Full Length Article

Influence of Ambient Ozone Pollution on Olive Leaf Gas Exchange Irrigated with Saline Water

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ABSTRACT

Saline water is often available for irrigation and ambient ozone pollution is significant in the Mediterranean region. These two factors may negatively affect cultivated plant productivity. We studied the effects of a combination of these stress factors on leaf gas exchange of young olive trees, a major crop in the above region. Two-years-old 'Konservolea' and 'Kalamata' olive plants grafted on seedling rootstock were grown in sand:perlite mixture irrigated with half strength Hoagland's solution containing or not 100 mM NaCl. In open top chambers, the plants received outside air with ambient ozone or charcoal–filtered air from April to September. Leaf gas exchange parameters and chlorophyll fluorescence were measured periodically. High ozone concentrations from May to September able to damage plants (daylight mean ozone concentration >60 nL L^{-1}) did not affect the olive leaf functions studied, showing that young olive trees are relatively resistant to ozone levels found around the Mediterranean region. Irrigation with 100 mM NaCl solution decreased stomatal conductance by around 45%, photosynthetic rate by more than 35% and Fv/Fm values below 0.75. Both studied olive cultivars showed similar behavior to salinity stress possibly due to the seedling rootstock on which both cultivars were grafted. The combination of salinity and ambient ozone stress did not result in any further effects to leaf gas exchange besides the ones from the salinity stress alone. © 2011 Friends Science Publishers

Key Words: *Olea europaea*; Photosynthesis; Stomatal conductance; Chlorophyll fluorescence

INTRODUCTION

Olive tree is considered as a moderately salt tolerant plant species (Bernstein, 1965; Maas & Hoffman, 1977). Nevertheless, salinity decreased olive leaf net photosynthetic rate (Ps) and dry matter productivity via CO_2 availability to chloroplasts as stomatal and mesophyll conductance decreased at numerous publications (Loreto *et al*., 2003). The extent of this Ps and stomatal conductance (g_s) reduction depend on cultivar, rootstock and salinity severity (NaCl concentration & duration under salinity) (Al–Yassin, 2004; Chartzoulakis, 2005). Rootstock may be important for salinity resistance in glycophytes, due to the ability of the roots to exclude or retain the Na^+ and Cl^- ions (Chartzoulakis *et al*., 2002; Levy & Syvertsen, 2004). All published work about salinity effects on olive has been conducted with own–rooted plants. Recently, own–rooted 'Kalamata' olive trees were considered more resistant to salinity than 'Konservolea' (synonymous to 'Amfissis') trees (Chartzoulakis *et al*., 2002). But these two main table olive cultivars in Greece are commercially available as grafted plants on olive seedling rootstock. Thus, their behaviour to salinity could be different from the own–rooted plants.

High tropospheric ozone (O_3) concentrations have been found around the Mediterranean region (Riga-Karandinos & Saitanis, 2005). These O_3 levels are often higher than the safe limits for plants (40 nL L^{-1} during the growing season) (WHO, 2000). High ambient O_3, a highly oxidizing molecule, can enter the leaves through the stomata and generate reactive oxygen species, which may negatively affect most of the cellular processes, cause cell damage and death and, thus, reduce leaf and plant productivity. Olive is a sclerophyllous evergreen species expected to have reduced O_3 sensitivity due to reduced mesophyll gas conductivity and increased antioxidant capacity (Bussotti & Gerosa, 2002). Actually, only one scientific team has worked with O_3 effects on two Italian olive cultivars. 'Frantoio' olive plants were severely affected by exposure to close–to–ambient concentrations of O_3 (100 nL L^{-1} for 5 h day^{-1} & 120 days) with reduced Ps and g_s, but 'Moraiolo' plants were more resistant to O_3 as Ps was not significantly affected due to reduced g_s (Minnocci *et al*., 1999; Vitagliano *et al*., 1999). A Spanish research group has published a comparative study on ozone effects to various Mediterranean forest species including wild olive seedlings. They found no effects from relatively high ozone exposure (ambient + 40 nL L^{-1} for 12 h day^{-1} five days per week & 24 months) to Ps and biomass accumulation (Ribas *et al*., 2005), but the same group had previously reported for the

same wild olive seedlings and shorter exposure to ozone (ambient + 40 nL L^{-1} for 9 h day^{-1} & 10 months) close to 20% reductions in plant height and stem growth (Inclan *et al.*, 1999).

