Effects of long-term ozone exposure on citrus: Chlorophyll *a* fluorescence and gas exchange

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Abstract

Three-years-old trees of Satsuma mandarin (*Citrus unshiu* [Mak.] Marc.) cv. Okitsu were exposed to O_3 fumigation during long term (one year) in open-top chambers. As a result of the treatment, chlorophyll *a* fluorescence and gas exchange parameters were modified with respect to trees growing in O_3-free conditions. Net photosynthetic rate and stomatal conductance decreased and intercellular CO_2 concentration increased according to a reduction of the non-cyclic electron flow and a lower capacity to reduce the quinone pool. O_3 also reduced the development of non-photochemical quenching preventing the dissipation of excess excitation energy and, therefore, generated several alterations in photosynthetic apparatus. All these effects were obtained in long-term exposure and higher O_3 concentration. In O_3 ambient conditions, the effects were minor.

Additional key words: chlorophyll fluorescence; *Citrus*; intercellular CO_2 concentration; net photosynthetic rate; ozone; stomatal conductance.

Introduction

Citrus is the major cultivar in the Mediterranean area and one of the most important fruit crop in the world. Its productivity is of great importance in many countries, especially in the Valencian area of Spain, representing 4 000 Tm in a year (www.gva.es).

Ozone is a secondary pollutant with effects on large areas of rural Europe. Many Mediterranean areas suffer a critical photochemical oxidant problem (Gimeno *et al.* 1999, Calatayud and Barreno 2000, 2001). Research upon O_3 effects has been developed on a wide range of plant groups, although there are few studies on citrus trees (Matsushima *et al.* 1985, Olszyk *et al.* 1992). However, limited knowledge of citrus physiology, biochemistry, and molecular biology make difficult understanding of mechanisms that underlie O_3 uptake by stomata.

In several studies, chlorophyll (Chl) *a* fluorescence induction kinetics and gas exchange characteristics have been used to look for the primary site of photosynthesis limitations by O_3 (Soja *et al.* 1998). The study of Chl *a* fluorescence provides information about light reaction of photosynthesis and serves as a non-invasive indicator of the status of photosynthic reaction centres (RCs). Under

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Abbreviations: AOT40 – accumulated O_3 exposure over 40 mm^3 m^{-3}; CFA – charcoal-filtered ozone-free air; Chl – chlorophyll; C_i – intercellular CO_2 concentration; F_m – maximum Chl fluorescence yield obtained with a dark-adapted sample; F_m' – maximum Chl fluorescence yield in irradiated samples; F_0 – minimum Chl fluorescence yield in the dark-adapted state; F_0' – Chl fluorescence yield by a brief interruption of actinic irradiation in the presence of far-red radiation; F_s – Chl fluorescence yield during actinic irradiation; F_v – (F_m – F_0) variable Chl fluorescence in the dark-adapted leaf; g_s – stomatal conductance to water vapour; NFA – non-filtered air; NFA+O_3 – non-filtered air with additional ozone; NPQ – non-photochemical quenching calculated from Stern-Volmer quenching; OTC – open-top chamber; PAR – photosynthetically active radiation; P_{Nmax} – photosynthetic activity at PAR saturation; PFD – photon flux density; PS2 – photosystem 2; q_P – photochemical quenching; RC – reaction centre; ϕ_{PS2} – quantum efficiency of PS2; %D – fraction of PAR absorbed that is dissipated in the PS2 antennae; %P – fraction of PAR absorbed that is utilised in PS2 photochemistry; %X – fraction of PAR absorbed that is neither used in photochemistry nor dissipated in the PS2 antennae.

stress conditions, photosynthetic quantum conversion declines and heat emission markedly increases (Lichtenthaler 1996). All these changes can be measured through estimation of Chl *a* fluorescence. Moreover, damage to the PS2 RCs can occur when absorption of excitation energy exceeds the capacity of plants for its dissipation (Demmig-Adams and Adams 1992, Foyer *et al*. 1994). O_3 can limit the capability of the plant to use photon energy and thus alter photosynthetic processes. Given that O_3 is taken up by stomata, gas exchange can be a useful tool to obtain information about stomatal conductance (g_s). This might be associated to modifications in the plasmalemmae of guard cells that affect the ionic relations of cells (Castillo and Heath 1990, Torsethaugen *et al.* 1999). Moreover, changes in carboxylation capacity can be observed with reduction in CO_2 assimilation under O_3 conditions, and this plays a main role in impairment of photosynthesis (Calatayud *et al*. 2002, Ciompi *et al*. 1997).

