BIOLOGIA PLANTARUM 51 (2): 313-321, 2007

Effects of elevated ozone on chlorophyll *a* fluorescence in symptomatic and asymptomatic leaves of two tomato genotypes

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Abstract

Two different genotypes of *Lycopersicon esculentum* Mill. (cv. Cuor di Bue, O_3-sensitive and line 93.1033/1, O_3-resistant) were treated with a single dose of ozone (150 mm^3 m^{-3} for 3 h). The PS 2 activity was examined by measurements of chlorophyll *a* fluorescence on symptomatic and asymptomatic leaves. Symptoms were evident on the 4th leaves from the bottom, in both genotypes, while the 2nd leaves of the line 93.1033/1 were asymptomatic. In these leaves, the net photosynthetic rate (P_N) did not change even if the F_v/F_m ratio significantly decreased. A strong reduction in P_N, mostly due to the stomatal closure, was observed in Cuor di Bue. The non photochemical quenching coefficient (q_{NP}) and the degree of PS 2 reaction centres closure ($1-q_P$) were higher, while the quantum efficiency of PS 2 photochemistry (Φ_{PS2}) and quantum efficiency of excitation energy capture ($\Phi_{exc.}$) were lower in O_3 treated leaves of both genotypes. The limitation of photosynthesis was shown also by a decrease in the parameter %P, which diminished compared to controls in both genotypes. The response of the two genotypes for the energy fraction dissipated as thermal energy in the PS 2 antennae (%D) was similar. The fraction of %P remained lower during the recovery in symptomatic leaves of the resistant line as compared to the controls, whereas %X, which represents the amount of light energy that is not utilized in photochemistry or dissipated in the PS 2 antennae, significantly rose in the asymptomatic leaves of this line and in both the leaves of Cuor di Bue. From data obtained we concluded that ozone affected the plants independently on the appearance of visible symptoms of injury because the leaves without visible symptoms of both the genotypes were negatively influenced.

Additional key words: *Lycopersicon esculentum*, net photosynthetic rate, quenching analysis.

Introduction

Ozone is one of the most dangerous phytotoxic air pollutants (Matyssek *et al*. 1997). Its toxicity is principally attributed to the oxidizing potential which, in plants, determines the production of the activated species of oxygen (AOS) (Schraudner *et al*. 1998, Rao and Davis 1999).

Photosynthesis is particularly sensitive to ozone (Heath 1994, Schraudner *et al*. 1997). The reduction in the net photosynthetic rate can be determined by a direct effect of O_3 on the stomatal opening (Robinson *et al*. 1998), but also by an alteration of the electron transport rate and the biochemical activity of the Calvin cycle (Calatayud and Barreno 2001, Calatayud *et al*. 2002, Degl'Innocenti *et al*. 2002b).

The responses of plants to ozone differ among species and also among cultivars of the same species (Guidi *et al*. 2000). In a previous work (Guidi *et al*. 2005) we have reported the differing O_3 sensitivity of two tomato

Received 17 March 2005, *accepted* 27 January 2006.

Abbreviations: c_i - intercellular CO_2 concentration; E - transpiration rate; ETR - electron transport rate; F_0 - minimum Chl fluorescence in dark adapted state; F_m - maximum Chl fluorescence in dark adapted state; F_m' = maximum Chl fluorescence with all PS 2 reaction centres closed in light adapted state; F_s - Chl fluorescence in steady state conditions; F_v - variable Chl fluorescence in dark adapted state; F_v' - variable Chl fluorescence in light adapted state; F_v/F_m - photochemical PS 2 photochemistry in dark adapted state; g_s - stomatal conductance to water vapour; PFD - photon flux density; P_N - net photosynthetic rate; PS 2 - photosystem 2; q_{NP} - non-photochemical quenching; q_P - photochemical quenching; Φ_{exc} - excitation capture efficiency of PS 2; Φ_{PS2} - actual PS 2 efficiency; %D - fraction of light absorbed in PS 2 that is dissipated in the PS 2 antenna; %P - fraction of light absorbed that is used in photochemistry; %X - fraction of light absorbed that is not used or dissipated in the PS 2 antenna.

Acknowledgements: this research was supported by the MIUR (National Project), Rome, Italy. The authors wish to thank SAIS, Cesena, FO (Italy) for providing the seeds.

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genotypes which are known also for their different susceptibility to pathogens: cv. Cuor di Bue, sensitive to *Verticillium* spp., *Fusarium* spp. and to the tobacco mosaic virus (TMV) and line 93.1033/1, resistant to the same pathogens. In that research we found that, in terms of visible symptoms of injury, the line 93.1033/1 showed resistance to ozone, while the pathogen-sensitive Cuor di Bue showed visible symptoms of a damage probably due to ozone. This different sensitivity to ozone was shown by seedlings which presented only two leaves.

The aim of this paper was the evaluation of the different responses of symptomatic and asymptomatic leaves of these two tomato genotypes to an acute ozone treatment in terms of changes in chlorophyll *a* fluorescence, including quenching analysis, and in gas exchange measurements.