As O_3 and salinity cause alterations in g_s (Hassan, 2004), the combination of these two stresses can result in: no interaction (in alfalfa, Olszyk *et al.*, 1988), synergistic, additive (in rice, Welfare *et al.*, 1996) or antagonistic effects (in wheat, Hassan, 2004) on leaf gas exchange. There is no work published on the effects of a combination of O_3 and salinity stresses in cultivated woody plant species.

As salinity and O_3 are common problems in the Mediterranean area and both pollutants act on leaf gas exchange through stomatal opening, we hypothesized that the presence of these two pollutants could have antagonistic effects on leaf productivity in olive. Thus, we studied the effects of O_3 and salinity separately and in combination on olive leaf gas exchange and water relations for two main table olive cultivars.

MATERIALS AND METHODS

Plant material and treatments: Two-years-old uniform (similar height, canopy & trunk diameter) olive plants (*Olea europaea* L.) of two Greek table olive cultivars 'Konservolea' and 'Kalamata' grafted on seedling rootstock (seeds were collected from wild olive plants growing freely in forests) were planted individually in 12–L pots containing sand–perlite mixture 1:1 v/v. Plants of each cultivar were lightly pruned and transferred to open–top chambers (OTCs) constructed as described by Heagle *et al.* (1983). Each OTC was constructed of an iron frame covered with a HDPE sheet (180 μm thickness, >80% light penetration) and was continuously ventilated with an air ventilation unit bringing ambient outside air into the chamber at 1600 $m^3 h^{-1}$. Air was distributed via perforated tubes 15 cm in diameter positioned at 70 cm above ground along the chamber walls (the lower level of tree canopy). Mean air temperatures for two years (2006, 2008) were 21.8°C for May, 28.2°C for June, 28.5°C for July, 28.1°C for August, 23.2°C for September and 22°C for the first half of October. For the above period, maximum and minimum air temperatures were 37°C and 13°C, respectively.

Six OTCs were used in this experiment, three chambers ventilated with charcoal–filtered air and the other three with outside air containing ambient ozone levels. Two to three times per week half of the pots of each cultivar (randomly chosen) inside each chamber received two L per pot of half strength Hoagland's solution containing 100 mM NaCl, while the rest of the pots received half strength Hoagland's solution. There were four pots per treatment in each chamber and from each cultivar. Ambient or charcoal–filtered air fumigation and salinity treatment were applied from April until October for two experimental years, 2006 and 2008.

To avoid salt shock, the NaCl–treated plants were initially given 25 mM NaCl and the concentration increased by 25 mM in weekly intervals reaching 100 mM NaCl at the end of April. The conductivity of drainage water (leachate) from pots was measured weekly. In order to avoid any salt accumulation in the pots, all pots were flushed with half strength Hoagland's solution twice per month keeping the leachate conductivity of the pots receiving NaCl close to 13 $mS cm^{-1}$.

Ozone monitoring: Ozone was monitored with two Eco Sensors ozone monitors (Model C–30ZX, Eco Sensors, Santa Fe, NM, USA), one placed in a chamber with charcoal–filtered air and another one in a chamber with non–filtered (ambient) air. The data from each ozone analyzer were logged in a data logger and collected weekly from May 1st to October 15th.

Leaf parameters measured: A portable photosynthesis unit (LCi Photosynthesis System, ADC BioScientific Ltd., Hoddesdon, Herts, UK) was used to monitor leaf net photosynthetic rate, transpiration rate, stomatal conductance, photosynthetically active radiation, leaf temperature and calculate leaf water use efficiency. The measurements were taken at monthly intervals from June to September during morning hours between 09:00 and 11:00 am on mature leaves fully exposed to light. Throughout the measurement periods, relative humidity was below 50%, photosynthetically active radiation was above 1200 μmol $m^{-2} s^{-1}$ and ambient CO_2 concentration was above 360 μL L^{-1}. During the same days, leaf chlorophyll fluorescence was also measured with the OS–30p fluorometer (ADC BioScientific Ltd., Hoddesdon, Herts, UK) after 30 min dark adaptation and application of saturating excitation light provided by a 660 nm solid state source. The ratio between variable and maximal fluorescence (Fv/Fm) was calculated.

