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Combined effects of cadmium and ozone on photosynthesis of *Lycopersicon esculentum*

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Abstract

Tomato (*Lycopersicon esculentum* Mill. cv. Pearson) plants were grown in growth chambers for 25 days with cadmium (Cd) and then exposed briefly to ozone (O_3). Gas exchange, chlorophyll *a* fluorescence, and pigment composition were analysed in leaves at the end of the treatment to assess the effects of a single pollutant and their combination on photosynthesis. The CO_2 assimilation rate was dramatically reduced in plants subjected to the combined treatment, while the single effect of Cd appeared less severe than that of O_3. The decline of CO_2 photoassimilation found in all O_3-exposed plants was attributed to both stomatal and nonstomatal limitations. Tomato plants seemed to detoxify Cd to a great extent, but this resulted in growth suppression. In response to O_3 exposure, the plants protected their photosystems by heat dissipation of excess energy *via* the xanthophyll cycle. Cd combined with O_3 affected adversely this cycle resulting in an increase in photosynthetic performance under the same experimental light conditions.

Additional key words: chlorophyll a fluorescence; de-epoxidation index; electron transport rate; gas exchange; tomato; xanthophyll cycle.

Introduction

Human activity over many centuries has determined a widespread, environmental contamination due to the leakage and accumulation of various pollutants. The effects of single stress factors on plants have been extensively studied. On the other hand, less information is available concerning the effects triggered by the interaction between more than one stressor. Not so many reports are focused on the effects of combined pollutants, *e.g.* O_3 and Cd, which involve different zones of the biosphere (Di Cagno *et al*. 2001, Guo *et al*. 2012, Castagna *et al*. 2013).

Similarly to O_3 treatment alone (Castagna *et al.* 2001, Guidi *et al.* 2010), Cd also induces severe alterations in photosynthesis in addition to a wide range of other toxic effects in plants (Di Cagno *et al*. 1999, Küpper *et al*. 2007). Thus, CO_2 assimilation (P_N) is inhibited both by O_3 exposure and by growth in the presence of Cd. Mechanisms underlying these processes remain only partially discussed and mostly unclear. Stomata closure seems to limit photosynthesis more than any other event within the chloroplasts following O_3 exposure (Fiscus *et al*. 2005). Cd taken up by plants is accumulated preferentially in the chloroplasts and alters their function by inhibiting chlorophyll (Chl) synthesis (Siedlecka *et al*. 1997) and CO_2 photoassimilation (Di Cagno *et al*. 1999). However, photosystem II (PSII) has been frequently identified as the main target (Zhou and Qiu 2005, Küpper *et al*. 2007).

Previously, we found that Cd and O_3 acted synergistically inducing a strong reduction in P_N in sunflower plants grown hydroponically in presence of Cd (20 μM for 15 d) in the nutrient solution and subsequently exposed to O_3 (0.160 μl L^{-1} for 2 h) (Di Cagno *et al*. 2001).

We have already characterized the behaviour of tomato (*Lycopersicon esculentum* Mill.) plant, cv. Pearson, subjected to high O_3 concentration for a short period (0.200 μl L^{-1} for 4 h; Castagna *et al*. 2007).

We found only a mild sensitivity to such O_3 treatment

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Abbreviations: *ANOVA* – analysis of variance; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DEPS – de-epoxidation index; DM – dry mass; *E* – transpiration rate; ETR – electron transport rate; F_0 – minimal fluorescence under dark-adapted condition; F_0' – minimal fluorescence under light-adapted condition; F_m – maximal fluorescence under dark-adapted condition; F_m' – maximal fluorescence under light-adapted condition; F_t – transient fluorescence; F_v – variable fluorescence under dark-adapted conditions; F_v' – variable fluorescence under light-adapted conditions; F_v/F_m – PSII maximum efficiency in dark-adapted state; g_s – stomatal conductance; NPQ – nonphotochemical quenching; PAR – photosynthetically active radiation; PFD – photosynthetic flux density; P_N – net CO_2 assimilation rate; PSII – photosystem II; Q_A – first electron acceptor from PSII; q_P – photochemical quenching; RH – relative humidity; Φ_{exc} – quantum efficiency of open PSII reaction centers; Φ_{PSII} – actual quantum yield of PSII.
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as evidenced by barely visible symptoms on leaves, although H_2O_2 accumulation and the activation of the O_3-induced signal transduction pathway occurred.

