

Grapevine varieties exhibiting differences in stomatal response to water deficit

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Abstract. Knowledge on variety traits and physiological responses to stress is still scarce in *Vitis vinifera* L., limiting the optimisation of irrigation and breeding for high water use efficiency. We have characterised five grapevine varieties using thermal imaging, leaf gas exchange, leaf morphology and carbon isotope composition. Plants of the varieties Aragonez, Trincadeira, Cabernet Sauvignon, Syrah and Touriga Nacional were grown in field conditions. Two experiments were performed. In Experiment I (2006), vines of Aragonez and Trincadeira were either well irrigated (WI, 80% ET_c), non-irrigated but rain fed (NI) or subjected to regulated deficit irrigation (RDI, 40% ET_c) and studied along the summer season. In Experiment II (2006 and 2007), vines of the five varieties were subjected to RDI (30–40% ET_c) and studied at veraison. In Experiment I, leaf temperature (T_{leaf}) correlated negatively with stomatal conductance (g_s) and leaf water potential (Ψ_{pd}). The inverse relationship between g_s and T_{leaf} was highly significant in the afternoon. In Experiment II, the different genotypes showed different T_{leaf} for similar Ψ_{pd} . Stomatal density did not correlate with g_s , suggesting that varieties have different stomatal control. Our results show that combined measurements of canopy temperature and Ψ_{pd} can aid in better understanding of stomatal regulation in different grapevine varieties. Such variation in stomatal regulation should be taken into account in determining irrigation strategies.

Additional keywords: genotypes, stomatal conductance to water vapour, thermal imaging, *Vitis vinifera*, water stress.

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Introduction

Vitis vinifera L. has a large genetic variability, with several thousands of varieties having been cultivated worldwide (Schultz and Stoll 2010). The species is generally considered as ‘drought avoiding’ and is well adapted to the Mediterranean climate (Schultz 2003; Schultz and Stoll 2010). However, the combination of soil water deficit, high vapour pressure deficit (VPD), high light intensities and high temperature during the summer decreases growth and yield with potential negative impact on berry quality (Chaves and Rodrigues 1987; Escalona *et al.* 1999; Flexas *et al.* 2002; Chaves *et al.* 2007, 2010; Flexas *et al.* 2010). Moreover, the large genetic heterogeneity of the species results in differences in the responses of varieties to drought, namely in terms of their leaf stomatal response (iso and anisohydric behaviour), photosynthetic assimilation and water use efficiency (Chaves *et al.* 1987, 2010; Bota *et al.* 2001; Medrano *et al.* 2003; Flexas *et al.* 2010). It also represents an opportunity for breeding for more efficient vines regarding water use; this is particularly

important in the context of scarce water resources, where the use of water saving strategies (deficit irrigation) in irrigated viticulture is required.

Growers need to optimise deficit irrigation strategies to guarantee a proper balance between vegetative growth and fruit load, and minimise negative effects of the imposed mild stress (Cifre *et al.* 2005; Girona *et al.* 2006; Costa *et al.* 2007). Assessment of plant water status and canopy temperature is a relevant approach to decide when and how much irrigation is required.

In recent decades, several imaging technologies were developed enabling the study of plant stress physiology remotely and non-destructively (Nilsson 1995; Chaerle and van der Straeten 2000; Jones 2004). Among them, thermal imaging has emerged as one of the most useful, being widely used in industry and in science (Kaplan 2007). In plant science, thermal imaging has become a feasible approach to measure the temperature of plants and indirectly assess leaf transpiration and plant water status. When stomata close, leaf transpiration

is reduced, leading to higher leaf temperatures as compared with leaves with open stomata (Jones 1999; Jones et al. 2002; Araus et al. 2008).

Thermal imaging has been used to screen genotypes with different stomatal conductance not only for the model plant *Arabidopsis thaliana* (L.) Heynh. (Merlot et al. 2002, 2007) but also for crops like wheat (Raskin and Ladyman 1988; James et al. 2008), chickpea (Kashiwagi et al. 2008), rice (Hirayama et al. 2006), barley (Sirault et al. 2009) and more recently, strawberry (Grant et al. 2012). Different studies have shown the feasibility of thermal imaging as a tool to monitor water stress and support irrigation scheduling of relevant crops like grapevine (Jones et al. 2002; Grant et al. 2006, 2007; Möller et al. 2007), cotton (Alchanatis et al. 2010), olive trees (Ben-Gal et al. 2009) and citrus (García-Tejero et al. 2011). However, screening varieties of grapevine showing different leaf temperature has not been explored.