Statistical analysis: Data from 2008 are shown (data from 2006 showed similar trend). Analysis of variance was conducted over three factors (cultivar, time & treatment) using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL). Means were separated by Duncan's test. Overall least significant differences at 5% were calculated and are shown.

RESULTS

Ambient ozone concentrations: Ozone concentration in Velestino area (Central Greece) was high during the entire period of measurements. Daylight mean ozone concentration from May 1st to October 15th exceeded 60 nL L^{-1} and maximum values were 72–93 nL L^{-1} inside the chamber with non–filtered air. AOT40 (cumulative exposure to ozone above 40 nL L^{-1}) values reached 54176 nL L^{-1} h for the mentioned period. In the chamber with filtered air, daylight mean ozone concentration was below 24 nL L^{-1} and maximum values reached 27 nL L^{-1}.

Salinity and ozone effects: Salinity significantly decreased olive leaf g_s (around 45%), transpiration rate (>30%) and Ps (>35%), but only slightly decreased WUE in both cultivars

studied throughout the measurement period (Table I & II). These reductions were similar in the two cultivars. At all times, air filtering to remove the high ambient ozone did not have any positive effect on leaf functioning.

Supporting the above, leaves from the no-NaCl with low or ambient ozone treated plants had Fv/Fm values above 0.8, showing no stress, while leaves from the high salinity treatments had Fv/Fm values lower than 0.75 after June showing stress due to NaCl (Table II).

DISCUSSION

The high ambient ozone concentrations measured in the rural area of Velestino have been found before for Volos, a nearby urban area (Riga-Karandinos & Saitanis, 2005). Actually, the ambient ozone concentrations found today in various rural or not Mediterranean areas are high enough (like in Velestino) to damage many plant species (WHO, 2000). Ozone was found to reduce gas exchange rates in various tree species with concentrations and AOT40 values significantly lower than in our study (Pye, 1988). Only the ozone that diffuses into a plant through the stomata to the photosynthesizing cells within a leaf impairs plant processes or performance. Thus, stomatal conductance is considered the regulatory point of plant sensitivity to ambient ozone (Reich, 1987). This stomatal conductance is relatively low in sclerophyllous evergreen broadleaf drought–resistant species such as olive (Larcher, 1995). Based on the above, olive is expected to be relatively tolerant to high ozone levels due to low stomatal and mesophyll conductance and the position and size of stomata (Ribas *et al.*, 2005). Nevertheless, Minnocci *et al.* (1999) found that g_s was substantially reduced in both olive cultivars studied (own–rooted) and Ps was reduced in only one of them after exposure to 100 nL L^{-1} for 5 h day^{-1} for 120 days reaching AOT40 around 36000 nL L^{-1} h. No conclusive results were found on wild olive seedling dry matter productivity from exposure to higher than our study's AOT40 values in Spain (Inclan *et al.*, 1999; Ribas *et al.*, 2005). Based on the data presented herein and other previously published (Abusafieh *et al.*, 2011), it is clear that the two cultivars used in our study (grafted on wild olive seedling rootstock) were not stressed or negatively affected in any significant way from the ozone levels found today in the Mediterranean region with AOT40 exceeding 54000 nL L^{-1} h over one growing period. This could be due, as described above, to low stomatal conductance, which would diminish ozone access to intercellular spaces and photosynthesizing cells, but also due to the high antioxidant capacity of olive leaves, which would protect the cells from oxidative damage. Thus, damage from ozone in olive seems to be genotype–dependent and may be affected from the rootstock, ozone concentration and duration of exposure to ozone, an estimation of which is AOT40 and possibly, biochemical factors associated with the leaf antioxidant capacity of each cultivar. In addition, although in all

experimental work the olive plants have been properly irrigated and fertilized, the farm cultivated olive plants are normally grown in dry climates where, during the summer months and even if irrigated promptly, transpirational demand during the hot daylight hours is high and stomatal conductance is very low. This coincides with the hours when ozone concentration is also highest. Thus, ozone can not significantly enter and accumulate in the olive leaves in levels high enough to cause damage, as it can happen to other woody species (Bussotti & Gerosa, 2002).