This study was addressed to determine if the Cd presence during tomato growing modified the photosynthetic responses to the acute O_3 treatment.

Materials and methods

Plants and experimental design: Seeds of *Lycopersicon esculentum* Mill. (cv. Pearson) were provided by Dr. Jennifer Petersen (*Tomato Genetic Resource Center, University of California*, Davis, CA, USA). Seeds were sown in watered sand. Seedlings were transplanted after 25 d into 12-cm-diameter pots filled with 2 kg of soil with 935, 17.5, and 47.5 g kg^{-1} (dry soil) of sand, silt, and clay, respectively. Organic carbon and pH of the soil were 23.4 g kg^{-1} and 6.8, respectively. Cd was added to the soil as $Cd(NO_3)_2$ by irrigation to provide 200 mg Cd^{2+} kg^{-1}(dry soil). The plants grown without Cd in a nutrient solution represented a control. The Cd concentration was chosen according to preliminary experiments (*data not shown*) and represented a sublethal dose for the tomato, cv. Pearson.

The plants were grown for 25 d in a growth chamber with temperatures of 25/20°C day/night, 60–80% of relative humidity (RH), and a 12-h photoperiod under *ca*. 400 μmol (photon) m^{-2} s^{-1} of photosynthetically active radiation (PAR) and at the O_3 concentration lower than 0.2×10^{-3} μL L^{-1}. At the end of the 25-d period, the plants grown with or without Cd were subdivided into 2 groups, and then half of them was exposed to O_3. Six plants were utilized for gas exchange and Chl *a* fluorescence and biochemical analysis (pigment content). The fully expanded leaves of the second youngest node from the bottom were used for these analyses as well as for determination of Cd concentration; this latter being measured also in the roots. Physiological analysis and pigment content were determined at the end of the Cd and/or O_3 treatment.

Ozone exposure: The O_3 treatment was performed in environment-controlled chambers (*Cavallo*, Milan, Italy). Ambient air supplied into the chambers was filtered by a charcoal filter. Temperature was maintained at 25 ± 2.5°C, RH at 83 ± 2.4%, and light intensity was about 400 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR). O_3 was generated by electric discharge with an air-cooled generator (*Fisher Model 500*, Meckenheim, Germany) supplied with pure oxygen and it was mixed with the air entering the chamber. The O_3 concentration was continuously analyzed with a photometric *ML8810 analyzer* (*Monitor Labs*, San Diego, USA). The plants grown with or without Cd were exposed to a single treatment of O_3 with a target concentration of 0.200 μl L^{-1} for 5 h.

Cd determination: Immediately after harvesting, leaves and roots were washed in distilled water and oven-dried for 36–48 h at 50°C until constant mass. Samples were ground to powder and mineralized with nitric oxide. Cd concentration was determined by atomic absorption spectrophotometry (*Perkin-Elmer AAnalist 100*, Norwalk, CT, USA) equipped with a *Perkin-Elmer INTENSITRON™* lamp (λ = 228.8 nm) and determined by using a calibration curve in the linear range of 0–2 mg(Cd) L^{-1}.

Gas-exchange measurements were carried out at the end of the growth with or without Cd and after the O_3 exposure using an open system (*CMS-400*; *Walz, Effeltrich*, Germany). For details of the experimental procedures *see* Guidi *et al*. (1997). During the measurements in an assimilation chamber, temperature was maintained at 25 ± 2.1°C, with RH 65 ± 7%, CO_2 concentration of 380 μmol mol^{-1}, and O_2 of 21%. The response of leaf photosynthetic CO_2 assimilation to irradiance [0–1,000 μmol(photon) m^{-2} s^{-1} PAR] was calculated using the *Smith*'s equation (Tenhunen *et al*. 1976). CO_2 assimilation rate (P_N), stomatal conductance to water vapour (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were determined at light-saturation level (about 800 μmol m^{-2} s^{-1}).