Materials and methods

Plant material: Seeds of two genotypes of *Lycopersicon esculentum* Mill. (cv. Cuor di Bue and line 93.1033/1, sensitive and resistant to some pathogens, respectively) were sown in a sterilized soil. The seedlings emerged 20 - 30 d later and were grown thereafter in a greenhouse (temperature of 25 ± 3 °C, relative humidity 75 ± 5 %, 12-h photoperiod at an irradiance of about 400 μmol m^{-2} s^{-1}). The tomato plants were grown to the 5th leaf stage and then placed in 2 growth chambers, one to permit ozone treatment, 24 h before the fumigation. In the experiment, we analyzed the 2nd (mature) and the 4th (young) leaves from the bottom. The measurements in symptomatic leaves were carried out on asymptomatic area.

Ozone fumigation: The O_3 treatments were performed in a controlled-environment chamber (*Cavallo*, Milan, Italy): more details of the O_3 exposure are reported in Guidi *et al* (2000). A single ozone fumigation was carried out for 3 h at an irradiance of 350 μmol m^{-2} s^{-1}, temperature of 25 ± 3.1 °C and relative humidity 81 ± 7 %. The plants were treated for 3 h with a single dose of O_3 (150 ± 6.7 and 150 ± 2.3 mm^3 m^{-3}, respectively for the first and second experiment) or maintained in charcoal-filtered air. After the end of the O_3 fumigation, ozonated plants were put in the same chamber with filtered air, where the control plants were placed.

Gas exchange analysis: Gas exchange measurements [net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (c_i)] were carried out at the end of the treatment on the second fully developed leaf from the bottom and using an open system (*CMS-400*, *Walz*, Effeltrich, Germany; for details see Guidi *et al*. 1997). Measurements were carried out at an irradiance of about 800 μmol m^{-2} s^{-1}, temperature 25 ± 2.3 °C, relative humidity 70 ± 5.2 %, CO_2 concentration 350 μmol mol^{-1} and O_2 concentration 21 %.

Chlorophyll fluorescence analysis: Modulated chlorophyll *a* fluorescence measurements were made with a *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany) on second and fourth leaves, counting from the bottom, at the end of the fumigation and 24 and 48 h after the end of O_3 treatment. Leaves were pre-darkened for 40 min in leaf-clips before measurement. Firstly a weak irradiance (< 0.1 μmol m^{-2} s^{-1}) was used to obtain basic fluorescence (F_0). Successively, a 1-s saturating pulse of white light (15 000 μmol m^{-2} s^{-1}) was given to determine maximum fluorescence (F_m) when all PS 2 reaction centres are closed in the dark. These parameters were used for the calculation of the F_v/F_m ratio which means the maximum photochemical capacity of PS 2. The saturation pulse method was used for the analysis of quenching components according to Schreiber *et al*. (1986). Intermittent, short-term illumination by sufficiently strong radiation causes a transient, but complete removal of photochemical quenching, prompting a corresponding increase in variable fluorescence, F_v to F_v'; the residual quenching is assumed to have non-photochemical nature. The photon flux density (PFD) of the actinic light was maintained at 300 μmol m^{-2} s^{-1} and a sequence of saturating flashes of white light 15 000 μmol m^{-2} s^{-1} was given firstly every 20 s and then increases to 40 s and then to 80 s. The overall time of illumination during the quenching analysis was 20 min. After the saturating pulse, the maximum fluorescence yield reached the F_m' value and the actinic radiation allowed both steady-state photosynthesis and steady-state fluorescence yield (F_s). Determination of quenching components q_P and q_{NP} was done as defined by Schreiber *et al.* (1986), *i.e.* $q_P = (F_m' - F_s)/F_v'$ and $q_{NP} = 1 - (F_v'/F_v)$. Minimum fluorescence in the light-adapted state (F_0') was determined immediately after turning off the actinic source in the presence of a far-red (7 μmol m^{-2} s^{-1}) background for 10-s to ensure maximum oxidation of PS 2 electron acceptors. The actual quantum yield of PS 2 photochemistry (Φ_{PS2}) and the quantum efficiency of open PS 2 reaction centres in light conditions ($\Phi_{exc.}$) were computed as $(F_m' - F_s)/F_m'$ and F_v'/F_m', respectively, as reported by Genty *et al*. (1989). Apparent rates of photosynthetic electron transport (ETR) were estimated as $(\Phi_{PS2} \times 0.5) \times (PPFD \times 0.8)$ where factor 0.5 represents the excitation of both PS 2 and PS 1 and factor 0.8 represents the average value for leaf absorbance.

The fraction of radiation absorbed that are dissipated in the PS 2 antennae (%D) and utilized in PS 2 photochemistry (%P) were estimated as $[1-(F_v'/F_m')] \times 100$ and $[(F_v'/F_m') \times q_P] \times 100$, respectively (Demmig-Adams and Adams 1996). It should be noted that although the parameter %P is proportional to Φ_{PS2}, both are still commonly used in the literature. The fraction of radiation absorbed by PS 2 which is not utilized in photochemistry

or dissipated in the PS 2 antennae (%X), was estimated from $[(F_v'/F_m') \times (1-q_P)] \times 100$, according to Demmig-Adams and Adams (1996).