Use of Chl *a* fluorescence and gas exchange measurements is an important indicator of the state of photosynthetic apparatus indicating not only changes in photosynthesis performance, but also allowing the localization of primary sites of damage (Guidi *et al.* 1997). The main objective of this study was to determine sensitivity or tolerance of Satsuma mandarin (*Citrus unshiu* [Mak.] Marc.) cv. Okitsu trees after long-term O_3 exposure (one year) in open top chambers (OTCs) under three air qualities: O_3-free (CFA), ambient ozone (NFA), and elevated O_3 concentration (NFA+O_3) during one year. Chl *a* fluorescence and gas exchange analysis were used to determine photosynthetic performance, thus contributing to understanding the mechanism of O_3 uptake in citrus trees.

Materials and methods

Site description: The experimental site was in the Centro de Capacitación Agraria (Generalitat Valenciana) at Carcaixent (39º7´N, 0º27´W, and 22.1 m a.s.l.) in a rural environment. The site is about 40 km south of Valencia on the Spanish Eastern Coast.

Plants: Three-years-old trees (100–125 cm tall) of Satsuma mandarin (*Citrus unshiu* [Mak.] Marc.) cv. Okitsu were used in the experiments. This cultivar belongs to the Satsuma Wase group, and shows moderate dwarfism. Eighteen uniform trees were selected, transferred from commercial soil to a mixture of sand and turf (85 : 15) into pots (150×60 cm), and grown up in a greenhouse at the Instituto Valenciano de Investigaciones Agrarias (Moncada, Valencia, Spain), with temperatures of 24–29 ºC at day and 16–18 ºC at night. After acclimation during six months, plants were transferred into nine OTCs (two plants per chamber) for twelve additional months. During the experiment, plants were irrigated twice a week with standard nutrient solution (half-strength Hoagland solution, modified by Bañuls *et al*. 1997). Environmental conditions during plant growth were as presented in Table 1 and the maximum daily photon flux density (PFD) ranged between 500 and 700 μmol m^{-2} s^{-1}.

OTC treatments: Potted plants were grown up in nine OTCs located at the Carcaixent Experimental Station. OTCs were based on the original design employed in the NCLAN (National Crop Loss Assessment Network) programme (see Heagle *et al.* 1973). Over the course of the growing season, three OTCs were ventilated continuously (24 h d^{-1}) by passing air through activated charcoal and dust filters (type *SF/Q* class *EU-3*, *Servifilter*, Zaragoza, Spain, and *EU-4*, *SF/CG*, and *RBAA2*, *FARR Europe*, Madrid, Spain) (CFA, ozone-free air). Three OTCs were ventilated with non-filtered air (NFA) and three ones received additional ozone (NFA+O_3). Ozone was generated electrically (ozone generator *S-3003*, *G.O.A.C.*, *Dasibi Co*., Glendale, CA, USA) using pure compressed oxygen, which was added to the ambient air. The flow of ozone-enriched air to the OTCs was regulated by a flow controller *(S-2000/D*, *Sir S.A*., Madrid, Spain). The O_3 additions were carried out from 09:00 to 15:00 GMT five days a week. The concentration of gaseous pollutants (O_3, nitrogen oxides NO and NO_2, and SO_2), wind speed (model *12005*, *Young Co*., Traverse City, MI, USA), wind direction (model *12002*, *Young*), air temperature and relative humidity (model *SKH 2013/1*, *Skye Instruments*, Powys, UK), and irradiance (model *SKP 215*, *Skye Instruments*) were all continuously monitored inside OTCs. The concentrations of O_3, NO_x, and SO_2 were checked using EPA-approved analyzers, specific to each pollutant (*Dasibi* models *1008*, *2108*, and *4108*, respectively). The ozone analyzer was calibrated on a twice-monthly basis with a *Dasibi* model *5008*.