Chl *a* fluorescence was assessed on leaves similar to those used for gas-exchange analysis by a pulse-amplitude modulation fluorometer (*PAM-2000, Walz*, Germany). The leaf was positioned with a clip at a constant distance and angle (60°) to the fibre optics of the fluorometer. Photosynthetic flux density (PFD) was determined close to the leaf surface by means of a microquantum sensor. Leaves were predarkened for 40 min before starting the measurements. The leaves were excited with a weak measuring beam to obtain minimum dark fluorescence yield, F_0 (0.1 μmol m^{-2} s^{-1}). A 0.8-s saturating pulse of white light was given to determine maximal fluorescence yield (F_m), when all PSII reaction centers are closed. These parameters were then used for the calculation of the F_v/F_m ratio, which indicates the maximum quantum efficiency of PSII photochemistry (Schreiber and Bilger 1993). Actinic light (about 420 μmol m^{-2} s^{-1}) was switched on and a saturating pulse (8,000 μmol m^{-2} s^{-1}) was applied at different intervals (10 pulses at 20-s intervals, 5 pulses at 40-s intervals, and finally 6 pulses at 80-s intervals) to determine maximum fluorescence yield in the light-adapted state (F_m'). The saturation pulse length was 1.2 s during the induction curve. The value of F_0' was obtained by switching off the actinic light, and giving a 3-s pulse of weak far-red light to obtain the fully oxidised state of PSII. The Chl fluorescence yield during actinic illumination was termed F_t. The coefficient of photochemical quenching, q_P, was calculated as $(F_m' - F_t)/(F_m' - F_0')$, while NPQ as $(F_m - F_m')/F_m'$ (Schreiber *et al*. 1986). Excitation pressure on PSII reflects the proportion of the primary, stable

quinone acceptor Q_A in the reduced state; it is calculated as $1 - q_P$ (van Kooten and Snel 1990). The quantum efficiency of open PSII reaction centers (Φ_{exc}) was calculated from the equation $(F_v'/F_m') = (F_m' - F_0')/F_m'$ (Genty *et al*. 1989). Another useful parameter is the quantum efficiency of the PSII photochemistry (Φ_{PSII}), which is determined in the light-adapted state as $(F_m' - F_t)/F_m'$ (Bilger *et al*. 1995). The apparent electron transport rate through PSII (ETR) was estimated according to Krall and Edwards (1992) as

$$ETR = \Phi_{PSII} \times PFD \times a \times f,$$

where a, the absorptivity of photosynthetic active radiation in the leaves, is assumed as 0.84, and f, the light distribution factor between PSI and II, is assumed 0.5.

Pigment analysis was performed on leaf discs of known area (1.13 cm^2) punched from leaves, which were previously utilised for fluorescence measurements, according to Castagna *et al*. (2001). Briefly, discs were ground in 100% HPLC-grade acetone in the presence of sodium ascorbate under dimmed room light, filtered through 0.2 μm *Minisart SRP15* 0.2 μm filters (*Sartorius,* Goettingen, Germany) and immediately analysed. The HPLC pigment separation was performed at room temperature with a *Zorbax ODS* column (*Agilent Technologies*, Santa Clara, CA, USA). The pigments were eluted using 100% solvent A (acetonitrile: methanol, 75:25, v/v) for the first 15 min, followed by a 2.5-min linear gradient to 100% solvent B (methanol:ethylacetate, 68:32, v/v) which continued isocratically until the end of the 32-min separation. The column was allowed to re-equilibrate in 100% solvent A for 10 min before the next injection. The flow rate was 1 mL min^{-1}. Pigments were detected by their absorbance at 445 nm and quantified by injecting known amounts of commercial standards (*Sigma-Aldrich*, St. Louis, MO, USA). The de-epoxidation index (DEPS) was calculated as:

$$[100 \times (V + 0.5\ A)/(V + A + Z)],$$

where V is violaxanthin, A is antheraxanthin and Z is zeaxanthin.

