

Ozone Effects on Fruit Productivity and Photosynthetic Response of Two Tomato Cultivars in Relation to Stomatal Fluxes

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Abstract

An Open-Top Chamber experiment on two tomato cultivars (cv. Oxheart and cv. San Marzano) was carried out in Curno (Northern Italy) between June and September 2007. Two ozone treatments were applied for a 3.5 months period: Non-Filtered OTC (NF-OTC, 95% of ambient ozone) and Charcoal-Filtered OTC (CF-OTC, 50% of ambient ozone). Diurnal cycles of porometry measurements were performed during the season and allowed to draw a stomatal conductance model for each cultivar in order to calculate the ozone stomatal fluxes taken up by plants. Assessments on fruits yield were performed during the season, taking into account the number of fruits, their fresh weight and their marketability. In addition, measurements on the chlorophyll fluorescence of photosystems were carried out to assess possible negative effects on photosynthetic efficiency. Despite the two cultivars absorbed a similar ozone stomatal dose during the season (with an 8% difference), their responses to ozone treatments were totally divergent in relation to both fruits yield and photosynthetic efficiency. Plants of cv. Oxheart grown in NF-OTCs showed significant yield loss in the total weight of fruits (-35.9%) which is exclusively related to a decrease in the number of fruits produced (-35.7% of total fruits; -30.6% of marketable fruits), since mean fresh weight of fruits remained unaffected. Moreover the same plants displayed low values (in comparison to CF-OTCs plants) of the photosynthetic efficiency index (PI_{abs}) during the most intense period of ozone stress (July) occurred in the flowering stage of plants and at the beginning of fructification. Plants of the cv. San Marzano had an opposite behaviour with an increase of the mean fresh weight of fruits in plants grown in NF-OTC (even if not statistically significant) and no difference in the number of fruits produced and in the values of photosynthetic efficiency.

Key-words: tomato, ozone fluxes, stomatal conductance, marketable fruits, chlorophyll fluorescence.

1. Introduction

Tropospheric ozone pollution is known to be an important risk factor causing negative impacts on crop yield in many parts of Europe and North America (Benton et al., 2000; Mauzerall and Wang, 2001; Pleijel et al., 2007). A constant increase of ozone concentration background levels has been recorded during the last decades in the most industrialized countries, particularly in the Mediterranean area, as a consequence of increasing emissions of anthropogenic precursors (Vingarzan, 2004; EEA, 2007). Starting

from the 80s, many experimental studies and International research programs have tried to assess ozone negative effects on vegetation and concluded that this pollutant is the main responsible of ecological and economical damages on crops, forests and semi-natural vegetation in almost every European and North-American Country (Fuhrer et al., 1997; UN/ECE, 2004) and in many developing Countries (Emberson et al., 2001).

Moreover, several investigations have repeatedly shown that ozone impact is more strict-

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ly linked to the ozone flux truly absorbed by plants through stomata rather than to the mere exposure to a given air concentration (Musselman and Massman, 1999; UN/ECE, 2004).

As a consequence, a large effort of the scientific community is aimed to the development of stomatal conductance models suitable for ozone-flux calculation or estimation, and to the definition of reliable ozone-flux response relationships for large scale risk assessments. Currently, this kind of relationships has been developed for wheat and potato grown in Northern Europe climatic conditions, since these crops have the major datasets available (Pleijel et al., 2002; Danielsson et al., 2003).

On the contrary, relatively few datasets are available from southern Europe, which can be used to derive ozone-flux relationships for the main sensitive Mediterranean crops (e.g. field-grown horticultural crops such as tomato, lettuce, spring onion and soft fruit), and the most recent Mapping Manual from UN/ECE (2004) still suggests to use a concentration-based critical level for horticultural crops calculated over a 3.5 months period. Moreover, Mediterranean Countries, and particularly Italy, show the highest ozone concentrations in Europe, due to the high levels of temperature and solar radiation during summer (EEA, 2006; Paoletti, 2006).