Statistical analysis: Two experiments were done in two different periods of the year (May - June and July 2003). For each experiment, 2 growth chambers were used. The chambers were equivalent, but one was fed with filtered air and the other with ozone. Ten-fifteen potted plants were put in each chamber. After treatment, two plants, similar in their growth, were chosen from each chamber and the 2nd and the 4th leaves from every plant were utilized. The data obtained from each experiment were pooled, in order to obtain 4 replicates for both leaves (2nd and 4th). Data were subjected to three way analysis of variance in which the age of leaves, ozone treatment and time represent the three factors of variability. Multiple comparison was conducted on significant ($P<0.05$) three-way interactions using least square means analysis. The significance of pairwise comparisons was determined by using a Bonferroni adjustment with $\alpha = 0.05/14 = 0.0036$ for three-way interaction.

Data measured as a percentage were angularly transformed previously to the *ANOVA*. For gas exchange analysis, the one way *ANOVA* was applied with the O_3 treatment as variability factor.

Results

Visual symptoms: The day after the end of the fumigation, visible symptoms of injury due to the presence of O_3 appeared on the young leaves on both genotypes. Symptoms were principally located on the third and fourth leaves, counting from the bottom, and were characterized by chlorotic and/or necrotic areas on the margins of the leaves.

Gas exchange: Measurements were carried out only in the asymptomatic leaves at the end of the O_3 fumigation. In the sensitive Cuor di Bue a significant decrease in P_N, accompanied by a reduction in g_s was observed following the ozone treatment (Table 1). Also E and c_i decreased in O_3-treated plants. In the resistant line 93.1033/1, no changes in P_N and c_i were observed after the O_3 fumigation, while a significant increase in g_s and E was recorded (Table 1).

Chlorophyll *a* fluorescence: Minimum fluorescence yield F_0 in line 93.1033/1 was significantly greater in ozonated leaves than in controls (Table 2) while no effects were found in relation to the time and leaf age. There were no significant two-way or three-way interactive effects of different treatments on F_0 in line 93.1033/1. Maximum fluorescence F_m and the ratio F_v/F_m significantly decreased because of the presence of O_3, with the major effects detected on leaf No. 4 at the end of the experiment (Fig. 1).

Ozone induced alterations in the values of F_0 and the ratio F_v/F_m in the 2nd and 4th leaves of Cuor di Bue, while no changes were evident in the values of F_m (Fig. 1, Table 2). The time factor affected maximum fluorescence in this tomato variety, but not the F_0 and F_v/F_m ratio. Considering the significant leaf age-ozone interaction ($P < 0.05$), the comparison showed that the air pollutant increased the F_0 in the symptomatic leaves compared to the 4th one (asymptomatic) but there was no effect of time. The three-way interaction was not significant for F_o, F_m and F_v/F_m in Cuor di Bue (Table 2).

The quenching coefficients ($1-q_P$) and q_{NP} of both genotypes were significantly higher in the ozonated leaves than in controls, according to time and leaf age treatments (Fig. 2, Table 2). In line 93.1033/1 the reduction state of Q_A, *i.e.* ($1-q_P$), significantly increased in both the leaves at the end of the fumigation and also during the recovery (Fig. 2). The behavior of q_{NP} coefficient was different, changing significantly in relation to the presence of O_3 and the time of treatment.

Table 1. Gas exchange parameters determined in two genotypes of *Lycopersicon esculentum* leaves exposed to a single dose of O_3 (150 mm^3 m^{-3} for 3 h). Controls were represented by plants maintained in filtered air. Measurements were carried out at the end of the fumigation (O_3). Analysis were carried out on the 2nd leaves counting from the bottom. Each data represents the mean of 4 replicates ± standard deviation. Analysis were made in saturating irradiance (about 800 µmol $m^{-2}s^{-1}$), temperature of 25 ± 2.3 °C, RH of 70 ± 5.2 % and 350 µmol(CO_2) mol^{-1}. P_N - net photosynthetic rate; g_s - stomatal conductance; E - transpiration rate; c_i - intercellular CO_2 concentration. Data are subjected to the one way *ANOVA*; significant differences between ozonated and controls at the $P < 0.05$ (*) and $P < 0.01$ (**)

Genotype	Treatment	P_N [µmol(CO_2) m^{-2} s^{-1}]	g_s [mmol(H_2O) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	c_i [µmol(CO_2) mol^{-1}]
93.1033/1	control	5.19 ± 1.31	238 ± 47.1	3.8 ± 0.78	302 ± 3.0
	O_3	6.22 ± 0.70	301 ± 69.2*	4.9 ± 1.12*	303 ± 4.3
Cuor di Bue	control	5.20 ± 0.81	254 ± 16.1	4.2 ± 0.16	304 ± 3.0
	O_3	3.80 ± 0.95*	143 ± 33.0**	2.4 ± 0.54**	295 ± 1.4**

Table 2. Analysis of variance for parameters of chlorophyll fluorescence kinetics in tomato leaves (93.1033/1 and Cuor di Bue). ns - not significant, * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

