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Effects of elevated CO_2 on grapevine (*Vitis vinifera* L.): Physiological and yield attributes

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Summary

During the 2004, 2005 and 2006 growing seasons, physiological and anatomical leaf characteristics and productivity were studied in field-grown grapevines (*Vitis vinifera* L.) cv. 'Touriga Franca' under ambient (C, 365 ± 10 ppm) or elevated carbon dioxide [CO_2] (E, 500 ± 16 ppm) under Open-top chambers (OTC-C and OTC-E, respectively). The elevated [CO_2] concentration increased net photosynthetic rate (A), intrinsic water use efficiency (A/g_s), leaf thickness, Mg concentration, C/N, K/N and Mg/N ratios and decreased stomatal density and N concentration. Nevertheless, stomatal conductance (g_s), transpiration rate (E), photochemical efficiency (F_v/F_m), leaf water potential, SPAD-values and Red/Far-red ratio transmitted by leaves were not significantly affected by [CO_2]. Meanwhile, there is no evidence for downward acclimation of photosynthesis and stomatal conductance. Yield, cluster weight and vigour showed an increase in elevated [CO_2] treatment but yield to pruning mass ratio was unaffected. Despite elevated [CO_2] stimulates grapevine photosynthesis and yield, more long-term studies, particularly at sub-optimal nutrient and water availability, are needed in order to reveal the grapevine responses to climate change in the Mediterranean area.

K e y w o r d s : Elevated [CO_2], grapevine, leaf anatomy, nutrient content, Open-Top Chamber, photosynthesis, pruning mass, *Vitis vinifera*, yield.

Introduction

According to the Intergovernmental Panel on Climate Change (IPCC 2007), the concentration of carbon dioxide [CO_2] in the atmosphere has been increasing since pre-industrial times and is expected to exceed 550 ppm by the middle of the twenty-first century as a direct result of human activities, primarily fossil fuel burning, cement production and modified land-use patterns. More frequent extreme weather is therefore predicted by most models, along with a significant increase of the summer air temperature and water stress, namely for regions with a Mediterranean-type environment (Tubiello *et al.* 2000). Expected changes in the climate of viticultural regions may alter significantly both the spectrum and the distribution of grape varieties currently used. In particular, shifts in precipitation patterns will affect most European regions, with increased risk of drought and, given this scenario, the consequences would be most dramatic for the Iberian Peninsula (Schultz 2000).

Few studies have been conducted in the field that quantified grapevine responses to elevated [CO_2] (Bindi *et al.* 1996, 2001 a and b, Bindi and Fibbi 2000). In general, they concluded that doubled [CO_2] in the atmosphere results in strong stimulation of yield without having any negative or positive repercussion on grapes at maturity stage. At physiological level, the state of the art is supported by research in others species with C_3 photosynthetic metabolism, whose results showed that increased atmospheric [CO_2] may lead to a short-term increase in net photosynthesis because the [CO_2] at the catalytic site limits the activity of ribulose bisphosphate carboxylase/oxygenase (Stitt 1991, Ainsworth and Rogers 2007), improve light, nutrient and water-use efficiency (Griffin and Seemann 1996) and may increase the leaf thickness and improve alterations in cell and chloroplast development (Robertson and Leech 1995). However, during long-term exposure (days to weeks), some studies report that these main effects may be partly lost as a result of down-regulation in photosynthesis (Faria *et al.* 1996), although long-term positive changes in carbon fixation have also been reported in some species (Campbell *et al.* 1990, Maroco *et al.* 2002). A high metabolic or storage sink capacity seems to be crucial for sustained photosynthetic response to [CO_2]. On the other hand, on a global scale, the increase of [CO_2] could result in a significant increase in air temperature and soil water evaporation, leading to shorter growth intervals, reduced yield and increased yield variability (Schultz 2000). Furthermore, other studies about the impacts of climate change on grape growing and wine production infer a reduction in frost occurrence, advanced initiation of growth in the spring and greater pest and disease pressure due to milder winters (Duchêne and Schneider 2005, Jones *et al.* 2005). Therefore, in vine regions with these climatic characteristics, it is fundamental to study the plant behaviour under the influence of each of these factors as simple variable or in interaction, in order to adjust the best strategy to cultivate the vines without loss of the yield quality.

