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ORIGINAL PAPER

Effects of Open-Top Chambers on physiological and yield attributes of field grown grapevines

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Abstract Touriga Franca grapevines were grown in Open-Top Chamber (OTC) and in outside plot (Exterior) for 3 years (2004–2006) to investigate the impact of these structures on climatic conditions and, consequently, on physiological and yield attributes. In general, CO_2 assimilation, stomatal conductance, carbohydrate concentration, maximum bulk modulus of elasticity, palisade parenchyma thickness, leaf mass per unit area and values of red/far-red ratio transmitted by leaves were lower, whereas intrinsic water use efficiency, SPAD-readings and osmotic potential at full turgor were higher in OTC leaves. However, OTC did not affect leaf water potential, maximum PSII photochemical efficiency, stomatal density and soluble proteins concentration. Also, there were no significant differences in C, P, Ca and Fe between treatments. Meanwhile, N and Mg were higher, whereas K concentration was lower in OTC leaves. The environmental conditions inside OTC provided a significant reduction in yield and Ravaz index of 2004, mainly due to a decrease in clusters weight. Regarding the vegetative growth parameters, OTC did not influence the pruning weight, but in 2006 the weight/shoot was significantly lower in OTC vines. In conclusion, the use OTC facility to study the impact of CO_2 enrichment was very expedite, but the extrapolation of results to the open-field environment must be prudent due to the OTC effect.

Keywords Grapevine · Leaf anatomy · Nutrient content · Open-Top Chamber · Photosynthesis · Pruning mass · *Vitis vinifera* L. · Yield

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Introduction

For the research of the effects of elevated CO_2 on plants, it is crucial to study plant responses at CO_2 concentrations which are representative of the ambient conditions predicted in the future (IPCC 2007). Particularly in Mediterranean viticultural areas, marked effects on current agroclimatic conditions are expected with significant changes on grapevine physiological behaviour, on yield interannual variability and respective wine quality and probably on spectrum and distribution of finest grape varieties (Schultz 2000).

Among the several trial experiments systems developed to simulate the response of field crops to the elevated levels of CO_2 or others atmospheric gases, that include leaf chambers, controlled-environment plant growth cabinets, greenhouses, sunlit, controlled-environment plant growth chambers, field air exclusion systems and free air stream gas enrichment systems (Bindi et al. 2001a, b), the Open-Top Chamber (OTC) system has been used in many climate change research (Norris et al. 1996; Bindi et al. 2002). In general, the relatively simple design and construction of OTC systems make them the most likely method to be used for long-term elevated CO_2 exposure of small to medium stature plants (Leadley and Drake 1993).

On other hand, OTC provides lower carbon dioxide costs and maintains the CO_2 concentrations at desirable target without significant spatial and temporal changes, compared with free air CO_2 enrichment (Drake and Peresta 1993; Leadley and Drake 1993). However, compared with outside plots, several researchers recognised that microenvironment conditions in OTCs differ from ambient conditions in quality and quantity of light and in temperature, vapour pressure deficit, atmospheric turbulence and potential evaporation (Sanders et al. 1991; Leadley and Drake 1993; Bindi et al. 2001b; Fangmeier et al. 2002). Consequently, these microclimatic alterations may significantly alter plant growth and development when compared with unchambered plants. Drake and Peresta (1993) reported that the chamber effect on physiological processes is important, but it is expressed differently and to different degrees depending on the local climate factors, chamber design, the plant species and the treatment applied. In this study, which is one part of an ongoing research about the effects of elevated CO_2 and interacting environmental variables on grapevines grown under Mediterranean field conditions, particularly in Port wine region (Portugal), we monitored various parameters related with grapevine physiology and yield to test the hypothesis that the OTC decreases photosynthesis and related metabolites, induces acclimation on leaf anatomy and causes vegetative and yield losses.

Materials and methods

Plant material and growth conditions

The experimental plot was part of a 3-year study on the effects of OTC CO_2 enrichment and interacting environmental variables on grapevines grown under Mediterranean field conditions. More details can be found in Gonçalves et al. (2009). Briefly, 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. cv. Touriga Franca grafted on 1103P. Grapevines were grown at a spacing of 2.0 × 1.2 m, cordon trained and spur-pruned at 10–12 buds per vine. Rows were north–south orientated.