Genotype		d.f.	F_0	F_m	F_v/F_m	$1\text{-}q_P$	q_{NP}	Φ_{PS2}	Φ_{exc}	ETR
93.1033/1	ozone	1	57.11***	75.81***	149.72***	282.06***	115.37***	449.38***	198.13***	286.99***
	time	2	2.71ns	4.60*	13.10***	14.64***	11.90***	25.93***	17.99***	10.87***
	age	1	0.02ns	31.25***	37.66***	0.20ns	0.08ns	0.99ns	19.56***	6.08*
	age×time	2	1.08ns	12.65***	12.90***	6.50**	0.74ns	1.81ns	2.34ns	1.75ns
	age×ozone	1	1.08ns	53.31***	50.59***	3.32ns	1.80ns	6.76*	17.49***	13.48***
	time×ozone	2	2.71ns	4.60*	13.10***	14.63***	11.90***	25.92***	17.99***	10.87***
	time×age×ozone	2	0.15ns	12.65***	12.91***	6.50**	0.74ns	1.81ns	2.34ns	1.65ns
Cuor di Bue	ozone	1	70.85**	0.69ns	13.07***	155.32***	248.39***	1721.55***	441.44***	516.19***
	time	2	0.13ns	5.74**	1.95ns	30.80***	2.00ns	60.76***	14.04***	58.00***
	age	1	0.01ns	0.06ns	0.003ns	0.58ns	10.12**	0.04ns	8.72**	0.35ns
	age×time	2	1.68ns	0.05ns	0.31ns	0.83ns	0.19ns	0.25ns	0.58ns	0.18ns
	age×ozone	1	6.92*	2.95ns	0.45ns	0.49ns	12.17**	1.84ns	0.41ns	0.08ns
	time×ozone	2	2.52ns	5.63**	2.34ns	23.67***	0.53ns	60.76***	14.04***	58.01***
	time×age×ozone	2	0.53ns	0.06ns	0.31ns	0.43ns	0.65ns	0.25ns	0.58ns	0.18ns

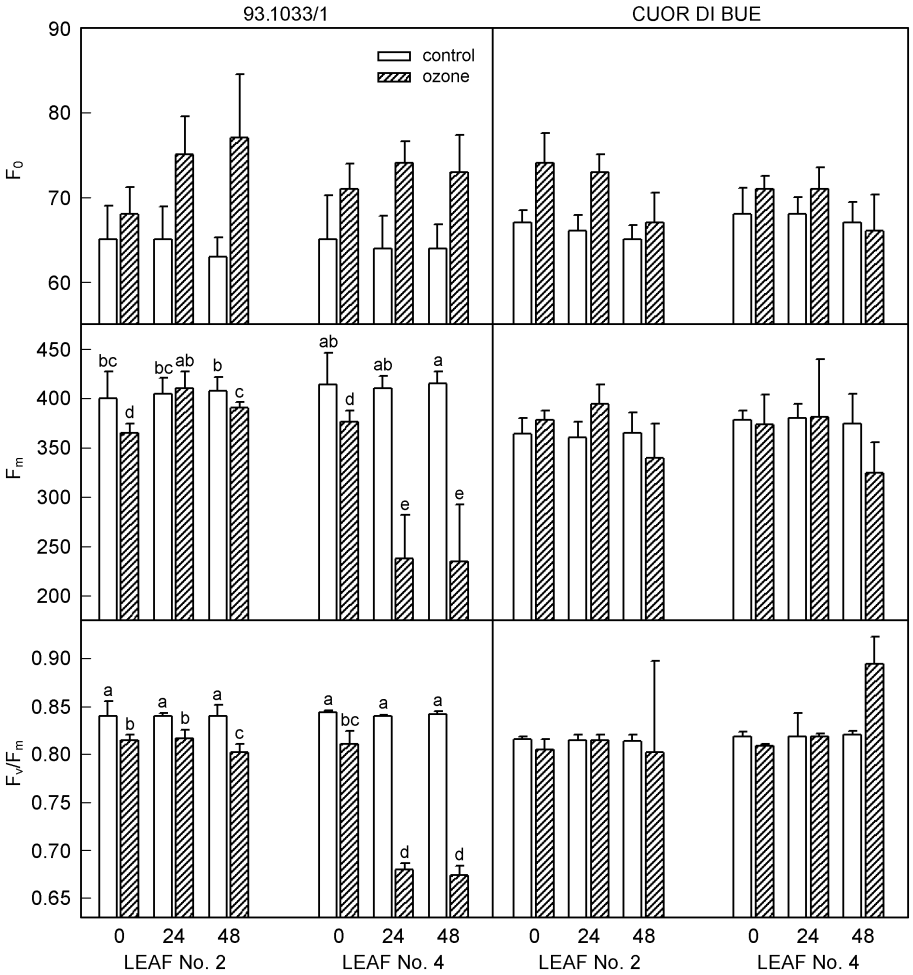

Fig. 1. Minimum and maximum fluorescence yields (F_0 and F_m) and the ratio F_v/F_m in the 2nd and 4th leaves, counting from the bottom, of *Lycopersicon esculentum* 93.1033/1 and Cuor di Bue. Plants were exposed to a single dose of O_3 (150 mm^3 m^{-3} for 3 h) (*grey bars*) or to charcoal-filtered air (*white bars*). The measurements were carried out at 0, 24 and 48 h after the end of the fumigation. Each value represents the mean of four replicates. *Bars* indicate the standard deviation. Different letters over the bars indicate significant differences between means in pairwise comparison within a three-way interaction using a Bonferroni adjustment ($P < 0.0036$).