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Red wine produced in Demarcated Douro Region (Oporto wine region) is one of the most important products for the Portuguese economy. To our knowledge, information about the variation in photosynthesis and yield of field-grown grapevine at elevated [CO_2] is limited. However, this information is relevant to predict the grapevine performance in a future scenario of climate change. Therefore, the main purpose of this study was to investigate the pattern of acclimation of grapevine physiology and yield responses to elevated [CO_2] in Open-Top Chamber (OTC).

Material and Methods

Plant material and growth conditions: The trial was carried out from 2004 to 2006 in a vineyard located at Vila Real (*Campus* of UTAD, 41°17'N, 7°44'W, 470 m above sea level, Baixo Corgo sub-region of Demarcated Douro Region, Northern Portugal). The vineyard was planted in 1997 with *Vitis vinifera* L. 'Touriga Franca' grafted on 1103 P. This cultivar is universally recognized as the finest grape for Porto and red wine. Grapevines were grown at a spacing of 2.0 x 1.2 m, cordon trained and spur-pruned at 10-12 buds per vine. Rows were north-south orientated. The canopy height and thickness was 1.1-1.2 m and 0.5-0.6 m, respectively. The soil was typical schistous and uniform across the experimental plot and effective root depth was about 90-110 cm. The climate is typically Mediterranean with mild rainy winters and long, hot and dry summers. Mean annual rainfall is 1112 mm, of which 12 % from June to September. The warmest months are July and August and the coldest are December and January, with mean daily temperatures of 21-22 °C and 6-7 °C, respectively. Mean annual sunshine value over a 30-year period is 2410 h, of which 49 % from June to September (data for the period 1961-1990, according to the database of Portuguese Meteorological Institute).

Plants were managed without irrigation and grown using standard cultural decisions as applied in commercial farmers. All water and nutrient inputs were continuously recorded, along with other environmental data.

Grapevines were grown in ambient (C, 365 ± 10 ppm) or elevated [CO_2] (E, 500 ± 16 ppm) under Open-top chambers (OTC-C and OTC-E, respectively). More details can be found in GONÇALVES *et al*. (2009). CO_2 was fumigated from sunrise to sunset between budbreak and harvest. Since there appears to be no, or only small positive (DAVEY *et al.* 2004) direct effects of CO_2 on leaf dark respiration, the reports in the literature appearing to be artefacts (JAHNKE and KREWITT 2002), so lack of CO_2 enrichment during the night is probably irrelevant.

Physiological measurements were made at midday of typical summer days on sun exposed and mature leaves at the middle of the shoots (usually between 8th and 11th nodes on the shoot axes). Two sampling periods were fixed: the first corresponding to veraison and the last corresponding to the ripeness period, when the accumulated [CO_2] effect was eventually more intense.

Gas exchange, chlorophyll a fluorescence and leaf water potential: Leaf gas-exchange rates were measured at natural incident PPFD using a portable gas exchange system (LC*pro*+, ADC, Hoddesdon, England). PPFD incident on the leaves was always greater than 1000 μmol m^{-2} s^{-1}, which is above light saturation point in these plants (FLEXAS *et al.* 2002). Net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration/ambient CO_2 ratio (C_i/C_a) were estimated from gas exchange measurements using the equations developed by von CAEMMERER and FARQUHAR (1981). The A/g_s ratio was used as intrinsic water use efficiency according to IACONO *et al.* (1998). Several times, leaf gas exchange of OTC-E treatment was measured at elevated [CO_2] (CO_2 injection system in operation) and immediately at ambient [CO_2] (CO_2 injection system shut-off), in order to evaluate the respective photosynthetic acclimation.

Photochemical efficiency of PSII of dark-adapted leaves (F_v/F_m) was measured on the same leaves used for gas exchange measurements by a pulse modulated fluorometer (FMS 2, Hansatech Instruments, Norfolk, England) as described by ÖQUIST and WASS (1988). Before measurements, leaves were adapted to dark for 30 to 45 min using light exclusion clips. Leaf water potential (Ψ) was determined with a pressure chamber (PMS, Oregon, USA). Measurements were performed on fully expanded leaves at predawn (1 h before sunrise) and at midday (between 14:00 and 15:00 h local time, just after gas-exchange measurements).

