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(E)MERGING DIRECTIONS ON AIR POLLUTION AND CLIMATE CHANGE RESEARCH IN MEDITERRANEAN BASIN ECOSYSTEMS

A dose-response relationship for marketable yield reduction of two lettuce (*Lactuca sativa* L.) cultivars exposed to tropospheric ozone in Southern Europe

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Abstract The present study investigated the response to ozone (O_3) of two cultivars (cv.'Romana' and cv. 'Canasta') of irrigated lettuce grown in an open-top chamber (OTC) experiment in Mediterranean conditions. Two different levels of O_3 were applied, ambient O_3 in non-filtered OTCs (NF-OTCs) and −40% of ambient O_3 in charcoal-filtered OTCs (CF-OTCs), during four consecutive growing cycles. At the end of each growing cycle, the marketable yield (fresh biomass) was assessed while during the growing periods, measurements of the stomatal conductance at leaf level were performed and used to define a stomatal conductance model for calculation of the phytotoxic ozone dose (POD) absorbed by the plants.

Results showed that O_3 caused statistically significant yield reductions in the first and in the last growing cycle. In general, the marketable yield of the NF-OTC plants was always lower than the CF-OTC plants for both cultivars, with mean reductions of −18.5 and −14.5% for 'Romana' and 'Canasta', respectively. On the contrary, there was no statistically significant difference in marketable yield due to the cultivar factor or to the interaction between O_3 and cultivar in any of the growing cycle performed.

Dose-response relationships for the marketable relative yield based on the POD values were calculated according to different flux threshold values (*Y*). The best regression fit was obtained using an instantaneous flux threshold of 6 nmol O_3 m^{-2} s^{-1} (POD_6); the same value was obtained also for other crops. According to the generic lettuce dose-response relationship, an O_3 critical level of 1 mmol O_3 m^{-2} of POD_6 for a 15% of marketable yield loss was found.

Keywords Ozone fluxes · Lettuce · POD · Stomatal conductance · Biomass · Dose-response relationship

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Introduction

Tropospheric ozone (O_3) is currently considered the most harmful atmospheric pollutant for natural vegetation and crops, and its actual high concentration levels in Europe are of remarkable concern (Vingarzan 2004; EEA 2015).

The photochemical formation of O_3 in the atmosphere depends on the presence of primary precursors such nitrogen oxides and volatile organic compounds (produced by anthropogenic activities and natural processes) and on favourable conditions of high solar radiation and temperature. These conditions can produce particularly high concentrations during the summertime in the Mediterranean environment, representing a serious threat for the health of forests (Ferretti et al. 2007), semi-natural vegetation ecosystems (Mills et al. 2007) and for the productivity of many crops and horticultural species, which can be particularly sensitive to this abiotic stress (Mills et al. 2011; González-Fernández et al. 2014; Gerosa et al. 2009a).

The harmful effects of O_3 on plants at biochemical and physiological level, including photosynthesis, growth and yield reductions, have been extensively documented in the last years (Wittig et al. 2009; Booker et al. 2009, Agathokleous et al. 2015). However, it is even assumed that plant response and sensitivity to O_3 might be species specific (Bussotti et al. 2005) and, in the case of crops, even cultivar dependent (González-Fernández et al. 2010; Monga et al. 2015).

On the other hand, many studies revealed that the severity of the phytotoxic response depends on the dose of O_3 entering the plant through stomata (the cuticular pathway can be considered negligible), which is not counteracted by the detoxification defence of the plant.

Ozone risk has for a long time been assessed relating biomass/growth reductions with cumulated exposure indices based exclusively on O_3 atmospheric concentrations (AOT40, accumulated ozone over a threshold of 40 ppb, Karenlampi and Skarby 1996), leading to the definition of concentration-based critical levels for vegetation protection which have been adopted by the European legislation. However, in the last decade, the scientific community and the UN-ECE (United Nations Economic Commission for Europe), within the framework of the CLRTAP (Convention on Long-Range Transboundary Air Pollution), have been working on the re-definition of ozone critical levels using the cumulated ozone stomatal flux to estimate the phytotoxic ozone dose (POD), since this approach takes into account the physiological and biological characteristics of plants and their capability to regulate the stomatal aperture in response to changing environmental conditions (Gerosa et al. 2009b; Emberson et al. 2000; Buker et al. 2015).

A further improvement to this approach leads to the definition of ozone critical levels based on POD_Y values, which considers the application of different 'Y' threshold values for O_3 instantaneous stomatal flux, to account for the presence of a detoxification metabolic defence that contrasts the negative effects of the pollutant.

Ozone flux-based response functions and critical levels for biomass loss based on POD_Y are currently available for some forest species in different European climate conditions and for some horticultural and agricultural crops (CLRTAP 2015; Grünhage et al. 2012; Mills et al. 2011).