Given these preliminary remarks, the objective of the present study was to evaluate the negative effects of ozone on crop yield, assessed as relative biomass reduction in ripe fruits, of two well-spread cultivars of tomato (*Lycopersicon esculentum* Mill., cv. "Hoxheart" and cv. "San Marzano") in relation to ozone exposure and ozone uptake as stomatal fluxes. Furthermore, a possible effect on the performance of the photosynthetic systems has been investigated with measurements on the fluorescence transient of the chlorophyll *a* in photosystems II.

Italy is the European country leader in the annual production of tomatoes with 6.3 M of tonnes and 122,000 ha of cultivated areas in 2006 (FAOSTAT, 2007), and with 2/3 of this production deriving from open-field cultivations; it is thus obvious that the assessment of a significant yield loss due to the high ambient levels of ozone may have important impacts and consequences also from an economical point of view.

Data reported from this experiment can be considered as a contribution for the definition

of an appropriate ozone flux-based critical level for the most sensitive horticultural crops at European level. Such critical level will take into account all the environmental conditions that influence stomatal conductance, and yield losses related to the ozone uptake.

2. Materials and methods

The experiment was carried out in summer 2007 in the Open-Top Chambers (OTC) research site of Curno Forest Nursery (lat. 45°70' N, long. 9°62' E, elev. 242 m asl) on the Northern edge of the Po Valley. Details concerning the construction parameters of the OTCs and the pollution conditions of the area have been reported in previous studies (Bussotti et al., 2007a, Gerosa et al., 2007). Experiments were made with two cultivars of *Lycopersicon esculentum* Mill., cv. Oxheart and cv. San Marzano, using four OTCs and two ozone exposure treatments: 50% ambient ozone in charcoal-filtered OTC (CF), 95% ambient ozone in non-filtered OTC (NF). Plants were transplanted on May 17 in single pots filled with a standard peat based substrate, and placed inside the OTCs from June 1 until September 15, when ripe fruits harvest was concluded. Five plants for each cultivar were grown in each OTC and optimal conditions of irrigation were maintained throughout the whole experiment period.

Ozone concentrations within each chamber were continuously monitored with an ozone automatic analyzer model 1308 RS (Dasibi Italia srl, Milano, I), via a solenoid valves switch system, which drew air through sampling points at the centre of each chamber at 90 cm a.g.l. The system was driven and controlled by a personal computer equipped with an NI-DAQ 6.9 I/O board and a Labview 6.1 program (National Instruments, Austin, Texas), purposely devised. Temperature and relative humidity were monitored in one filtered OTC using a Vaisala 50Y sensor (Vaisala, Vantaa, Finland). At the same time, all relevant meteorological data from a two-level tower (3m and 6m) were continuously monitored and recorded by a CR10X datalogger (Campbell Sci., Logan, Utah) equipped with an AM16/32 relay multiplexer and an SDM-SW8A switch closure interface (Campbell Sci., Logan, Utah).

2.1 Stomatal conductance measurements and modelling

Stomatal conductance to water vapour (g_w) was measured with a cycling diffusive porometer AP-4 (Delta-T Devices LTD, Cambridge, UK). The instrument works by measuring the time it takes for a leaf to release sufficient water vapour to change the relative humidity in a small chamber by a fixed amount. This is compared with a calibration plate of known resistance in order to derive the stomatal resistance or conductance of the leaf. The instrument was calibrated before each series of measurements. Measurements were performed on five different days from July 17 to September 5, with four diurnal cycles starting from 8:00 AM until 5:30 PM. Leaf stomatal conductance was measured in the median portion of the lamina, on one light-exposed leaf of each plant in all the OTCs.