SPAD and R/FR readings: Chlorophyll concentration per area was determined non-destructively using a SPAD-502 meter (Minolta, Japan). This instrument uses measurements of transmittance of radiation in the red and near-infrared wavelengths to derive a numerical SPAD value which is related to the quantity of chlorophyll present in the leaf. SPAD-readings were carried out in the field in the same leaf samples used for gas exchange measurements. The quantum ratio (R:FR ratio) transmitted by leaves was determined in the field under clear sky at midday using a 660/730 nm sensor (Skye Instruments, Wales). Transmitted radiation was measured normal to the plane and immediately under the leaf, positioned with its surface perpendicular to the Sun.

Leaf anatomical traits: Anatomical studies and tissues measurements were performed on six healthy, sun-exposed, fully expanded leaves. The thickness of leaf blade, palisade and spongy parenchyma, upper and lower epidermis were measured in leaf cross sections prepared for microscopic examination. Sections were taken from the middle of the leaves to avoid differential thickness along the leaf. To make stomatal impressions, one coat of polish (colodium) was applied on the abaxial leaf surface only because *Vitis vinifera* leaves are hypostomatous. The part of the leaflet used in this study was midway between the tip and the base of the leaflet and avoided the area in the vicinity of main vein. The polish was then carefully peeled off with forceps, mounted on a microscope slide and covered with a cover slip and examined under a light microscope. The number of stomata were determined for six peels per treatment.

Leaf mass area and element concentration measurement: The leaf mass per unit area

(LMA, g m^{-2}) was calculated according to Dijkstra (1989), measuring the leaf area (LICOR 3100, Lincoln, NE, USA) and respective dry mass by oven-dried at 70 ºC to constant mass. Afterwards, leaves were ground and analyzed using standard procedures at the University of Trás-os-Montes e Alto Douro Soil Analysis Laboratory. Briefly, C content was determined with an elemental analyzer by ignition at 1,100 ºC followed by the measurement of evolved CO_2 by near infrared detection. N and P were determined by molecular absorption spectrophotometry, after digestion with H_2SO_4 and H_2O_2 (Mills and Benton Jones 1996). Plant concentration of other elements was determined by atomic absorption spectrophotometry (Ca, Mg and Fe) or by flame emission photometry (K), after digestion with HNO_3 and $HClO_4$ (Mills and Benton Jones 1996). Element concentration was calculated on a dry mass basis.

Yield, vegetative growth and Ravaz index: At harvest, the total number of clusters per vine was counted and the total fruit weight was determined per vine using a hand held balance. The weight per cluster was calculated by dividing the total fruit weight per vine by the number of clusters. During winter pruning all shoots were cut to two node spurs. From these shoots the pruning weight of each vine was determined with a hand-held balance. The weight per shoot was calculated by dividing the total pruning weight by the number of shoots. The fruit weight to pruning weight ratio (Ravaz index) was determined using yield and pruning weight per vine.

Statistical analysis: Data were processed using a one-way ANOVA test to determine the main effect of $[CO_2]$ treatment. Differences were considered statistically significant or tendency for significant when $P < 0.05$ or $0.05 < P < 0.10$, respectively.

Results

Elevated $[CO_2]$ enhanced net photosynthetic rate (Tab. 1). However, this effect was not significant when the CO_2 injection system was shut-off and leaf gas exchanges measured in ambient $[CO_2]$. In elevated $[CO_2]$ conditions, no consistent effect was observed for g_s, E and C_i/C_a. Indeed, the values of these parameters were statistically lower or similar in leaves grown in elevated $[CO_2]$ than in ambient $[CO_2]$, whereas A/g_s had an opposite behaviour since the values were predominantly more elevated in OTC-E (Tab. 1).

The maximal efficiency of excitation energy capture by open PSII reaction centres (F_v/F_m) and leaf water potential (Ψ) measured at either midday (Tab. 1) or predawn (data not shown) did not significantly change under elevated $[CO_2]$. This behaviour was consistent during the 3 growing seasons.

The influence of $[CO_2]$ on leaf thickness was shown in Tab. 2. Total lamina thickness was thicker (14 % in 2004 and 4.5 % in 2005) in OTC-E. In 2004, this was due to a thicker palisade and spongy parenchyma (21 % and 19 %, respectively), and thus the palisade/spongy parenchyma ratio was not significantly altered, while in 2005 it was mainly due to a thicker spongy parenchyma (9 %), giving consequently a lower palisade/spongy parenchyma ratio. Meanwhile, no significant effects of elevated $[CO_2]$ were observed on upper and lower epidermis thickness (data not shown). The positive effect of elevated $[CO_2]$ on leaf thickness is related with higher leaf mass per unit area (+15 %, $P = 0.099$, Tab. 3). Furthermore, leaves developed with high $[CO_2]$ had lower stomatal density than leaves developed under ambient $[CO_2]$. Despite the effect of $[CO_2]$ in