Statistical analysis: The experiment was performed twice and 40 plants were utilized in each experiment. Means and standard deviations (± SD) were calculated from pooled data of three replicates for each experiment. Cd concentration data were tested by *Student*'s *t*-test. Other physiological and biochemical data were subjected to one-way analysis of variance (*ANOVA*) using *Graphpad Prism Version 3 Software* (San Diego, USA). Percentage values of DEPS were angularly transformed before the analysis of variance. When the *F* value of *ANOVA* was significant, the least significant difference (LSD) was calculated for P=0.05.

Results

Cd accumulation and plant development: As expected at the end of the cultivation period, Cd-treated plants displayed a significantly higher Cd concentration than controls, at both root and leaf level (Table 1). Although most Cd was retained in the roots, where Cd concentration was 86-fold higher than in the control plants, translocation to the above ground organs occurred, leading to a significant Cd accumulation also at the leaf level, *i.e.* from 2.5 to 82.4 μg g^{-1}(DM) in control and Cd-treated plants, respectively (Table 1). The ratio of root/leaf Cd concentration was high, reaching a value of about 4.6 in the Cd-grown plants.

Cd uptake and subsequent accumulation in the below- and aboveground organs negatively affected plant development. Both leaves and roots reduced their growth, which was particularly evident at the root level (Fig. 1). Despite this growth reduction, leaf lamina did not show visible symptoms of damage attributable to Cd treatment.

Table 1. Cadmium concentration in different organs of *Lycopersicon esculentum* Mill. cv. Pearson grown for 25 d with (Cd) or without (control) Cd in a growth chamber in which the O_3 concentration was $< 0.2 \times 10^{-3}$ μL L^{-1}.

	Cd concentration [mg g^{-1}(DM)]	
	leaf	root
Control	2.5 ± 0.26	4.4 ± 3.44
Cd	82.4 ± 7.38	382.9 ± 54.00
P	**	***

Gas-exchange analyses were performed at the end of the growth with or without Cd both before and after the O_3 exposure. In the case of plants grown with Cd, additional O_3 treatment did not cause any leaf lesions.

Growth with Cd influenced negatively the P_N inducing a decrease in light-saturation level (Fig. 2). The O_3 treatment induced the more pronounced effect in all plants grown with or without Cd. The reduction in P_N was accompanied by a significant decrease in respiratory carbon loss in leaves of the plants grown with Cd and exposed to O_3 as the dark respiration rate showed a significant reduction compared with the controls (P<0.001).

The leaf gas exchange measured under light-saturation conditions showed that the decrease in P_N was induced by a significant reduction in g_s in plants grown with or without Cd but exposed to O_3 (Fig. 3). The g_s of O_3-exposed leaves was about 69% lower than that of the controls; the reduction was more pronounced in Cd+O_3 leaves (–77%). No differences were found between g_s in controls and in the plants grown with Cd (Fig. 3*B*). Similar pattern was observed

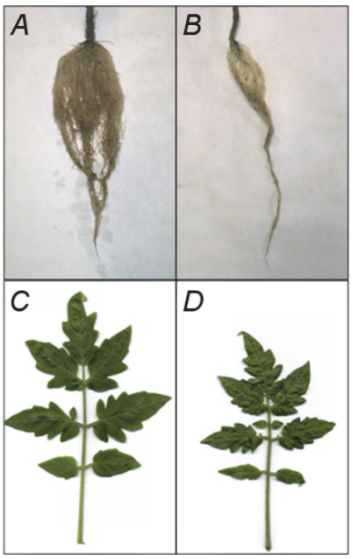

Fig. 1. Roots and leaves of *Lycopersicon esculentum* Mill. cv. Pearson at the end of the growth in filtered air without (*A*,*C*) or with (*B*,*D*) Cd in the soil.