However, among the three horticultural crops for which flux-based response functions were derived (bean, lettuce and tomato), only the function for tomato was sufficiently robust for the derivation of critical levels (González-Fernández et al. 2014), but it should be noted that this crop is the least ozone sensitive of the three and the use of this critical level to quantify impacts on all horticultural crops may lead to an underestimation of damage extent (CLRTAP 2015).

Among the leafy vegetables, lettuce (*Lactuca sativa* L.) is commercially and economically the most important crop of the group. It is by far the most valuable fresh market vegetable in Northern America and in Europe, with an estimated value of 1.86 and 2.57 billions USD as a 10-year average between 2004 and 2013 (FAOSTAT 2016).

Lettuce also represents the most relevant horticultural leafy crop for many Southern Europe countries. Although these countries account, on a 10-year mean basis, for 62% of the total European production (FAOSTAT 2016) and for 65% of the total area dedicated to lettuce cultivation in Europe, they are also characterized by high levels of O_3 concentrations in the summer season (European Environmental Agency 2015) which can seriously reduce the yield, the quality and the production value of many agricultural and horticultural species (Fumagalli et al. 2001; González-Fernández et al. 2014).

Lettuce was one of the first species recognized for being highly susceptible to O_3-induced oxidative stress injury (Middleton et al. 1950, Middleton 1956; Reinert et al. 1972), but its sensitivity/tolerance to O_3 in terms of visible leaf injuries and yield reduction often depends on the cultivar (Temple et al. 1986). Many studies carried out during the last years have highlighted several negative effects of O_3 on lettuce at biochemical and physiological levels (Calatayud et al. 2002; Calatayud and Barreno 2004; Goumenaki et al. 2010) and in terms of yield and biomass reductions (Goumenaki et al. 2007, Goumenaki and Barnes 2009). However, older studies conducted in North America did not find any statistically significant yield reduction in lettuce due to O_3 levels (Temple et al. 1990; McCool et al. 1987).

Regarding the definition of an ozone flux-based critical level for biomass/yield reduction in *L. sativa*, the only available study in the literature (Goumenaki et al. 2007) presented a dose-response relationship based on open-top chamber (OTC) experiments carried out in Northern Europe climate conditions with two cultivars ('Paris Island' and 'Salad Bowl') and open field and controlled chamber experiments carried out in Greece with one cultivar ('Paris Island').

The hypothesis tested with this study is that in Southern Europe, the current high levels of O_3 during the summer can cause significant yield reductions in irrigated lettuce and that the extent of the O_3 damage for this important horticultural crop could depend on the cultivar and genotype used.

The objectives of this paper are the following: (i) to bring new evidences on the harmful effect of O_3 on the marketable yield of two lettuce cultivars, (ii) to contribute in parameterizing a stomatal conductance (g_s) model for O_3 stomatal flux calculation in lettuce, (iii) to define a dose-response relationship based on POD_Y which could be used at a European level for the ozone risk assessment and (iv) to test different 'Y' threshold values for the POD_Y calculation in order to identify the most reliable (best fitting) dose-response relationship.

Materials and methods

Experimental set-up and plant material

The experiment was carried out in the OTC experimental site of Curno (BG, Northern Italy) on two cultivars of *L. sativa* (L.), 'Romana' and 'Canasta'. Four growing cycles (GCs) were performed between June and September 2007, in the following dates: 6 June–3 July, 5 July–1 August, 3–27 August and 28 August–20 September. The OTCs (3 m diameter, 2.4 m height) were constructed according to the scheme described by Heagle et al. (1973) and surrounded with crystal PVC panels. Twenty pots (30-L volume) filled with standard commercial soil were prepared for each cultivar, and two seedlings were transplanted in each pot at the beginning of each growing cycle.

Four OTCs were used for the experiment, two of them were equipped with charcoal filters in order to reduce the O_3 concentration inside by up to 60% of the ambient O_3 concentration, while the other two OTCs received non-filtered ambient air. Five pots of the cultivar 'Romana' and five pots of the cultivar 'Canasta' were randomly assigned to each OTC.

Automatic irrigation to maintain the soil water content close to field capacity was applied during the whole experiment, in order to avoid water stress confounding effects on plant response to O_3.

Due to adaptation problems after plantlet transplantation in the pots, the third GC of cv. 'Romana' was ruined and data from this growing cycle could not be used.

Ozone and meteorological variable monitoring

The hourly O_3 concentrations within the OTCs were continuously monitored with an O_3 analyser (model 1308, DASIBI, Italy) that received air samples from the four chambers (10 min per hour of sampling for each OTC) through a solenoid valve system controlled by a PC with a dedicated LabVIEW (National Instruments, USA) program. The O_3 analyser was calibrated before and after the experiment.