The major aims of our study were to characterise the transpirational behaviour of different *V. vinifera* genotypes in response to soil water deficit and to assess the potential of thermal imaging as a tool to monitor plant water status and stomatal aperture (stress monitoring).

Two independent field experiments were conducted combining thermal imaging, leaf gas exchange, leaf morphology and carbon isotope analysis. In Experiment I, we monitored plant water status and leaf gas exchange along the summer season in plants of Aragonez and Trincadeira subjected to different irrigation treatments. In Experiment II, we characterised the transpirational behaviour of five different grapevine genotypes (Trincadeira, Aragonez, Cabernet Sauvignon, Syrah and Touriga Nacional) growing in the field under regulated deficit irrigation.

Materials and methods

Location and climate

Two experiments were conducted at a commercial vineyard (Monte Seis Reis) located in Estremoz (38°48'N, 7°29'W), South Portugal in 2006 and 2007. The climate is Mediterranean, with hot, dry summers and mild air temperatures with precipitation concentrated during autumn and winter (Fig. 1). When comparing the 2 years, 2006 was found to be the hottest with mean summer temperatures, 1.5–2°C higher than those measured in 2007 (Fig. 1; Table 1). The year 2006 was also characterised by lower precipitation in the winter and spring than in 2007 (Fig. 1). This may have contributed to a

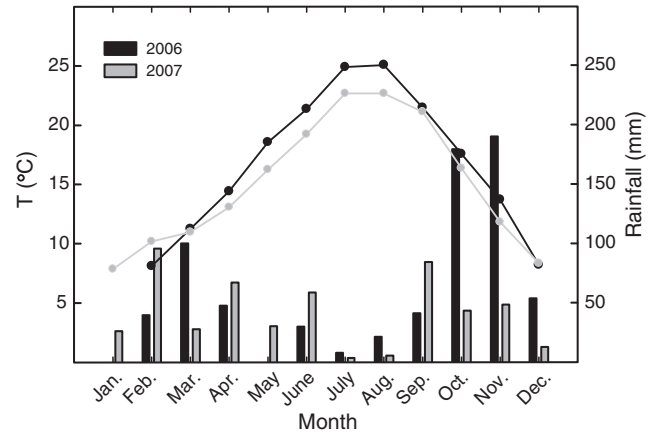


Fig. 1. Variation of the mean air temperature (T , lines) and precipitation (Rainfall, bars) measured for the years 2006 and 2007.

more severe summer drought stress experienced by plants in 2006 than in 2007.

Climate data on precipitation, solar radiation, air RH, air temperature, potential evapotranspiration (ET_0) and wind speed were obtained from an automatic weather station located near the vineyard (38°52'N, 07°31'W) (COTR 2007). Vapour pressure deficit (VPD) was calculated from measurements of ambient temperature and humidity and expresses the evaporative demand of the atmosphere.

The soil is derived from schist with a variable depth (1.0–1.5 m). Soil horizons present a silty clay loam texture with the following average characteristics: clay 34.2%; silt 30.4%; sand 35.4%; organic matter 2.0%; pH of 7.1. The total soil available water, up to 1.0 m depth, was 124.2 mm, determined as the difference between field capacity and permanent wilting point.

Plant material

In Experiment I, two grapevine (*Vitis vinifera* L.) varieties were studied: 'Aragonez' (syn. Tempranillo) (ARA) and 'Trincadeira' (TRI). In Experiment II, we compared five varieties: 'Touriga Nacional' (TOU); 'Aragonez' (ARA); 'Syrah' (SYR); 'Trincadeira' (TRI); and 'Cabernet Sauvignon' (CAB). All vines were 6–7 years old and were grafted on the rootstock 1103-P. They were planted at a density of 4000 plants ha⁻¹, with a spacing of 2.5 × 1.0 m. Vines were trained on a bilateral Royat Cordon system using a vertical shoot positioning with a

Table 1. Global radiation (R_G), mean air temperature (T_{mean}), wind speed at 2 m above ground surface (u_{2m}), vapour pressure deficit (VPD), reference evapotranspiration (ET_0) measured at different dates of thermal imaging measurements conducted in Experiment I (2006) and in Experiment II (beginning of August 2006 and 2007)

Source: Centro Operativo e de Tecnologia de Regadio (COTR 2007)