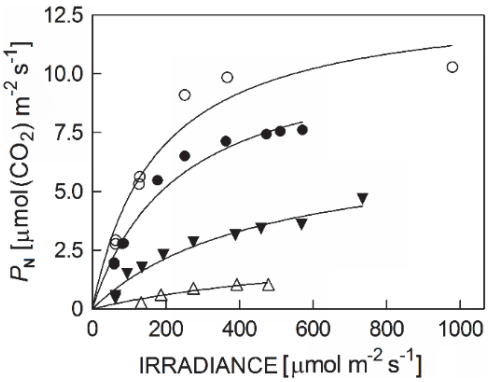

Fig. 2. Light dependency of CO_2 assimilation rate in leaves of *Lycopersicon esculentum* Mill. cv. Pearson measured at the end of the growth period in: plants grown in filtered air without (*open circle*) or with (*closed circle*) Cd, plants grown without Cd and then exposed to a single treatment of O_3 (0.200 ± 0.010 0.160 µl L^{-1} for 5 h; *closed triangle down*), plants subjected to Cd and then exposed to O_3 (*open triangle up*). Measurements were made at 25°C at a CO_2 concentration of 380 µmol mol^{-1} and $O_3 < 0.2 \times 10^{-3}$ µL L^{-1}. Each value represents the mean of 6 replicates.

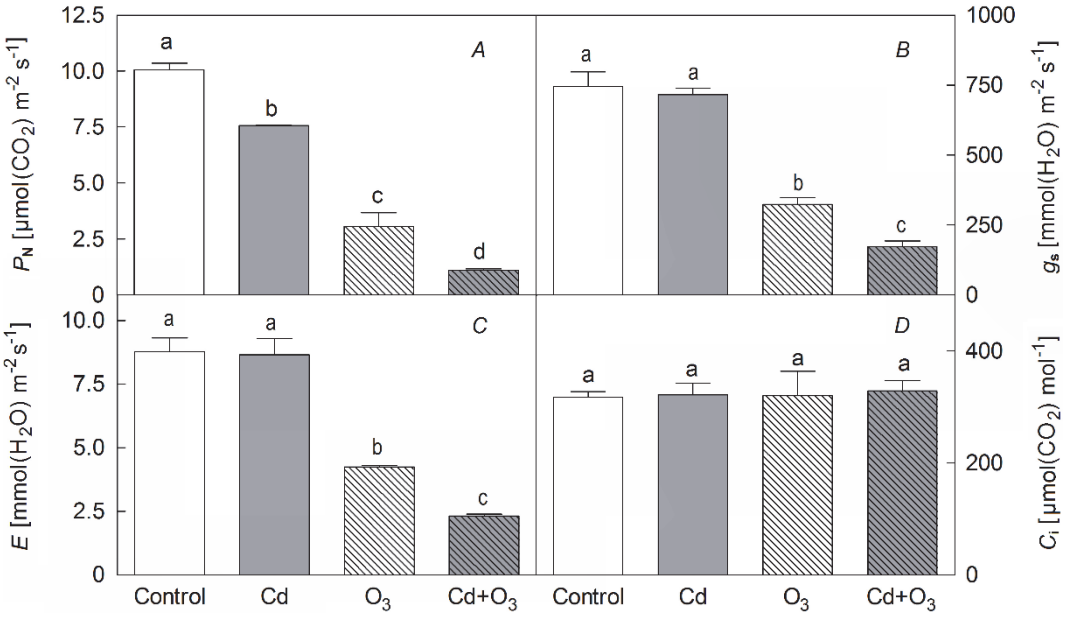

Fig. 3. *A:* Net CO_2 assimilation rate (P_N), *B:* stomatal conductance to water vapour (g_s), *C:* transpiration rate (E), and *D:* intercellular CO_2 concentration (C_i) in leaves of *Lycopersicon esculentum* Mill. cv. Pearson determined at the end of the growth period in: plants grown in filtered air with (Cd; *grey bars*) or without (Control; *white bars*) Cd, plants grown without Cd and then exposed to a single pulse of O_3 (0.200 ± 0.010 µL L^{-1} for 5 h; *white bars with diagonal lines*), plants subjected to Cd and then exposed to O_3 (Cd+O_3; *grey bars with diagonal lines*). Measurements were made at 25°C with a CO_2 concentration of 380 µmol mol^{-1} in the absence of O_3 and under light-saturated level (about 800 µmol m^{-2} s^{-1}). Each value represents the mean of 6 replicates with SD. Data were subjected to one-way *ANOVA* and when the F ratio was significant, $LSD_{0.05}$ was calculated. For each parameter *different letters* indicate statistically significant differences.

