves stimulation of detoxifying systems for eliminating superoxide radicals. The result in this study was in agreement with some other investigations (Singh et al., 2009, 2018). EDU-induced increase in SOD activity was previously reported in poplar trees, while other studies have reported a decrease in SOD activity in other plants treated with EDU (Lee and Bennett, 1982), which indicated the potential species difference in scavenging reactive oxygen species (ROS). Increased SOD activity is related with the detoxification of ROS resulting in reduced accumulation of ROS in EDU treated plants (Pandey et al., 2014; Singh et al., 2018).

ABA in plants has been identified as being important in abiotic stress signaling and adaptive physiological response (Danquah et al., 2014; Kundu and Gantait, 2017). Usually, the production and accumulation of ABA can be induced in plants exposed to high O_3 concentration, which may be helpful in enhancing the ability of stress tolerance (McAdam et al., 2017), as shown in our previous study in leaves of *Ginkgo biloba* exposed to high O_3 concentration (Li et al., 2011). However, the opposite result was found in this study where elevated O_3 decreased significantly ABA content, compared to ambient air. The conflicting finding obtained in our study might be related to plant species and the concentration and duration of O_3 exposure, especially the timing of ABA measurement following the initiation of O_3 fumigation during the experiment. In fact, the similar results were reported that O_3 decreased ABA content in some other plants (Li et al., 2007; Mao et al., 2017). Nonetheless, the significant increase of ABA content of EDU-treated plants in this study relative to distilled water controls under O_3 fumigation may contribute to the higher resistance of EDU-pretreated plants against O_3 damage through ABA regulation of stomatal closure (Ludwikow et al., 2009; McAdam et al., 2017). Actually, EDU did not affect stomatal behavior; it can reduce ozone toxicity by its deposition on leaf surfaces as a foliar fertilizer or reacting with ozone before entering into the leaf (Ashrafuzzaman et al., 2018; Oksanen, 2018).

As one of the ozone precursors, VOC may scavenge O_3 and help provide protection of the photosynthetic apparatus against oxidative stress (Loreto and Velikova, 2001). In this study, we found that 120 $\mu g/m^3$ O_3 significantly decreased VOC emission rate. This was in agreement with some studies that O_3 decreased VOC emission rate in other plants (Velikova et al., 2005; Calfapietra et al., 2007). However, the results in our previous studies showed that elevated O_3 increased VOC emission rates of some urban tree species (Xu et al., 2012; Xu et al., 2015). The contradictory findings in our study might be associated with the difference in species and age of plants (Li et al., 2009; Paoletti et al., 2009). In current study, VOCs emission rate in EDU-pretreated

plants was higher than non EDU-retreated plants. This association between improved antioxidant capacities increased VOCs emission rate by EDU application is a novel finding. As far as we know, this is first study that EDU promotes the VOCs emission of plants, which may be a beneficial plant response under elevated O_3, probably due to the abiotic chemical reaction of EDU with ozone (Ashrafuzzaman et al., 2018). Generally, elevated O_3 decreases *Pn*, *gs* and *Tr*, which may exert an adverse effect on VOCs emission. In other word, the emission rate of VOCs is greatly associated with photosynthetic performance of plants. By the analysis of Pearson correlation, we found that VOCs emission rate showed a significant positive correlation with *Pn*, *gs*, *Tr*, WUE, *Fv/Fm*, ΦPSII, and ABA concentration (Table 2), respectively. EDU application in this study improved photosynthetic performance, increased ABA accumulation and promoted the VOCs emission of plants regardless of O_3 fumigation. Actually, VOCs was positively correlated with ABA concentration in leaves, as they shared a common biosynthetic pathway (Barta and Loreto, 2006). VOCs emission from plants could enhance O_3 formation in the surrounding environment, especially in the places with high NOx level (Li et al., 2008). Therefore, much more research is needed to understand the benefits associated with application of EDU to alleviate the O_3 stress on plants, particularly for the adult tree species playing an important role in sustainable forest management and silviculture of China under the microclimate conditions including regional O_3 pollution (Wang et al., 2018a, 2018b).

4. Conclusions

The current study confirmed that EDU alleviated the adverse effect on foliar visible injury induced by elevated O_3 and exerted a protective function on photosynthetic apparatus in leaves *P. alba* "*Berolinensis*." EDU mitigated the oxidative stress of elevated O_3 by maintaining low lipid peroxidation, and high antioxidant enzyme activity and ABA content in plants. EDU significantly increased VOCs emission rate regardless of O_3 treatment, which may help protect the photosynthetic apparatus against oxidative stress under elevated O_3. Further studies are needed to understand the benefits and risks of using EDU as a chemical protectant.

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