Ozone exposure during each GC was calculated as AOT0 and AOT40 (accumulated ozone over a threshold of 0 and 40 ppb, respectively), by summing up all of the exceedances of the hourly O_3 concentrations above 0 and above 40 ppb during the daylight hours (when global radiation was higher than 50 W/m^2).

Ancillary measurements of air temperature and relative humidity (50Y, Campbell Scientific, USA) and soil water content (EC5, Decagon Devices, USA) were performed in each open-top chamber. Two photosynthetically active radiation sensors (LI-190, LICOR, USA) were placed in one charcoal-filtered (CF) and one non-filtered (NF)-OTC. Precipitation (52,202 rain gauge, R.M. Young, USA) was measured inside one of the OTCs and outside at a distance of 10 m in the same experimental field (ARG100 Environmental Measurements, UK).

Stomatal conductance measurements and ozone stomatal flux calculation

Leaf level measurements of g_s to water vapour were performed on four different dates during the experiment with a dynamic diffusion portable porometer (AP4 Delta-T Devices, UK).

Three cycles of measurements were performed at different times during each measurement day, in the morning (between 8 and 10 AM), at midday (between12 AM and 2 PM) and in the afternoon (between 4 and 6 PM). During the measurement cycles, one g_s measurement for each pot was performed on the abaxial surface of a sunlit leaf. A total of 240 measurements for each cultivar was available for the parameterization of the g_s models.

Before each measurement cycle, the portable porometer was calibrated according to the relative humidity value that was detected inside the OTCs.

The stomatal O3 dose was calculated by applying a g_s multiplicative model (Jarvis 1976) and a 'big-leaf' O3 deposition scheme (CLRTAP 2015). Hourly g_s to water was modelled according to the following equation:

$$g_s = g_{\max} * f_{\mathrm{PAR}} * \min(f_{\mathrm{PHEN}}; f_{\mathrm{AOT0}}) * \max(f_{\min}; f_{\mathrm{T}} * f_{\mathrm{O3}} * f_{\mathrm{VPD}} * f_{\mathrm{SWP}} * f_{\mathrm{TIME}})$$

where $g_{\max}$ is the maximum g_s to water; the function $f_{\min}$ represents the minimum g_s to water, expressed relatively to $g_{\max}$, which occurs during daylight hours; the other f functions (all ranging from 0 to 1) describe the relative effect on the gmax of phenology (f_{PHEN}), seasonal accumulated ozone during the diurnal hours (f_{AOT0}), ozone concentration (f_{O3}), hour of the day (f_{TIME}) and the environmental conditions registered inside the OTCs such as PAR (f_{PAR}), air temperature (fT), vapour pressure deficit (f_{VPD}) and soil water potential (f_{SWP}). The modifying functions f_{T}, f_{PAR}, f_{VPD}, f_{AOT0} and f_{O3}were defined by boundary layer analysis, based on the values corresponding to the 98th percentile of the g_s relative to $g_{\max}$ plotted for each class of values of the variable considered (T, VPD, SWC and PAR).

The modifying function related to soil water potential (f_{SWP}) was set to 1 because the soil in the pots was maintained close to the field capacity with automatic irrigation. The modifying function f_{PHEN} was set to 1 because lettuce growth is characterized by continuous emergence of leaves. The modifying function f_{TIME} was parameterized according to what was suggested by Goumenaki et al. (2007) for their experiment.

The $g_{\max}$ was calculated separately for each cultivar as the 90th percentile of the g_s measurements that were performed during the experiment. A specific parameterization related to each cultivar studied was obtained, and a third generic parameterization for lettuce in general was obtained summarizing the previous two and using the mean g_{max} of the two cultivars as a generic lettuce g_{max}.

The meteorological data recorded inside the OTCs and collected during each GC were used to run the model. Once the g_s to water was obtained, the O_3 stomatal flux was calculated from the O_3 concentration measured inside the OTCs with a 'big-leaf' resistive scheme (CLRTAP 2015), which includes the O_3 deposition on the leaf (cuticles) and branch surfaces, represented by a resistance to O_3 deposition of 2500 s/m per unit of surface area index (SAI = green + senescent leaf area index). LAI and SAI were both set to 1.

Finally, the POD_Y was calculated for each GC of the experiment by integrating the O_3 stomatal flux for the whole duration of the GC, with the application of different detoxifying thresholds Y (from 0 to 9 nmol O_3 m^{-2} s^{-1}) as suggested by the Mapping Manual (CLRTAP 2015).

Marketable yield and dose-response relationship

At the end of each growing cycle, all the plants were harvested and the aboveground fresh biomass, representing the marketable yield of the crop, was assessed separately for each pot.