Table 1

Net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), internal CO_2 concentration/ambient CO_2 ratio (C_i/C_a), intrinsic water use efficiency (A/g_s), ratio of variable to maximum fluorescence (F_v/F_m) and leaf water potential (Ψ) at midday of grapevines grown at elevated (OTC-E) and ambient (OTC-C) CO_2. Values represent the mean ± S.E. (n = 10 in 2004 and n = 8 in 2005 and 2006) and the *P* value represent the Probability of F ratio of CO_2 effect

Year	Phenological stage	Treatment	A ($\mu mol \cdot m^{-2} s^{-1}$)	g_s ($mmol \cdot m^{-2} s^{-1}$)	E ($mmol \cdot m^{-2} s^{-1}$)	C_i/C_a	A/g_s ($\mu mol \cdot mol^{-1}$)	F_v/F_m	Ψ (MPa)
2004	Veraison	OTC-E*	20.44 ± 1.37	538.0 ± 41.8	6.69 ± 0.17	0.72 ± 0.01	38.7 ± 2.9	0.51 ± 0.04	-1.12 ± 0.04
		OTC-C	15.08 ± 0.67	556.6 ± 63.0	7.06 ± 0.28	0.72 ± 0.01	28.4 ± 2.5	0.46 ± 0.01	-1.16 ± 0.03
		P value	0.006	0.810	0.284	0.707	0.022	0.248	0.530
	Ripeness	OTC-E*	7.66 ± 0.60	78.0 ± 6.0	3.05 ± 0.16	0.57 ± 0.03	100.3 ± 7.8	0.54 ± 0.01	-1.33 ± 0.03
		OTC-C	4.92 ± 0.38	111.2 ± 7.7	3.68 ± 0.18	0.71 ± 0.01	44.6 ± 2.3	0.54 ± 0.02	-1.42 ± 0.07
		P value	0.001	0.003	0.016	< 0.001	< 0.001	0.928	0.266
		OTC-E**	4.02 ± 0.28	77.4 ± 4.9	2.77 ± 0.16	0.67 ± 0.01	52.5 ± 3.10	-	-
		OTC-C	3.15 ± 0.58	82.8 ± 6.1	2.82 ± 0.14	0.75 ± 0.04	37.5 ± 7.7	-	-
		P value	0.258	0.531	0.843	0.137	0.140		
2005	Ripeness	OTC-E*	11.06 ± 0.85	198.6 ± 6.6	5.37 ± 0.12	0.62 ± 0.03	56.1 ± 5.0	0.38 ± 0.04	-1.35 ± 0.03
		OTC-C	8.77 ± 0.46	184.3 ± 3.9	5.18 ± 0.06	0.67 ± 0.02	47.5 ± 3.1	0.46 ± 0.01	-1.37 ± 0.04
		P value	0.041	0.113	0.174	0.125	0.169	0.152	0.734
2006	Ripeness	OTC-E*	14.93 ± 0.50	456.4 ± 51.9	7.43 ± 0.31	0.75 ± 0.01	24.8 ± 3.2	0.76 ± 0.02	-1.44 ± 0.12
		OTC-C	12.69 ± 0.56	712.6 ± 72.0	10.40 ± 0.30	0.80 ± 0.01	12.5 ± 1.7	0.80 ± 0.02	-1.49 ± 0.07
		P value	0.012	0.013	0.001	0.014	0.008	0.120	0.723

* CO_2 enrichment system in operation.
** CO_2 enrichment system shut-off.

Table 2

Leaf tissue thickness, stomatal density, SPAD-readings and Red/Far Red ratio transmitted by leaves of grapevines grown at elevated (OTC-E) and ambient (OTC-C) CO_2 measured in ripeness stage (August of 2004 and 2005). Values represent the mean ± S.E. (n = 6) and the *P* value represent the Probability of F ratio of CO_2 effect