Gas exchange measurements were performed after six months of treatment and at the end of the growing season (12 months) using an IRGA (*LICOR-6400*, *LI-COR*, Lincoln, NE, USA). The method consisted of an open system equipped with a sensor head, a leaf chamber, two Peltier thermoelectric coolers, and sample and reference gas analysers. The leaf chamber was provided with a Gallium Arsenide Phosphide (GaAsP) PAR that produces the required irradiances for the measurements with a red-blue PAR source. Air was supplied by a flow controller and all measurements were taken when operating at a constant air flow rate of 500 μmol s^{-1}. Within the leaf chamber, environmental conditions did not show statistical differences between measurements during the study. Average temperature was 18.02±1.50 ºC, relative humidity (RH) was 60.6±5.0, and

leaf-to-air vapour pressure deficit (VPD) was 0.6±0.1. Regarding stability conditions and measurements taken, *LI-6400* automatically measures and computes net photosynthetic rate (P_N), providing continuously a coefficient of variation of the last ten monitored values. Photosynthesis values were taken into account only when the coefficient of variation for each measurement was lower than 1 %. Determinations were first performed by increasing PFD to estimate saturating values for citrus external mature leaves. In the following measurements, all determinations were performed at 700 μmol m^{-2} s^{-1}, the minimum saturating PFD observed in the initial experiments. The gas exchange parameters determined at saturating PFD were: net photosynthetic rate, P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}], stomatal conductance to water vapour, g_s [mol(H_2O) m^{-2} s^{-1}], and intercellular CO_2 concentration, C_i [μmol(CO_2) mol^{-1}(air)]. Gas exchange determinations were performed between 10:00 and 11:00 ($n \geq 3$ leaves per plant). Measurements were repeated on similar leaves for 6 months and a year after the beginning of treatments.

Chl *a* fluorescence measurements: After six months and at the end of the growing period (1 year), Chl *a* fluorescence was measured at ambient temperature *in situ* in the OTCs, using a portable fluorometer (*PAM-2000, Walz*, Effeltrich, Germany). Mature citrus leaves without visible injury symptoms were darkened for 30 min prior to measuring. The ambient conditions were similar to gas exchange and did not change between different measurement days. The leaves were darkened for 30 min prior to measurement. The minimum (dark) fluorescence, F_0, was obtained upon excitation of leaves with a weak beam from a light-emitting diode. The maximum fluorescence (F_m) was determined following a 600-ms pulse of saturating white radiation. The yield of variable fluorescence (F_v) was calculated as $F_m - F_0$. Following 2 min of dark re-adaptation, actinic "white" radiation [230 μmol(photon) m^{-2} s^{-1}] was switched on and saturating pulses [8 000 μmol(photon) m^{-2} s^{-1}] were applied at 60 s intervals for 15 min to determine maximum fluorescence yield during actinic irradiation (F_m'), the level of modulated fluorescence during a brief interruption of actinic irradiation in the presence of far-red radiation (F_0'), and the Chl fluorescence yield during actinic radiation (F_s). Calculation of quenching due to non-photochemical dissipation of absorbed photon energy (NPQ) was determined at each saturating pulse, according to the equation $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkman 1991). The coefficient for photochemical quenching, q_P, which represents the fraction of open PS2 RCs, was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (Schreiber *et al*. 1986). The quantum efficiency of PS2 photochemistry, ϕ_{PS2}, closely associated with the quantum yield of non-cyclic electron transport, was estimated from $(F_m' - F_s)/F_m'$ (Genty *et al*. 1989). The fraction of radiation absorbed dissipated in the antenna (%D) and utilized in PS2 photochemistry (%P) were estimated as $1 - (F_v'/F_m') \times 100$ and $(F_v'/F_m')\ q_P \times 100$, respectively (Demmig-Adams *et al*. 1996). The fraction of absorbed radiation by PS2 neither used in photochemistry nor dissipated in the PS2 antennae (%X) was estimated as $(F_v'/F_m')\ (1 - q_P) \times 100$ (Demmig-Adams *et al*. 1996). In addition, Chl *a* fluorescence parameters (ϕ_{PS2}, q_P, and NPQ) at the range of actinic radiation 40–910 μmol (photon) m^{-2} s^{-1} were estimated after 12 months, and the saturating pulses were applied at 5-min intervals after acclimation period for each actinic radiation. Chl *a* fluorescence values were obtained from 2 leaves per plant, in a total of 12 replicates per air quality treatment.

Statistical analysis: Variance analyses (ANOVA) were performed on experimental data, statistical significance ($p<0.05$), and judged by the least significant differences (LSD) method. Appropriate transformation of percentage data ($\log p/100 - p$) was shown before applying LSD. Statistical analyses were performed using the statistical package *SPSS* (*SPSS*, Chicago, IL, USA). The values obtained were independently analysed after 6 and 12 months of treatment due to different flushes in the trees.