Parameter	Experiment I			Experiment II	
	28–29 June 2006	1–2 August 2006	29–30 August 2006	8 August 2006	9 August 2007
R_G (MJ m ⁻² day ⁻¹)	27.9–28.5	25.3–26.0	27.4–27.4	24.7	25.0
T_{mean} (°C)	20.0–22.9	23.6–25.0	27.6–28.0	28.4	26.9
u_{2m} (m s ⁻¹)	1.2–1.1	1.0–1.0	1.1–1.0	1.0	1.0
VPD (kPa)	0.85–1.16	1.18–1.28	2.19–2.42	2.45	2.32
ET_0 (mm day ⁻¹)	3.9–4.2	4.1–4.3	5.6–6.0	5.2	4.6

pair of movable wires and spur pruned. All vines were uniformly pruned to 12–14 nodes per vine. Vines from the varieties ARA and TRI were grown side by side. Plants of the remaining varieties were grown in nearby plots with similar sun exposure and soil characteristics. The standard cultural practices of the region were applied by keeping the natural soil cover during the season. Shoots were trimmed at ~30 cm above the higher movable wire, once or twice between bloom and veraison.

Irrigation treatments

Experiment I was conducted during the summer of 2006. The vines of the varieties ARA and TRI were subjected to three irrigation treatments: well irrigated (WI, 80% ET_c), non-irrigated but rain fed (NI) and regulated deficit irrigation (RDI, 40% ET_c). Watering was applied according to crop evapotranspiration (ET_c) and soil water content. ET_c was estimated from the ET_o , using the crop coefficients (K_c) proposed by the FAO (1998).

Experiment II was conducted during two consecutive summers: 2006 and 2007. The vines from the five varieties studied (ARA, TOU, SYR, TRIN and CAB) were grown under RDI (30–40% ET_c) conditions. In 2006, RDI was around 40% ET_c . RDI irrigation started on the 19 June and finished mid August (DOY=227). Irrigation amount was adjusted in both WI and RDI taking into consideration precipitation events (Fig. 1). By mid August a short interruption of irrigation occurred by a technical default in the WI treatment. In 2007, RDI irrigation started on the 26 June and was arrested on the 11 August. RDI was between 30–40% of ET_c to adjust to the less stressful conditions compared with 2006. For details on the water applied (mm) see Table 2. Drip irrigation lines were positioned along plants' row and consisted of pressure compensating 2.5 L h⁻¹ emitters at 1.0 m spacing, one per vine, positioned between two contiguous vines.

Measurements

Soil and plant water status

Soil water content was monitored with a capacitance probe (Diviner 2000TM; Sentek Environmental Technologies, Kent Town, SA, Australia). Predawn leaf water potential (Ψ_{pd}) was

Table 2. Amount of water applied (mm) and fraction of transpirable soil water (FTSW, %, measured at 1 m depth), on 1 August 2006 and on 6 August 2007) to plants of the different genotypes of the Experiment II, Aragonese (ARA), Trincadeira (TRI), Syrah (SYR), Cabernet (CAB) and Touriga Nacional (TOU) subjected to RDI conditions since 2006

Year	<i>Vitis vinifera</i> genotype	Water applied (mm)	FTSW
2006	TRI	126	52
	TOU	145	47
	CAB	145	47
	SYR	121	29
	ARA	86	40
2007	TRI	32	55
	TOU	28	57
	CAB	28	57
	SYR	36	43
	ARA	63	45

measured at 0300–0400 hours solar time using a pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR).

In Experiment I, Ψ_{pd} was measured periodically from the 29 June until the 1 September 2006. In Experiment II, Ψ_{pd} was measured during the first 2 weeks of August in 2006 and 2007. Here, 4–6 plants located in three different rows were used and three leaves were harvested per plant.

Canopy temperature and thermal indices

In order to assess vines canopy temperature, we used in both experiments a thermal imager (IR Snapshot 525, Infrared Solutions, Minneapolis, MN), which consisted in a 120 × 120 pixel line scan imager operating in the wavebands 8–12 μm, with a noise equivalent temperature difference (NETD) of 0.1°C at 30°C and an accuracy of 2°C or 2% of reading. Infrared (IR) images were taken from the sunlit side of the canopy (Jones *et al.* 2002). Background temperature was determined as the temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest with the emissivity set at 1.0 (Jones *et al.* 2002). Emissivity for measurements of grapevine leaves/canopies was set at 0.96 (Grant *et al.* 2006; Leinonen *et al.* 2006). Images were taken at a distance of ~1–1.5 m from the canopy of interest resulting in an IR image representative of a surface of 0.25 m² (0.05 × 0.05 m) of the canopy foliage. Visible digital images were taken immediately after infrared measurements to aid subsequent analysis of thermograms in the SnapView Pro software (Infrared Solutions), as previously described in literature (Grant *et al.* 2006; Leinonen *et al.* 2006). By using the IR image software, we have selected the sunlit portions of leaves on the basis of the correspondent visible images and we corrected them for spatial calibration drift by subtracting a correspondent reference image of an isothermal surface (the lens cap), as suggested in the literature (Jones *et al.* 2002; Grant *et al.* 2006). Aluminium markers were placed over the canopies of interest and within the thermal camera's field of view as means to geo-reference the visible and thermal images.

