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Ameliorating effects of three kinds of antioxidants to ozone-polluted painted nettle (*Coleus blumei* Benth.)

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Abstract

Ground concentration of ozone (O_3) causes serious threat to plants. In order to protect sensitive plants from O_3 pollution, many kinds of antioxidants were assessed in previous studies. In this study, effects of O_3 fumigation (a single spike of 120 ± 20 nmol mol^{-1} for four hours) on an ornamental species (*Coleus blumei*) was examined in open-top chambers. Before the O_3 treatment, plants were sprayed respectively either with a solution of three different antioxidants [Na-ascorbate (NaAsA), kinetin (KIN), and spermidine (Spd)] or with distilled water to compare their protective effects to plants. Our results revealed that O_3 fumigation impaired the plasma membrane, decreased chlorophyll (Chl) content, inhibited photosynthesis, induced photoinhibition and photodamage, and caused visible injury. Spraying with KIN, NaAsA or Spd ameliorated the decrease of the Chl content and photosynthetic capability, the impairment of membrane, and visible injury under O_3 fumigation. The plants treated with KIN showed the best ability to mitigate the injury caused by O_3.

Additional key words: chlorophyll *a* fluorescence; gas exchange; kinetin; Na-ascorbate; spermidine.

Introduction

Ground concentration of ozone (O_3) is created by the photochemical reactions between volatile organic compounds (VOC) and oxides of nitrogen (NO_x) (Paoletti *et al*. 2010, Bortolin *et al.* 2014). Due to increases of NO_x and VOC, surface O_3 has increased remarkably in current summer seasons (Royal Society 2008). The surface one-hour average O_3 concentration in many regions of China reached 150 nmol mol^{-1} (Shao *et al.* 2006). It has been well reported that elevated O_3 caused serious damages to crops, natural vegetation, and ecosystems (Ashmore 2005, Hayes *et al.* 2007).

Stomata is the main path how O_3 enters into the leaf. After the entrance, O_3 dissolves in the apoplast and reactive oxygen species (ROS) are produced (Castagna and Ranieri 2009). Protein and lipid components of plasma membrane are attacked by ROS, causing cell ion leakage (Overmyer *et al.* 2003). Because of the change in leaf pigment contents or cell death, visible injury sometimes can be observed in leaves of O_3-polluted plants in the form of chlorotic or necrotic lesions (Paoletti *et al.* 2014).

In order to protect sensitive vegetation from O_3 pollution, many kinds of chemical protectants were applied and evaluated (Didyk and Blum 2011). So far, mitigating effects of ethylene diurea (EDU) on O_3-polluted plants are the most studied (Paoletti *et al.* 2009a; Feng *et al.* 2010), but it has long been reported that EDU had dose-dependent toxicity to plants (Manning and Vardaro 1973). Therefore, the effects of natural antioxidants are being widely examined. There have been many studies about exogenous application of ascorbic acid and its salts to alleviate O_3-induced injury, such as visible injury or membrane leakage (Zheng *et al.* 2000, Didyk and Blum

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Abbreviations: Car – carotenoids; CF – charcoal-filtered air; C_i – intercellular CO_2 concentration; Chl – chlorophyll; CK – control; E – transpiration rate; EC – electrical conductivity; ETR – electron transport rate; F_o – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; MDA – malondialdehyde; NPQ – nonphotochemical quenching; OTCs – open-top chambers; P_N – net photosynthetic rate; ROS – reactive oxygen species; VOCs – volatile organic compounds; Y_{II} – the effective quantum yield of PSII photochemistry; Y_{NPQ} – quantum yield of light-induced nonphotochemical fluorescence quenching; Y_{NO} – quantum yield of nonregulated heat dissipation and fluorescence emission.
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2011). It has also been reported that application of cytokinin such as kinetin (KIN) could act as antioxidant to cope with oxidative damage such as O_3 pollution (Tomlinson and Rich 1973, Runeckles and Resh 1975, Rattan 2004). Cytokinins mitigate the loss of Chl by retarding the breakdown of chloroplasts (Sobieszczuk-Nowicka *et al*. 2009). Polyamines, such as putrescine, spermidine (Spd), and spermine, can scavenge free radicals to inhibit O_3-caused injury in tomato and O_3-sensitive tobacco Bel W3 (Ormrod and Beckerson 1986, Bors *et al.* 1989). However, it has not yet been clarified which kind of antioxidants shows a better mitigating effect.

Coleus blumei is a perennial garden plant, cultivated worldwide for decoration because of its colorful foliage (Tsushima and Sano 2015). It belongs to the family Lamiaceae. Its foliage color variation is mostly dependent on the intensity of sunlight which changes the balance of Chls and anthocyanins (Nguyen and Cin 2009). Ozone pollution often occurs under high light and temperature when the ornamental value of *C. blumei* is the highest. In previous studies, some cultivars of *C. blumei* were considered to be moderately sensitive to O_3, while others seemed to be tolerant (Adedipe *et al*. 1972, Semeniuk and Heggestad 1981).

The objectives of this research were: (*1*) to assess effects of acute O_3 pollution on the common used cultivar 'Wizard Sunset' of *C. blumei* in order to verify the O_3 sensitivity of this cultivar and whether an acute exposure could reduce its ornamental value by inducing foliar injury, (*2*) to compare the mitigating effects of different kinds of antioxidants on possible O_3-caused injury. The results of this study could probably provide some hints to the protection of ornamental crops in the O_3-polluted areas.

Materials and methods

Plant material: *C. blumei* Benth. was selected for this experiment. Seeds of the 'Wizard Sunset' cultivar were bought from *China National Tree Seed Corporation*. The cultivar is characterized by beautiful red leaves and is one of the most commonly used garden plants in China. The seeds were sown in pots (10 cm in diameter) filled with a mixture of vermiculite and peat (1:2, v/v) on 26 March, 2015. After germination, the seedlings were irrigated every day to field capacity. The plants were cultivated in the greenhouse until each seedling had four pairs of fully expanded leaves on 9 July, 2015.