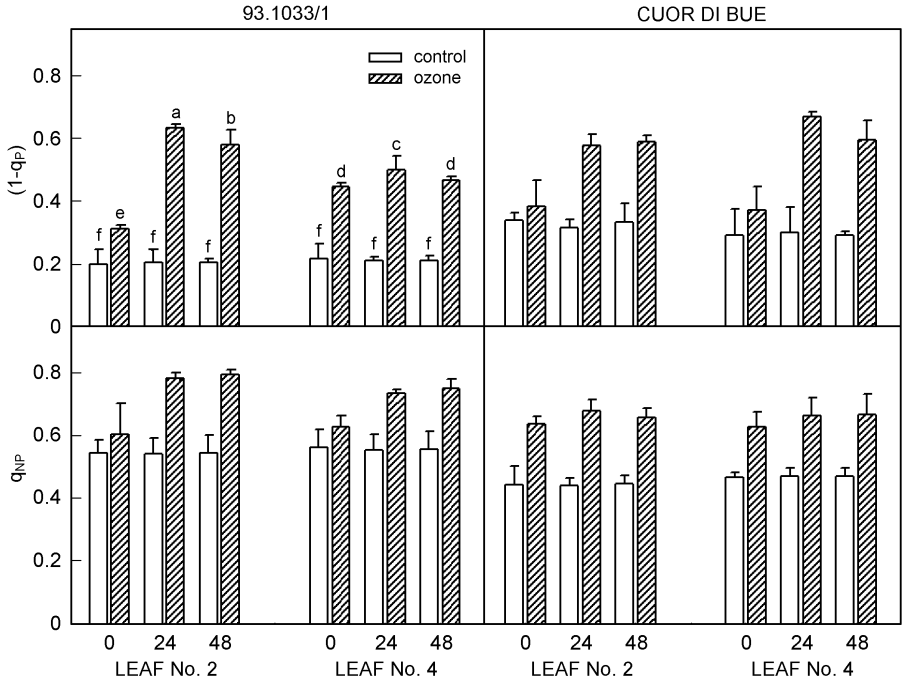

Fig. 2. Reduction state ($1-q_P$) and the non photochemical quenching coefficient (q_{NP}) in the 2nd and 4th leaves, counting from the bottom, of *Lycopersicon esculentum* 93.1033/1 and Cuor di Bue. Plants were exposed to a dose pulse of O_3 (150 mm^3 m^{-3} for 3 h) (*grey bars*) or to charcoal-filtered air (*white bars*). The measurements were carried out at 0, 24 and 48 hafter the end of the fumigation. Each value represents the mean of four replicates. *Bars* indicate the standard deviation. Different letters over the bars indicate significant differences ($P < 0.0036$).

The interaction between these two factors was also significant (Table 2). There were no other significant two-way or three-way interactive effects of treatments on the q_{NP} coefficient in line 93.1033/1.

In Cuor di Bue a significant effect on ($1-q_P$) was attributable to ozone and time and also the interaction between these two factors was significant (Table 2). This parameter did not change in relation to time and no effects were recorded for the other two-way or three-way interactive effects of treatments (Table 2). Non-photochemical quenching q_{NP} was significantly higher in the 4th leaf of Cuor di Bue, while no differences were found in relation to time. Two-way interaction between ozone and leaf age was significant while time × ozone and the three-way interaction had no reflections in q_{NP}.

The actual PS 2 efficiency (Φ_{PS2}) significantly decreased following of ozone treatment in both the genotypes (Table 2, Fig. 3), with major effects recorded the days after the end of the treatment. However, there was no significant difference between the 2nd and 4th leaf in both the genotypes and the two-way interaction between leaf age and time was not significant. In line 93.1033/1 the interaction between leaf age and ozone and time × ozone was significant, with major effect attributable to ozone treatment in the 2nd leaf on the day after the end of the exposure to the pollutant (Table 2, Fig. 3). In Cuor di Bue the parameter Φ_{PS2} significantly changed only in relation to time without effects due to the leaf age.

The values of $\Phi_{exc.}$, which reflect the excitation capture efficiency of open PS 2 centres in the light, significantly decreased both in symptomatic and asymptomatic leaves of Cuor di Bue following the O_3 exposure compared to controls, as well as the line 93.1033/1 (Table 2, Fig. 3). The decrease in $\Phi_{exc.}$ was strong during the days after the end of the fumigation (Fig. 3). There were significant two-way interactive effects of treatments (leaf age × ozone and ozone × time) on $\Phi_{exc.}$ values in line 93.1033/1, while no significant three-way interactive effects were observed. In Cuor di Bue only the two-way interaction between time and ozone was significant (Table 2).

The electron transport rate significantly decreased also in both symptomatic and asymptomatic leaves of the line 93.1033/1 at the end of fumigation without recovering when plants were put in O_3-free air. Comparisons showed that ozone significantly decreased ETR in both the 2nd and the 4th leaves in line 93.1033/1, but there was no significant interactive three-way effect (Table 2). In Cuor di Bue the three factors of variability (ozone, leaf age and time) significantly reduced ETR values (Table 2, Fig. 3) and the only interactive effect was between leaf age and ozone (Table 2).