Salinity in the absence or presence of ambient ozone reduced leaf productivity via reductions in g_s and mesophyll CO_2 conductance, which negatively affected each other, and, finally, leaf Ps and dry matter productivity, as described by Loreto *et al.* (2003). Under salt stress, besides the reduced g_s, olive leaves become thicker and more succulent (Bongi & Loreto, 1989). Increasing leaf thickness may further reduce the mesophyll conductance by extending and making more tortuous the CO_2 pathway toward the chloroplast (Evans *et al.*, 1994). Low CO_2 availability to chloroplasts will reduce Ps and Fv/Fm as found in our study. The reduction in Fv/Fm can be ascribed as down regulation of PSII that reflects the protective or regulatory mechanism to avoid photodamage of photosynthetic apparatus (Demmig-Adams, 1992).

In the extensive literature about salinity tolerance of olive, the 100 mM NaCl in the irrigation solution is the critical concentration of NaCl to damage olives (Chartzoulakis, 2005). Salt-tolerant cultivars maintained their leaf Ps, when irrigated with 100 mM NaCl solution for a short period of time, but Ps and g_s significantly decreased at 200 mM NaCl (Tattini *et al.*, 1995). In our study, the duration of salinity stress resembled field conditions, when saline water is available for irrigation over an entire season and the studied table olive cultivars grafted on wild olive rootstock showed significant reductions in leaf functioning. Thus, the certain combination of seedling rootstock and table olive cultivars used in our study can be considered quite sensitive to salinity as far as leaf productivity is concerned. But our olive plants showed no macroscopic damage (leaf scorching, leaf drop) due to salinity in any of the experiments.

In a previous study with young own–rooted olive trees (Chartzoulakis *et al.*, 2002); 'Kalamata' plants were found more tolerant to NaCl than 'Konservolea' plants, due to lower g_s and Ps of 'Kalamata' leaves. In our study, with the same as above cultivars but grafted on the same seedling rootstock, there were no differences in the gas exchange properties and thus NaCl tolerance between the two cultivars. This was probably due to the seedling rootstock, which seemed to increase the leaf productivity of 'Kalamata' trees and improve the NaCl tolerance of 'Konservolea' trees. It is expected and is well documented that roots play a substantial regulating role in Na^+ and Cl^- ion uptake and transport to leaves (Tattini *et al.*, 1994; Chartzoulakis *et al.*, 2002; Levy & Syvertsen, 2004).

Table I: Changes in leaf photosynthetic rate (Ps, μmol m^{-2} s^{-1}), transpiration rate (Trans., mmol m^{-2} s^{-1}) and water use efficiency (WUE, mmol CO_2/mol H_2O) during the summer period for 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock. Overall least significant differences (LSD) at 5% level are included

Treatment		'Konservolea'			'Kalamata'		
	Month	Ps	Trans.	WUE	Ps	Trans.	WUE
-NaCl LowO$_3$	June	13.0 a	3.67 ab	3.72 c	14.8 a	4.15 a	3.59 b
	July	12.6 ab	3.79 a	3.32 cd	12.4 bc	3.78 ab	3.29 bc
	August	11.4 c	3.75 a	3.06 d	11.8 c	3.14 cd	4.13 a
	September	11.6 bc	2.64 cd	4.47 b	11.4 c	3.23 c	3.67 b
-NaCl AmbO$_3$	June	12.3 b	3.51 ab	3.54 cd	13.0 b	3.99 ab	3.29 bc
	July	12.2 b	3.61 ab	3.38 cd	11.9 c	3.47 bc	3.47 bc
	August	11.2 c	3.35 b	3.45 cd	11.5 c	3.72 b	3.14 cd
	September	11.7 bc	2.84 c	4.14 bc	11.7 c	2.82 d	4.20 a
+NaCl LowO$_3$	June	7.7 d	2.45 cd	3.15 d	8.2 d	2.81 d	2.96 cd
	July	7.5 d	2.27 d	3.34 cd	7.8 de	2.39 e	3.29 bc
	August	6.5 f	1.62 e	4.04 bc	7.0 e	2.28 e	3.21 bc
	September	6.8 ef	1.59 e	5.04 a	6.8 e	2.36 e	2.90 cd
+NaCl AmbO$_3$	June	7.8 d	2.46 cd	3.17 d	8.3 d	3.01 cd	2.76 d
	July	7.3 de	2.30 d	3.24 d	7.2 e	2.31 e	3.16 c
	August	6.3 f	1.68 e	3.78 c	6.9 e	2.31 e	3.16 c
	September	6.8 ef	1.65 e	4.13 bc	6.7 e	2.29 e	2.96 cd
	LSD$_{0.05}$	0.7	0.4	0.5	0.7	0.4	0.4

