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Interactions between nitrogen fertilization and ozone in watermelon cultivar Reina de Corazones in open-top chambers. Effects on chlorophyll *a* fluorescence, lipid peroxidation, and yield

A. CALATAYUD[*,***], F. POMARES[*], and E. BARRENO[**]

Horticulture Department and Natural Resources Department, Valencian Institut for Agricultural Research (I.V.I.A.), Agriculture, Fisheries and Food Authority, Generalitat Valenciana, Ctra. Moncada-Náquera km. 4.5,
46113 Moncada, Valencia, Spain[*]
University of Valencia, Faculty of Biological Sciences, Department of Botany, C/Dr. Moliner, 50,
46100 Burjasot, Valencia, Spain[**]

Abstract

Watermelon (*Citrillus lanatus*) plants were grown for two consecutive years in open-top chambers with three different ozone concentrations (O_3-free air, O_3 ambient, and air with additional O_3; CFA, NFA, and NFA+O_3) and three nitrogen fertilizer concentrations [0, 14.0, and 29.6 g N per pot; N0, N1, and N2). There was an interaction between ozone and N fertilizer for the major parameters studied. O_3 and N2 treatments led to a significant decrease in maximum efficiency of photosystem 2 (PS2) photochemistry (F_v/F_m), and induced a significant decrease in the actual quantum yield of PS2 (Φ_{PS2}), due mainly to the increased closure of PS2 reaction centres (q_P) and to an increase in the non-photochemical quenching (NPQ). On the other hand, these plants exhibited an increased susceptibility to photoinhibition, which could be associated with an increased fraction of reduced Q_A. An increase in lipid peroxidation indicated that damage was occurring at the membrane levels. High N concentration enhanced the detrimental effects of ozone on the fluorescence parameter induction and lipid peroxidation. All these negative alterations led to a decreased yield.

Additional key words: *Citrollus lanatus*; photochemical and non-photochemical quenching; photoinhibition; photosystem 2; quantum yield.

Introduction

Many rural Mediterranean areas suffer from a severe photochemical oxidant problem. Ozone is the major phytotoxic air pollutant associated with crop damage (Gimeno *et al.* 1999, Calatayud *et al.* 2002a,b). Ozone impacts are species dependent (Barnes *et al.* 1999) and strongly influenced by cultivation methods (Fuhrer 2003) and climatic factors (Krupa *et al.* 1995). An increase in N fertilization may trigger short-term increases in plant photosynthesis and biomass production, but an excess of N can disturb normal plant metabolism, induce mineral imbalance, reduce frost hardiness, and render plants more vulnerable to other environmental stresses (Utriainen and Holopainen 2001). Therefore, a possibility exists that crops will be adversely affected by elevated O_3 concentration and by excess soil N. Some studies indicate that plants exhibiting low N content may be less susceptible to O_3. Brewer *et al.* (1961) reported a reduction in O_3-induced visible injury when *Spinacea oleracea* and

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[***] Corresponding author; fax: 34-6-3424001, e-mail: angeles.calatayud@ivia.es

Abbreviations: AOT40, accumulated O_3 exposure over 40 μm m^{-3}; CFA, charcoal-filtered ozone-free air; Chl, chlorophyll; 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, level of Chl fluorescence yield a brief interruption of actinic irradiation in the presence of far-red irradiation; F_s, Chl fluorescence yield during actinic irradiation; $F_v = (F_m - F_0)$, variable Chl fluorescence in the dark-adapted leaf; MDA, malondialdehyde; NFA, non-filtered air; NFA+O_3, non-filtered air with additional ozone; NPQ, non-photochemical quenching calculated from Stern-Volmer quenching; OTCs, open-top chambers; q_P, photochemical quenching; RC, reaction centre; TBA, thiobarbituric acid; TCA, trichloroacetic acid; UN-ECE, United Nations Economic Commission for Europe; Φ_{PS2}, quantum efficiency of PS2; $\Phi_{exc,}$ intrinsic efficiency of open PS2 reaction centres.