Following the ozone treatment, in the resistant line 93.1033/1 the amount of radiation absorbed by antennae and utilized in photochemistry (%P) significantly decreased during the time of the experiment (Table 3, Fig. 4), while no differences were found between the 2nd and 4th leaves. Only the two-way interactive effect of ozone × time was significant. A similar response was observed for %P in Cuor di Bue (Table 3).

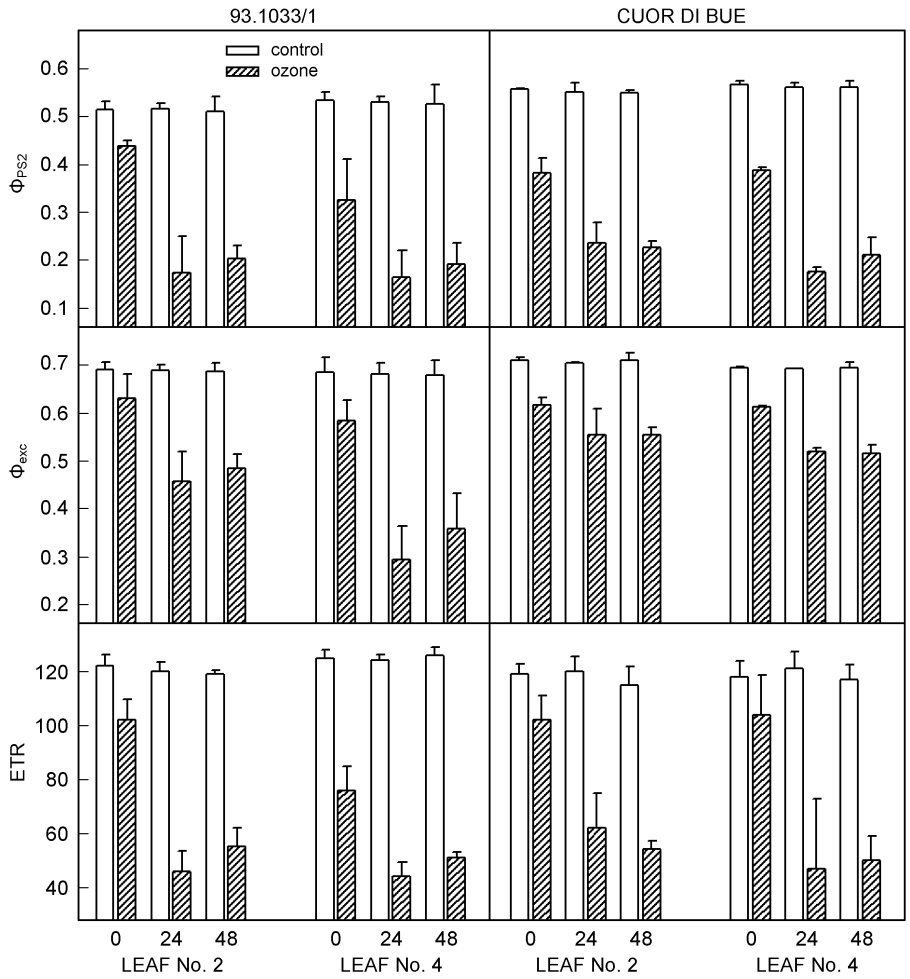

Fig. 3. Actual photochemical efficiency (Φ_{PS2}), intrinsic PS 2 efficiency ($\Phi_{exc.}$) and electron transport rate (ETR) in the 2nd and 4th leaves of *Lycopersicon esculentum* 93.1033/1 and Cuor di Bue. Plants were exposed to a single dose of O_3 (150 mm^3 m^{-3} for 3 h) (*grey bars*) or to charcoal-filtered air (*white bars*). The measurements were carried out at 0, 24 and 48 h after the end of the fumigation. Each value represents the mean of four replicates. *Bars* indicate the standard deviation.

The amount of dissipated radiation absorbed (%D), significantly increased in both the leaves of line 93.1033/1 following the ozone exposure and the increase was more marked the days after the end of fumigation. The highest values were recorded 24 h after the end of O_3 exposure in the 4th leaf when %D reached values of about 70 % (Table 3, Fig. 4). No interactive three-way effects were detected in this line. In Cuor di Bue %D increased significantly in both leaves after the ozone fumigation with a stronger increase during the recovery in O_3-free air (Table 3, Fig. 4). The interactive effect of time × ozone was significant, whereas the other two- and three-way effects were not significant (Table 3).

Finally, the amount of radiation absorbed, which is neither used for PS 2 photochemistry nor dissipated in the PS 2 antennae (%X), significantly increased in line 93.1033/1 at the end of the fumigation with ozone (Table 3). During the recovery time (24 and 48 h after the end of the fumigation) %X high without differences attributable to the leaf age in line 93.1033/1 (Table 3), while in Cuor di Bue, the %X significantly increased in both leaves in plants exposed to ozone, but no differences were found in relation to the leaf age (Table 3). The only interactive effects was between leaf age and ozone as %X increased in the 4th leaf (Fig. 4).