Table II: Changes in leaf stomatal conductance (g$_s$, mol m^{-2} s^{-1}) and leaf chlorophyll fluorescence ratio Fv/Fm over the summer period for 'Konservolea' and 'Kalamata' olive trees grafted onto seedling rootstock. Overall least significant differences (LSD) at 5% level are included

Treatment		'Konservolea'		'Kalamata'	
	Month	g$_s$	Fv/Fm	g$_s$	Fv/Fm
-NaCl LowO$_3$	June	0.18 a	0.82 a	0.21 a	0.83 a
	July	0.17 ab	0.83 a	0.18 b	0.83 a
	August	0.16 b	0.82 a	0.14 d	0.82 ab
	September	0.13 cd	0.83 a	0.15 cd	0.83 a
-NaCl AmbO$_3$	June	0.15 b	0.83 a	0.20 a	0.83 a
	July	0.16 b	0.83 a	0.16 c	0.81 bc
	August	0.14 c	0.82 a	0.15 cd	0.82 ab
	September	0.12 d	0.82 a	0.13 d	0.82 ab
+NaCl LowO$_3$	June	0.09 e	0.80 b	0.11 e	0.80 c
	July	0.08 ef	0.73 d	0.09 f	0.74 d
	August	0.08 ef	0.75 c	0.09 f	0.75 d
	September	0.06 f	0.75 c	0.08 f	0.75 d
+NaCl AmbO$_3$	June	0.09 e	0.80 b	0.12 de	0.80 c
	July	0.09 e	0.73 d	0.08 f	0.74 d
	August	0.08 ef	0.75 c	0.12 de	0.74 d
	September	0.07 f	0.76 c	0.08 f	0.75 d
	LSD$_{0.05}$	0.019	0.02	0.019	0.02

Values with similar letter(s) within a column are not significantly different at P=5% by Duncan's Multiple Range Test

Actually, we found similar Na$^+$ ion concentrations in the leaves of the two cultivars, when salinity was applied (1.16 & 1.15 g kg^{-1} dry matter for 'Konservolea' and 'Kalamata' leaves, respectively). Even though the rootstock may have the most significant effect on salt tolerance of olives, no previous work has been published on the salinity sensitivity of grafted olive plants. Grafting economically important olive cultivars on resistant easy-to-root cultivars or selections should be further studied, as a way to reduce the negative consequences on plant productivity from saline irrigation water.

Our results showed no interaction between salinity and ozone in any of the physiological parameters studied. Similar results were obtained in alfalfa (Olszyk *et al.*, 1988). Our hypothesis was that salinity and ozone will act antagonistically as salinity reduces stomatal conductance and this would cause reduced intercellular ozone levels and thus, reduced oxidative damage. On the contrary, it was possible that these two stress factors, as both cause oxidative damage to cells, could act synergistically and result in even more damage than each factor separately. This has actually been found in rice (Welfare *et al.*, 1996). Nevertheless, the ambient ozone levels today were not found to negatively affect olive leaf gas exchange even in the presence of high NaCl concentration, another significant stress factor.

CONCLUSION

In conclusion, similar significant reductions in the leaf gas exchange parameters were found in 'Konservolea' and

Kalamata' olive trees irrigated with 100 mM NaCl solution possibly due to the seedling rootstock on which the two cultivars were grafted. The presence of relatively high ambient ozone concentrations over the summer period (more than 5 months) did not affect olive leaf gas exchange. The combination of salinity and ambient ozone stress did not modify the olive leaf physiological parameters any further than the salinity stress alone.

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