Ten grapevines were grown under OTC (12.0 × 2.5 × 2.5 m and constructed of 1 mm polyethylene film with a 75% light transmittance) and other ten grapevines were grown in outside OTC (Exterior). Several sensors connected to a logger from deltaT devices were installed to monitor the climate variables inside and outside of the OTC. The climate variables were acquired and/or controlled with a sampling interval of 30 s, being the storage time of 5 min.

Physiological and anatomical measurements were made on sun exposed (within OTC this condition was guaranteed at midday) and mature leaves at the middle of the shoots (usually between 8th and 11th nodes on the shoot axes).

Yield and vegetative growth parameters were obtained on three consecutive years (2004, 2005 and 2006). Since the physiological data showed a similar tendency during the 3 years of study, only the more representative days of the first year (2004) are presented (2 July and 14 August).

Gas exchange, chlorophyll *a* fluorescence and leaf water relations

Leaf gas-exchange rates were measured at natural incident photosynthetic photon flux density (PPFD) using a portable gas exchange system (LC*pro*+, ADC, Hoddesdon, UK). Incident PPFD on the leaves was always greater than 1,000 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). To eliminate possible effects of air humidity and temperature on transpiration, the A/g_s ratio, rather than the A/E ratio, was used as an estimate of intrinsic water use efficiency (Iacono et al. 1998).

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, UK) as described by Öquist and Wass (1988). Before measurements, leaves were adapted to dark for 30–45 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). Care was taken to minimise water loss during transfer of the leaf to the chamber by enclosing it in a plastic bag immediately after excision. Pressure–volume (PV) curves were done on fully hydrated leaves, using the pressure chamber technique (Tyree and Hammel 1972) according to Moutinho-Pereira et al. (2007). Briefly, leaves were collected early in the morning and brought back to the laboratory in containers partially filled with distilled water. After the leaves reach full saturation and at periodic intervals, the leaves were weighted and immediately Ψ was evaluated using a pressure chamber. Pressure–volume curves were drawn, and osmotic potential at full turgor (Ψ_π) was determined by

extrapolation of a first order regression fitted to the linear portion of the $1/\Psi$ versus relative water content relationship. The maximum bulk modulus of elasticity (ε_{max}) was estimated from $\Delta\Psi_p/\Delta F$, where $\Delta\Psi_p$ represent the change in turgor (Ψ_p) for change in respective relative symplasmic water content (ΔF, actual symplasmic water expressed as percentage of symplasmic water at full turgor) over the portion of the curve, where the pressure–volume relationship was approximately linear (the first of four points of the Ψ_p vs. F relationship), according to Rodrigues et al. (1993).

Foliar metabolic assays

All metabolic compounds analyses were made with leaf discs taken at midday (just after leaf water potential measurements) from six fully expanded leaves per treatment.

Soluble sugars (SS) were extracted by heating leaf discs in 80% ethanol, according to Irigoyen et al. (1992) and analysed by the reaction of 0.2 ml of the alcoholic extract with 3 ml fresh anthrone and placed in a boiling water bath for 10 min. After cooling, the absorbance at 625 nm was determined. After the extraction of the soluble fractions, the solid fractions were used for starch analysis. Starch (St) was extracted with 30% perchloric acid, according to Osaki et al. (1991), and its concentration was determined by the anthrone method as described above. Glucose was used as standard for both soluble sugars and starch.

The amount of total soluble proteins (SP) was quantified using the method of Bradford (1976). Leaf discs were homogenised in a grinding medium that contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 100 mM PMSF and 2% (w/v) PVP. Bovine serum albumin was used as a standard.

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 Olympus IX 51 microscopic examination (Olympus Optical Co., GmbH, Hamburg, Germany), using image analysis software Cell* (Soft Imaging System GmbH, Hamburg, Germany). 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 was 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 was determined for six peels per treatment.

SPAD and R/FR readings

Chlorophyll concentration per area was estimated 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 the others 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, UK). Transmitted radiation was measured normal to the plane and immediately under the leaf, positioned with its surface perpendicular to the sun.

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 (LI-COR 3100, Lincoln, NE, USA) and respective dry mass by oven-dried at 70°C to constant mass. Afterwards, leaves were ground and analysed using standard procedures at the University of Trás-os-Montes e Alto Douro Soil Analysis Laboratory. Briefly, C content was determined with an elemental analyser by ignition at 1,100°C 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 per vine was determined. 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. 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 analysed using the SuperANOVA statistical package (Abacus Concepts Inc., Berkeley, CA, USA). One-way analysis of variance and Fisher's test were used to establish significant differences at $P \leq 0.05$ between treatment means.