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Beta vulgaris were grown under low N, in comparison to plants receiving high N. Ozone induced reductions in biomass at high N concentrations, but not at low N concentrations (*Raphanus sativus* – Pell *et al*. 1990, *Pinus taeda* – Tjoelker and Luxmoore 1991). Reduced foliar biomass can cause significant declines in yield. Cardoso-Vilhena and Barnes (2001) reported that O_3 exposure reduced both the growth and photosynthetic rate in plants supplied with 4 and 14 mM NO_3^- [$Ca(NO_3)_2$]; however, O_3 had no impact on these parameters in plants supplied with low N (1.5 mM NO_3^-). Therefore, there are contradictory results for the ozone×nitrogen relationship (Maurer *et al*. 1997, Bielenberg *et al.* 2001) because although all plants demand N, they differ greatly in their requirements, not only in terms of the concentration that promotes optimum growth, but also in their capacity to use the N that is in the soil solution.

Watermelon is an important crop in Spain. This crop is not only considered as susceptible to O_3 injury but decreases plant relative growth rate, flower production, and fruit number (Reinert *et al.* 1992, Fernández-Bayon *et al*. 1993, Gimeno *et al.* 1999). The cultivated surface is 18 000 ha, with a production of 723 000 t, of which the Valencian Community produces 15 %, mainly in open-field conditions. We used the cv. Reina de Corazones, a seedless watermelon, which is an important cv. in the Valencia area with a great commercial value.

The Valencia region is characterized by a high ozone concentration (>40 mm^3 m^{-3}) in spring and summer (Calatayud *et al.* 2002b). Obviously, an elevated deposition of N fertilizer, a major plant nutrient, modifies the plant response to ozone. To test this hypothesis, we investigated the potential effects of ozone and nitrogen fertilizer supply [0, 14, and 29.6 g(N) per pot, i.e. N0, N1, N2] and their interactive effects using open-top chambers (OTCs) and the mentioned cultivar. The determined parameters, chlorophyll (Chl) *a* fluorescence, lipid peroxidation, and productivity, were chosen to explore the capacity for non-radiative energy dissipation, electron transport rate, and the proportion of closed reaction centres (RCs) in photosystem 2 (PS2), damage in membranes, and changes in productivity under different ozone and N concentrations.

Materials and methods

Plants: The experiments were performed in the years 2000 and 2001 under the same experimental conditions and dates. Seeds of watermelon, *Citrullus lanatus* (Thumb.) Matsum cv. Reina de Corazones (*Petoseed*, El Ejido, Almeria, Spain) were germinated in vermiculite, and kept in a greenhouse for 3 weeks in the Estación Experimental Agraria de Carcaixent (Valencia, Spain). The greenhouse environmental conditions during plant growth were as follows: 15–25 ºC (day), 10–15 ºC (night); a relative humidity of 50–95 %; photosynthetically active radiation of day-time maximum at plant height in the glasshouse of 500–700 μmol m^{-2} s^{-1}. When the first four leaves were fully expanded, the plants were transferred to large pots (7 000 cm^3) containing a commercial soil mixture (*Terraplan*t, *BASF*, Uchte, Germany) and transferred into OTCs where they remained for 90 d (mid June–mid September) during 2000 and 2001. During the growth period, plants were watered daily. Each OTC contained three plants, one for each N concentration in order to avoid them shading each other during a long exposure time inside the OTC.

OTC treatments: Potted plants were grown in 9 OTCs located at the Carcaixent Experimental Station. OTCs were based on the original design employed in the NCLAN 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 (CFA, ozone-free air), three OTCs were ventilated with non-filtered air (NFA), and three received additional ozone (NFA+O_3) (for details see Calatayud *et al.* 2002b).

Applied nitrogen: Each of the CFA, NFA, and NFA+O_3 treatments were combined with three nitrogen concentrations added as ammonium nitrate (33.5 % of N) to the pots. This fertilizer was split weekly over the growing season according to the following pattern: week 1 in OTCs: 0 %, weeks 2–4: 13 %, weeks 5–7: 24 %, weeks 8–10: 36 %, and weeks 11–13 (at the end of growing season): 21 %, which was considered adequate for watermelon requirements (Pomares *et al.* 2002). There were no losses of N by leaching through the pots because the irrigation was regulated to avoid the water loss through runoffs. By taking the main N inputs into account (fertilizer, irrigation water, and substrate) the N rates added were equivalent to 140 (N0), 280 (N1), and 436 (N2) kg(N) per ha, corresponding to adequate, moderate, and higher levels than those normal for watermelon requirements (Pier and Doerge 1995, Pomares *et al*. 2002).