In order to calculate stomatal fluxes, the stomatal conductance for each species has been modelled using the classical Jarvis multiplicative approach (Jarvis, 1976):

$$g_w = g_{\max} * [f_T * f_{\text{PAR}} * f_{\text{VPD}}]$$

The g_{\max} for water value has been chosen by taking the 95th percentile of all the valid stomatal conductance measurements.

The limiting functions f_T , f_{PAR} , f_{VPD} , which give a value between 0 and 1, were characterized by *borderline analysis*, using the points corresponding to the 98th percentile of g_{rel} (conductance value relative to the g_{\max}) plotted for each class of values of environmental variable considered (T, VPD, PAR). It was thus possible to make the modelling less dependent on outliers representing measurements of doubtful quality. No limiting function for soil water content was considered because constant irrigation was provided during the whole season (pots were irrigated every two days).

2.2 Calculation of ozone stomatal fluxes and ozone exposure

Ozone stomatal fluxes were calculated for a top canopy leaf according to Emberson et al. (2000) and Karlsson et al. (2000):

$$F_{\text{st},\text{O}_3} = [\text{O}_3] / (R_{b,\text{O}_3} + 1.68/g_w)$$

where 1.68 accounts for the different diffusivity of water in air with respect to the ozone. The

sub-laminar ozone resistance R_{b,O_3} was obtained using Unsworth (1984) equation:

$$R_{b,\text{O}_3} = k * (d/u)^{1/2}$$

where the empirical coefficient k is set to a value of 132, as supported by Thom's 1975 measurements, d is the mean leaf width for each specie, and u is the mean wind speed at top canopy level.

Ozone dose was calculated summing up all the semi-hourly stomatal fluxes from June 1, when pots were placed in OTCs, to September 15 when the experiment was concluded. In the same period of 3.5 months, as suggested by UN/ECE (2004), ozone exposure during daylight hours was calculated by means of the AOT40 index (Kärenlampi and Skärby, 1996).

2.3 Chlorophyll a fluorescence measurements

Photosynthesis parameters were measured on 17 and 24 July, 2 and 13 August and 5 September. All measurements were performed with a HandyPEA fluorimeter (Hansatech Instruments, Norfolk, UK) between 10 and 11 AM (in order to avoid the hottest hours in the summer) on two leaves, chosen at random, for each individual. Measurements were taken in subapical position and with a good exposure to light. Before each measurement, the leaves were dark adapted for 30 min with leaf clips. The rising transients were induced by a red light (peak at 650 nm) of 600 Wm⁻² provided by an array three light-emitting diodes. Data recorded and JIP-test parameters (Strasser et al., 2000; Strasser et al., 2004) were processed through Biolyzer 3.0 software (by Ronald M. Rodriguez, Bioenergetics Laboratory, Geneva, CH).

2.4 Fruits yield assessment

Tomato fruits were weekly harvested, starting from July 28, until the end of experiment (September 15). For each plant, fruits were counted, weighed and ranked for their marketability. Ripe fruits with visible lesions and/or having weight < 10 g were considered non-marketable. Data for each cultivar and ozone treatment were analyzed by one-way ANOVA for a completely randomized design.

3. Results

3.1 Meteorological conditions and ozone exposure

Trends and mean values of the main meteorological parameters, during the 3.5 months experiment period, are reported in figure 1 and table 1. For a large part of this period daily mean temperatures were between 20 and 25 °C, with the exception of the last two weeks of July, characterized by sunny warm and dry conditions and by daily mean temperatures slightly below 30 °C. Seasonal rainfalls (Tab. 1) were mostly concentrated in August (142 mm) with highly dry conditions in June (21 mm) and typical values in July (73 mm, occurred during the first days of the month).