The ozone dose-effect relationships on the marketable yield of lettuce were obtained with a linear regression of the mean relative effect observed in each OTC during each GC and the POD value calculated for the same OTC. The relative effect (i.e., the relative yield RY) was calculated as the ratio between the observed mean marketable yield obtained in each OTC for each cultivar and their calculated values under hypothetical conditions of null O_3 uptake (Gerosa et al. 2012; Fuhrer et al. 1994). The latter values were estimated as the intercept of the linear regression of the observed mean marketable yield with the relative POD value calculated in the same OTC.

Statistics

The statistical significance of the differences in marketable yield due to the O_3 treatments and to the used cultivar was assessed with a two-way analysis of variance (ANOVA), considering the single pot as the statistical unit, because no significant difference in environmental conditions was detected between the OTCs. The ANOVA was performed separately for each GC with $\alpha = 0.05$ (differences with $p < 0.05$ were assumed to be significant).

The normal distribution of the marketable yield data of each GC was verified by the Shapiro-Wilk W test and by normal probability plots. The assumption of homoscedasticity of the data was verified with the Levene's test.

The significance of the regressions that were used to define the dose-response relationships for each cultivar and for the generic lettuce species was checked with the F test and the goodness of fit with the calculation of the determination coefficient R^2.

The comparison of the slopes of the regressions was performed with a parallelism analysis by means of Student's t test with $\alpha = 0.01$ (Ireland 2010).

The validity of the regressions was tested by verifying the normal distribution of errors with the Kolmogorov-Smirnoff test and the homoscedasticity of error variance.

All of the tests were performed with Statistica 8.0 software (StatSoft Inc., USA).

Results

Climatic conditions and ozone exposure

Climatic and meteorological conditions during the different GCs were quite similar, with the only exception of GC2 that was characterized by a mean temperature slightly higher than the other GCs and GC4 that, conversely, had slightly lower mean temperatures and a minimum temperature well below the values registered during the other GCs (Table 1). The second GC had also a remarkable higher mean VPD with respect to other cycles.

Table 2 reports the mean O_3 concentrations and the AOT40 index that were measured during the different GCs in the two O_3 treatments (CF and NF), considering only the diurnal daytime (from 8 AM to 8 PM), since during the night-time, O_3 filtration and fumigation were not active.

Table 1 Main meteorological variables during the four growing cycles of the experiment

		Unit	GC1	GC2	GC3	GC4
T	Mean	°C	22.68	26.50	22.63	19.80
	Max	°C	32.42	37.49	31.78	29.46
	Min	°C	14.58	12.87	13.33	9.97
VPD	Mean	kPa	1.18	2.21	1.27	1.02
	Max	kPa	3.05	4.97	3.34	3.12
PAR	Mean of daily max	μmol m^{-2} s^{-1}	1468	1635	1520	1326

Table 2 Mean ozone concentration and cumulated exposure (expressed as AOT40) during the four growing cycles of the experiment, in the two different O_3 treatments

	O_3 treatment	Unit	GC1	GC2	GC3	GC4
$[O_3]$ mean[a]	CF	ppb	20.6	32.1	25.6	16.9
	NF	ppb	32.9	54.4	41.4	30.8
$[O_3]$ max	NF	ppb	65	106	76	68
AOT40	CF	ppb.h	32	710	91	0
	NF	ppb.h	705	5073	2247	662

GC growing cycle, *CF* charcoal-filtered OTC, *NF* non-filtered OTC, *AOT40* accumulated ozone over the threshold of 40 ppb

[a] Calculated between 8 AM and 8 PM

The mean O_3 concentrations in NF-OTCs were between 30.8 and 54.4 ppb. A maximum hour concentration of 106 ppb was observed during the GC2. The O_3 filtration efficiency in the CF-OTCs was around 40% of the NF-OTC concentrations monitored during the daytime. This was sufficient to keep the AOT40 index widely lower than the critical level of 3.000 ppb.h suggested by the UN-ECE for vegetation protection (although this level is calculated on a 3-month base) in the CF-OTCs. On the contrary, this critical level was exceeded in the NF-OTCs during the GC2 (5073 ppb.h) although the integration time for the AOT40 accumulation was only 27 days.

Marketable yield

The measurements of the plant fresh biomass (marketable yield) performed at the end of each GC resulted mainly related to the registered climatic conditions. The most productive harvests were those of the central part of the summer season (GC2 and 3 for 'Canasta', GC2 for 'Romana', Fig. 1) characterized by optimal conditions of solar radiation and temperature.

Biomass production for the two cultivars was similar and comparable, with a mean value for the three common GCs (GC1, 2 and 4) of 144 and 140 g in the CF-OTCs of 'Canasta' and 'Romana', respectively. As reported in Table 3, there was no statistically significant difference in the yield due to the cultivar factor, while O_3 caused a significant negative effect on lettuce marketable yield for GC1 and GC4. The interaction between O_3 and cultivar did not cause any significant effect on the marketable yield of plants (Table 3, 'O_3 × cultivar' effect).