Treatments: The experiment was conducted in six open-top chambers (OTCs, 1.8 m in diameter and 2.4 m in height) at the Horticultural Research Station, Northeast Agricultural University, China. During the experiment, the max/min relative humidity and temperature in the OTCs were 60/35% and 30/18°C, respectively. The daily average PPFD inside the OTCs was 600 μmol m^{-2} s^{-1}. The gas-dispensing system of the OTCs was designed according to Uprety (1998) and the ventilation tube was adjusted 10 cm higher than the height of plants. Twenty potted seedlings were randomly placed in each OTC on 9 July, 2015. After adaptation for six days, every five plants in each OTC were sprayed with Na-ascorbate (NaAsA) (1×10^{-3} mol l^{-1}), KIN (1×10^{-3} mol l^{-1}), Spd (1×10^{-3} mol l^{-1}), or distilled water until dripping. The concentrations of the antioxidants were selected according to Didyk and Blum (2011). After 12 h, three chambers were ventilated with O_3, where concentration was maintained at 120 ± 20 nmol mol^{-1} (10:00 – 14:00 h) for 4 h, and the other three chambers were treated with charcoal-filtered (CF) air on 15 July 2015. Such O_3 concentration was intended to simulate a single spike of O_3 pollution, similar to those occurring during summer at urban area of Shanghai or suburban Tianjin, China (Ran *et al*. 2009, 2012). Ozone was produced by an O_3 generator (*CF-KG1*, *Beijing Sumsun EP Hi-Tech.*, Beijing, China) and bubbled through distilled water (Zhang *et al*. 2010). Glass tube rotameters were used to control the flow of injected O_3. Ozone concentrations in the OTCs were monitored once every 5 min at the height of plant canopy using an O_3 analyzer (*Model 205, 2B Technologies Inc.*, Boulder, Colorado, USA).

Visible injury: After 4 h of O_3 exposure, leaf injuries were immediately recorded. Six plants per treatment by O_3 or CF were randomly selected. The percentage of injured area (mottled or necrotic) on the first pair of fully expanded leaves in each plant was assessed by three surveyors using a 5% and 1% step scale when visible injury was above and below 5%, respectively (Paoletti *et al*. 2009b) .

Gas exchange: Instantaneous gas exchange was measured on one leaf of the first or second pair of fully expanded leaves from 08:30 to 11:30 h on the day after O_3 exposure using a portable photosynthesis system (*Li-6400*, *Li-Cor*, USA). Six plants per treatment in O_3 or CF were randomly selected. The light intensity and the air temperature inside the leaf chamber were set to 600 μmol(photon) m^{-2} s^{-1} and 25 ± 0.5°C, respectively. Ambient CO_2 concentration (380 ± 10 μmol mol^{-1}) was used as reference. Net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were recorded, and the ratio of intercellular/ambient CO_2 concentration (C_i/C_a) was calculated.

Chl *a* fluorescence: Six plants of each treatment in O_3 or CF were selected for Chl *a* fluorescence measurement using a *PAM-2500* fluorometer (*Heinz Walz*, Effeltrich, Germany) from 08:00 to 11:00 h on 17 July 2015. For each measurement, the middle part of one leaf of the first or second pair of fully expanded leaves was dark-adapted with a leaf clip (*DLC-8*, *Heinz Walz*, Effeltrich, Germany) for 20 min. Parameters of Chl *a* fluorescence were measured on the adaxial leaf surface immediately after the

dark adaptation. Fluorescence induction curves were automatically recorded using the slow kinetic program in the saturation pulse analysis mode. The minimal (F_o) and maximal fluorescence (F_m) in the dark-adapted state were obtained with a modulated light [630 nm, 0.1 μmol(photon) m^{-2} s^{-1}] and a 0.8 s saturating pulse [630 nm, 10,000 μmol(photon) m^{-2} s^{-1}], respectively. After 40 s, an actinic irradiation [630 nm, 196 μmol(photon) m^{-2} s^{-1}] was turned on. Then a saturating pulse was imposed every 20 s until 5 min to determine the maximal fluorescence in the irradiation-adapted state (F_m'). The maximal quantum yield of PSII photochemistry [$F_v/F_m = (F_m - F_o)/F_m$], the electron transport rate (ETR = PAR × 0.84 × 0.5 × Y_{II}), the effective quantum yield of PSII photochemistry [$Y_{II} = (F_m' - F)/F_m'$], the quantum yield of nonregulated heat dissipation and fluorescence emission ($Y_{NO} = F/F_m$) and the quantum yield of light-induced nonphotochemical fluorescence quenching ($Y_{NPQ} = F/F_m' - F/F_m$) were calculated according to Kitajima and Butler (1975), Genty *et al.* (1989), and Genty *et al.* (1996).

Leaf pigments: The first pair of fully expanded leaves was selected for sampling. Each sample was a pool of two leaves from two plants. Four samples per treatment were gathered (n = 4). Leaf samples were ground with quartz sand, calcium carbonate powder, and ethanol (95%, v/v). Then the extracts were filtered to 25-ml brown volumetric flask. The final constant volume was kept at 25 ml. The absorbance was measured using a UV-visible spectrophotometer (*T6 New Century*, China) at 470, 649, and 665 nm. The concentration was calculated according to Hao *et al*. (2004) as follows:

Chl *a* = 13.95 OD_{665} − 6.88 OD_{649};
Chl *b* = 24.96 OD_{649} − 7.32 OD_{665};
Car = (1000 OD_{470} − 2.05 Chl *a* − 114.8 Chl *b*)/245.

Electrical conductivity (EC): The first pair of fully expanded leaves was selected for sampling. Each sample was a pool of two leaves from two plants. Four samples per treatment were gathered (n = 4). Fresh samples (0.2 g) were cut as discs of uniform size. Then the sample was placed in test tubes containing 20 ml of double distilled water in two sets. One set (EC1) was kept at 100°C in boiling water bath for 20 min and the other set (EC2) was maintained at 25°C for 24 h. Using a conductivity meter (*Delta 326*, *Mettler-Toledo*, Switzerland), the values of electrical conductivity of the two sets were measured, and the relative conductivity was calculated as (EC2/EC1) × 100%.

Malondialdehyde (MDA) content: The first pair of fully expanded leaves was selected for sampling. Each sample was a pool of two leaves from two plants. Four samples per treatment were collected (n = 4). Fresh sample (0.2 g) was ground. Then it was homogenized with trichloroacetic acid (10%). The homogenates were centrifuged at 4,000 × *g* for 10 min. The supernatants (1 ml) were mixed with 5 ml of thiobarbituric acid (0.6%) and then maintained in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 4,000 × *g* for 10 min. The absorbances of supernatants were measured using a UV-visible spectrophotometer (*T6 New Century,* China) at 450, 532, and 600 nm. The MDA content was calculated according to Hao *et al*. (2004) as:

$[(OD_{532} - OD_{600}) \times 6.45 - 0.56 \times OD_{450}]/0.2$.