Table 1 reports also, for each month, mean concentrations of ozone, maximum values registered and AOT40 values in the two ozone treatments (NF-OTC and CF-OTC). It is evident that during July, warm and dry conditions (and low rainfalls) strongly enhanced the photochemical processes that lead to the formation of tropospheric ozone, which maintained a monthly concentration average higher than the

phytotoxic threshold (40 ppb) and an AOT40 monthly value corresponding to the 60% of the total seasonal value. In figure 2 we reported the seasonal trend of AOT40 index confronting the two different ozone treatments used. In the NF-OTCs, the final value of AOT40 was 8162 ppb h, far beyond the concentration-based critical level of 6000 ppb.h suggested by UN/ECE (2004), which was reached on August 5. In the CF-OTCs on the contrary, the AOT40 index was below 1000 ppb h, thanks to a good performance of the charcoal filters (Fig. 3). Seasonal curve in the NF-OTCs displays a sigmoidal course with a large part of accumulation concentrated between the first days of July and the middle days of August.

3.2 Stomatal conductance model and ozone fluxes

A database of 700 measurements of abaxial surface stomatal conductance to water vapour was used to draw two stomatal conductance models, one for each cultivar. The maximum stomatal conductances (g_{max}) individuated for the two cultivars are reported in table 2 (values are re-

Table 1. Monthly average values for the main meteorological and ozone parameters.

	T (°C)	UR (%)	Rain (mm)	Mean O3 ppb*	Max O3*	AOT40 NF ppb.h	AOT40 CF ppb.h
June	21.9	64.6	21	23.9	58.0	374	23
July	25.2	44.5	73	43.5	104.0	4952	708
August	22.7	61.2	142	33.2	79.0	2401	193
September**	20.4	51.5	0	25.7	69.0	435	0

* Values reported are from ozone sampling points of Non-Filtered OTC.

** Values calculated only for the first 15 days of the month.

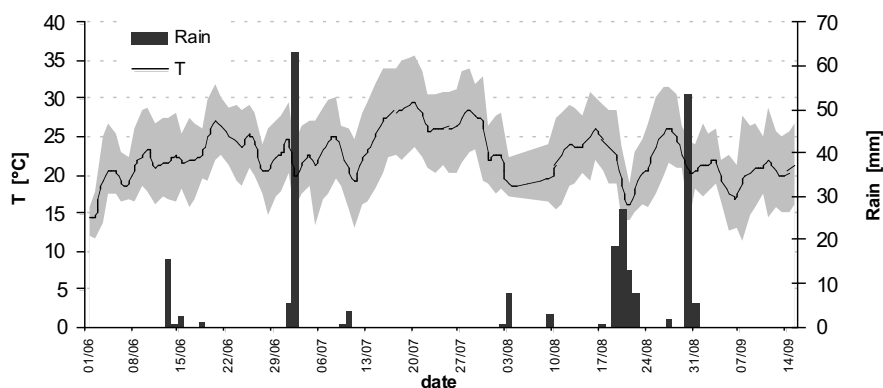


Figure 1. Daily mean temperature and rain during the experiment period. Grey area is delimited by maximum and minimum daily temperature.

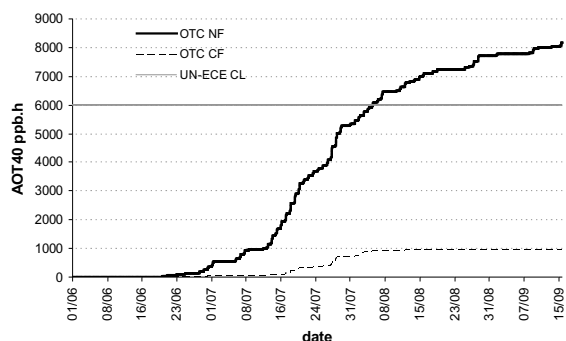


Figure 2. AOT40 index during the growth season in Charcoal Filtered (CF) and Non-Filtered (NF) Chambers. Grey line is the UN-ECE Critical Level (CL) for tomato.

ferred to Projected Leaf Area). Table 2 also reports the equations of the limiting functions f_{PAR} , f_T , f_{VPD} and the values of their parameters obtained by the boundary-line analysis (Fig. 4). These models were “fed” with the meteorological parameters referred to the top canopy leaves in order to calculate the cumulative ozone stomatal fluxes (AF_{ST}) reported in figure 5. Despite a 22% difference in the g_{max} values of the two cultivars, the seasonal ozone stomatal doses were quite similar: 39.66 and 36.63 $mmol\ m^{-2}\ s^{-1}$ in NF-OTCs, respectively for cv. Oxheart and cv. San Marzano, 20.57 and 19.84 in CF-OTCs.