Results

Air quality: During the growth period, SO_2 and NO_x concentrations remained low (less than 2 mm^3 m^{-3} for SO_2 and 8 mm^3 m^{-3} for NO_x). The accumulated hourly ozone exposure, when the concentration was over 40 mm^3 m^{-3} (AOT40) between sunrise and sunset, are shown in Table 1. After 6 months, AOT40 in the NFA+O_3 chambers was 4.5-fold higher than in the NFA ones. This proportion was slightly higher after 12 months (5.1). Ozone concentration in CFA chambers never reached the threshold of 40 mm^3 m^{-3}. The environmental conditions were similar in all treatments during the time of measurements (Table 1).

Gas exchange: After six months and 1 year into the OTCs, net photosynthetic rate (P_{Nmax}) (Table 2) was depressed in NFA+O_3 leaves with a reduction by about 10 and 19 % in comparison to control trees (CFA-6-months and CFA-12-months). No change was detected in NFA plants. g_s was affected in NFA+O_3 plants at 1 year of exposure with a decrease by about 41 % in respect to CFA. An increase in C_i was observed after 12 months in NFA and NFA+O_3 treatments.

Chl *a* fluorescence was analysed after 6 and 12 months in OTCs under different air qualities (Table 2). The maximum quantum yield of PS2 photochemistry, F_v/F_m,

was affected in NFA and NFA+O_3 plants after 12 and 6 months of exposure, respectively, with significant differences and percentage declines of 9 % in comparison to CFA. F_v/F_m was also adversely affected (with significant differences) in NFA+O_3 after O_3 exposure for 12 months, with a decline of 19 % in respect to CFA. The decline in this ratio was mostly a result of a decrease in the maximum Chl fluorescence yield in dark-adapted leaves (F_m). F_0 was similar in all treatments and was not affected by O_3. The changes in fluorescence parameters in the last saturating pulse of the kinetic fluorescence induction with actinic irradiation of 230 μmol(photon) m^{-2} s^{-1} are shown in Table 2. The actual quantum yield (ϕ_{PS2}) of the PS2 electron transport showed a significant reduction in NFA and NFA+O_3 leaves after 12-month-treatment in comparison to controls (9 and 25 %, respectively). The ozone affected q_P in leaves of citrus significantly after 12 months of NFA+O_3 treatment. The fraction of photons absorbed by PS2 antennae used in photochemistry (%P) only significantly decreased in NFA+O_3 treated plants after 12-months-O_3 fumigation. The fraction thermally dissipated in the antennae (%D) was similar among treatments and the fraction neither used in photochemistry nor dissipated in the antennae

Table 1. Summary of O_3 air quality and environmental conditions in the open top chambers during growing season of citrus plants. $AOT40_{6m}$ and $AOT40_{12m}$, accumulated ozone exposure over 40 mm^3 m^{-3} during daylight after 6 and 12 months in the OTCs, respectively; T_{mean}, mean air temperature [ºC]; T_{min} and T_{max}, minimum and maximum air temperatures [ºC], respectively; and RH [%], mean air relative humidity. Treatments: plants treated with filtered ozone-free air (CFA), non-filtered air (NFA), or non-filtered air with additional ozone (NFA+O_3).

Treatment	$AOT40_{6m}$	$AOT40_{12m}$	T_{mean}	T_{min}	T_{max}	RH
CFA	0	0	13.0	8.0	17.6	72.2
NFA	3 063	6 995	13.1	8.2	18.0	72.3
NFA+O_3	13 895	36 210	13.0	8.1	17.8	72.3