Statistical analysis: All data were analyzed using *SPSS* (*version 12*, *SPSS*, Chicago, IL, USA). The normal distribution of data was checked by the *Kolmogorov-Smirnov*'s *d* test. Two-way analysis of variance (*ANOVA*) was used to identify the effect of O_3 treatment, antioxidant type, and their interactions. Means of each parameter among different treatments were compared by post-hoc *Duncan*'s test. Relative change of each parameter was expressed as percentage difference between O_3-exposed (O_3) plants and charcoal-filtered air (CF) plants, (O_3 – CF)/CF × 100%.

Results

Visible injury: The plants in the CF air exhibited no visible injury. Under O_3 fumigation, marginal burn and irregular lesions between the veins over the adaxial leaf blade were found (Fig. 1). The average percentage of visible injured surface over the first fully expanded leaves were significantly lower after all antioxidant treatments compared with the controls sprayed with distilled water (CK) (Table 1). The most effective antioxidant treatment was KIN (–89% of injury relative to CK), followed by NaAsA (–68%) and Spd (–51%).

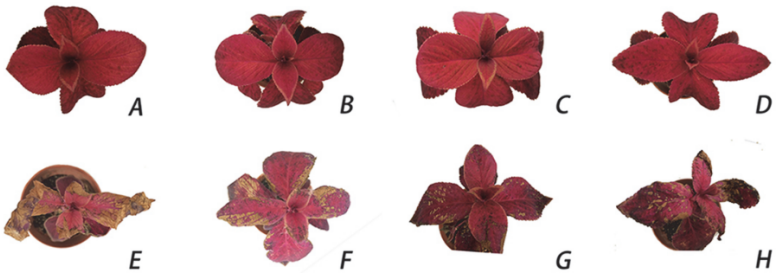

Fig. 1. Leaf visible injury of *Coleus blumei* 'Wizard Sunset' after being exposed to charcoal-filtered air (CF) or acute O_3 fumigation (O_3). Plants sprayed with distilled water (CK) under C (*A*); Na-ascorbate (NaAsA) under CF (*B*); kinetin (KIN) under CF (*C*); spermidine (Spd) under CF (*D*); CK under O_3 (*E*); NaAsA under O_3; (*F*); KIN under O_3 (*G*); Spd under O_3 (*H*).

Table 1. Average percentage of visible injury on the first fully expanded leaves in plants of *Coleus blumei* sprayed with distilled water (CK), Na-ascorbate (NaAsA), kinetin (KIN), and spermidine (Spd) after being exposed to elevated O_3 (120 nmol mol^{-1}) or charcoal-filtered air (CF) for 4 h. Results are shown as means ± SE (n = 6 plants). *Different letters* show significant differences between treatments (*Duncan* test, $P \leq 0.05$).

Treatment	CF visible injury [%]	O_3 visible injury [%]
CK	0	57.50 ± 1.11^{a}
NaAsA	0	18.33 ± 1.05^{c}
KIN	0	6.67 ± 1.05^{d}
Spd	0	28.33 ± 1.05^{b}

Leaf pigments: Ozone significantly decreased the content of Chl *a*, total Chl, and Car relative to controls in CF, but did not affect the Chl *b* content (Fig. 2). Due to the stable Chl *b* content, the relative loss of total Chl was probably caused by the decrease of Chl *a*. Compared with the plants treated with CK, the lowest relative losses of Chl *a* and total Chl were recorded in the plants sprayed with KIN, followed by NaAsA and Spd. Due to such differences in the response of the antioxidant-treated plants to O_3, there were significant interactions between O_3 fumigation and antioxidants in Chl *a* and total Chl. Compared with CF, the plants with KIN and NaAsA showed the stable Car contents under O_3 exposure, while decreases of Car content were found in the plants of CK and Spd.