Discussion

This experiment was carried out on plants which had 5 leaves and the O_3 fumigation induced visible symptoms of injury only in the young leaves of both genotypes (the 4th from the bottom). This indicates that the sensitivity of the plants to ozone fumigation was related to the age of the leaf, as the young leaves of both genotypes showed

Table 3. Analysis of variance for energy distribution parameters determined by using chlorophyll fluorescence measurements determined in leaves tomato (93.1033/1 and Cuor di Bue). For each source of variation the value and significance of the F ratio are reported (ns - not significant, * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$).

Genotype		d.f.	%P	%D	%X
93.1033/1	O_3	1	356.19*	191.13***	19.77***
	time	2	1.24*	19.05***	13.89***
	age	1	4.65ns	11.24**	3.93***
	age×time	2	5.91ns	0.71ns	0.76ns
	age×O_3	1	10.08ns	8.76**	0.46ns
	time×O_3	2	0.76***	19.05***	13.89***
	time×age×O_3	2	5.28ns	0.71ns	0.76ns
Cuor di	ozone	1	189.14***	238.03***	20.08***
Bue	time	2	18.94***	8.38**	9.43**
	age	1	1.82ns	4.99*	0.05ns
	age×time	2	0.57ns	0.31ns	1.00ns
	age×O_3	1	0.32ns	0.20ns	0.11ns
	time×O_3	2	14.72***	8.38**	8.08**
	time×age×O_3	2	0.24ns	0.31ns	0.04ns

symptoms. In order to evaluate if changes in PS 2 activity were related to the effects of ozone on photosynthesis, chlorophyll *a* fluorescence measurements were carried out in leaves with visible symptoms and leaves without them of both genotypes. As both genotypes were grown in the same conditions, the effects of ozone also in the asymptomatic leaves, could be present.

Results obtained from gas exchange analysis indicate that the asymptomatic (the 2nd counting from the bottom) leaves of Cuor di Bue showed an alteration in P_N mainly due to a reduced g_s. The behavior of the resistant genotype 93.1033/1 was different: no changes were recorded in the P_N, whereas the g_s significantly increased. The data obtained indicate that the greater O_3 tolerance of line 93.1033/1 is not attributable to lower absorption of the pollutant. These results agree with the findings of Degl'Innocenti *et al*. (2002a) in the tobacco-tolerant cultivar BelB and also with those of Pell and Pearson (1983) and Langebartels *et al*. (1991), who found that O_3 did not influence stomatal closure.

Acute O_3 exposure caused a significant decrease in the dark-adapted variable-to-maximum fluorescence yield ratio (F_v/F_m) only in the young and symptomatic leaves of

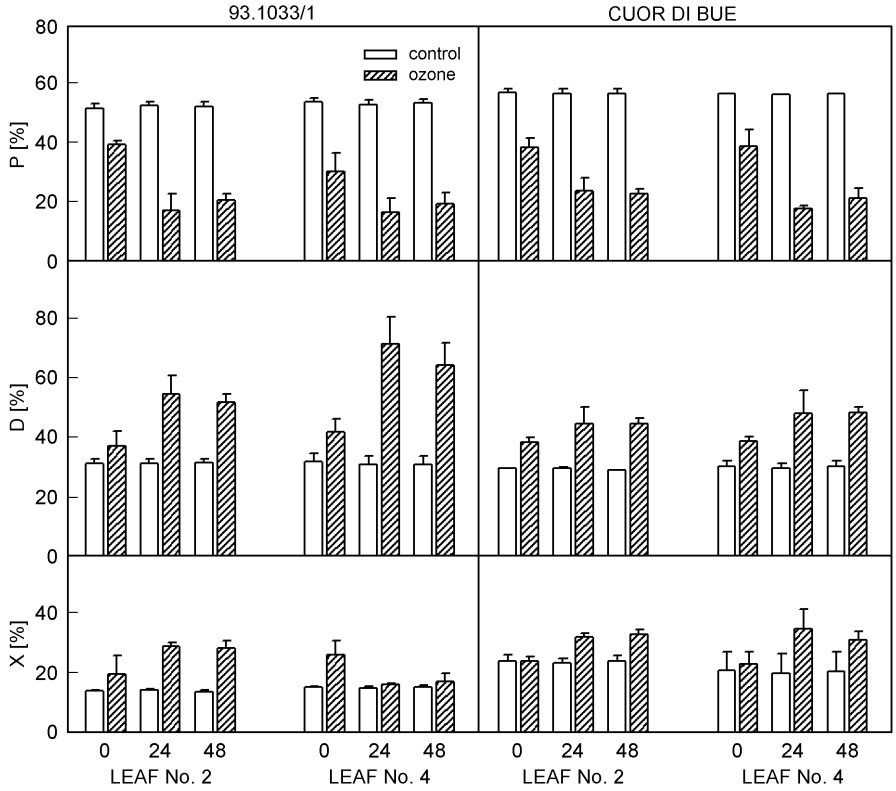

Fig. 4. Fraction of absorbed radiation utilized in PS 2 photochemistry (%P), fraction of absorbed radiation dissipated in the PS 2 antennae (%D) and absorbed radiation that is not utilized in photochemistry or dissipated in the PS 2 antennae (%X) in the 2nd and 4th leaves of *Lycopersicon esculentum* 93.1033/1 and Cuor di Bue. Plants were exposed to a single dose of O_3 (150 mm^3 m^{-3} for 3 h) (*grey bars*) or to charcoal-filtered air (*white bars*). The measurements were carried out at 0, 24 and 48 h after the end of the fumigation. Each value represents the mean of four replicates. *Bars* indicate the standard deviation. Data are angularly transformed previous of the application of *ANOVA* test.

the resistant line 93.1033/1, while the effects were minor in the asymptomatic leaves which showed also an unaffected P_N. The decline in F_v/F_m observed in leaves of this genotype was related to the increase in F_0 and a decrease in F_m values. This indicates that the photochemistry of PS 2 and its ability to reduce the primary acceptor Q_A were affected by ozone, as already reported (Calatayud and Barreno 2001).