The general trend of the AF_{ST} during the season was significantly different from the AOT40 trend reported in figure 3 being more regular with a quite constant increasing in both treatments.

3.3 Fruits yield

The total and commercial yields, expressed as fresh weight of ripe fruits, were significantly affected by ozone treatment only in cv. Oxheart (Tab. 3). Plants of this cultivar grown in NF-OTCs show large decrease in fresh weight values of total fruits and marketable fruits, with significant differences in comparison to the plants grown in CF-OTCs (-35.9% and -30.87% respectively). The reduced yield was mainly caused by a reduction in fruits number rather than fruits mean fresh weight, which remained totally unaffected (-0.33%). The percentage of non-commercial fruits was approximately 17% and 7% of the total fruits harvested respectively in CF-OTCs and NF-OTCs.

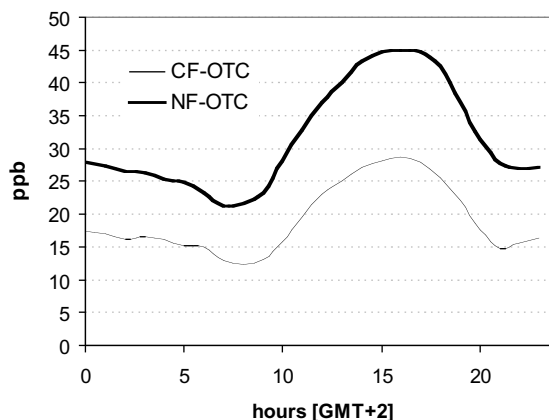


Figure 3. Mean daily ozone concentration in Charcoal-Filtered OTCs (CF-OTC) and in Non-Filtered OTCs (NF-OTC) during the growing season (1 June - 15 September).

Table 2. Summary of the coefficients values for the stomatal conductance models of the two tomato cultivars.

Equation function	Parameter	Oxheart	San Marzano	Unit
	g_{max} (P.L.A.)	1.425	1.825	cm/s
$f_{PAR} = 1 - e^{-a \cdot PAR}$	a	0.020	0.025	adim.
$f_T = \frac{(T - T_{min})}{(T_{opt} - T_{min})} \cdot \left[\frac{(T_{max} - T)}{(T_{max} - T_{opt})} \right]^b$	T_{opt}	27	27	°C
	T_{max}	39	37	°C
	T_{min}	13	15	°C
	b	0.857	0.833	adim.
$f_{VPD} = \frac{(1 - 0.1) \cdot (c - VPD)}{(c - d)} + 0.1$	c	5.53	3.87	KPa
	d	1.8	1.25	KPa

Notes: PAR is the photosynthetically active radiation expressed as $\mu mol_{photons} \ m^{-2} \ s^{-1}$; T_{min} and T_{max} are the range of temperature where the stomatal conductance is above its minimum value, and T_{opt} is the temperature where g reaches g_{max} , with all the temperatures expressed in °C; VPD is the atmospheric water vapour pressure deficit expressed in KPa.