Results

Environmental conditions during the growth period were typical of a Mediterranean summer: maximum temperature around 30°C; total hours of sunshine from June to September of about 50% of the year; midday vapour pressure deficit (VPD_{midday}) ranging from 1.8 to 3.7 kPa; rain falls specially during the winter months, except in 2006 where it was around 220 mm from June to September, i.e. about 20% of the total rainfall in the year (Table 1). Inside the OTC, the average night temperature was lower (−0.45°C) from June to September than in the unchambered plot. In opposition, at midday the average temperature and VPD were higher (+4.1°C and +0.9 kPa, respectively). Air CO_2 concentration was similar in both treatments, and PPFD was attenuated 20–30% by OTC until mid-morning and from mid-afternoon, but at midday the degree of attenuation was irrelevant (data not shown). The predawn leaf water potential values, which is presumed to express the equilibrium between soil water potential and leaf water potential of the plant when the plant has covered its need for water after the moisture loss of the previous day (Katerji and Hallaire 1984), suggests that during the summer grapevines were grown under good soil water availability in both treatments (Table 2). Similar water potential trend was obtained by Moutinho-Pereira et al. (2004).

Leaf gas-exchange parameters recorded at midday in typical summer days were affected by OTC (Table 2). In general, A, g_s and E were lower, whereas A/g_s was higher in OTC ambient leaves. The impact of OTC on midday leaf water potential (Ψ_{midday}) and F_v/F_m ratio was generally not significant, although the values were slight higher in Exterior plot (Table 2). Meanwhile, the analysis of bulk leaf water relations showed that Exterior leaves exhibited consistently lower Ψ_π and higher ε_{max} than OTC leaves (Table 3). At foliar metabolic level, OTC system caused significant decrease in SS and St, but it had no effect in SP concentration (Table 3). The decreased of carbohydrate

Table 1 Mean monthly and annual maximum and minimum air temperatures (T_{max} and T_{min}, respectively), midday air vapour pressure deficit (VPD_{midday}), total rainfall and number of sunshine hours during the growing period of the 3 years of measurements (data from the meteorological station inside the study area)

	T_{max} (°C)		T_{min} (°C)		VPD_{midday} (kPa)		Rainfall (mm)	Sunshine (h)
	Exterior	OTC	Exterior	OTC	Exterior	OTC		
2004								
June	30.0	34.7	15.9	15.5	2.94	4.02	0.4	342.6
July (2 Jul)[a]	30.8 (30.3)	35.2 (35.4)	13.9 (13.7)	13.4 (12.7)	3.33 (3.47)	4.38 (4.70)	0.3 (0)	343.3 (14.2)
August (14 Aug)[a]	27.5 (31.4)	31.4 (35.3)	14.0 (13.8)	13.8 (13.7)	2.21 (2.87)	3.03 (3.78)	68.7[b] (0)	284.7 (11.8)
September	25.8	29.8	13.5	13.4	1.76	2.52	24.2	244.1
Annual	18.9	–	7.8	–	1.36	–	670.6	2,527.2
2005								
June	30.0	34.4	12.7	12.3	3.21	4.20	14.8	340.4
July	32.4	35.6	15.7	14.9	3.73	4,44	8.9	345.1
August	33.9	38.1	15.1	14.8	3.40	3.91	2.4	347.0
September	27.6	30.8	11.8	11.6	2.51	3.42	24.5	321.6
Annual	19.5	–	7.6	–	1.57	–	569.9	2,719.6
2006								
June	29.5	33.7	14.0	13.4	2.85	3.82	74.1	290.3
July	32.5	36.7	12.5	12.0	3.56	4.70	26.5	319.1
August	32.0	35.6	14.9	13.9	3.68	4.60	31.9	320.8
September	26.7	31.3	13.3	12.9	2.34	3.36	91.8	261.4
Annual	19.3	–	8.3	–	1.52	–	1,121.3	2,349.1

[a] Within parenthesis represent the climatic values recorded on the 2 days that were physiologically discussed

[b] 55% of this rain fell from 8 to 11 August 2004

Table 2 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) and maximum photochemical efficiency of PSII (F_v/F_m) at midday, and predawn and midday leaf water potential (Ψ_{pd} and Ψ_{md}, respectively) of grapevines grown in OTC and Exterior