Chl *a* fluorescence measurements: At the end of the growing period, Chl *a* fluorescence was measured at ambient temperature *in situ* in the OTCs, using a portable fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany). Mature leaves without visible injury symptoms were darkened for 30 min prior to measuring. 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 light”. The variable fluorescence yield (F_v) was calculated as $F_m - F_0$. After 2 min of dark re-adaptation, actinic “white light” [330 μmol(photon) m^{-2} s^{-1}] was switched on and a saturating pulse [14 000 μmol(photon) m^{-2} s^{-1}] was applied at 60 s intervals for 15 min to determine: (*1*) maximum fluorescence yield during actinic irradiation (F_m'), (*2*) modulated fluorescence during a brief interruption of actinic irradiation in the presence of far-red radiation (F_0'), and (*3*) Chl *a* fluorescence yield during actinic irradiation (F_s). Quenching due to non-photochemical dissipation of absorbed photon energy (NPQ), was calculated at each saturating pulse, using 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). Φ_{PS2}, closely associated with the quantum yield of non-cyclic electron transport, was estimated using $(F_m' - F_s)/F_m'$), and the intrinsic PS2 efficiency, Φ_{exc}, was calculated as F_v'/F_m' (Genty *et al.* 1989). Chl *a* fluorescence determinations were obtained from *n* = 2 leaves per plant, in a total of 6 replicates by air quality and N concentrations.

Photoinhibition was induced at ambient temperature and under ambient air conditions using leaves similar to those used in Chl *a* fluorescence measurement. Intact leaves were exposed to an irradiance of 1 800 μmol m^{-2} s^{-1} for 30 min, after which the leaves were dark-adapted, and the recovery of the Chl *a* fluorescence ratio F_v/F_m was determined (Calatayud *et al.* 1997). Photoinhibitory treatments were obtained from *n* = 2 leaves per plant, in a total of 6 replicates by air quality and N concentrations.

Lipid peroxidation extent was estimated at the end of the growing period using malondialdehyde (MDA) as an indicator, according to the protocol adapted for leaves by Heath and Packer (1968), employing the modifications suggested by Dhindsa *et al.* (1981). Lipid peroxidation analyses were obtained from *n* = 3 leaves per plant, in a total of 9 replicates by air quality and N concentrations.

Statistical analyses were performed using the statistical package *SPSS* (Chicago, IL, USA). The experimental design was a 3×3 factorial design (three N treatments and three O_3 concentrations) replicated once each year. Treatment effects on variables were assessed using a variance analysis (ANOVA) with air quality (CFA, NFA, and NFA+O_3) and nitrogen concentrations, and with interactions between factors. Due to significant air quality×N interactions, this combination was tested on variables using a Tukey`s test calculated at the 5 % level as a *post hoc* test after ANOVA. Analyses were performed for each year separately. There were no significant differences between data for replicate chambers.

Results

Air quality: During the growth period, ozone concentration attained potentially phytotoxic levels, which were high during both growing seasons. By contrast, the SO_2 and NO_x concentrations were low, below 2 and 5 mm^3 m^{-3}, respectively (see Table 1) using several commonly used indices to describe O_3 air quality: the accumulated ozone exposure over 40 mm^3 m^{-3} (AOT40) between sunrise and sunset; the number of hours exceeding 40 mm^3 m^{-3} threshold (H40), and a highest hourly average peak of O_3 (O_{3max}) during the course of both growing seasons. The O_3 concentrations exceeded the UN-ECE critical level for the protection of crop yield (set at an AOT40 of 3 000 mm^3 m^{-3} h^{-1}) based on the AOT40 during daylight hours for the consecutive 3-month period (Fuhrer *et al.* 1997) in both the years 2000 and 2001. The AOT40 values were higher in 2000 than in 2001. The ratio between average high-peaks for NFA+O_3 and NFA were 2.40 for 2000 and 1.98 for 2001. CFA simulated a non-polluted environment and never exceeded the threshold of 40 mm^3 m^{-3}.

Mean growing season air temperature, and maximum and minimum air temperatures inside the OTCs were similar in both 2000 and 2001 (Table 1). The daily mean relative humidity inside the OTCs was 69 % in 2000 and 72 % in 2001. Midday PPFDs (10.00 to 13.00 h) were 1 600 μmol m^{-2} s^{-1} in 2000 and 1 657 μmol m^{-2} s^{-1} in 2001.