for E (Fig. 3*C*). C_i was not influenced by either Cd or O_3 treatment at the light-saturation level (Fig. 3*D*).

Chl *a* fluorescence: The F_v/F_m ratio decreased significantly to 0.76 in all O_3-treated plants, while no differences were found in the plants grown in Cd presence (Table 2) and in the plants subjected to both stresses as compared with controls.

Values of $1 - q_P$ increased in the plants grown with Cd

Table 2. Chlorophyll *a* fluorescence parameters recorded in the leaves of *Lycopersicon esculentum* Mill. cv. Pearson grown for 25 d with or without Cd (Cd or control, respectively) in a growth chamber in which the O_3 concentration was $<0.2 \times 10^{-3}$ μL L^{-1}. At the end of the growth with or without Cd plants were exposed to 0.200 μL L^{-1} of O_3 for 5 h (Cd+O_3 and O_3 respectively). Each value represents the mean of 6 replicates ± SD. Data were subjected to one-way *ANOVA* test and, when the treatment was significant, $LSD_{0.05}$ was calculated. Means followed by *different letters* indicate significant differences at the $P<0.05$ level (LSD *post-hoc* test).

	Control	Cd	O_3	Cd+O_3
F_v/F_m	0.81 ± 0.08^A	0.81 ± 0.02^A	0.76 ± 0.04^B	0.78 ± 0.02^{AB}
$(1 - q_P)$	0.15 ± 0.06^B	0.23 ± 0.07^A	0.27 ± 0.04^A	0.23 ± 0.05^A
NPQ	0.41 ± 0.02^C	0.47 ± 0.04^C	1.40 ± 0.35^A	0.89 ± 0.01^B
Φ_{PSII}	0.58 ± 0.06^A	0.51 ± 0.11^{AB}	0.41 ± 0.04^B	0.49 ± 0.04^B
ETR	47.3 ± 3.6^A	41.4 ± 8.6^{AB}	33.6 ± 3.6^B	38.3 ± 4.1^B

Table 3. Pigment concentration [μmol m^{-2}(leaf area)] and activation state of the xanthophyll cycle (DEPS index), calculated as [100 × (V + 0.5 A)/(V + A + Z)] in leaves of *Lycopersicon esculentum* cv. Pearson grown for 25 d with or without Cd (Cd or control, respectively) in a growth chamber in which the O concentration was $< 0.2 \times 10^{-3}$ μL L^{-1}. At the end of the growth with or without Cd plants were exposed to 0.200 μL L^{-1} of O_3 for 5 h (Cd+O_3 and O_3, respectively). Each value represents the mean (± SD) of 6 measurements. Data were subjected to one-way *ANOVA* test and, when the treatment was significant, $LSD_{0.05}$ was calculated. For each parameter, *different letters* indicate statistically significant differences at the $P<0.05$ level (LSD *post-hoc* test). The absence of letters indicates no significance of *F* ratio. V – violaxanthin; A – antheraxanthin; Z – zeaxanthin.