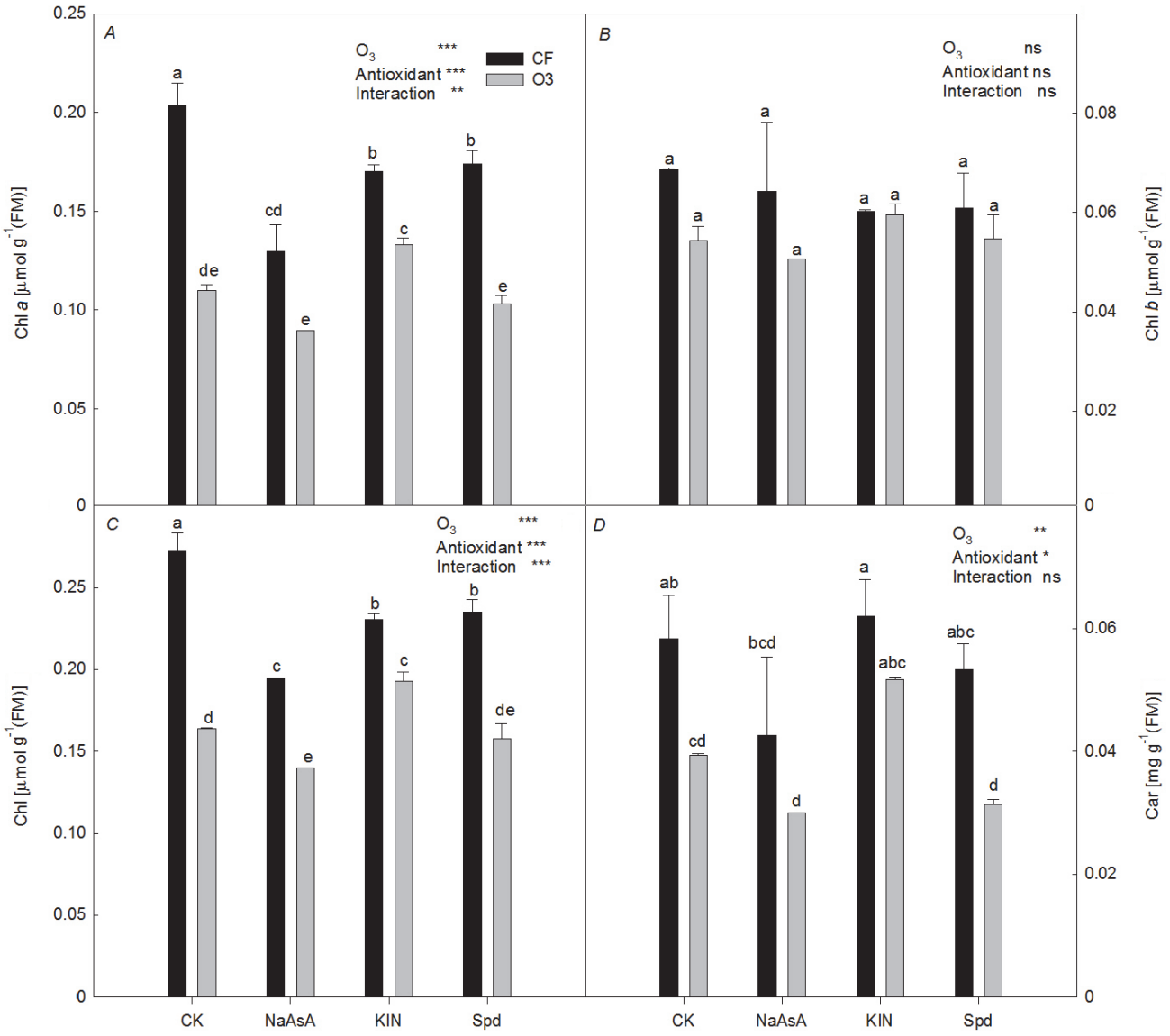

Fig. 2. The contents of chlorophyll (Chl) *a* (*A*), Chl *b* (*B*), total Chl (*C*), and carotenoid (Car, *D*) of *Coleus blumei* sprayed with distilled water (CK), Na-ascorbate (NaAsA), kinetin (KIN) or spermidine (Spd) after being exposed to charcoal-filtered air (CF) or acute O_3 fumigation (O_3). Data are means + SE. *Different letters* show significant differences between bars (n = 4 pooled foliar samples, *Duncan*'s test, $P \leq 0.05$). Results of a two-way *ANOVA* are shown in the inset, *** – $P \leq 0.001$; ** – $P \leq 0.01$; * – $P \leq 0.05$; ns – $P > 0.05$.

Membrane integrity: The significant increases of electrical conductivity (EC) and MDA content were indicative of plasma membrane damage caused by O_3 fumigation (Fig. 3). There were significant interactions between O_3 fumigation and antioxidant spraying. The plants of CK had higher relative increases of EC and MDA compared to the plants sprayed with antioxidants under the O_3 treatment. Under O_3 fumigation, the increases of EC and MDA (26 and 27%, respectively) in the KIN-sprayed plants were much lower than those in NaAsA-sprayed (39 and 49%, respectively) and Spd-sprayed plants (54 and 97%, respectively).

Gas exchange: P_N, g_s, and E were all significantly inhibited after O_3 fumigation, while the ratio of C_i/C_a increased (Fig. 4). In CF air, antioxidants significantly decreased the P_N, g_s, and E. However, the P_N of plants with KIN was the highest under the elevated O_3. The interactions between the O_3 treatment and antioxidant spraying were very significant. In plants treated with KIN, the relative decrease of P_N was much lower than that in the other antioxidant treatments after O_3 exposure. The g_s and E of plants treated with KIN were not significantly different after O_3 fumigation and CF air. However, the plants with other antioxidant treatments had lower g_s and E under elevated O_3 compared to KIN. The relative increase of C_i/C_a in the plants with KIN was lower than that of other antioxidant treatments.

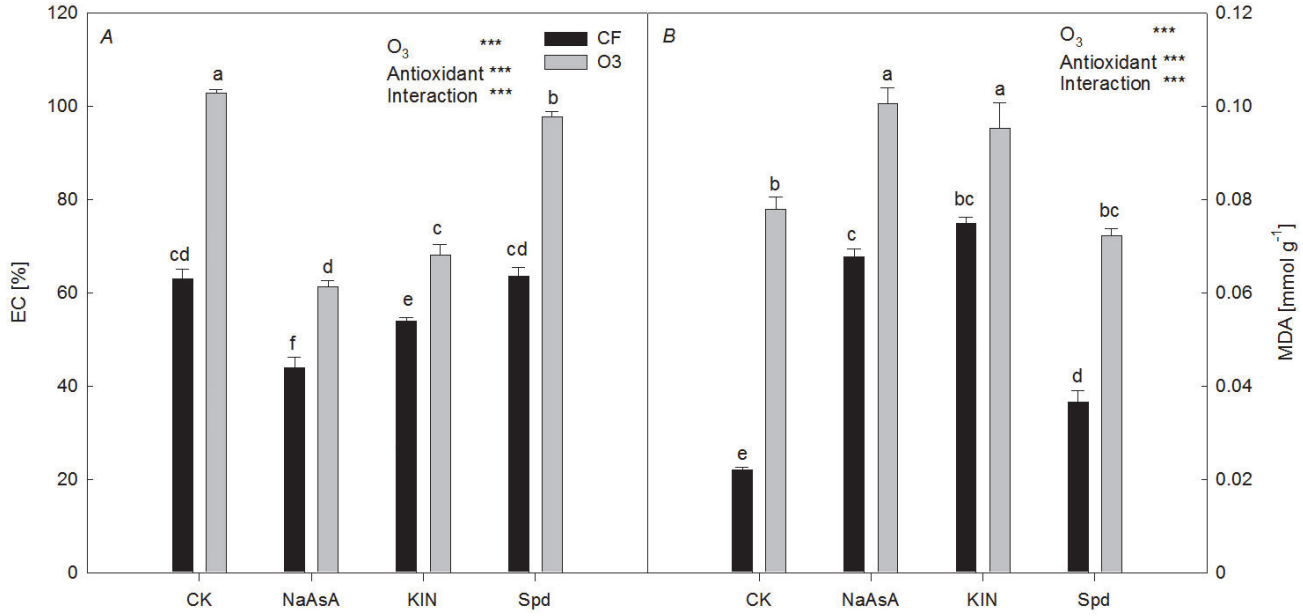

Fig. 3. Electrical conductivity (EC, *A*) and malondialdehyde content (MDA, *B*) of *Coleus blumei* sprayed with distilled water (CK), Na-ascorbate (NaAsA), kinetin (KIN) or spermidine (Spd) after being exposed to charcoal-filtered air (CF) or acute O_3 fumigation (O_3). Data are means + SE. *Different letters* show significant differences between bars (n = 4 pooled foliar samples, *Duncan*'s test, $P \leq 0.05$). Results of a two-way *ANOVA* are shown in the inset, *** – $P \leq 0.001$.

Chl *a* fluorescence: Elevated O_3 concentration significantly decreased F_v/F_m, ETR, and Y_{II} relative to CF, while increased Y_{NO} and Y_{NPQ} (Fig. 5). There were marked interactions between O_3 fumigation and exogenous antioxidants for F_v/F_m, ETR, Y_{II}, and Y_{NO}. The relative losses of F_v/F_m, ETR, and Y_{II} in the plants treated with KIN were much lower than those with other antioxidants (Fig. 5*A–C*). The Y_{NO} in plants treated with KIN increased by 34% which was much lower than the increases after other antioxidant treatments (Fig. 5*E*). Compared with CF, the Y_{NPQ} in plants sprayed with CK increased, while no difference was found in the plants of other treatments under O_3 (Fig. 5*D*).