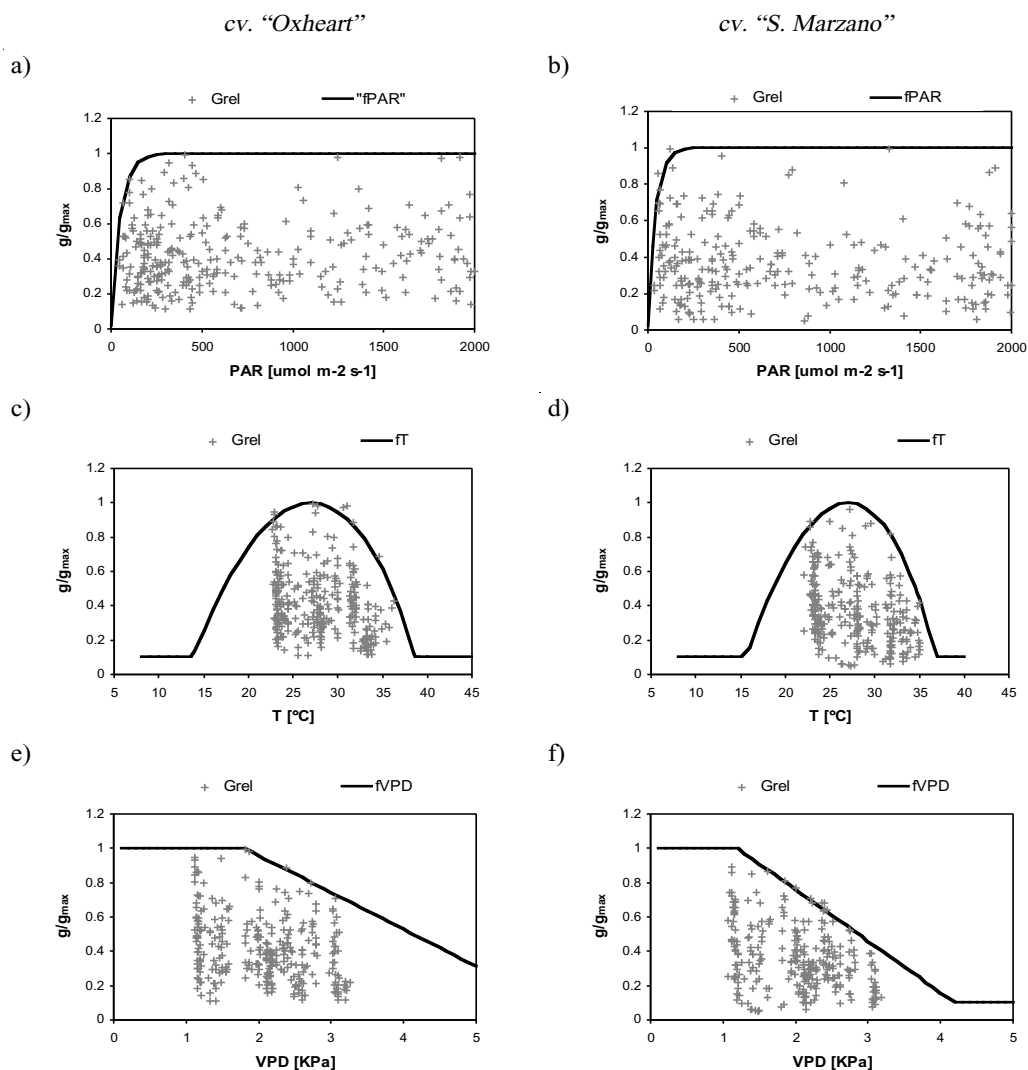


Figure 4. The f functions for the stomatal conductance models identified by boundary line analysis. Figures a), c) and e) refers to the “Oxheart” cultivar, while b), d), f) to the cultivar “S. Marzano”. $fPAR$, fT and $fVPD$ describes the dependence of the stomatal conductance to light, temperature and air vapour pressure deficit, respectively.

On the contrary, fruits yield in cv. San Marzano displayed opposite results in comparison to Oxheart. Plants grown in NF-OTC show an increase in total yield and marketable yield respectively of 20.46% and 28.21%. These differences were related to the mean fresh weight of fruits (+29.15% in NF-OTC) rather than the fruits number which remained substantially identical (-0.74% for marketable fruits).