Pigment	Control	Cd	O_3	Cd+O_3
Chlorophyll *a*	315.7 ± 10.5	299.6 ± 11.9	281.5 ± 11.9	247.1 ± 23.0
Chlorophyll *b*	96.2 ± 10.1	96.7 ± 4.2	90.8 ± 4.9	80.8 ± 8.3
Neoxanthin	7.9 ± 0.6	7.7 ± 0.8	7.0 ± 0.4	6.4 ± 0.8
Lutein	35.6 ± 2.3	34.0 ± 5.1	31.7 ± 1.2	29.1 ± 4.1
V+A+Z	11.9 ± 0.8	12.9 ± 0.8	11.3 ± 0.3	11.0 ± 0.8
Total xanthophylls	55.4 ± 3.6	54.6 ± 6.2	49.9 ± 1.9	46.6 ± 5.6
β-carotene	88.1 ± 3.6	97.1 ± 6.9	84.5 ± 4.0	74.1 ± 8.8
Total carotenoids	143.5 ± 7.2	151.8 ± 10.2	134.4 ± 5.9	120.6 ± 14.4
DEPS index [%]	38.5 ± 5.6^C	44.5 ± 5.1^C	182.9 ± 21.4^A	117.9 ± 9.4^B

and/or exposed to O_3 treatment, while NPQ increased significantly in the O_3-treated leaves and in the leaves subjected to both treatments (Table 2). The ϕ_{PSII} was slightly reduced in the plants grown with Cd and significantly in the plants exposed to O_3 or to Cd+O_3 (Table 2). A similar pattern was observed for values of ETR, while Φ_{exc} was reduced only in the plants subjected to O_3 or to both treatments.

Leaf pigment concentration: Chl *a* and Chl *b* concentration did not change significantly following Cd treatment or O_3 exposure applied alone or in the combination (Table 3). Neoxanthin, lutein, and β-carotene, as well as by total carotenoids and total xanthophylls (Table 3) behaved similarly. The content of xanthophyll cycle pigments, *i.e.* the sum of V, A, and Z, was unaffected by either Cd and O_3 stress, while the DEPS increased by O_3 (+375%) and by the combined treatment Cd+O_3 (+ 206%) (Table 3).

Discussion

The growth of tomato plants in soil contaminated with Cd induced the accumulation of this element both in leaves and roots. In the roots, the increase in Cd content was even more pronounced in comparison with the leaves (Table 1). Retention/immobilization of high amount of Cd in the root tissue might represent an important protection mechanism against the diffusion of this heavy metal to green tissues. However, the significant increase in Cd concentration detected also in leaves supported the occurrence of an efficient root to shoot metal translocation. Despite consistent Cd accumulation and marked reduction in leaf development, neither visible symptoms of chlorosis or necrosis nor marked effects at photosynthetic level were evident. This suggested the onset of efficient metal compartmentalisation and/or detoxificant-repair mechanisms. The translocation of Cd to leaves implied an efficient transport, which is linked to *E* and thereby to g_s. In effect, the Cd presence did not influence g_s and *E,* both showed values similar to the controls (Fig. 3) suggesting that no alterations in stomata behaviour occurred in these plants. Accordingly, the reduction in P_N observed in Cd-treated plants was not linked to stomatal limitation as it was also indicated by the unchanged C_i values.

It is well known that Cd, like other heavy metals, influences light- and dark reactions of photosynthesis. Di Cagno *et al.* (1999) found alterations in Φ_{PSII}, q_P,

nonphotochemical quenching, and Φ_{exc} without changes in F_v/F_m in sunflower plants treated with 10 or 20 μM Cd. On the other hand, Mobin and Kahn (2007) observed a reduction in CO_2 photoassimilation in two mustard cultivars subjected to 100 mg(Cd) kg^{-1}(soil) (Mobin and Khan, 2007). Our results showed that F_v/F_m ratio did not change in Cd-treated leaves. This was in contrast with the results found by other authors in hyperaccumulating plants (Küpper *et al*. 2007, Mobin and Khan 2007), but it was in agreement with our previous research (Di Cagno *et al*. 1999). We postulated that short term exposure of tomato plants to Cd affected only moderately the reoxidation rate of the primary acceptor of PSII as evidenced by the slight increase in $1 - q_p$ parameter in the early stage of growth (Table 2). This increment induced only minor changes in ETR and Φ_{PSII} and the effect of these changes determined the reduction in CO_2 photoassimilation. In accordance with the absence of leaf chlorosis, Cd-treated leaves had the Chl content similar to the controls (the slight decrease was not statistically significant) indicating that the heavy metal neither inhibited biosynthesis nor induced oxidation of Chl (under the experimental conditions applied in this experiment) as reported by other authors (Krupa *et al*. 1993, Krupa 1999, Castagna *et al.* 2013). Definitively, Cd reduced the plant growth without strong negative effects at the level of photosynthesis.