Table 1. Summary of O_3 air quality and environmental conditions in the OTCs, charcoal-filtered ozone-free (CFA), non-filtered air (NFA), and non-filtered air with ozone fumigation (NFA+O_3) during both growing seasons of the watermelon plants cv. Reina de Corazones. AOT40, accumulated ozone exposure over 40 mm^3 m^{-3} during daylight hours; H40, number of hours exceeding 40 mm^3 m^{-3}; $[O_3]_{max}$, high-peak mean of O_3 for daylight hours; T_{mean}, mean 24 h air temperature [ºC]; T_{min}, minimum air temperature [ºC]; T_{max}, maximum air temperature [ºC]; RH [%], air relative humidity; and PPFD, midday photosynthetic photon flux density. Means for 3 OTCs with a SE≤3 %.

Year	Treatment	AOT40	H40	$[O_3]_{max}$	T_{mean}	T_{min}	T_{max}	RH	PPFD
2000	CFA	0	0	10	24.1	21.0	28.2	70	1620
	NFA	6276	592	54	24.4	21.0	27.8	69	1600
	NFA+O_3	35583	745	131	24.4	21.2	28.0	69	1650
2001	CFA	0	0	8	24.5	21.0	28.5	70	1634
	NFA	5103	480	55	24.5	21.0	27.0	72	1657
	NFA+O_3	32547	618	109	24.6	21.2	28.0	71	1660

Productivity: The fruit yields of both years are shown in Table 2. Under the CFA treatment, they decreased with the N concentration in 2000, but the opposite trend was found in 2001. Under the NFA treatment, the relative yield decreased considerably in the second year (55–42 %), the values for first year decreased slightly with respect to CFA+N0. On the other hand, under the NFA+O_3 treatment the fruit yield decreased because of the N1 and N2 concentrations, reaching those reductions of N2 yield relative to CFA+N0 of 59 and 36 % in the first and second experiment, respectively.

Table 2. Total fruit yield [kg] for the watermelon cv. Reina de Corazones treated with different O_3 concentrations, charcoal-filtered ozone-free (CFA), non-filtered air (NFA), and non-filtered air with ozone fumigation (NFA+O_3), and N concentrations (N0, N1, and N2).

Treatment	N	Yield	
		2000	2001
CFA	N0	14.60	9.88
	N1	13.71	9.48
	N2	12.44	12.47
NFA	N0	11.38	4.18
	N1	11.32	5.42
	N2	13.53	4.85
NFA+O_3	N0	13.26	10.70
	N1	10.81	4.83
	N2	8.64	3.57

Chl *a* fluorescence was measured at the end of the growing period in both years. F_v/F_m ratio mainly decreased in leaves exposed to NFA+O_3 and N2 in both years with significant differences compared to other treatments. The values of F_v/F_m were similar in CFA and NFA for all the N treatments in the year 2000, except NFA+N2 in 2001 (Fig. 1). In the initial period of irradiation, Φ_{PS2} (Fig. 2) increased until a steady-state was reached. The initial induction velocity and steady-state values corresponding to both the CFA and NFA treatments in all the N supplies (Fig. 2*A*,*B*,*D*,*E*) were higher than those obtained under NFA+O_3 (Fig. 2*C*,*F*). The minor Φ_{PS2} values were obtained in NFA+O_3 and with the N2 concentration in the year 2000 (Fig. 2*C*) followed by the values obtained in 2001 under the same treatments (Fig. 2*F*). The fraction of open PS2 RCs, as indicated by q_P (Fig. 3), decreased during the onset of actinic irradiation, and increased during the irradiation period, and an apparent steady-state was reached after 5 min of irradiation. The values of q_P in the steady-state were similar for the CFA and NFA leaves for all the N concentrations (Fig. 3*A*,*B*,*D*,*E*). In accordance with the minor Φ_{PS2} values detected in NFA+O_3 in the N2 variant, q_P values were also minor for this treatment (Fig. 3*C*,*F*). NPQ is a good estimate of the amount of energy dissipated non-radiatively by a plant. In all treatments, NPQ (Fig. 4) initially rose, which was followed by relaxation until it reached steady-state values, approximately 8 min after the onset of actinic irradiation. The NPQ in CFA values were similar in all the N applied (Fig. 4*A*,*D*), except for CFA+N2 in the year 2001, when the values increased. For plants grown in NFA with the N2 concentration, NPQ values were higher than with N0 and N1 (Fig. 4*B*,*E*). The maximum NPQ values were obtained in NFA+O_3 leaves at N_2 in both years (Fig. 4*C*,*F*). In this situation, the leaves lost a higher proportion of absorbed photons in the form of heat instead of them being used to drive photosynthetic transport.

