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## Effects of Ozone Concentration on Cabbage (*Brassica oleracea* L.) in a Rural Mediterranean Environment

By

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**Key words:** Cabbage, chlorophyll fluorescence, lipid peroxidation, ozone, solute leakage.

### Summary

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The effects of three levels of O<sub>3</sub> air quality on a variety of cabbage grown in Valencia (Spain) was studied. The investigation was based on modulated chlorophyll *a* fluorescence, lipid peroxidation and solute leakage. Increased O<sub>3</sub> exposure reduced F<sub>v</sub>/F<sub>m</sub> as well as photochemical quenching. This alteration may be associated with an injured plasmalemma and increased ion leakage at increased O<sub>3</sub> concentration. TBARS analysis confirmed the hypothesis that O<sub>3</sub> modified the membrane structure. These alterations in membrane structure reduced the ability to develop photochemical quenching and non-photochemical quenching. These results suggest that O<sub>3</sub> has adverse effects on cabbage grown in the Valencia region during the winter.

### Introduction

The mechanisms of O<sub>3</sub> toxicity in plants have not been completely defined, but O<sub>3</sub> or its radical by-products impair membrane functions resulting in reduced photosynthesis, accelerated foliar senescence and premature leaf abscission (FARAGE & al. 1991, PELL & al. 1992, SOLDATINI & al. 1998). Photosystem II (PSII) activity is believed to play a regulatory role in the response of photosynthesis to environmental changes (BAKER & al. 1994). The photochemistry of PSII has been extensively studied using chlorophyll *a* fluorescence induction. There are

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many reports about the effects of  $O_3$  on photosynthetic energy conversion (SOLDATINI & al. 1998, CALATAYUD & BARRENO 2000, 2001). All these reports clarify the modified photosynthetic performance as photoinhibition, over-reduction of photosystem reaction centres or heat dissipation induced by  $O_3$ . The primary site of action of  $O_3$  is quite probably the plasmalemma with consequent modifications to membrane structure and function (HEATH 1988, GUIDI & al. 1999, CALATAYUD & BARRENO 2001) which in turn may alter photosynthetic metabolism.

The aim of the present work was to determine the sensitivity of cabbage var. "Caramba" grown in open top chambers (OTC's) in the Mediterranean area (Valencia, Spain) during the winter to  $O_3$ . Although there is evidence that many rural zones in the Mediterranean area suffer photochemical problems especially during the spring and summer little is known of the sensitivity of winter-grown crops to  $O_3$ . We report here on the effects of  $O_3$  on chlorophyll a fluorescence, lipid peroxidation and solute leakage.

## Material and Methods

The experimental site was established at the "Centro de Capacitación Agrária (Generalitat Valenciana)" Carcaixent (39°7'N, 0°27'W and 22.1 m above sea level) in a rural environment. The site is about 40 km south of Valencia on the Spanish Mediterranean Coast.

Cabbage seeds (*Brassica oleracea* L.) var. "Caramba" were germinated in vermiculite and maintained in a greenhouse. Four-week-old seedlings were transplanted into large pots containing a commercial soil mixture (Terraplant, BASF, Uchte, Germany) and transferred into OTC's where they remained for 30 days (from the middle of January to the middle of February). During the growth period plants were watered daily.

Potted plants were grown in 9 OTC's, based on the original design employed in the NCLAN programme (see HEAGLE & al. 1973). Over the course of the growing season, three OTC's were CFA, ozone-free air, three OTC's were ventilated with non-filtered air (NFA) (CALATAYUD & BARRENO 2001) and three received additional ozone (NFA+ $O_3$ ). Ozone was generated using pure electrically discharged compressed oxygen (ozone generator S-3003, G.O.A.C., DASIBI) added to air. The flow of ozone-enriched air to the OTC's was regulated by a flow controller. The ozone additions took place between 10 h to 15 h GMT five days a week.

At the end of the growth period chlorophyll fluorescence was measured at ambient temperature in situ in the OTC's, using a portable fluorometer (PAM-2000, Walz, Effeltrich, Germany), see for more information in CALATAYUD & al. 2002. The extent of lipid peroxidation was estimated using malondialdehyde (MDA) as an indicator (HEATH & PARKER 1968, DHINDSA & al. 1981). Leaves used were without visible symptoms at the end of the growth period. The measurement of solute leakage was estimated according to the protocol employed by NALI & al. 1998. Twenty disks taken from six plants for each air treatment were placed in 10 ml ultrapure water (Millipore, Milli-Q Plus) and shaken at room temperature. Conductance measured is expressed as a percent of total electrolyte leakage (DIJAK & ORMROD 1982).