Discussion

Visible injury is an important index for O_3 effect assessment, especially for ornamental plants such as *C. blumei*. In this study, the leaves were damaged seriously after the short-term O_3 fumigation at high but realistic concentration, suggesting that this cultivar is very sensitive to O_3 pollution and may be seriously injured by the O_3 spikes occurring in urban environments (Ran *et al*. 2009, 2012). In previous study, selected cultivars of *C. blumei* showed a big genetic variability in response to O_3 (Semeniuk and Heggestad 1981). We demonstrated here that there are also some ozone-sensitive cultivars in *C. blumei*. Ozone sensitivity assessment of commercial

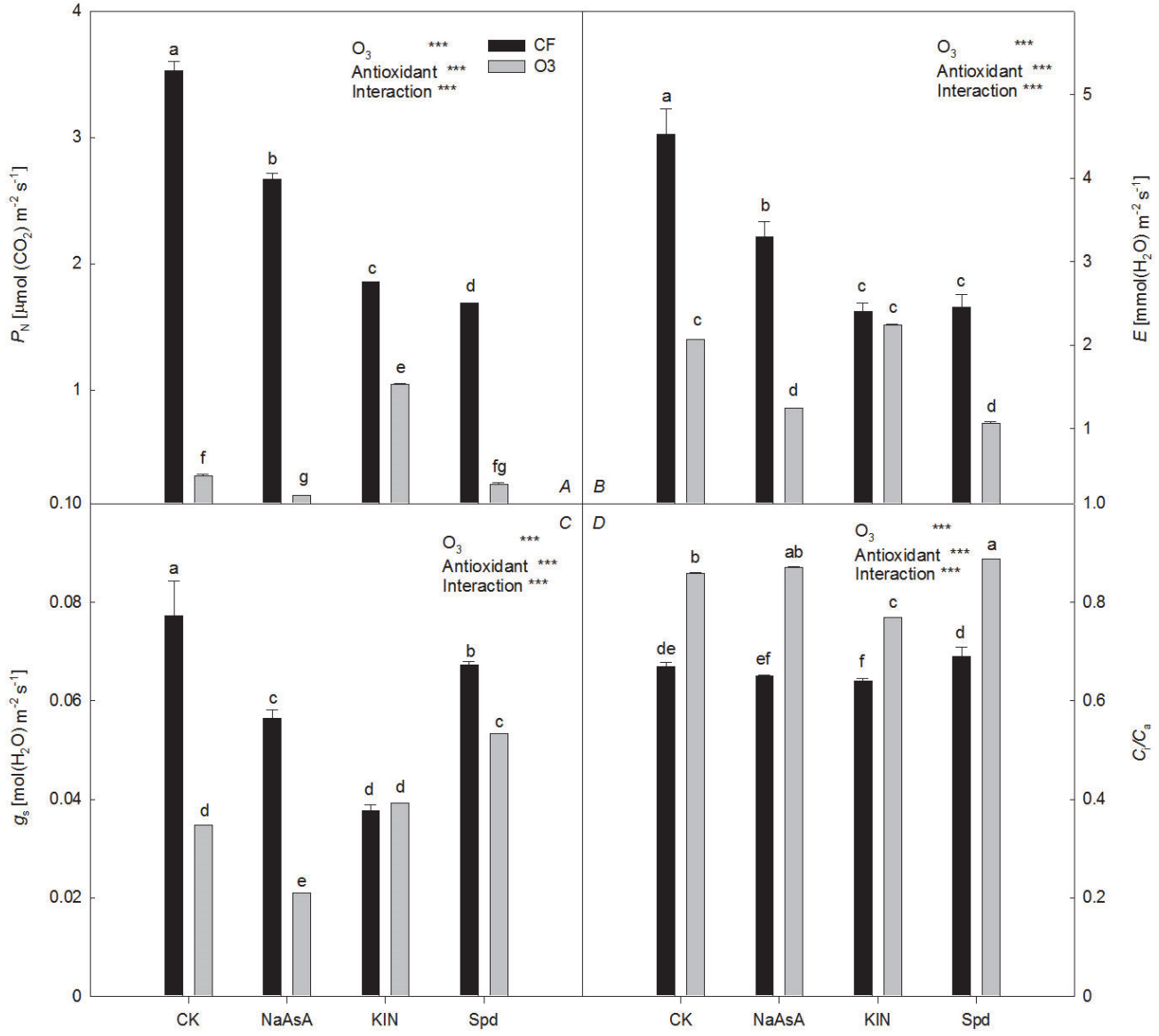

Fig. 4. Net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), transpiration rate (*E*, *C*) and the ratio of intercellular/ambient CO_2 concentration (C_i/C_a, *D*) of *Coleus blumei* sprayed with distilled water (CK), Na-ascorbate (NaAsA), kinetin (KIN), and spermidine (Spd) after being exposed to charcoal-filtered air (CF) or acute O_3 fumigation (O_3). Data are means + SE. *Different letters* show significant differences between bars (n = 6 plants, *Duncan*'s test, $P \leq 0.05$). Results of a two-way *ANOVA* are shown in the inset, *** – $P \leq 0.001$.

cultivars in ornamental crops is very important before extensive application in O_3-polluted areas. It has been well demonstrated that the integrity and permeability of plasma membranes of plants could be damaged by O_3 (Heath and Castillo 1988). The content of MDA, which is the end-product of lipid peroxidation, is considered as a biomarker of oxidative stress (Liu *et al*. 2015). In this study, the plasma membrane was attacked by O_3 or O_3-induced ROS. Then electrolyte leakage and membrane lipid peroxidation occurred, which was indicated by the significant increase of EC and MDA content. The degradation of Chl *a* and Car were enhanced by O_3. These results are in accordance with other studies (Bortolin *et al*. 2014). Under O_3 fumigation, F_v/F_m significantly decreased, suggesting photoinhibition (Thwe *et al*. 2014). Such severe inhibition led to photo-damage as indicated by the increase of Y_{NO}. Furthermore, a dramatic reduction in P_N was found, together with stomatal closure and reduced transpiration, while C_i/C_a significantly increased, suggesting nonstomatal limitations to P_N (Zhang *et al*. 2010).