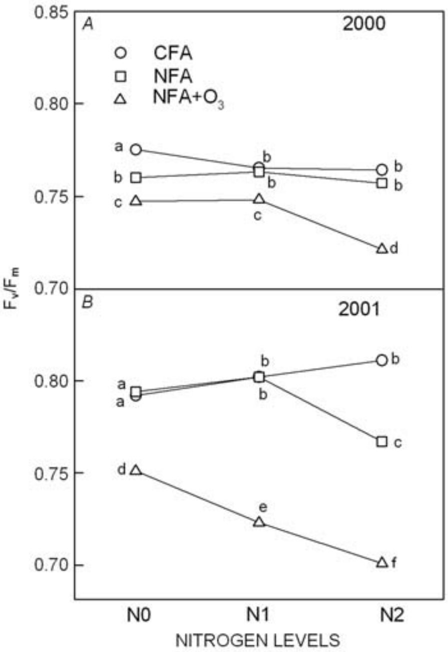

Fig. 1. Maximum quantum yield of PS2 photochemistry (F_v/F_m) on mature leaves of the watermelon cv. Reina de Corazones in charcoal-filtered ozone-free (CFA), non-filtered air (NFA), and non-filtered air with ozone fumigation (NFA+O_3) with three nitrogen concentrations: N0, N1 and N2 during the years 2000 and 2001. Means for $n = 6$. Symbols in one graph followed by the different letters are statistically different at $p<0.05$ (Turkey`s test) due to the interaction between air quality and N concentrations.

Photoinhibition: The extent of F_v/F_m recovery was similar in the CFA leaves under all the N concentrations (Fig. 5*A*,*D*). In NFA, minor recovery occurred in plants supplied with the N2 (Fig. 5*B*,*E*), of which the major differences occurred in 2001 (Fig. 5*E*). Under the NFA+O_3 treatment, the plants never reached a 100 % recovery; the minor recovery occurred in N2 plants (40 %) in both years (Fig. 5*C*,*F*).

Lipid peroxidation was measured as MDA content in watermelon leaves subjected to different air qualities and N concentrations. Lipid peroxidation increased in leaves after exposure to NFA and NFA+O_3 (Fig. 6). The N

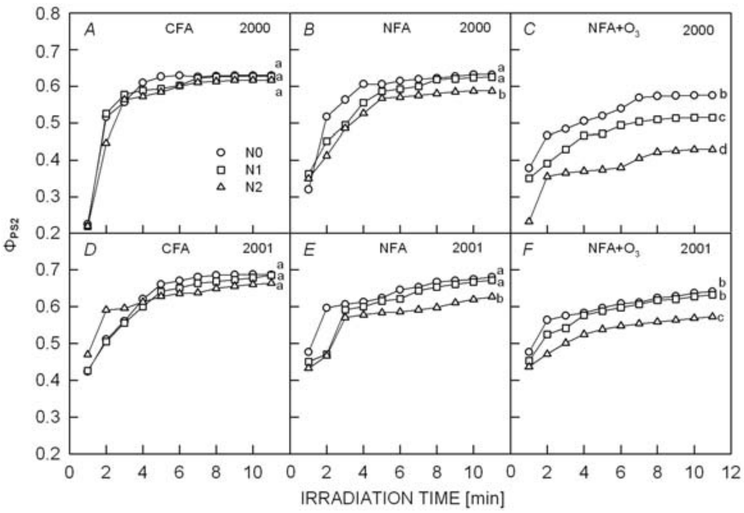

Fig. 2. Time courses of the quantum efficiency of PS2 (Φ_{PS2}) at actinic irradiance of 330 μmol(photon) m^{-2} s^{-1} in watermelon leaves cv. Reina de Corazones during the years 2000 (*A, B, C*) and 2001 (*D, E, F*) in CFA (*A*, *D*), NFA (*B*, *E*), and NFA+O_3 (*C, F*) with three nitrogen concentrations: N0, N1, and N2. Means for *n* = 6. *Different letters* indicate significant differences in the means ($p<0.05$) due to the interaction between air quality and N concentrations at the end of the actinic irradiation (Tukey`s test) at final point.