The marketable yield of the NF-OTC plants was always lower than that of the CF-OTC plants, with mean reductions of −14.5 and −18.5% for 'Canasta' and 'Romana', respectively (Fig. 1).

The most pronounced decrease was observed for GC1 (−20.3 and −30.6% for 'Canasta' and 'Romana', respectively).

The negative effect of O_3 on biomass production was statistically significant ($p < 0.05$) in GC1 and GC4, while the yield reductions due to O_3 were not significantly different in the two cultivars (Table 3).

Maximum g_s and g_s model

The g_s measurements performed during the experiment were used to calculate the g_{max} value for the two studied cultivars. Two g_{max} values of 730 and 850 mmol H_2O m^{-2} s^{-1} were calculated for 'Canasta' and 'Romana', respectively. These values resulted remarkably higher than those reported by Goumenaki et al. (2007) for the cultivar 'Paris Island' calculated from open field measurements in Greece (198 mmol H_2O m^{-2} s^{-1}) but were not so far from those reported by the same authors (Goumenaki et al. 2009) for a laboratory experiment on 'Paris Island' and 'Granada' cultivars (550 and 490 mmol H_2O m^{-2} s^{-1}, respectively). Calatayud et al. (2002) during their experiments found a mean g_s value of 423 mmol H_2O m^{-2} s^{-1} for a Romaine type cv. (similar to our 'Romana' cultivar), while the g_{max} value was not reported.

The formulations of the different *f* functions included in the three g_s models (see the 'Stomatal conductance measurements and ozone stomatal flux calculation' section) and the relative

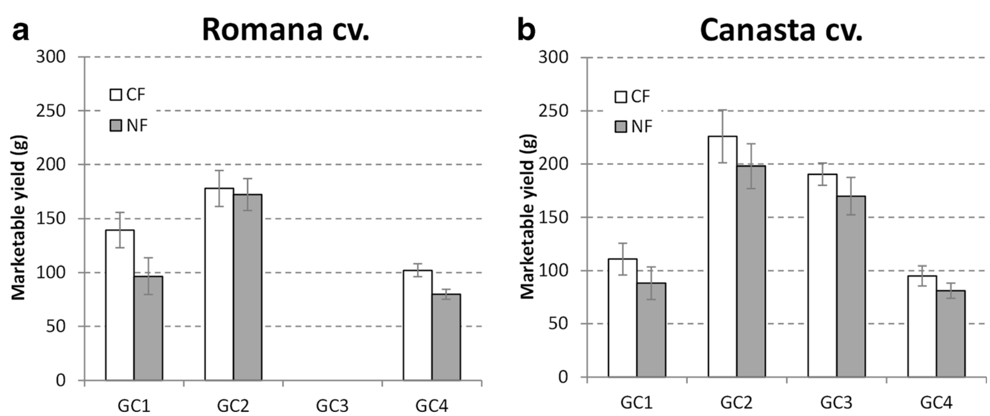

Fig. 1 Marketable yield for 'Romana' (**a**) and 'Canasta' (**b**) cultivars during the four growing cycles (GCs) in the two different O_3 treatments. *GC* growing cycles, *CF* charcoal-filtered OTCs, *NF* non-filtered OTCs

Table 3 Results of the ANOVA test performed for each GC

	O_3		Cultivar		O_3 × cultivar	
	p value	Signif.	*p* value	Signif.	*p* value	Signif.
GC1	0.0483	*	0.2562	n.s.	0.5305	n.s.
GC2	0.4012	n.s.	0.0704	n.s.	0.5747	n.s.
GC3[a]	–		–		–	
GC4	0.0137	*	0.6831	n.s.	0.5545	n.s.

n.s. not significant

$*p < 0.05$

[a] Due to growing problems with the cultivar 'Romana', the ANOVA test could not be performed for this GC

parameterization are reported in Table 4, while Fig. 2 reports the graphic shape of the *f* functions for the lettuce generic g_s model.

The shape of the functions f_{PAR}, f_T and f_{VPD} are identical to that proposed by the Mapping Manual (CLRTAP 2015), while the f_{PHEN} and the f_{SWP} functions were set to 1, because plants were continuously producing new leaves and were kept irrigated to field capacity during the GCs.

Two functions regarding the effect on g_s of the instantaneous O_3 concentration (f_{O3}) and of the cumulated O_3 exposure (f_{AOT0}) from the beginning of each GC have been introduced in the g_s models. The function f_{O3} (Table 4, Fig. 2) provides a linear reduction of g_{max} when O_3 concentrations go over a threshold of 65 ppb for the generic model, leading to a minimum value of g_s (corresponding to the f_{min} constant value), when O_3 concentration reaches a value of 102 ppb.