Year	Treatment	Thickness (μm)				Stomatal density (per·mm^{-2})	SPAD-readings	Red/Far-red transmitted
		Total thickness	Palisade parenchyma	Spongy parenchyma	Palisade/ spongy ratio			
2004	OTC-E	171.2 ± 5.2	56.4 ± 2.5	78.0 ± 3.2	0.736 ± 0.034	130.0 ± 3.5	45.4 ± 1.5	0.339 ± 0.013
	OTC-C	150.2 ± 6.7	46.7 ± 2.1	65.5 ± 4.8	0.735 ± 0.043	168.1 ± 6.4	44.9 ± 1.6	0.315 ± 0.027
	P value	0.033	0.013	0.056	0.994	< 0.001	0.815	0.397
2005	OTC-E	293.9 ± 3.3	88.1 ± 2.0	152.3 ± 1.9	0.579 ± 0.01	165.7 ± 4.0	45.1 ± 0.9	0.066 ± 0.003
	OTC-C	281.3 ± 3.7	90.5 ± 1.8	139.7 ± 2.7	0.650 ± 0.02	193.8 ± 5.1	44.0 ± 0.8	0.058 ± 0.003
	P value	0.023	0.40	0.002	0.012	< 0.001	0.397	0.087

leaf anatomical characteristics, the SPAD-values and Red/Far-red ratio transmitted by leaves were not significantly changed (Tab. 2).

Tabs 3 and 4 present the leaf mineral composition and its relationships. In general, there were no statistically significant differences in the carbon and mineral nutrient concentrations, with the exception of N and Mg concentrations, which were lower and higher, respectively, in OTC-E leaves. In terms of mineral relative composition, the C/N, K/N and Mg/N ratios were significantly increased by elevated [CO_2] (Tab. 4), whereas the C/Mg ratio had a tendency to decrease in high [CO_2]. All other calculated ratios were similar.

The yield per vine showed a clear tendency to increase in elevated [CO_2] in the 3 growing seasons ($P < 0.1$) and this increase was mainly due to an increase in the average cluster weight (Tab. 5). The pruning weight always was superior in OTC-E, but only in 2005 that increase was significant (+ 58 %, Tab. 5). For this effect the number of shoots had a minor contribution since there was no significant difference between the two treatments in 2004 and 2005. Consequently, in 2005, the vigour, expressed by weight per shoot, was higher (+ 42.2 %) in vines grown under elevated [CO_2]. In all three years, no significant differences between the two treatments were recorded for Ravaz index.

Discussion

The results of this study on mature field-grown grapevines indicate that an increase in atmospheric [CO_2] to the levels predicted for 2050 affect leaf gas exchange. Net CO_2 assimilation rate was significantly higher in grapevines grown in elevated than in ambient [CO_2], corroborating the results obtained with several C_3 species (ROGERS and DAHLMAN 1993). On the other hand, this positive effect disappeared when the CO_2 injection system was switched off and the grapevines were measured at ambient [CO_2]. According to EPRON *et al.* (1996), this behaviour reveals the lack of photosynthetic acclimation. Moreover, GUNDERSON and WULLSCHLEGER (1994) refer that in most of the tree species exposed to elevated [CO_2] for a period of weeks or months there is a decline in net photosynthesis when plants are measured at ambient [CO_2], expressing a photosynthetic acclimation process often related with source-sink imbalance. In our experiment, this evidence was not shown, since the vegetative and reproductive growth of the grapevines growing in field conditions has higher transport capacity and large sinks for additional carbohydrates produced by elevated [CO_2], nullifying the down-regulation in photosynthesis, which is evident for example in potted plants with constrained root growth. This hypothesis was supported by experiments in *Quercus petraea* (EPRON *et al.* 1994). Our results demonstrate that grapevines had the capacity to use a CO_2-induced surplus of carbohydrate into structural growth or long-term storage, specifically in higher pruning weight, thicker leaves and slightly higher yield production, without imbalance of the yield to pruning mass ratio. Plant growth generally increases at elevated [CO_2] along with increased photosynthesis (CENTRITTO *et al.* 1999 a, MELGAR *et al.* 2008).