Spraying by antioxidants changed the responses of *C. blumei* to O_3 fumigation. In CF air, the Chl *a* and total Chl contents of plants sprayed with antioxidants were lower than those of plants sprayed with distilled water. Although many researches have demonstrated that the exogenous application of antioxidants should increase the

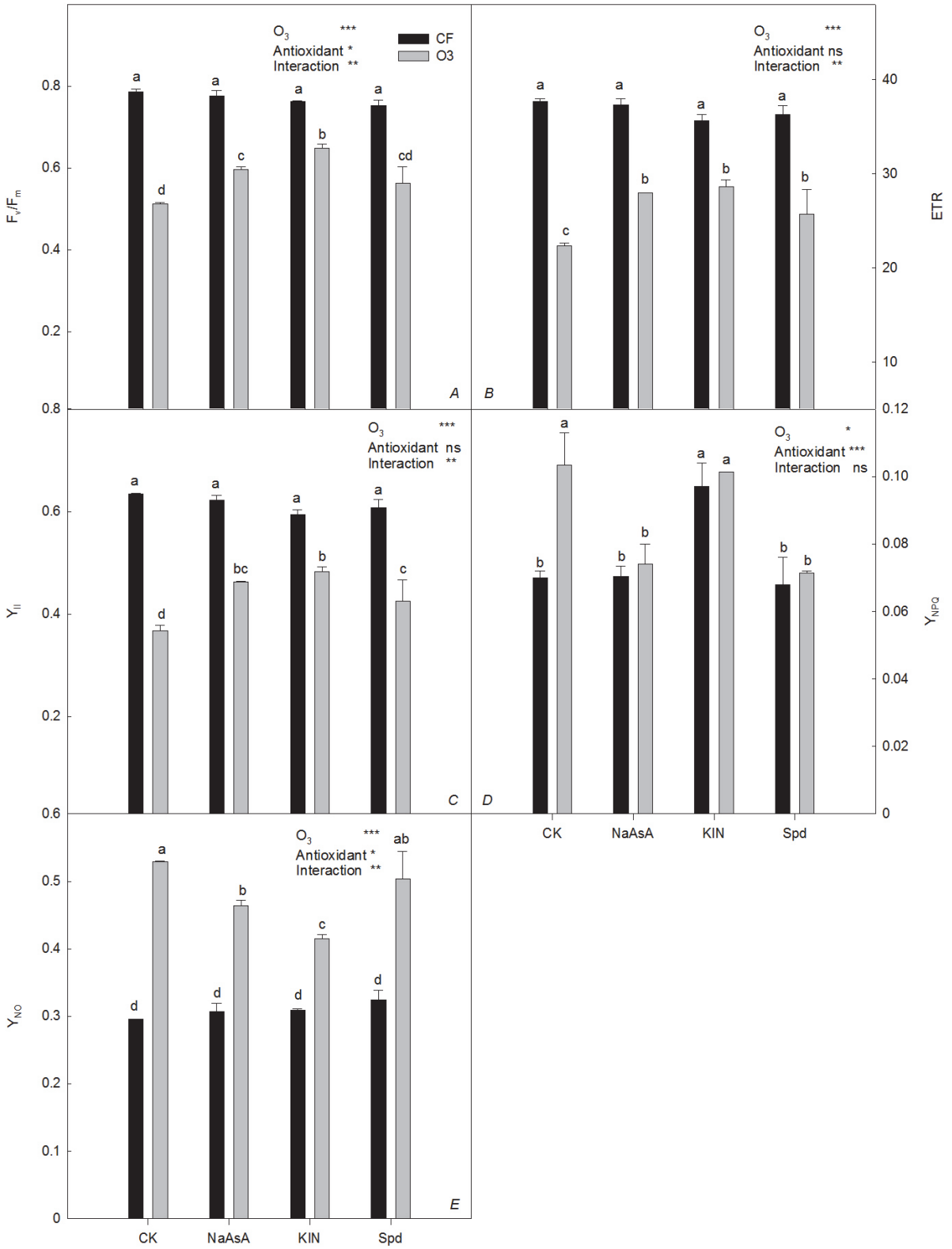

Fig. 5. The maximal photochemical quantum yield of photosystem II (F_v/F_m, *A*), electron transport rate (ETR, *B*), effective quantum yield of photosystem II (Y_{II}, *C*), quantum yield of light-induced nonphotochemical fluorescence quenching (Y_{NPQ}, *D*) and quantum yield of nonregulated heat dissipation, and fluorescence emission (Y_{NO}, *E*) of *Coleus blumei* sprayed with distilled water (CK), Na-ascorbate (NaAsA), kinetin (KIN), and spermidine (Spd) after being exposed to charcoal-filtered air (CF) or acute O_3 fumigation (O_3). Data are means + SE. *Different letters* show significant differences between bars (n = 6 plants, *Duncan*'s test, $P \leq 0.05$). Results of a two-way *ANOVA* are shown in the inset, *** – $P \leq 0.001$; ** – $P \leq 0.01$; * – $P \leq 0.05$; ns – $P > 0.05$.