3.4 Photosynthetic efficiency

Data from the five measurement campaigns carried out during the experiment were analysed and screened by ANOVA for repeated mea-

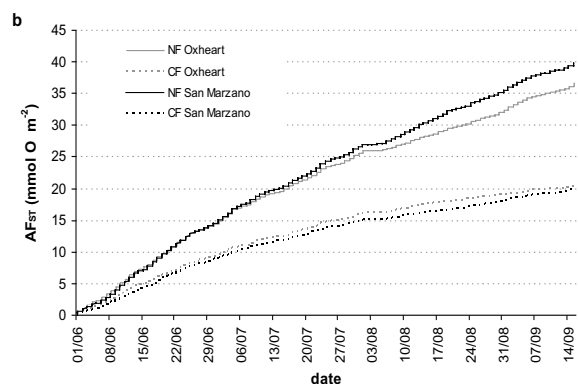


Figure 5. Accumulated stomatal fluxes (AF_{ST}) during the growing season in Charcoal Filtered (CF) and Non-Filtered (NF) OTCs for the two tomato cultivars.

Table 3. Fruits yield for the two tomato cultivars in the Charcoal-Filtered (CF) and Non-Filtered ozone treatments.

	Ozone Treat.	AOT40 ppb.h	AF _{ST} O ₃ mmolm ⁻²	Total yield		Marketable yield		
				Fruits fresh weight (g)	Fruits number	Fruits fresh weight (g)	Fruits mean fresh weight (g)	Fruits number
<i>Oxheart</i>								
	CF	924	20.57	10333.0	90	8968.6	119.6	75
	NF	8162	36.63	6621.6	56	6199.6	119.2	52
Difference				-35.90%*	-37.77*	-30.87%**	-0.33%	-30.66%
<i>San Marzano</i>								
	CF	924	19.84	4734.2	187	3955.2	29.5	134
	NF	8162	39.66	5703.2	169	5071.0	38.1	133
Difference				+20.46%	-9.62%	+28.21%	+29.15%	-0.74%

* p < 0.1.

** p < 0.05.

surements. However scarce significance in the differences between the two ozone treatments was found for the main fluorescence parameters usually assessed, as Fv/Fm, Kp and Kn.

Figure 6 reports the ratio NF/CF for the Performance Index based on absorption (PI_{abs}, Clark et al., 2000) which expresses the overall photosynthetic efficiency of the plant. Plants of cv. Oxheart grown in NF-OTCs had low values of PI_{abs} in the first three measurement campaigns, between July 17 and August 3, and presented a large increasing in the last two campaigns, strongly exceeding the CF plants values. In the same figure are also reported the hourly means of ozone concentration for the time period during which fluorescence measurements were performed. The first three measurement assessments were carried out during a long period of high ozone concentrations that began to decrease after the first days of August.

Plants of cv. San Marzano on the contrary show, for the two ozone treatments, quite identical values during the whole season with a slight difference only in the first assessment (July 17), when NF plants had a higher level of photosynthetic efficiency than CF plants.

4. Discussion

The two stomatal conductance models showed fairly high values of g_{max}, but it should be noted that in our experiment g_{max} was determined on measurements carried out on plants grown in conditions of maximum conductivity (i.e.

non-limiting water supply), and this definitively contributed to the high values. Nevertheless, these values can be considered consistent with other studies on tomato carried out with the same instrument (Sobeih et al., 2004) in non-limiting water conditions.