At the photochemical level, F_v/F_m ratio was unaffected by elevated [CO_2], which suggest that there is no evidence of 'down-regulation' of photosynthesis with respect to

Table 3

Leaf mass per unit area (LMA) and element contents on leaf dry mass basis of grapevines grown at elevated (OTC-E) and ambient (OTC-C) CO_2 measured in August 2004. Values represent the mean ± S.E. (n = 6) and the *P* value represent the Probability of F ratio of CO_2 effect

Treatment	LMA (g·m^{-2})	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})	Fe (mg·kg^{-1})
OTC-E	83.6 ± 2.6	507 ± 6.6	21.5 ± 0.4	1.56 ± 0.08	6.80 ± 0.56	17.0 ± 1.3	3.94 ± 0.83	158 ± 7.6
OTC-C	72.6 ± 5.0	497 ± 16.3	23.7 ± 0.7	1.63 ± 0.18	5.78 ± 0.34	19.2 ± 1.3	2.01 ± 0.23	152 ± 8.9
P value	0.099	0.582	0.023	0.758	0.150	0.269	0.048	0.590

Table 4

Element content ratios on leaf dry mass basis of grapevines grown at elevated (OTC-E) and ambient (OTC-C) CO_2 measured in August 2004. Values represent the mean ± S.E (n = 6) and the *P* value represent the Probability of F ratio of CO_2 effect

Treatment	C/N	P/N	K/N	Ca/N	Mg/N	Fe/N	C/K	C/Mg	C/P	C/Ca	K/Mg
OTC-E	23.6 ± 0.6	0.073 ± 0.003	0.32 ± 0.03	0.79 ± 0.07	0.185 ± 0.040	0.007 ± 0.000	76.5 ± 4.8	163.4 ± 36.3	328.4 ± 17.7	30.7 ± 2.7	2.35 ± 0.74
OTC-C	21.1 ± 0.8	0.068 ± 0.006	0.24 ± 0.01	0.81 ± 0.07	0.086 ± 0.012	0.006 ± 0.000	87.1 ± 4.2	271.3 ± 45.7	320.1 ± 30.0	26.6 ± 2.2	3.19 ± 0.60
P value	0.023	0.524	0.028	0.826	0.040	0.120	0.129	0.094	0.817	0.257	0.395

maximum photochemical efficiency of PSII inferred from chlorophyll fluorescence and reinforce the above explanation related with leaf gas exchange results. Some studies have suggested that growth in an elevated $[CO_2]$ atmosphere can decrease the photochemical energy use and increase the probability of 'down-regulation' or photoinhibition of photosynthesis but under limiting nitrogen conditions (Rogers *et al.* 1996, Hymus *et al.* 2001).

Despite the increase on A, there was not a clear change on g_s in elevated $[CO_2]$. Probably, in our experimental conditions, the drought intensity imposed by vapour pressure deficit (VPD ≈ 1.3 to 2.7 KPa) and soil water availability ($\Psi_{predawn}$ ≈-0.2 to -0.3 MPa) was not sufficiently severe for an acclimation of g_s to elevated $[CO_2]$. Vu *et al.* (2002) reported that the magnitude of stomatal response to elevated $[CO_2]$ was species-specific and smaller in trees than in annual crops. On the other hand, Medlyn *et al.* (2001) also found that this response was stronger in water stressed plants. The greater effect of elevated $[CO_2]$ on A than on g_s contributes for an improvement in A/g_s. Under elevated $[CO_2]$, especially when soil moisture becomes the limiting factor (Chaves and Pereira 1992), the increase in A/g_s may be even more important than the increase in photosynthesis. Usually there is a clear positive relationship between the photosynthetic capacity and leaf N status, because a large fraction of the leaf N is in the photosynthetic apparatus (Zhang *et al.* 2008). The N concentration in leaves grown at the elevated $[CO_2]$ decreased about 9 %, relatively to OTC-C leaves, but this lower value had no effects on net CO_2 assimilation rate, probably because elevated $[CO_2]$ increased the Rubisco activity (Faria *et al.* 1996). The reductions in N concentration under elevated $[CO_2]$ may be the result of a larger leaf area (data not shown) and woody tissues (pruning mass). This result is consistent with *Quercus suber* exposed to elevated $[CO_2]$, where the increase in carbohydrates, *per si*, might have resulted in N dilution (Maroco *et al.* 2002). Despite the significant difference in N concentration between the two treatments, we found that these results were not reflected in the SPAD values, taken as a good indicator of N status in leaves (Netto *et al.* 2005). The absence of $[CO_2]$ effect on SPAD values showed that the atmospheric $[CO_2]$ enrichment did not affect chlorophyll content, corroborating the findings of Centritto *et al.* (1999 b).