The function f_{AOT0} considers a linear decrease of g_s (more gradual than f_{O3}) when the AOT0 (accumulated ozone over 0 ppb during the daylight hours, 8 AM–8 PM) exceeds 2000 ppb.h.

The use of a function f_{AOT0} was already introduced by Pleijel et al. (2002) for the parameterization of a g_s model to calculate O_3 stomatal flux in a potato, and its use was also adopted by the Mapping Manual. Although our formulation of the f_{AOT0} is different from that of Pleijel et al. (2002), the limiting effecst on g_{max} is quite similar.

Finally, in order to account for the g_s decline in the late afternoon, we used the function f_{TIME} introduced by Goumenaki et al. (2007) in their work on lettuce.

The regression of predicted vs. measured hourly mean conductance is summarized in the following equation for the generic lettuce model:

$$g_{predicted} = 0.8594\, g_{measured} + 56.05$$

which presents an R^2 of 0.9172 ($p < 0.001$).

The lettuce generic g_s model had a good performance with a slight tendency to underestimate low conductance and overestimate high conductance.

Regarding the cultivar-specific g_s models, the Romana g_s model ($g_{predicted} = 0.8105\, g_{measured} + 97.98$) showed the lowest goodness of fit ($R^2 = 0.7425$), while the Canasta g_s model

Table 4 The parameterization of the limiting functions used for the g_s models of Romana and Canasta cultivar and for the g_s model of a generic lettuce

Equation function	Parameter	Unit	Cv Romana	Cv Canasta	Generic lettuce
	$g_{w\,max}$	mmol m^{-2} s^{-1} (PLA)	850 (1)	730 (1)	790 (2)
f_{min} = const.		adim.	0.05	0.05	0.05
f_{PHEN} = const.		adim.	1	1	1
$f_{PAR} = 1 - e^{-a_{Light} \cdot PAR}$	a_{Light}	adim.	0.015	0.005	0.005
$f_T = \max\left\{f_{min}; \frac{(T-T_{min})}{(T_{opt}-T_{min})} \cdot \left[\frac{(T_{max}-T)}{(T_{max}-T_{opt})}\right]^b\right\}$	T_{opt}	°C	39	42	42
	T_{max}	°C	30	33.5	31.5
	T_{min}	°C	10	15	10
	b	adim.	0.4500	0.4594	0.4884
$f_{VPD} \min\left\{1; \max\left[f\min; \frac{(VPD_{max}-VPD)}{(VPD_{max}-VPD_{min})}\right]\right\}$	VPD_{min}	kPa	3.2	3.3	3.2
	VPD_{max}	kPa	5.3	5.5	5.3
f_{SWP} = const.		adim.	1	1	1
$f_{O_3} = \min\left\{1; \max\left[f\min; \frac{(O_{3max}-O_3)}{(O_{3max}-O_{3min})}\right]\right\}$	O_{3min}	ppb	60	65	65
	O_{3max}	ppb	100	102	100
$f_{AOT0} = \min\left\{1; \max\left[f\min; \frac{(AOT0_{max}-AOT0)}{(AOT0_{max}-AOT0_{min})}\right]\right\}$	$AOT0_{min}$	ppb.h	2000	2000	2000
	$AOT0_{max}$	ppb.h	35,000	35,000	35,000
$f_{TIME} = 1/(1 + 10^{12} e^{-100 time})$ if time < 14:00[a]					
$f_{TIME} = 1/(1 + 9 * 10^{-8} e^{-20.4 time})$ if time > 14:00[a]					

[a] From Goumenaki et al. (2007)

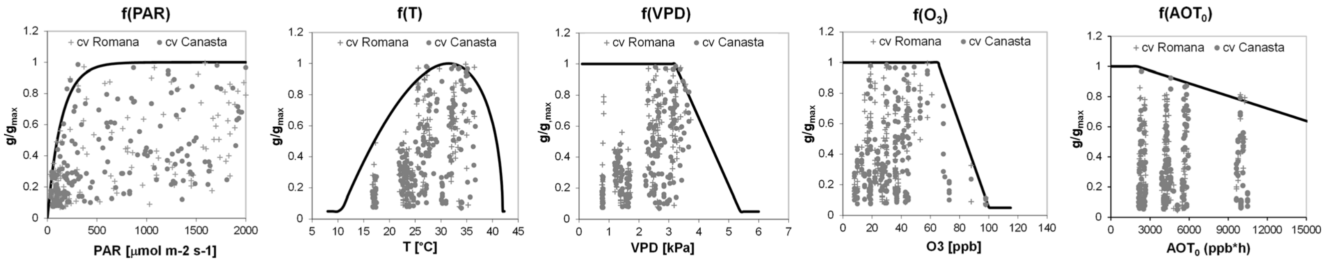

Fig. 2 Limiting functions for the stomatal conductance generic model for lettuce

($g_{predicted} = 0.8081\ g_{measured} + 38.41$) evidenced a better performance, similar to the generic model one ($R^2 = 0.9160$).