Parameter	2 July 2004			14 August 2004		
	OTC	Exterior	P value	OTC	Exterior	P value
A (μmol m^{-2} s^{-1})	15.08 ± 0.67	18.62 ± 0.79	0.006	4.92 ± 0.38	6.32 ± 0.40	0.020
g_s (mmol m^{-2} s^{-1})	556.6 ± 63.0	999.8 ± 106.7	0.005	111.2 ± 7.7	165.4 ± 9.7	<0.001
E (mmol m^{-2} s^{-1})	7.06 ± 0.28	6.56 ± 0.24	0.207	3.68 ± 0.18	4.20 ± 0.16	0.040
C_i/C_a	0.72 ± 0.01	0.74 ± 0.01	0.076	0.71 ± 0.01	0.72 ± 0.01	0.350
A/g_s (μmol mol^{-1})	28.4 ± 2.5	19.5 ± 1.8	0.015	44.61 ± 2.32	38.89 ± 2.28	0.097
F_v/F_m	0.46 ± 0.01	0.54 ± 0.03	0.029	0.54 ± 0.02	0.56 ± 0.01	0.280
Ψ_{pd} (MPa)	−0.28 ± 0.01	−0.30 ± 0.01	0.437	−0.29 ± 0.01	−0.29 ± 0.03	0.866
Ψ_{md} (MPa)	−1.16 ± 0.03	−1.09 ± 0.08	0.428	−1.42 ± 0.07	−1.25 ± 0.04	0.066

Values represent the mean ± SE ($n = 10$), and P values represent the probability calculated by one-way ANOVA

Table 3 Osmotic potential at full turgor (Ψ_π), maximum bulk modulus of elasticity (ε_{max}) and biochemical parameters of the grapevine leaves grown in OTC and Exterior measured in August 2004

Parameter	OTC	Exterior	P value
Ψ_π (MPa)	−1.24 ± 0.07	−1.48 ± 0.06	0.037
ε_{max} (MPa)	1.84 ± 0.90	4.80 ± 0.65	0.037
SP (mg dm^{-2})	29.17 ± 4.64	39.05 ± 5.02	0.263
SS (mg dm^{-2})	45.86 ± 3.14	57.04 ± 3.29	0.037
St (mg dm^{-2})	22.87 ± 2.16	40.35 ± 3.88	0.003
SS/St	2.10 ± 0.24	1.47 ± 0.16	0.069

Values represent the mean ± SE ($n = 6$), and P values represent the probability calculated by one-way ANOVA

SP soluble proteins; *SS* soluble sugars; *St* starch content; *SS/St* soluble sugars/starch ratio

Table 4 Leaf tissues thickness, stomatal density, SPAD-readings and red/far red ratio transmitted by grapevine leaves grown in OTC and Exterior measured in August 2004

Parameter	OTC	Exterior	P value
Total thickness (μm)	150.2 ± 6.7	165.8 ± 3.3	0.063
Palisade parenchyma (μm)	46.7 ± 2.1	57.3 ± 1.1	0.001
Spongy parenchyma (μm)	65.5 ± 4.8	73.9 ± 3.7	0.195
Palisade/spongy ratio	0.735 ± 0.04	0.793 ± 0.04	0.369
Stomatal density (per mm^{-2})	168.1 ± 6.4	186.9 ± 8.9	0.109
SPAD readings	45.1 ± 1.8	40.2 ± 1.5	0.048
Red/far-red transmitted	0.32 ± 0.03	0.47 ± 0.06	0.034

Values represent the mean ± SE ($n = 6$), and P values represent the probability calculated by one-way ANOVA

Table 5 Leaf mass per unit area (LMA) and foliar nutrients concentration on dry mass basis of grapevines grown in OTC and Exterior measured in August 2004

Parameter	OTC	Exterior	P value
LMA (g m^{-2})	72.6 ± 5.0	85.9 ± 2.7	0.049
C (g kg^{-1})	497 ± 16.3	477 ± 8.7	0.308
N (g kg^{-1})	23.7 ± 0.7	21.7 ± 0.7	0.077
P (g kg^{-1})	1.63 ± 0.18	1.63 ± 0.08	0.976
K (g kg^{-1})	5.78 ± 0.34	8.23 ± 0.48	0.002
Ca (g kg^{-1})	19.2 ± 1.3	16.8 ± 0.6	0.138
Mg (g kg^{-1})	2.01 ± 0.23	1.30 ± 0.11	0.022
Fe (mg kg^{-1})	152 ± 8.9	180 ± 17.5	0.172

Values represent the mean ± SE ($n = 6$), and P values represent the probability calculated by one-way ANOVA

concentration inside OTC was more pronounced in St (43.2%) than in SS (19.5%). Thus, the SS/St ratio was slightly higher in OTC than in Exterior plot.