Chl *a* and Chl contents, there are still some findings showing that these leaf pigments could be reduced by KIN (Pazurkiewicz-Kocot *et al.* 2011). Under O_3 fumigation, the plants sprayed with KIN exhibited the highest values of Chl *a* and Chl compared to other treatments. This result indicated that there was a relative lower loss of Chl *a* and Chl in plants with KIN. It has been reported that exogenous application of KIN improved biosynthesis of Chl and/or downregulated pigment degradation in salt-stressed *Nigella sativa* and Cd-stressed *Solanum melongena* (Shah 2011, Singh and Prasad 2014). Our results were similar to these findings. Addition of exogenous polyamines could restrain the loss of pigments during leaf senescence or under abiotic stresses such as UV (Besford *et al*. 1993, Jantaro *et al*. 2014). In this study, the lower degradation of Chl *a* and total Chl in plants with Spd provided another evidence. The loss of Chl influenced P_N. In CF air, the P_N of plants sprayed with antioxidants were lower than that of plants sprayed with distilled water. This result is in accordance with other researches where the exogenous application of spermidine decreased the P_N of tomato seedlings (Yu *et al*. 2016). Under high O_3 exposure, the plants sprayed with KIN had the highest values of Chl *a* and Chl compared with other treatments. Under high O_3 exposure, the plants sprayed with KIN had the highest P_N and a relatively lower decrease of P_N caused by O_3 pollution. This result is similar to the reports that exogenous supply of cytokinin improved photosynthesis under water deficit or Cd stress (Pospíšilová *et al*. 2000, Singh and Prasad 2016). We found that the degrees of photoinhibition and photodamage, as indicated by F_v/F_m and Y_{NO}, respectively, were mitigated by the spraying with Spd. Study in spinach showed that Spd is associated with LHC and PSII (Kotzabasis *et al*. 1993). The reduction in 2,6-dichlorophenol indophenol (DCIP) photoreduction of PSII due to water stress was alleviated by Spd pretreatment (Duan *et al*. 2006). It has been suggested that foliar application of 10 $\times 10^{-3}$ mol l^{-1} NaAsA alleviated the decline in photosynthesis caused by elevated O_3 (Zheng *et al*. 2000). However, our results showed no mitigating effect on P_N, which probably due to the lower concentration of NaAsA we used. Compared with plants sprayed with distilled water, the plants treated with KIN showed lower increases in EC and MDA contents caused by O_3 fumigation, indicating that KIN spraying could protect the plasma membrane from the ROS attack. These results are consistent with the finding that foliar applied KIN decreased the electrolyte leakage induced by salt stress (Kaya *et al*. 2010). Although the role of endogenous ascorbic acid in O_3 scavenging is unclear (Booker *et al*. 2012, Frei *et al*. 2012), many studies showed that exogenous ascorbic acid and its salts reduced membrane damage in plants under O_3 pollution (Didyk and Blum 2011). Our results are in agreement with these reports. It has been found that Spd pretreatment could alleviate the increases of electrolyte leakage and MDA of plasma membranes caused by salinity stress or water stress (Roy *et al*. 2005, Duan *et al*. 2006). Here, we demonstrated that Spd was also effective for keeping the stability of membrane under O_3 stress.

This was the first study that compared the O_3-protecting ability of the antioxidants, such as NaAsA, KIN, and Spd. The plants treated with NaAsA or Spd showed higher increases of EC and MDA contents than the plants with KIN, but had lower increases than those treated with distilled water, which suggests that KIN had the best mitigating effect. The plants sprayed with KIN exhibited the best ability to alleviate degradation of chloroplasts. Furthermore, KIN alleviated the photoinhibition and photodamage better than NaAsA or Spd. The relative decrease in P_N of the plants sprayed with KIN was obviously ameliorated, but the other antioxidants did not show the ameliorating effects.

To summarize, a short-term O_3 fumigation caused serious visible foliar injury in the commonly used commercial cultivar of *C. blumei.* Therefore, its ornamental value was obviously reduced. Moreover, O_3 impaired the plasma membrane, lowered the contents of Chl and Car, inhibited the photosynthesis, triggered photoinhibition and photodamage. Spraying with KIN, NaAsA or Spd ameliorated the negative effects of O_3. KIN proved the best ability to mitigate the damages caused by O_3, followed by NaAsA and Spd.

References

Adedipe N.O., Barrett R.F., Ormrod D.P.: Phytotoxicity and growth responses of ornamental bedding plants to ozone and sulfur dioxide. – J. Am. Soc. Hortic. Sci. **97**: 341-345, 1972.

Ashmore M.: Assessing the future global impacts of ozone on vegetation. – Plant Cell Environ. **28**: 949-964, 2005.

Besford R.D., Richardson C.M., Campos J.L. *et al.*: Effect of polyamines on stabilization complexes in thylakoid membranes of osmotically stressed oat leaves. – Planta **189**: 201-206, 1993.

Booker F.L., Burkey K.O., Jones A.M.: Re-evaluating the role of ascorbic acid and phenolic glycosides in ozone scavenging in the leaf apoplast of *Arabidopsis thaliana* L. – Plant Cell Environ. **35**: 1456-1466, 2012.

Bors W., Langebartels C., Michel C. *et al.*: Polyamines as radical scavengers and protectants against ozone damage. – Phytochemistry **28**: 1589-1595, 1989.

Bortolin R.C., Caregnato F.F., Divan A.M. *et al.*: Effects of chronic elevated ozone concentration on the redox state and fruit yield of red pepper plant *Capsicum baccatum*. – Ecotox. Environ. Safe. **100**: 114-121, 2014.

Castagna A., Ranieri A.: Detoxification and repair process of ozone injury: From O_3 uptake to gene expression adjustment. – Environ. Pollut. **157**: 1461-1469, 2009.

Didyk N.P., Blum O.B.: Natural antioxidants of plant origin against ozone damage of sensitive crops. – Acta Physiol. Plant. **33**: 25-34, 2011.

Duan H.G., Yuan S., Liu W.J. *et al*.: Effects of exogenous spermidine on photosystem ii of wheat seedlings under water stress. – J. Integr. Plant Biol. **48**: 920-927, 2006.

Feng Z., Wang S., Szantoi Z. *et al*.: Protection of plants from ambient ozone by applications of ethylenediurea (EDU): a meta-analytic review. – Environ. Pollut. **158**: 3236-3242, 2010.

Frei M., Wissuwa M., Pariasca-Tanaka J. *et al*.: Leaf ascorbic acid level – is it really important for ozone tolerance in rice? – Plant Physiol. Bioch. **59**: 63-70, 2012.

Genty B., Briantais J-M., Baker N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Genty B., Harbinson J., Cailly A.L. *et al*.: Fate of excitation at PS II in leaves: the non-photochemical side. – In: The Third BBSRC Robert Hill Symposium on Photosynthesis, 31 March to 3 April 1996, Abstract P28. Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield 1996.

Hao Z., Chang J., Xu Z.: [Plant Physiology Experiment.] Pp. 106-108. Harbin Inst. Technol. Press, Harbin 2004. [In Chinese]

Hayes F., Jones M.L.M., Mills G. *et al*.: Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. – Environ. Pollut. **146**: 754-762, 2007.

Heath R.L., Castillo F.J.: Membrane disturbances in response to air pollutants. – In: Schulte Hostede S., Darrall N.M., Blank L.W. *et al.* (ed.): Air Pollution and Plant Metabolism. Pp. 55-75. Elsevier Appl. Sci., London 1988.

Jantaro S., Baebprasert W., Piyamawadee C. *et al*.: Exogenous spermidine alleviates UV-induced growth inhibition of *Synechocystis sp*. pcc 6803 *via* reduction of hydrogen peroxide and malonaldehyde levels. – Appl. Biochem. Biotech. **173**: 1145-1156, 2014

Kaya C., Tuna A.L., Okant A.M.: Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. – Turk. J. Agric. For. **34**: 529-538, 2010.

Kitajima M., Butler W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. Biophys. Acta **376**: 105-115, 1975.