POD_Y and dose-response relationship

The dose-response relationships for the marketable relative yield based on the POD_Y values calculated for different '*Y*' values are reported in Table 5. The best regression fit ($R^2 = 0.4476$, $p < 0.01$) was obtained using an instantaneous flux threshold of 6 nmol O_3 m^{-2} s^{-1}; the same threshold value was already adopted by the Mapping Manual (CLRTAP 2015) for the calculation of the O_3 critical levels in spring wheat, potato and tomato (Pleijel et al. 2007; González-Fernández et al. 2014).

The dose-response relationship defined for the POD_6 calculated with the generic lettuce is showed in Fig. 3**a.** Based on this linear regression, a POD_6 value of 1 mmol O_3 m^{-2} accounts for a 14.6% reduction of the marketable yield in lettuce.

The cultivar-specific dose-response relationships are presented in Fig. 3b ('Romana') and c (Canasta). The cv. Canasta showed a dose-response relationship with a better goodness of fit than the cv. 'Romana' (0.7580 vs 0.3685). The comparison between the two linear regressions obtained

Table 5 Regression coefficient values of the dose-response relationships for generic lettuce calculated with different '*Y*' threshold values. The highest value is highlighted in italic characters. *POD* phytotoxic ozone dose, *RY* relative yield

Y value for POD (nmol O_3 m^{-2} s^{-1})	Equation	R^2
0	RY = −0.077*POD_0 + 1	0.2433
1	RY = −0.082*POD_1 + 1	0.2944
2	RY = −0.089*POD_2 + 1	0.3389
3	RY = −0.098*POD_3 + 1	0.3744
4	RY = −0.101*POD_4 + 1	0.4071
5	RY = −0.125*POD_5 + 1	0.4371
6	RY = −0.146*POD_6 + *1*	*0.4476*
7	RY = −0.175*POD_3 + 1	0.4416
8	RY = −0.216*POD_8 + 1	0.4231
9	RY = −0.278*POD_9 + 1	0.3813

shows that 'Canasta' resulted in slightly more O_3 sensitive than 'Romana', showing a more marked decrease of the relative yield for each POD_6 unit (−17.8%). However, according to the test of parallelism, this difference was not statistically significant.

Discussion

With a marketable yield loss of 14.6% for each unit of POD_6 absorbed, lettuce resulted much more vulnerable than other crop species for which O_3 dose-response relationships were already been defined.

Tomato, for example, presented a 2.6% decrease of fruit yield for each unit of POD_6 absorbed (González-Fernández et al. 2014), while in potato and spring wheat (Pleijel et al. 2007), the relative yield losses were 1.3 and 3.8%, respectively, for the same POD_6 value.

Despite the vulnerability in terms of marketable yield loss, lettuce plants did not show any visible leaf symptoms in any of the GC performed, and this result is in contrast with the findings of other authors (Calatayud et al. 2002; Calatayud and Barreno 2004; Goumenaki et al. 2009).

It is worth noticing that the '*Y*' threshold value of 6 nmol O_3 m^{-2} s^{-1} for POD_Y calculation, which gave the best regression performance for the dose-response relationship, is the same threshold already determined and suggested for other crops in the Mapping Manual (CLRTAP 2015). This result, on the one hand, confirms that the POD_6 is a good choice to infer O_3 critical levels in crop species and, on the other hand, represents a solid proof that the annual crop species have higher detoxifying capacity in comparison to forest species that present a '*Y*' threshold of 1 nmol O_3 m^{-2} s^{-1} (Karlsson et al. 2007).

The results of our experiment are comparable to the findings of Goumenaki et al. (2007), which reported for their lettuce cultivars a relative yield reduction due to O_3 of 4.6% for each unit of POD_0 absorbed. In our case, the dose-response relationship based on POD_0 (Table 5) highlights a 7.7% of relative yield loss. This difference could be likely due to the different climatic conditions between the two experiments. Both