The effects induced by O_3 were quite different in tomato plants. There was a strong and significant reduction in P_N and it was linked to a reduction in g_s albeit the C_i did not change (Fig. 3). These results indicated that simultaneously stomatal and nonstomatal limitations were involved in the changes of CO_2 assimilation. In addition, the F_v/F_m ratio was significantly reduced in the leaves exposed to O_3 indicating that light reactions were also negatively influenced by O_3. The Φ_{PSII} and the Φ_{exc} decreased significantly following the O_3 exposure in tomato leaves. The Φ_{exc} parameter can be interpreted as a measure of the relative integrity of the PSII reaction centers, because its decrease is associated with downregulation of PSII or damage to the reaction centres (Baker and Oxborough 2004). The Φ_{PSII} is nonlinearly related to the oxidation state of the Q_A pool and changes in Φ_{PSII} can be attributed to changes downstream of PSII, including Rubisco and the Calvin cycle (Baker and Oxborough 2004).

Results obtained from gas exchange and Chl fluorescence measurements indicate that O_3 induced an alteration in biochemical processes of photosynthesis which in turn enhanced photoinhibition (reduction in F_v/F_m) as previously found by Crous *et al*. (2006) and Flowers *et al*. (2007).

Under the photoinhibiting conditions, O_3-treated tissues activated the xanthophyll cycle to dissipate the surplus of light energy intercepted and absorbed by the antennae as already reported in many plant species (Alonso *et al*. 2001, Castagna *et al*. 2001, Scebba *et al*. 2003, Pellegrini *et al*. 2011). The O_3-induced activation of the xanthophyll cycle, indicated by the increase in the DEPS index (Table 3) and in NPQ (Table 2). This suggests that tomato thylakoids actively reacted to O_3, in an attempt to alleviate the excitation pressure on the reaction centres.

When tomato plants were subjected to combined stress, some differences were observed with respect to what occurred when plants were exposed to Cd or O_3 alone. The strong reduction in CO_2 assimilation was recorded and it was attributed to both stomatal and nonstomatal limitations (g_s decreased and C_i unchanged). However, tomato plants subjected to both Cd and O_3 stress showed lower ability to activate mechanisms aimed to dissipate excess excitation energy. In fact, NPQ increased in the plants subjected to both stresses, but to a lower extent as compared with those observed in the O_3-treated leaves. On the other hand, the DEPS index increased significantly as compared with the controls, but at a lower extent as compared with the plants treated with O_3.

Although this work was not addressed to evaluate the defensive strategies set up in response to Cd, this metal is known to stimulate the cell antioxidant machinery (Di Toppi and Gabrielli 1999, Di Cagno *et al*. 2001, Ranieri *et al*. 2005). Thus, we could postulate that the plants grown in Cd-enriched soil possessed a greater antioxidant potential that enabled them to cope better with O_3-triggered oxidative stress. On the other hand, nonphotochemical quenching *via* the xanthophyll cycle represents a protective tool that preserves thylakoid membranes from lipid peroxidation (Demmig-Adams and Adams 1996). In this context, it is worth noticing that photosynthesis was generally less affected by Cd treatment than by O_3 exposure. Moreover, Cd-treated leaves did not develop any sign of Cd toxicity, only reduced their growth, which apart from a diminished CO_2 assimilation might originate from an enhanced demand for carbon compounds used in detoxification-repair processes.

Conclusion: Tomato plants seemed to adapt partially to Cd stress as indicated by the reduced growth and photosynthetic rate without consistent alterations of Chl fluorescence parameters. Under O_3 exposure, tomato plants suffered major constraints at the photosynthetic level despite the active heat dissipation of excess energy *via* the xanthophyll cycle. These downregulated mechanisms were less efficient when tomato plants were subjected to both the stresses.

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