Total leaf thickness was slightly higher in the Exterior plants, mainly due to a thicker palisade parenchyma (Table 4). However, the palisade/spongy ratio was not significantly affected by OTC. The stomatal density had a tendency to be lower (−10%) in OTC leaves. Moreover, the SPAD-readings and red/far-red ratio transmitted by mature and sunlit leaves were, respectively, lower and higher in the Exterior leaves. In accordance with total leaf thickness, leaf mass per unit area values were 18.2% higher in Exterior than in OTC leaves (Table 5).

Concerning the leaf nutrient concentration, there were no significant differences in C, P, Ca and Fe concentration between Exterior and OTC ambient leaves (Table 5). Meanwhile, Mg was lower and N slightly lower, whereas K concentration was 42.4% higher in Exterior plot, contributing for a significantly higher ratios of K/N and K/Mg in Exterior leaves (58.3 and 109%, respectively), relatively to OTC leaves.

The environmental conditions inside OTC provided a significant reduction in yield of 2004, due to a decrease in

Table 6 Yield, cluster number and weight, shoot number, pruning weight, weight per shoot and yield weight/pruning weight ratio (Ravaz index) of grapevines grown in OTC and Exterior in the 3 years

Parameter	2004			2005			2006		
	OTC	Exterior	*P* value	OTC	Exterior	*P* value	OTC	Exterior	*P* value
Yield (kg/vine)	2.80 ± 0.26	4.54 ± 0.32	0.004	7.24 ± 0.52	6.86 ± 0.38	0.627	5.61 ± 0.55	7.46 ± 0.94	0.127
Cluster (no/vine)	9.7 ± 1.1	12.3 ± 0.7	0.065	21.1 ± 1.6	18.3 ± 0.8	0.110	15.9 ± 1.3	15.8 ± 1.8	0.975
Cluster weight (g)	303.7 ± 22.1	371.6 ± 16.1	0.029	353.5 ± 27.3	371.1 ± 11.2	0.493	351.5 ± 20.8	468.5 ± 25.4	0.003
Shoot (no/vine)	11.9 ± 0.9	12.0 ± 0.4	0.878	13.8 ± 0.9	13.1 ± 0.5	0.498	18.8 ± 1.7	14.3 ± 0.9	0.024
Pruning weight (kg)	0.81 ± 0.12	0.83 ± 0.08	0.863	0.67 ± 0.07	0.59 ± 0.04	0.400	0.76 ± 0.13	0.79 ± 0.08	0.865
Weight per shoot (g)	68.7 ± 8.9	67.7 ± 4.9	0.921	47.9 ± 4.3	44.7 ± 2.6	0.568	39.8 ± 4.8	55.0 ± 5.1	0.048
Ravaz index	3.8 ± 0.4	5.9 ± 0.4	0.003	12.2 ± 1.9	12.4 ± 0.6	0.928	8.4 ± 1.1	9.7 ± 1.0	0.393

Values represent the mean ± SE ($n = 10$), and *P* values represent the probability calculated by one-way ANOVA

number and weight of clusters (Table 6). In 2005, yield and associated parameters were similar for both treatments OTC. In 2006, a similar trend was observed as in 2004, mainly due to a significant lower weight per cluster in OTC vines. In terms of vegetative growth, OTC did not influence the pruning weight. However, in 2006, the average weight per shoot was significantly higher in Exterior vines, contributing greatly to this the lower number of shoots per vine. In relation to Ravaz index, the OTC effect was significant only in 2004, noting a higher value on Exterior vines, although well below the values obtained in the following 2 years, mainly due to a higher production per vine.