Table 2. Effects of different air quality (CFA, NFA, and NFA+O_3) on gas exchange parameters and chlorophyll (Chl) *a* fluorescence parameters of citrus leaves. Measurements were carried out at ambient CO_2 and O_2 concentrations (345 cm^3 m^{-3} and 21 %, respectively) and at saturating PFD (700 μmol m^{-2} s^{-1}). P_{Nmax}, photosynthetic activity at saturation irradiance [μmol(CO_2) m^{-2} s^{-1}]; g_s, stomatal conductance to water vapour [mol(H_2O) m^{-2} s^{-1}]; C_i, intercellular CO_2 concentration [μmol(CO_2) mol^{-1}]; F_0, minimum Chl fluorescence in the dark-adapted state; F_m, maximum Chl fluorescence in the dark-adapted state; F_v/F_m, maximal photochemical efficiency; ϕ_{PS2}, quantum efficiency of PS2; q_P, photochemical quenching; %P, the fraction of photons utilized in PS2 photochemistry; %D, the fraction of photons dissipated in the antenna; %X, the fraction of absorbed photons by PS2 neither used in photochemistry nor dissipated in the PS2. The parameters were compared after 6 and 12 months in open-top chambers. Values are means of 18 (gas exchange) or 12 (fluorescence) samples. For comparison of means, variance analysis (ANOVA) followed by the least significance differences (LSD) test, calculated at 95 % confidence level, was performed. The obtained values were independent statistical analyses at 6 and 12 months. Values at 6 or 12 months in a column followed by the same letter indicate no significant differences.

Treatment		P_{Nmax}	g_s	C_i	F_o	F_m	F_v/F_m	ϕ_{PS2}	q_P	%P	%D	%X
6 months	CFA	4.2a	0.105a	337a	0.226a	0.790a	0.705a	0.513a	0.839a	51a	40a	9a
	NFA	4.5a	0.100a	326a	0.229a	0.807a	0.715a	0.495a	0.810a	49a	40a	11a
	NFA+O_3	3.8b	0.120a	338a	0.236a	0.711b	0.648b	0.473a	0.802a	46a	42a	12a
12 months	CFA	4.8a	0.118a	321a	0.242a	0.810a	0.700a	0.490a	0.855a	49a	42a	9a
	NFA	4.4a	0.096a	348b	0.259a	0.732a	0.644b	0.446ab	0.804a	44ab	46a	10a
	NFA+O_3	3.9b	0.070b	345b	0.241a	0.557b	0.569c	0.368b	0.723b	39b	46a	15b

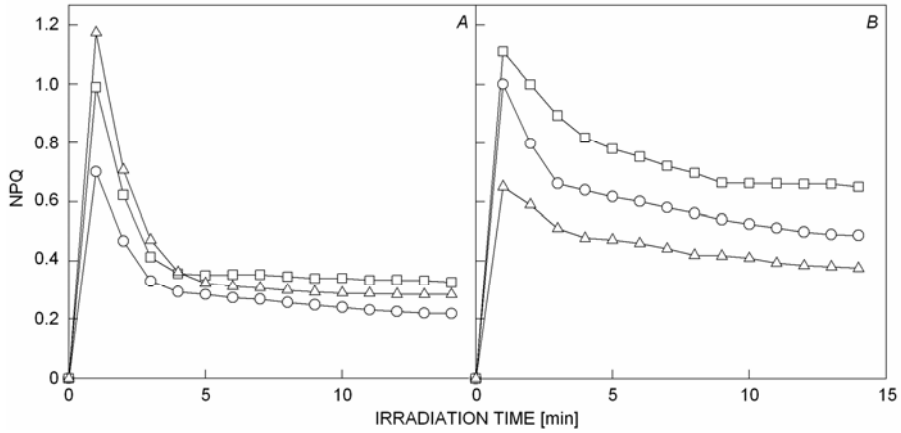

Fig. 1. Variations in non-photochemical quenching (NPQ) during fluorescence induction kinetics in citrus leaves at 6 (*A*) and 12 months (*B*) in open top chambers. Symbols denote: trees in charcoal-filtered ozone-free (CFA) (○), non-filtered air (NFA) (□), and non-filtered air with ozone fumigation (NFA+O_3) (△). Means for n = 12 and ±SE<10 %.

(% X) showed significant differences in NFA+O_3 treatment after 12 months in OTCs (increase by 167 %).

Fig. 1 shows the variations of NPQ during fluorescence induction kinetics after 6 (Fig. 1*A*) and 12 (Fig. 1*B*) months of treatment. Immediately following actinic irradiation, NPQ experienced a quick rise and reached their maximum values within 1 min. In Fig. 1*A*, the maximum value was obtained for NFA+O_3 leaves, thereafter NPQ declined until it reached steady-state values after approximately 6 min of actinic irradiation. The values in the steady-state were lower in CFA and NFA+O_3 than in NFA (33 and 13 %, respectively).

After twelve months (Fig. 1*B*) in the OTCs, O_3 fumigation decreased the maximal NPQ of the plants, modifying the NPQ relaxation pattern, and decreasing the values in the steady-state.