By contrast to foliar N concentration, C, P, K, Ca and Fe concentrations were unaffected by elevated $[CO_2]$, whereas the Mg content significantly increased. This increment in leaves was also reported by Marinari *et al.* (2007) in a poplar $[CO_2]$ experiment and was associated with an increase of total canopy transpiration. According to these authors, a high Mg content in the leaves has a key role in proton pumping ATPase, important for phloem loading and respective carbohydrate partitioning. By calculating the ratio between elements, nutrient unbalance was detected for C/N, K/N and Mg/N ratios. The increment of these ratios in elevated $[CO_2]$ might be the combined result of N dilution effect by higher C sequestration and an increase in K and Mg uptake by roots.

At the anatomical level, the elevated $[CO_2]$ was associated with a long-term reduction in stomatal density (Tab. 2), which is in accordance with the hypothesis of adaptive modifications in stomata number (Woodward 1987, Woodward and Kelly 1995). Moreover, grapevines grown under $[CO_2]$ enrichment presented thicker leaves mainly due to greater palisade and/or spongy parenchyma in the transversal plane. The leaf mass area analysis also reflected this tendency, confirming the assumption that leaf mass per unit area and leaf thickness are closely related (Aranda *et al.* 2004, Gonçalves *et al.* 2008) and the close effect of elevated $[CO_2]$ on leaf anatomical structure. Rogers *et al.* (1983) also reported an increase in the thickness of all the cell layers in leaves of *Pinus taeda* and *Liquidambar styraciflua* grown in elevated $[CO_2]$ and Radoglou and Jarvis (1990) have found similar increases in the leaves of four poplar clones. The increase in leaf thickness is the result of greater cell enlargement, which is sensitive to $[CO_2]$ whereas cell division apparently is not. Cell enlargement affects the internal leaf surface available for the absorption of CO_2 and is likely to have also consequence for photosynthesis (Nobel 1977). In fact, an increase in mesophyll thickness represents a greater cell wall area for CO_2 diffusion and so should tend to decrease liquid-phase resistance (Mediavilla *et al.* 2001).

Conclusions

The results of three years of experiments under field grown conditions showed that the rising in atmospheric

Table 5

Yield, cluster number and weight, shoot number, pruning weight, weight per shoot and yield weight/pruning weight ratio (Ravaz index) of grapevines grown at elevated (OTC-E) and ambient (OTC-C) CO_2 in three years. Values represent the mean ± S.E. (n = 10) and the *P* value represent the Probability of F ratio of CO_2 effect

Year	Treatment	Yield ($kg \cdot vine^{-1}$)	Cluster ($n^o \cdot vine^{-1}$)	Cluster weight (g)	Shoot ($n^o \cdot vine^{-1}$)	Pruning weight (kg)	Weight per shoot (g)	Ravaz index
2004	OTC-E	4.20 ± 0.75	12.1 ± 2.2	367.3 ± 54.2	11.9 ± 0.7	1.04 ± 0.13	87.1 ± 8.2	4.0 ± 0.4
	OTC-C	2.80 ± 0.26	9.7 ± 1.1	303.7 ± 22.1	11.9 ± 0.9	0.81 ± 0.12	68.7 ± 8.9	3.8 ± 0.4
	P value	0.062	0.295	0.241	0.974	0.213	0.167	0.765
2005	OTC-E	9.21 ± 1.06	21.1 ± 2.1	436.7 ± 25.3	15.4 ± 1.1	1.06 ± 0.15	68.1 ± 7.3	9.2 ± 1.2
	OTC-C	7.24 ± 0.52	21.1 ± 1.6	353.5 ± 27.3	13.8 ± 0.9	0.67 ± 0.07	47.9 ± 4.3	12.2 ± 1.9
	P value	0.096	0.990	0.047	0.252	0.023	0.025	0.227
2006	OTC-E	8.43 ± 1.47	20.7 ± 2.7	406.0 ± 33.0	26.4 ± 2.6	1.03 ± 0.12	39.0 ± 3.0	8.2 ± 0.8
	OTC-C	5.61 ± 0.55	15.9 ± 1.3	351.5 ± 20.8	18.8 ± 1.7	0.76 ± 0.13	39.8 ± 4.8	8.4±1.1
	P value	0.069	0.105	0.167	0.025	0.159	0.889	0.888

$[CO_2]$ during photoperiod influence several anatomical and physiological leaf traits and stimulate grapevine photosynthesis and yield. There is no evidence for a downward acclimation of photosynthesis and stomatal conductance. More long-term studies, special at sub-optimal nutrient and water availability, are needed in order to reveal the grapevine responses to climate change in the Mediterranean area.

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