Discussion

Among the existing methods for analyzing the effects of elevated [CO_2] on plant growth (growth chambers, greenhouses, free air [CO_2] enrichment), the OTC approach was the selected method to develop this project due to robust design and simplicity and to lower costs. However, several researchers recognised that chambers interfered with natural micrometeorological conditions and consequently with crop growth and development (Sanders et al. 1991; Bindi et al. 2001b; Fangmeier et al. 2002). Briefly, the results from this experimental device confirmed that the microclimate within OTCs, independently of [CO_2] effect, was characterised by lower irradiance with a higher proportion of diffuse to direct radiation, higher temperature, higher and lower humidity at night and during the day, respectively, and lower wind speed, with important consequences on physiological and yield attributes.

Exterior vines always exhibited higher A and g_s than OTC vines at midday, when solar radiation reached its highest levels (around 2,000 μmol m^{-2} s^{-1}), and the measured OTC leaves were transient exposed to full sunlight (Table 2). Despite some uncertainty of C_i estimated from gas exchange measurements due to possible 'patchy' stomatal responses (Terashima 1992), the C_i/C_a ratio was not significantly different between treatments (Table 2), indicating that both stomatal and non-stomatal limitations of photosynthesis increased in OTC as compared with Exterior leaves (Downton et al. 1987). However, in OTC leaves the reduction of A was less than the reduction of g_s, increasing consequently the A/g_s. This strengthens the hypothesis that the higher limitation of photosynthesis within OTC was mainly due to stomatal mechanism (Flexas et al. 1998). To reinforce this argument, no consistent changes were detected in maximum photochemical efficiency of PSII (given by F_v/F_m in dark-adapted leaves) and in leaf soluble protein concentration (mostly related with the function of the photosynthetic machinery) between treatments (Tables 2, 3, respectively). However, it is important to note that the low midday F_v/F_m values ($\sim$0.50) observed in both treatments and dates, compared to the 0.80 determined at predawn (data not shown), revealed the occurrence of a dynamic photoinhibition, which seemed to be effective in protecting the photosynthetic apparatus from the high risk of photodamage in OTC and Exterior leaves occasioned by high light intensities and temperatures at midday (Souza et al. 2004). Thus, the excessive excitation energy is deflected away from PSII and dissipated harmlessly, primarily as heat (Osmond 1994). Down-regulation of photochemical efficiency around midday has also been shown in well-watered olive plants grown under Mediterranean field conditions (Bacelar et al. 2007). Despite the low midday F_v/F_m values, both treatments were able to maintain the leaf gas exchange at relatively high rates in the early summer period (2 July), suggesting that grapevines did not suffer severe water stress. Moreover, the leaves were at high physiological status, in relation to the leaves analysed in the mid-summer (14 August), where their functionality may be limited by the cumulative effects of summer stress and eventually with increasing leaf age (Poni and Intrieri 2001).

The highest stomatal limitation within OTC did not cause a proportional reduction in transpiration rate and a significative more favourable vine water status (i.e., less negative Ψ_{md}) (Table 2). This may indicate that the major environmental effects are compensatory, e.g. reduction of light intensity within OTC is compensated by increased temperature and vapour pressure deficit (Table 1). On the other hand, the absence of a positive association between g_s and E may be attributable to elevated sensibility of E to ambient temperature and humidity (which difference is considerable between inside and outside OTC) than of g_s (Düring 1994; Leidi et al. 1999). Drake and Peresta (1993) reported that the chamber environment may reduce or increase plant water stress depending on the interaction of a number of factors, such as radiation, temperature, boundary layer conductance and vapour pressure deficit, which must be evaluated separately in each case.

Exterior leaves exhibited always the highest values of SS and St contents, being the OTC effect more expressive to St than to SS (Table 3). These results were associated with the finding that the photosynthetic activity was higher in Exterior leaves and also suggests a slight delay in the sucrose transport to other grapevine organs. Meanwhile, the SS accumulation, between others metabolites and inorganic ions (Patakas and Noitsakis 2001; Patakas et al. 2002), contributed to an active osmotic adjustment (i.e. decreased of Ψ_π at full turgor) in Exterior leaves, relatively to OTC leaves. Additionally, the higher starch accumulation in Exterior leaves reduces the osmotic volume of the mesophyll cells which increase the concentration of osmotically active solutes only by itself (Ackerson 1981). These adjustments of Exterior leaves would enable them to maintain water potential at slightly higher levels than OTC leaves, despite external environmental conditions, mainly high light and atmospheric turbulence.