A negative impact of ozone on fruits yield of cv. Oxheart was clearly detected and related to a reduction in the number of fruits produced rather than the mean fresh weight. This latter parameter was not affected by ozone treatment, thus indicating that once the fructification process has started it is regularly carried on and concluded. The reduction in the total number of ripe fruits is also consistent with other results obtained for watermelon (Gimeno et al., 1999), beans (Schenone et al., 1992; Gerosa et al., submitted) and cucumber (Khan and Khan, 1999) and there is evidence that crop yield responses to ozone exposure are growth-stage dependent (Younglove et al., 1994; Stewart et al., 1996; Drogoudi and Ashmore, 2000). In this case it can be hypothesized the existence of an adverse effect of ozone on flowering or a higher susceptibility of plants during the period between flowering and fruit set, as suggested by other authors (Younglove et al., 1994; Calvo et al., 2007). Moreover in the same period the Performance Index (PI_{abs}) of NF-OTC plants displayed lower values in comparison to CF-OTCs plants (Fig. 6) and high levels of ozone concentration have been monitored in July, during the flowering stage of plants and at the beginning of fructification (the first harvest was on July

28). This decrease in the photosynthetic efficiency indicates a stress condition not adequately contrasted by the metabolic defence system at cellular and physiological level, and reveals negative effects of ozone directly on the photosynthetic processes, as suggested by other studies (Heath, 1994; Schraudner et al., 1997; Calatayud and Barreno, 2001; Degl'Innocenti et al., 2002). These impacts lead to a decrease of the energy available for flowering thus determining a decrease in the number of fruits.

On the contrary the San Marzano cultivar did not show ozone negative effects on fruit yield (both total and commercial) but a possible stimulating effect was detected, even if not statistically significant. In fact the NF-OTC plants of this cultivar showed a better photosynthetic efficiency, revealed by higher PI_{abs} relative values, than the CF-OTC plants starting already from the first assessment till the end of the season. The increase in photosynthetic efficiency as a response to oxidative stress has been already described by other authors (see e.g. the review of Bussotti et al., 2007b) and may generally enable the plants to respond to the need of metabolites, in order to feed the acclimatization process (detoxification and repair).

In many studies different responses to ozone have been explained by differences in stomatal conductance, and thus by the different ozone dose taken up by plants (Beyers et al., 1992; Panek and Goldstein, 2001; Fagnano and Mero-la, 2007; Gerosa et al., 2007). In this case, however, the different response of the two cultivars should be attributed to a genetically different sensitivity to ozone, since the cumulative stomatal ozone uptakes (AFst) were quite identical at the end of the season. Moreover the seasonal trends of ozone uptake were practically identical, thus excluding an effect of different timing in ozone dose accumulation. A seasonal difference of 8% in stomatal fluxes seems too low to explain such divergent responses in fruits yield.

The ozone tolerance of cv. San Marzano suggests a more efficient detoxification system at cellular level of this cultivar with respect to the other, which allows these plants to contrast the oxidative stress. The resistance to ozone has been often associated to high levels of antioxidants enzymes and compounds in the apoplast and the cytoplasm of foliar cells and to the ca-

capacity of plants to increase the activity of such metabolic defence under oxidative stress (Kangasjarvi et al., 1994; Ranieri et al., 1996; Rabotti and Ballarin-Denti, 1998; Langebartels et al., 2002; Maggio et al., 2007). The cv. San Marzano, that is a pool of ecotypes selected in Campania Region in 30s, seems to be more adapted to warm climates in which high temperature and sun radiation are frequently occurring with high ozone concentrations, as typical of Mediterranean area and particularly in Southern Italy.

5. Conclusions

Tomato cv. Oxheart is sensitive to ozone in relation to fruits yield while cv. San Marzano does not show particular susceptibility to this pollutant. Ozone impacts on cv. Oxheart affects the number of fruits (both total and marketable) rather than the size and the mean weight of fruit. Yield losses seem related to negative effects on photosynthetic efficiency occurring during flowering process and immediately before fructification. Quantification of fruits yield losses and calculation of cumulated ozone fluxes during the growing season, together with analogous results from other countries, may contribute to the definition of an appropriate flux-based critical level for horticultural crops. However results from this experiment give evidence that intraspecific variability of response to ozone can be as important as interspecific variability.

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