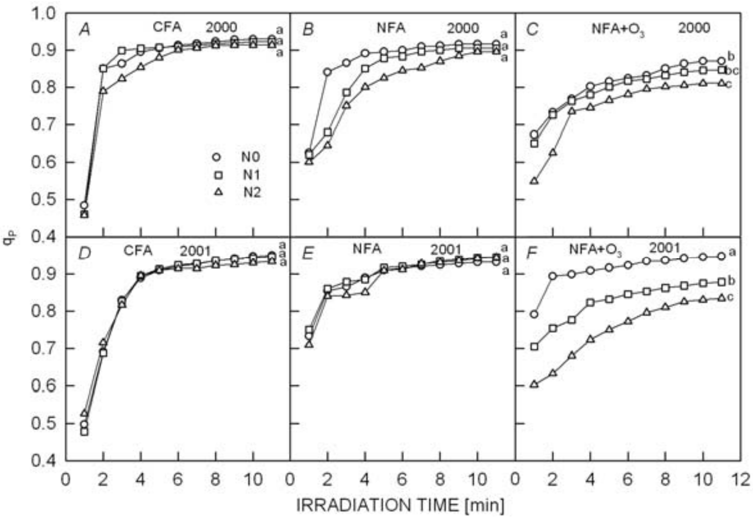

Fig. 3. Quenching analysis of photochemical quenching (q_P) at actinic irradiance of 330 μmol(photon) m^{-2} s^{-1} in watermelon leaves cv. Reina de Corazones during the years 2000 (*A, B, C*) and 2001 (*D, E, F*) in CFA (*A*, *D*), NFA (*B*, *E*), and NFA+O_3 (*C, F*) with three nitrogen concentrations: N0, N1, and N2. Means for *n* = 6. *Different letters* indicate significant differences in the means ($p<0.05$) due to the interaction between air quality and N concentrations at the end of the actinic irradiation (Tukey`s test) at final point.

treatments also affected an increase in the MDA content. The main increase was obtained in both NFA+O_3 and N_2 in both years, although the MDA content was higher in 2001.

Discussion

In the present study, ambient concentrations of O_3 on the Eastern coast of Spain (a major centre of horticultural production in the Mediterranean) substantially exceeded the present UN-ECE critical guidelines of crops protection. Furthermore, O_3-induced yield suffered losses in both years, which were higher when the maximum N concentration was applied. The plant response indicates that the inhibitory effect of O_3 on the photosynthesis induction reactions depends on the N concentration, and seems to confirm the presence of an interaction between the two factors with effects on the Chl *a* fluorescence parameters, lipid peroxidation, and the total yield.

The F_v/F_m ratio indicates the photochemical efficiency of PS2, a higher decrease in this parameter occurring in

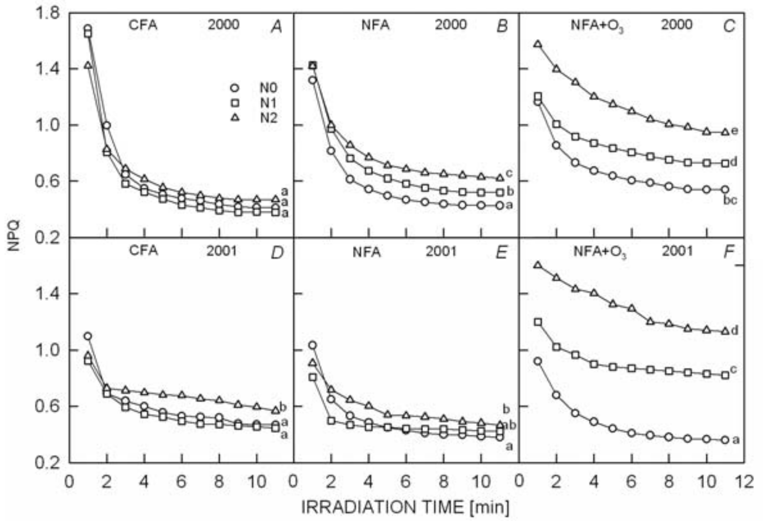

Fig. 4. Variations in non-photochemical quenching (NPQ) during fluorescence induction at actinic irradiance of 330 μmol(photon) m^{-2} s^{-1} in watermelon leaves cv. Reina de Corazones during the years 2000 (*A, B, C*) and 2001 (*D, E, F*) in CFA (*A, D*), NFA (*B, E*), and NFA+O_3 (*C, F*) with three nitrogen concentrations: N0, N1, and N2. Means for $n = 6$. *Different letters* indicate significant differences in the means ($p<0.05$) due to the interaction between air quality and N concentrations at the end of the actinic irradiation (Tukey`s test) at final point.