In several fruit tree species, the photosynthetic capacity is often related to leaf anatomical characteristics, which is influenced by ambient irradiance (Reich et al. 2000; Le Roux et al. 2001; Gonçalves et al. 2008). Indeed, because of the lower growth irradiance conditions in the OTC, leaves developed here were more like shade-type, with slight lower leaf thickness, mainly due to significantly thinner palisade parenchyma layer, than leaves developed outside of the OTC (more sun-type leaves) (Table 4). This pattern response was also reflected by the LMA differences between two treatments (Table 5), which support the assumption that leaf thickness and LMA are positively correlated (Aranda et al. 2004). However, the high-light growth conditions of the Exterior leaves may have contributed to lower SPAD values and higher transmittance in the red to near-infrared ratio (Table 4), in accordance with Daughtry et al. (2000).

From a nutrient management perspective, assumed special relevance the markedly lower Mg and slightly lower N concentration in Exterior leaves (Table 5). Since there is a close relationship between total chlorophyll content, leaf spectral properties and the concentration of these elements (Ayala-Silva and Beyl 2005; Netto et al. 2005), these lower values may be an indicator of some chlorophyll photo-oxidative destruction caused by high PPFD. However, we assume that the lower Mg and N concentration in Exterior leaves had no significant effect on carbon metabolism efficiency. In contrast, foliar K concentration increased significantly in Exterior vines, contributing to higher K/N and K/Mg ratios. This ability of Exterior leaves would enable them to decrease the Ψ_π, reinforcing the osmotic adjustment effect developed by SS accumulation. Several authors refer that it is usually the potassium that accumulates when inorganic ions are utilised for osmotic adjustment (Turner and Jones 1980; Morgan 1992; Patakas et al. 2002). This preference, particularly in drought-stressed grapevines, is justified by Patakas et al. (2002) as an important strategy to reduce the energetic costs of osmotic adjustment, although exacerbating the Mg deficiency, by K/Mg antagonism (Coutinho et al. 1984).

Changes of leaf structure in response to light environment not only include differences in leaf thickness and spectral properties but also bring about changes in cell wall elasticity (Bacelar et al. 2006). In our study, ε_{max} was higher (i.e. higher tissue rigidity) in Exterior vines (Table 3), reflecting changes on cell wall composition (Munoz et al. 1993). This modification can result in an increase in the gradient in water potential between the soil and the plant, thereby promoting a more effective water uptake from drying soils and/or accelerating recovery after re-watering (Bowman and Roberts 1985). In addition to these anatomical alterations, the lower radiation within the OTC also contributed to a slight stomatal density reduction (Table 4). Some studies demonstrated a high impact of lower irradiance levels induced by OTC on the reduction of the stomatal density, mainly when these light conditions, exacerbated by the 'greenhouse' effect, occur during the early leaf development (Sanders et al. 1991; Beerling and Chaloner 1993).

Finally, there was a tendency to a decrease in the yield of OTC (Table 6), probably induced by more favourable environmental conditions (less wind and particularly high relative humidity at night and early morning) to the development of the Botrytis bunch rot, a disease caused by the fungal pathogen *Botrytis cinerea* with considerable loss of grape yield and wine quality. Also it should be noted that the clusters of Touriga Franca variety are very sensitive to this disease. This problem, highly significant in 2004 and with negative consequences in the Ravaz Index, was minimised in 2005 and 2006 by a close control with

conventional fungicides. Particularly, in the last year the yield within OTC decreased 24.8%, but not significantly, mainly due to a lower cluster weight (25%) and a lesser cluster number by shoot (23.2%). In a complementary study using wines obtained from these grapes, Gonçalves et al. (2009) reported that OTC wine, possibly due to a lower yield and higher temperature, had significantly higher alcohol, pH, and total anthocyan and polyphenol concentrations and lower fixed acidity, but no significant differences in total antioxidant activity were found. The vigour, express by weight per shoot, decreased about 27.6%, probably due to a higher competition between shoots per vine (18.8 vs. 14.3 in OTC and Exterior vines, respectively) and eventually due to a lower photosynthetic activity (Table 2).

In conclusion, the results of this study reveal that grapevine anatomy, physiology, biochemistry and productivity were modified by particular microclimate conditions that were created in the OTC. Therefore, the extrapolation of results from OTC experiments to the free-air conditions must be done with special attention in studies related with the physiological impact of CO_2 enrichment or others atmospheric gases.

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