## Results

During the growth period  $SO_2$  and  $NO_x$  concentrations were low (less than 2 nl l<sup>-1</sup> for  $SO_2$  and 8 nl l<sup>-1</sup> for  $NO_x$ ). The twelve-hour (daylight hours) monthly mean concentrations of  $O_3$  inside the elevated  $O_3$  chambers (NFA+ $O_3$ ) (mean for

30 days was  $65 \text{ nl l}^{-1}$ ) was about twice that of ambient concentrations of  $\text{O}_3$  (NFA mean for 30 days was  $30 \text{ nl l}^{-1}$ ). The mean concentration of  $\text{O}_3$  in the CFA chambers was  $10 \text{ nl l}^{-1}$ . The environmental conditions were similar in all treatments.

Table 1 shows the leaf chlorophyll *a* fluorescence characteristics of the leaves subjected to CFA, NFA and NFA+ $\text{O}_3$  treatments at the end of the growing period (30 days). The maximum quantum yield of PSII photochemistry,  $F_v/F_m$  (variable/maximum fluorescence at light saturation) was adversely affected by  $\text{O}_3$  exposure (NFA+ $\text{O}_3$ ). The changes in fluorescence parameters in the steady-state level are shown in the Table 1. The actual photochemical efficiency ( $\phi_{\text{PSII}}$ ) and the fraction of open PSII centres ( $q_p$ ) showed a significant reduction in NFA+ $\text{O}_3$  (24% with respect to control, CFA). This decrease is less in NFA treated leaves but significant (11% with respect to CFA). NPQ (non-photochemical quenching of chlorophyll *a* fluorescence) is considered to be a good estimate of the energy dissipated non-radiatively by plants. NPQ decrease in leaves NFA and NFA+ $\text{O}_3$  both are significant with respect to CFA.

The MDA concentration was increased significantly in NFA and NFA+ $\text{O}_3$  leaves with respect to control leaves (Table 1). Solute leakage of leaf disks increased with  $\text{O}_3$  level. The increase was 34% in NFA and 72% in NFA+ $\text{O}_3$  with respect to the control (Table 1).

Table 1. Chlorophyll fluorescence parameters, MDA concentration ( $\text{nmol MDA g}^{-1} \text{ f.w.}$ ) and solute leakage (% of total) determined in the leaves of *Brassica oleracea* var. "Caramba" after 30 days in open top chambers. Air quality are: CFA, air filtered; NFA air non-filtered and NFA+ $\text{O}_3$ , air non-filtered and additional ozone. Values are means of 8 samples. For comparison of means, variance analysis (ANOVA) followed by the least significance differences (LSD) test, calculated at 95% confidence level, was performed. Values followed by the same letter indicate no significant differences.

Treatment	$F_v/F_m$	$\phi_{\text{PSII}}$	$q_p$	NPQ	MDA	% solute leakage
CFA	0.786a	0.580a	0.870a	0.868a	62a	18a
NFA	0.728ab	0.518b	0.725b	0.725b	85b	24b
NFA+ $\text{O}_3$	0.675b	0.442c	0.687c	0.687c	112c	31c

## Discussion

The exposure of plants to ambient  $\text{O}_3$  (NFA) and fumigation (NFA+ $\text{O}_3$ ) caused a decrease in all chlorophyll fluorescence parameters, and an increase in a lipid peroxidation and solute leakage.

The  $F_v/F_m$  ratios indicate the photochemical efficiency of PSII and a decrease in this parameter is a reliable sign of photoinhibition (KRAUSE 1988). In the NFA and NFA+ $\text{O}_3$  this ratio decreased, indicating that plants became more light sensitive. The decline of  $F_v/F_m$  which may result from an increase in NPQ, photo-damage of PSII centres or both (OSMOND & al. 1993), and the decline in  $F_v/F_m$  were attributable to PSII stress, because the NPQ parameter was adversely affected by  $\text{O}_3$  ambient (NFA) or fumigation (NFA+ $\text{O}_3$ ). The low values of  $F_v/F_m$  were re-

lated with a decrease of  $q_p$ , which measures the degree of closure of centres affected by ozone in NFA and NFA+O<sub>3</sub> indicating that O<sub>3</sub> decreased the capacity for re-oxidizing Q<sub>A</sub> during actinic illumination. This means that O<sub>3</sub> increased excitation pressure on PSII and contributed to the closure of PSII reaction centres (CALATAYUD & al. 1999, CALATAYUD & BARRENO 2001). NPQ is related with a dissipative process highly dependent on membrane integrity (WALTER & HORTON 1991), the lower values of NPQ in NFA and NFA+O<sub>3</sub> plants with respect to CFA may be due to lower rates of  $\phi_{PSII}$  and damage in thylakoid membranes as a consequence of substantial oxidative stress amongst other things. An increase in MDA concentration, which represents the state of membrane lipid peroxidation and an increase in solute leakage confirmed this hypothesis.

To summarize, ambient and elevated O<sub>3</sub> concentrations in winter induced alterations in chlorophyll *a* fluorescence parameters in cabbage plants, increased lipid peroxidation and caused a cellular leakage into intercellular spaces.

#### A c k n o w l e d g e m e n t s

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