In Cuor di Bue, only a mild increase in the values of F_0 was observed, indicating that O_3 induced modifications at the level of the antenna pigments or in the photochemical efficiency of the reaction centers of PS 2. These perturbations determined only slight changes in the ratio of F_v/F_m.

The increase of $(1\text{-}q_P)$ in symptomatic and asymptomatic leaves of both the genotypes suggests that ozone caused a decrease in the fraction of open PS 2 reaction centers (Krause and Weiss 1991). This could be attributable to a slow re-oxidation of Q_B, which may be due to the inhibition of Calvin cycle activity, as already reported about ozonated leaves of tobacco species (Degl'Innocenti *et al*. 2002a). However, we found a reduction in P_N only in the asymptomatic leaves of Cuor di Bue at the end of the fumigation, which was mainly attributable to stomatal closure. These results indicate that the first limitation to the P_N response to O_3 in Cuor di Bue is at stomatal level. Then the reduction in stomatal conductance determined a diminished availability of CO_2 at mesophyllic level. Also Calatayud *et al*. (2003) reported that in spinach there were no apparent effects on the ratio F_v/F_m even if the CO_2 assimilation rate significantly decreased. Similar results have been obtained by other authors in other plant species (Gimeno *et al*. 1999).

The non photochemical quenching coefficient q_{NP} was higher in leaves showing visual symptoms, but increased also in leaves that remained free of visible injury. The increased q_{NP} in both the genotypes following ozone treatment corresponds to a higher activation of defense and reparative mechanisms of non-photochemical nature.

The efficiency of excitation energy capture by open PS 2 reaction centers (Φ_{exc}) significantly changed in both the leaves of the two genotypes. Although the decrease in excitation capture efficiency is prevalently accompanied with an increase in energy dissipation in the antenna (Demmig-Adams *et al*. 1995), the mechanisms whereby ozone induces the reduction of this parameter are unknown, as reported also by Carrasco Rodriguez and Del Valle-Tascon (2001).

Also the Φ_{PS2}, closely correlated with the quantum yield of non-cyclic electron flow (Genty *et al*. 1989), was reduced in both tomato genotypes and both symptomatic and asymptomatic leaves by O_3 exposure. It indicates an inhibition of electron chain and was confirmed also by the reduction of %P, *i.e*. the amount of radiation absorbed by PS 2 antennae and used for photochemistry. The reduction in Φ_{PS2} is due both to the decline of q_P and the increase in q_{NP}.

The data obtained in this work seem to indicate that the different response of plants to ozone varies also in relation to the leaf age. In fact, these two tomato genotypes showed a different response when exposed to a single dose of ozone in terms of visible symptoms of injury, but also at a physiological level, as shown by the chlorophyll *a* fluorescence measurements. P_N was reduced only in Cuor di Bue while no change was observed in line 93.1033/1, in which mechanisms aimed at limiting the damage to the photosynthetic apparatus can be involved. On the other hand, a similar response was already observed in an O_3-tolerant cultivar of clover (Degl'Innocenti *et al*. 2003). Many hypotheses have been proposed to explain the effects of ozone on plants (Pell *et al*. 1997, Schraudner *et al*. 1997). In this article we suggest that ozone-induced effects are different in two tomato genotypes. In the O_3-resistant line 93.1033/1 the strong decrease in the optimal photochemical PS 2 efficiency (F_v/F_m) is linked to an increase in $(1\text{-}q_P)$, which determined also a large fraction of PS 2 reaction centres that are incapable of stable charge separation. This decreased ability of Q_A re-oxidation may be due to the large fraction of excitation energy which was dissipated mostly to heat (increased q_{NP}) and no differences were found between leaves with or without symptoms. In Cuor di Bue, the F_v/F_m ratio did not change significantly due to the presence of O_3, but the efficiency of PS 2 photochemistry in conditions of a steady state of photosynthesis was significantly affected. Even in Cuor di Bue, the leaf age did not significantly affect the response of the plants to the pollutant.

In conclusion, the data obtained in this work show that ozone affected the plants independently on the appearance of visible symptoms of injury because the leaves without symptoms of both the genotypes were negatively influenced. Also line 93.1033/1, which in a previous report was considered as resistant to O_3, showed symptoms on the 4th leaf and alteration in the photochemical efficiency of PS 2. Considering that in this genotype a stimulation of the phenylpropanoid metabolism was observed (Guidi *et al*. 2005), we can suggest that a defence O_3-induced mechanism of response was involved in line 93.1033/1.

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