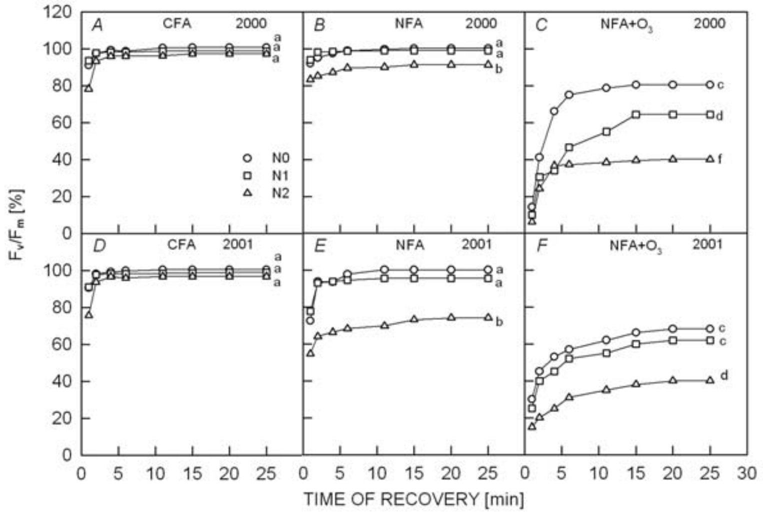

Fig. 5. Recovery of maximum quantum efficiency of PS2 (F_v/F_m) subsequent to 30 min treatment at 1 800 μmol m^{-2} s^{-1} in watermelon leaves cv. Reina de Corazones during the years 2000 (*A, B, C*) and 2001 (*D, E, F*) in CFA (*A, D*), NFA (*B, E*), and NFA+O_3 (*C, F*) with three nitrogen concentrations: N0, N1, and N2. Means for $n = 6$. *Different letters* indicate significant differences in the means ($p<0.05$) due to the interaction between air quality and N concentrations at the end of the actinic irradiation (Tukey`s test) at final point.