Changes in fluorescence parameters with regard to different actinic irradiations are shown in Fig. 2. The quantum yield (ϕ_{PS2}; Fig. 2*A*) and the fraction of PS2 RCs opened (q_P; Fig. 2*B*) experienced a gradual decrease with the different actinic irradiances; the effect was similar in NFA and CFA. The major decrease occurred in NFA+O_3 leaves. NPQ (Fig. 2*C*) increased markedly with larger actinic irradiances in CFA and NFA leaves. In NFA+O_3 leaves, NPQ increased until 400 μmol(photon) m^{-2} s^{-1} after decline to reach approximately the zero value with the highest actinic irradiance.

Discussion

Ozone fumigation affected photosynthesis performance in citrus trees (*Citrus unshiu* [Mak.] Marc.) cv. Okitsu) growing in OTCs after 12 months. High ozone concentration induced stomatal closure, decrease in P_{Nmax}, and increase in C_i. The P_{Nmax} measured in control leaves (CFA) matched the values of 4 to 8 μmol(CO_2) m^{-2} s^{-1} reported by Bañuls *et al*. (1997) and Pereira *et al*. (2000), under normal environmental conditions. The lower P_{Nmax} in *Citrus* spp. compared with other fruit trees (*i.e*. apple, peach, or grapevine) is related to high resistance to CO_2 diffusion in the stomata to the carboxylation sites (Lloyd *et al*. 1992, Syvertsen and Lloyd 1997). Citrus exposed to elevated O_3 showed a decrease in CO_2 assimilation carried out by a reduction in g_s. Stomatal closure may constitute an important mechanism for avoidance of injury to internal tissue (Kock *et al.* 1998, Guidi *et al*. 2001). In fact, a reason for tolerance to various pollutants in Satsuma madarin was the highly effective stomatal closure (Matsushima *et al*. 1985). Both stomatal limitations and altered mesophyll CO_2 fixation (increase in C_i) seem to contribute to the decrease in P_{Nmax}. Decrease in CO_2 fixation by O_3 exposure has been reported by many authors (Farage *et al.* 1991, Guidi *et al.* 1997, Calatayud *et al*. 2002). The reduction in photosynthesis may be caused by inhibition of mesophyll photosynthetic capacity (Degl´Innocenti *et al.* 2003) and this effect could be confirmed by a decline of F_v/F_m ratio (Calatayud *et al*. 2002) in citrus trees exposed to NFA+O_3 fumigation and NFA. The reduction in F_v/F_m was due to a decline in the F_m values, indicating that the photochemistry of PS2 and its ability to reduce the primary acceptor Q_A were affected by ozone (Ciompi *et al*. 1997, Guidi *et al*. 1997, Calatayud and Barreno 2001). The decline in F_v/F_m might be a result of an increase in non-photochemical process in the light-harvesting antennae of PS2 associated with a photochemical quenching down-regulation, photodamage of PS2 RCs, or both (Osmond *et al*. 1993). The significant decrease in F_v/F_m at the end of the growing station in NFA+O_3 could be explained by PS2 stress, because NPQ was adversely affected by ozone. In NFA after 12-months-exposure, the NPQ increase correlated with photosynthetic quantum conversion decrease

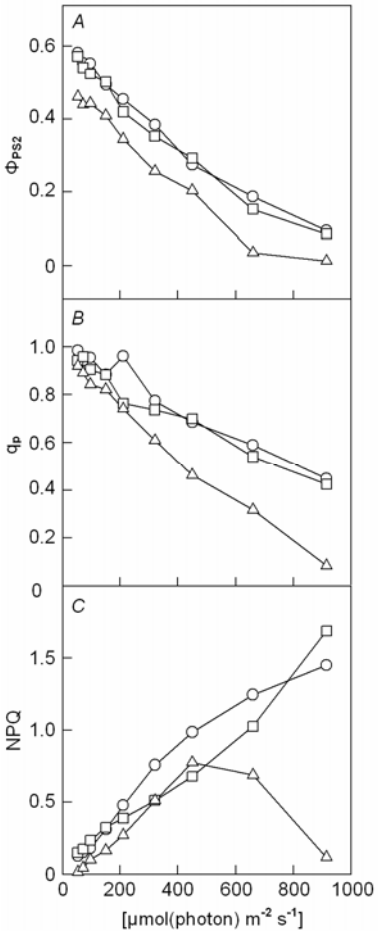

Fig. 2. Changes in chlorophyll *a* fluorescence parameters: ϕ_{PS2} (*A*), q_P (*B*), and NPQ (*C*) after applying different actinic irradiances at the end of the experiment (12 months). Symbols denote: trees in charcoal-filtered ozone-free (CFA) (○), non-filtered air (NFA) (□), and non-filtered air with ozone fumigation (NFA+O_3) (△). Means for n = 12 and ±SE<10 %.