Kotzabasis K., Fotinou C., Roubelakis-Angelakis K.A. *et al.*: Polyamines in the photosynthetic apparatus: Photosystem II highly resolved subcomplexes are enriched in spermine. – Photosynth. Res. **38**: 83-88, 1993.

Liu X., Sui L., Huang Y. *et al*.: Physiological and visible injury responses in different growth stages of winter wheat to ozone stress and the protection of spermidine. – Atmos. Pollut. Res. **6**: 596-604, 2015.

Manning W.J., Vardaro P.M.: Suppression of oxidant injury on beans by systemic fungicides. – Phytopathology **63**: 1415-1416, 1973.

Nguyen P., Cin V.D.: The role of light on foliage colour development in coleus (*Solenostemon scutellarioides* (L.) Codd). – Plant Physiol.Bioch. **47**: 934-945, 2009.

Ormrod D.P., Beckerson D.W.: Polyamines as antiozonants for tomato. – Hortic. Sci. **21**: 1070-1071, 1986.

Overmyer K., Broshé M., Kangasjärvi J.: Reactive oxygen species and hormonal control of cell death. – Trends Plant Sci. **8**: 335-342, 2003.

Paoletti E., Castagna A., Ederli L. *et al*.: Gene expression in snapbeans exposed to ozone and protected by ethylenediurea. – Environ. Pollut. **193**: 1-5, 2014.

Paoletti E., Contran N., Manning W.J. *et al*.: Use of the antiozonant ethylenediurea (EDU) in Italy: Verification of the effects of ambient ozone on crop plants and trees and investigation of EDU's mode of action. – Environ. Pollut. **157**: 1453-1460, 2009a.

Paoletti E., Ferrara A.M., Calatayud V. *et al*.: Deciduous shrubs for ozone bioindication: *Hibiscus syriacus* as an example. – Environ. Pollut. **157**: 865-870, 2009b.

Paoletti E., Schaub M., Matyssek R. *et al.*: Advances of air pollution science: from forest decline to multiple-stress effects on forest ecosystem services. – Environ. Pollut. **158**: 1986-1989, 2010.

Pazurkiewicz-Kocot K., Kita A., Haduch A.: The effect of kinetin on the chlorophyll pigment contents in leaves of *Zea mays* L. seedlings and accumulation of some metal ions. – Inzynier. Ochr. Srodow. **4**:397-409, 2011.

Pospíšilová J., Synková H., Rulcová J.: Cytokinins and water stress. – Biol. Plantarum **43**: 321-328, 2000.

Ran L., Zhao C.S., Geng F.H. *et al*.: Ozone photochemical production in urban Shanghai, China: Analysis based on ground level observations. – J. Geophys. Res. **114**: D15301, 2009.

Ran L., Zhao C.S., Xu W.Y. *et al*.: Ozone production in summer in the megacities of Tianjin and Shanghai, China: a comparative study. – Atmos. Chem. Phys. **12**: 7531-7542, 2012.

Rattan S.I.S.: N6-furfuryladenine (kinetin) as a potential anti-ageing molecule. – J. Antiaging Med. **5**: 113-116, 2004.

Roy P., Niyogi K., SenGupta D.N. *et al*.: Spermidine treatment to rice seedlings recovers salinity stress induced damage of plasma membrane and PM-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. – Plant Sci. **168**: 583-591, 2005.

Royal Society: Ground-level Ozone in the 21st Century: Future Trends, Impacts and Policy Implications. – Science Policy Report.1508, 2008.

Runeckles V.C., Resh H.M.: Effects of cytokinins on responses of bean leaves to chronic ozone treatment. – Atmos. Environ. **9**: 749-753, 1975.

Semeniuk P., Heggestad H.E.: Differences among *Coleus* cultivars in tolerance to ozone and sulfur dioxide. – J. Hered. **72**: 459-460, 1981.

Shah S.H.: Kinetin improves photosynthetic and antioxidant responses of *Nigella sativa* to counteract salt stress. – Russ. J. Plant Physl.+ **58**: 454-459, 2011.

Shao M.,Tang X.Y., Zhang Y.H. *et al*.: City clusters in China: Air and surface water pollution. – Front. Ecol. Environ. **4**: 353-361, 2006.

Singh S., Prasad S.M.: Growth, photosynthesis and oxidative responses of *Solanum melongena* L. seedlings to cadmium stress: Mechanism of toxicity amelioration by kinetin. – Sci. Hortic.-Amsterdam **176**: 1-10, 2014.

Singh S., Prasad S.M.: Kinetin ameliorates cadmium induced toxicity on growth, pigments and photosynthesis by regulating antioxidant potential in tomato seedlings. – Int. J. Sci. Eng. Appl. Sci. **2**: 1-10, 239-243, 2016.

Sobieszczuk-Nowicka E., Wieczorek P., Legocka J.: Kinetin affects the level of chloroplast polyamines and transglutaminase activity during senescence of barley leaves. – Acta Biochim. Pol. **56**: 255-259, 2009.

Thwe A.A., Vercambre G., Gautier H. *et al*.: Response of photosynthesis and chlorophyll fluorescence to acute ozone stress in tomato (*Solanum lycopersicum* Mill.). – Photosynthetica **52**: 105-116, 2014.

Tomlinson H., Rich S.: Anti-senescent compounds reduce injury and steroid changes in ozonated leaves and their chloroplasts. – Phytopathology **63**: 903-906, 1973.

Tsushima T., Sano T.: First report of *Coleus blumei viroid 5* infection in vegetatively propagated clonal coleus cv. 'Aurora black cherry' in Japan. – New Disease Rep. **32**: 7, 2015.

Uprety D.C.: Carbon dioxide enrichment technology: Open Top Chambers a new tool for global climate research. – J. Sci. Ind. Res. **57**: 266-270, 1998.

Yu H., Zhao W., Wang M. *et al.*: The exogenous application of spermidine alleviates photosynthetic inhibition and membrane lipid peroxidation under low-light stress in tomato (*Lycopersicon esculentum* Mill.) seedlings. – Plant Growth Regul.**78**: 413-420, 2016.

Zhang L., Xu H., Yang J.C. *et al*.: Photosynthetic characteristics of diploid honeysuckle (*Lonicera japonica* Thumb.) and its autotetraploid cultivar subjected to elevated ozone exposure. – Photosynthetica **48**: 87-95, 2010.

Zheng Y., Lyons T., Ollerenshaw J.H. *et al*.: Ascorbate in the leaf apoplast is a factor mediating ozone resistance in *Plantago major*. – Plant Physiol. Biochem. **38**: 403-411, 2000.