plants grown in higher O_3 concentration (Guidi *et al.* 2001, Calatayud *et al.* 2002a,b), and when a higher N concentration was applied (Shangguan *et al.* 2000). A decrease in this ratio is a reliable sign of photoinhibition, which indicates that these plants become more light-sensitive (Krause 1988). On the other hand, F_v/F_m recovery, following a photoinhibitory treatment, was more highly impaired in NFA+O_3 plants under both the N1 and N2. The sensitivity to photoinhibition is governed by various factors, such as the capacity to repair the photoinhibition PS2 during irradiation, the size of the light-harvesting antenna of PS2, and how efficiently harmless excitation energy is dissipated (Krause 1988). Photoinhibition has been associated with the inactivation of many more PS2 RCs, given an accumulation of a higher proportion of non-Q_B-reducing PS2 RCs (Lu and Zhang 2000). The key characteristics of these non-Q_B-reducing centres is the inhibition of electron transport from Q_A^- to Q_B (Cao and Govindjee 1990) that leads to an increase in the fraction of the Q_A reduction state, thus resulting in a lower q_P in the NFA+O_3 plants. A greater susceptibility to photoinhibition is not apparently related to less protective, non-photochemical quenching (Demmig-Adams 1990). According to our results, a higher NPQ was observed in plants grown in NFA+O_3 and under both the N1 and N2 conditions in both years, indicating that a higher proportion of absorbed photons was lost as heat instead of being used to drive photosynthesis (lower photochemical quenching). Photochemical quenching of the Chl *a* fluorescence, q_P, is related to redox states of the primary electron acceptor, Q_A (Fuhrer *et al.* 1997), and also to the fraction of open PS2 RCs. q_P decreased in all treatments at the onset of actinic irradiation, which can be attributed to the initial reduction of Q_A, and which then went on to increase during the irradiation period reflecting the oxidation of Q_A, and an apparent steady-state condition was found after approximately 6 min of irradiation. The values in the steady-state were minor under the N2+NFA+O_3 conditions, causing an increase in the reduction state of the PS2 acceptor, Q_A, indicating a decrease in the fraction of the open PS2 (Krause and Weis 1991), that suggests a down-regulation of the open PS2 RCs (Genty *et al.* 1989) in both years. This means that both O_3 (Calatayud *et al.* 2002a,b) and N increased (Shangguan *et al.* 2000) the excitation pressure on PS2, and contributed to the closure of the PS2 RCs. This fact diminishes the possibility of electron transport from PS2 to PS1 and beyond (Seaton and Walker 1990). In our results, Φ_{PS2} was closely correlated with the quantum yield of non-cyclic electron transport (Genty *et al.* 1989), was reduced by the interaction of O_3 and N in both years, and may be a mechanism to down-regulate photosynthetic electron transport so that the production of ATP and NADPH are in equilibrium with the decreased CO_2 assimilation capacity such as occurs in lettuce and maize (Lu and Zhang 2000, Calatayud *et al.* 2002b). Simultaneously, NPQ initially increased at the onset of the actinic irradiation as a result of electron transport initiation (Owens 1994) and of the formation of ΔpH. Subsequently, NPQ began the relaxation associated with both the ATP synthesis and CO_2 fixation. NPQ in NFA+O_3 with both the N_1 and N_2 rose quickly, but a slow relaxation occurred at the moment of steady-state, and the values of either the CFA or NFA plants were not reached. Observing that O_3 prevents the relaxation of membrane energization *via* NPQ during the fluorescence curve induction is a further confirmation of the Calvin cycle activity inhibition (Guidi *et al.* 1997). In our experiments, the higher inhibition of quenching NPQ was associated with an interaction of excess of N and O_3. In many studies, an increase in the thermal dissipation in the PS2 antennae (increase in NPQ) has been seen, and it competes with the excitation energy transfer from the PS2 antennae to the PS2 RCs, thus resulting in a decrease in the efficiency of the excitation energy capture by the open PS2 RCs (Φ_{exc}) (Genty *et al.* 1990, Demmig-Adams *et al.* 1996). We obtained a significant decrease in Φ_{exc} (data not shown). All results on Chl *a* fluorescence parameters are in accordance with the explanation for a greater sensitivity to O_3 of those plants grown with a higher N supply rate.

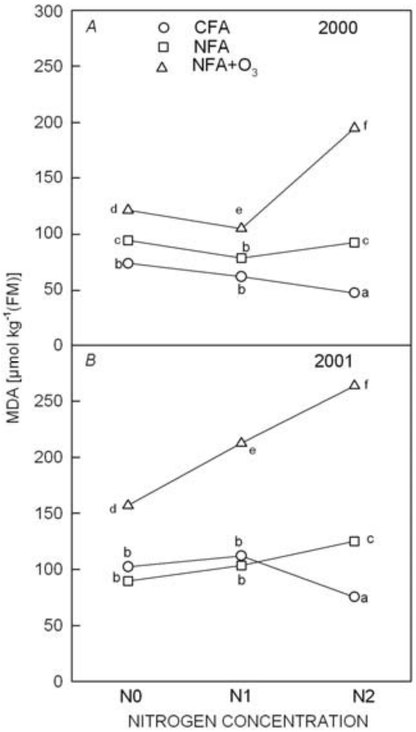

Fig. 6. Leaf malondialdehyde (MDA) content in watermelon leaves subjected to different treatments during the years 2000 (*A*) and 2001 (*B*) in CFA, NFA, and (NFA+O_3) with three nitrogen concentrations: N0, N1, and N2. Means for $n = 9$. Different letters in each graph indicate significant differences in the means ($p<0.05$) due to the interaction between air quality and nitrogen concentrations (Tukey`s test).

Changes in fluorescence yield under O_3 and N treatments can be associated with alterations in membrane properties. The MDA content, which represents the state of membrane lipid peroxidation, and which has been correlated with the degree of O_3 exposure to plants (Ranieri *et al.* 1996) was higher in the NFA+O_3 plants and it was in this group where maximum effects occur with the higher N concentration, confirming oxidative stress. The measurable effects of O_3 on the watermelon yield were not detected in plants grown at lower N supply. Only when the higher O_3 concentration is combined with the higher N concentrations, does the relative yield decrease occur in both years.

This is the first evidence that the interaction between ozone and N fertilizer alters the Chl *a* parameters, increases lipid peroxidation, and decreases watermelon yields.

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