favouring NPQ at the expense of photochemical utilization of excitation energy (lower Φ_{PS2}). This effect might be related to several mechanisms in photoprotective processes (Guidi *et al*. 2001, Calatayud *et al*. 2002).

Fluorescence quenching under steady-state conditions showed that, after 12 months in NFA+O_3, q_P significantly decreased indicating that O_3 decreased the capacity for re-oxidizing Q_A during actinic irradiation. The leaves under NFA+O_3 might have decreased their ability for non-photochemical quenching development and, as a consequence, the electron transport chain was closer to saturation, explaining the lower photochemical values found. We found that Φ_{PS2} was closely correlated with the quantum yield of non-cyclic electron transport (Genty *et al*. 1989), and was reduced by O_3. A decrease in q_P and Φ_{PS2} may correlate with a decrease in the proportion of available excitation energy used in photochemistry (Havaux *et al*. 1991). In this circumstance, non-photochemical processes must be increased to guarantee excitation energy dissipation. This occurs in first minutes of dark-light transition after 6 months in OTCs. After 12 months in OTCs under NFA+O_3, the capacity to NPQ formation decreased. The maximum value decreased and the quenching relaxation was less than in NFA and CFA. This may be due to lower rates of non-cyclic electron transport associated with a decreased ability to establish ΔpH across thylakoid membranes. Other factors might be also related (*i.e*. decreased zeaxanthin content).

According to these results, the distribution of energy in PS2 showed differences among treatments. Long-term O_3 fumigation limited the fraction of photons absorbed in the PS2 antennae used in photochemistry (decreased %P). The energy fraction dissipated as thermal energy in the PS2 antennae was similar in all treatments, and subsequently, the %X fraction was increased in NFA+O_3 leaves. This increase in %X may lead to de-excitation of Chl singlet (Demmig-Adams *et al.* 1996) and makes lower energy dissipation in the PS2 antennae and a lower fraction of the excitation energy can be utilized for photochemistry (Calatayud and Barreno 2004).

When plants are exposed to O_3, an additional stress as an amount of excess PAR absorbed by leaves can lead to a depression in efficiency of PS2 (Powles 1984, Krause 1994). Damage to the PS2 RCs might occur when the absorption of excitation energy exceeds the capacity of plants for its dissipation (Demmig-Adams and Adams 1992). When citrus leaves were exposed to increased actinic irradiance, responses depended upon air quality. Photochemical quenching decreased in all treatments parallel to actinic radiation increases. The lower q_P levels exhibited by NFA+O_3 plants in comparison with other treatments indicated that O_3 decreased the capacity for re-oxidizing Q_A during actinic radiation. In this sense, O_3 probably increased excitation pressure ($1 - q_P$) on PS2 and contributed to the closure of PS2 reaction centres (Calatayud and Barreno 2001). NPQ consists of an energy dissipation process, regulating the quantum yield of PS2 and constituting the major mechanism against damage to the photosynthetic apparatus (Horton and Hague 1998). In CFA and NFA leaves, NPQ increased concomitantly to actinic radiation increases, indicating the occurrence of effective non-radiative energy dissipation processes. In NFA+O_3 leaves, the increase in NPQ occurred until 400 μmol(photon) m^{-2} s^{-1} after this radiation and NPQ strongly decreased, suggesting that the photosynthetic apparatus was not able to dissipate completely the excess of excitation energy. Alterations to the PS2 RCs may take place (Chaumont *et al*. 1995).

The effects of O_3 in citrus leaves were mainly obtained after 12 months of exposure, suggesting a certain tolerance to O_3. Elevated ozone exposure induced inhibition of photosynthesis by enhanced g_s and a decrease in mesophyll activity as demonstrated by the increase in C_i and alterations in the light reactions of photosynthesis measured by Chl fluorescence parameters.

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