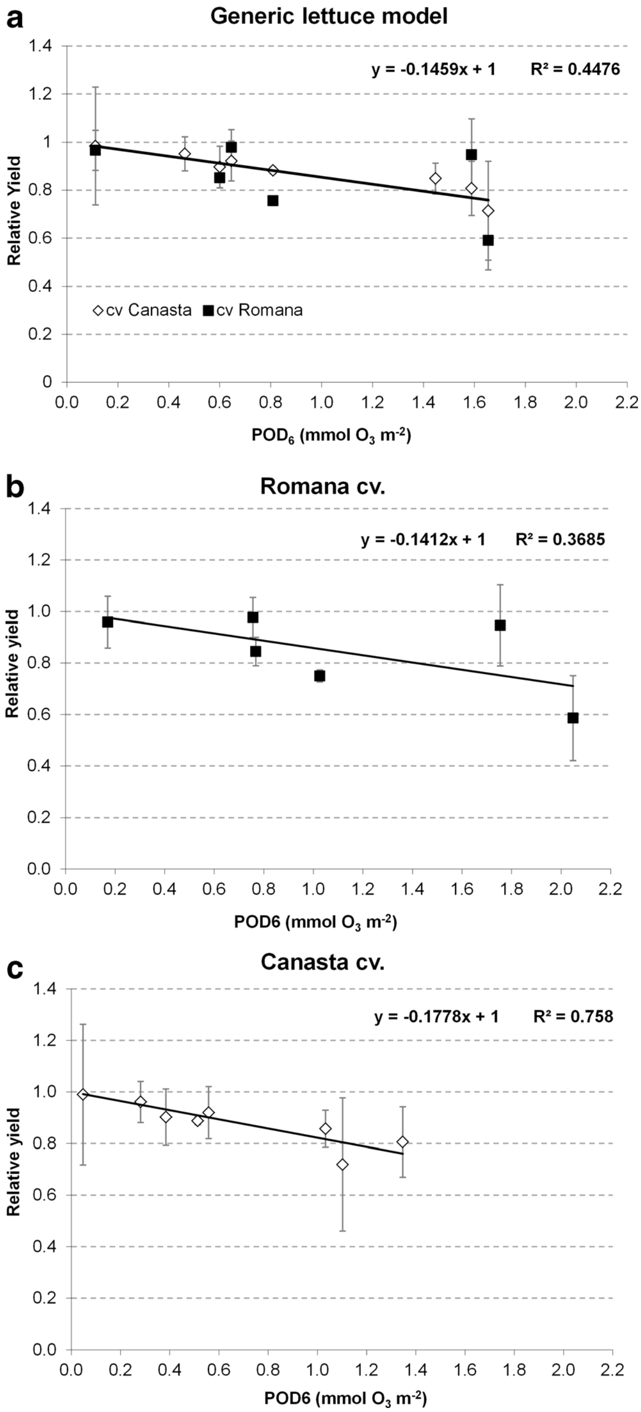

Fig. 3 Dose-response relationships based on POD_6 and relative marketable yield of lettuce calculated using a g_s generic model (**a**) and the cultivar-specific g_s models for 'Romana' (**b**) and for 'Canasta' (**c**)

experiments, however, highlight that in Southern Europe, current high levels of O_3 during the summertime could potentially cause significant marketable yield losses, particularly when water availability is not a limiting factor for g_s and O_3 uptake (in irrigated cultivation fields, for example).

The initial hypothesis about a possible influence of the cultivar on the O_3 sensitivity/tolerance of lettuce was not confirmed by our results (Table 3); however, further studies on other lettuce cultivars are necessary to draw certain conclusions.

Since the parallelism test performed on the two cultivar-specific dose-response relationships (Fig. 3b, c) did not provide a significant result at a level $\alpha = 0.01$, it was possible to define a single dose-response relationship for lettuce that included both cultivars.

According to this generic lettuce dose-response relationship (Fig. 3a), it is possible to establish an O_3 critical level of 1 mmol O_3 m^{-2} of POD_6 for a marketable yield loss of 15%, based on an average integration period of 25 days (the mean duration of the GCs). Considering a 5% yield loss, as suggested by the Mapping Manual for other crops, this critical level should be set to 0.34 mmol O_3 m^{-2}.

However, it is important to underline that this experiment was performed with the application of automatic irrigation of plants, thus providing non-limiting environmental conditions for g_s and O_3 uptake and representing the worst case scenario compared to field limiting conditions.

Actually, most of the cultivated species in the Mediterranean region grow in water-limited rain-fed areas where drought stress in some cases might mitigate O_3 effects on crops (Feng et al. 2008).

Conclusions

This study confirms that lettuce is an O_3-sensitive horticultural crop and that current levels of O_3 in Southern Europe could have a harmful effect on the marketable yield of this species under optimal conditions of irrigation.

The threshold of 6 nmol O_3 m^{-2} s^{-1} for POD_Y calculation was found to have the strongest relationship with marketable yield loss, suggesting a POD_6 critical level of 1 mmol O_3 m^{-2} s^{-1} for a 15% reduction of marketable yield on a mean integration time of 25 days.

New parameterizations of g_s models for the two lettuce cultivars and for lettuce in general were defined. Data obtained from this experiment could be used for the identification and adoption of O_3 critical levels for horticultural species at a European level.

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