In Experiment I, canopy temperature of NI, WI, RDI vines of the varieties ARA and TRI was assessed over the summer of 2006. We measured plants in two consecutive days to shorten measurements duration and minimise errors related to the rise of temperature of the thermal imager or eventual drift of environmental conditions; thermal imaging measurements were done on the 28–29 June (DOY = 179–180), on the 1–2 August (DOY = 213–214) and on the 29–30 August (DOY = 241–242). In Experiment I, thermal images were taken from four vines per irrigation treatment (two vines per day) in the morning (0900–1100 hours, in 2006) and in early afternoon (1330–1530 hours in 2006 and 2007). In Experiment II, we measured canopy temperature only at veraison (8 August 2006 and 9 August 2007) and in early afternoon. Here, 3–4 plants per variety were selected in different rows.

Thermal indices

To reduce meteorological interference in the interpretation of T_{leaf} measurements in relation to stomatal opening, we have calculated two thermal indices: the index of stomatal conductance (I_G) and the crop water stress index (CWSI). The index $I_G = (T_{dry} - T_{leaf}) / (T_{leaf} - T_{wet})$, is theoretically proportional

to stomatal conductance of water vapour (Jones *et al.* 2002). The CWSI = $(T_{\text{dry}} - T_{\text{leaf}})/(T_{\text{dry}} - T_{\text{wet}})$ (Idso 1982) commonly varies between 0 and 1. Values close to 0 indicate a fully transpiring leaf/crop (no stress) whereas values close to 1 indicate a non-transpiring leaf/crop (maximum stress). The T_{dry} and T_{wet} values used to estimate the thermal indices in Experiment I corresponded to the leaf temperatures of NI and WI plants, respectively, as previously suggested in the literature (Grant *et al.* 2006; Jones *et al.* 2009). NI and WI plants were measured just before measuring RDI vines.

Individual leaf gas exchange and Chl *a* fluorescence

In both experiments, we measured leaf stomatal conductance to water vapour (g_s) and net assimilation (A_n) immediately after thermal imaging measurements. We measured 3–4 light exposed leaves within the area of the canopy shown by the thermal camera. A total of 3–4 plants per genotype per treatment were used. Leaf gas exchange was determined with a portable infrared gas-analyser (Li-Cor 6400, Li-Cor, Lincoln, NE, USA) equipped with a transparent 6 cm² leaf chamber, with uncontrolled block temperature and CO₂, and air flow rate set at 500 μmol s⁻¹.

In Experiment II, we also generated light response curves of A_n for the five genotypes using the Li-Cor 6400 with a chamber 6400–02B (Li-Cor Inc.) equipped with a LED red/blue light source. The curves were obtained by measuring A_n at steady-state under different PPFDs (2000 to 0 μmol m⁻² s⁻¹), at constant air CO₂ (360 μL L⁻¹), a block temperature set at 25°C and an air flow rate set at 500 μmol s⁻¹. Measurements were conducted between 0900 and 1300 hours on six plants per variety and one leaf per plant.

Dark adapted measurements of Chl *a* fluorescence included ground state fluorescence (F_o), maximal fluorescence (F_m) and maximum quantum yield of the PSII system ($F_v/F_m = (F_m - F_o)/F_m$). Chl *a* fluorescence was measured in both experiments on 4 August 2006 at pre-dawn (0300–0400 hours solar time) with a Li-Cor 6400 equipped with a fluorometer (6400–40; Li-Cor Inc.).

Leaf carbon isotope composition $\delta^{13}C$

At the end of August of 2006 and 2007, leaves were randomly harvested from six plants under study and oven-dried for 3 days at 70°C. Six leaves were harvested per variety. The dry leaf samples were ground to a fine homogeneous powder and 1 mg was used to determine carbon isotope composition ($\delta^{13}C\%$). Analysis was done using a Europa Scientific ANCA-SL Stable Isotope Analysis System (Europa Scientific Ltd, Crewe, UK). Carbon isotopic composition was expressed as:

$$\delta^{13}C\% = ((R_s - R_b)/R_b) \times 1000, \quad (1)$$

where R_s is the ratio ¹³C/¹²C of the sample and R_b is the ¹³C/¹²C of the Pee Dee Belemnite standard.

Leaf morphology and chlorophyll content

The individual leaf area and dry weight and the specific leaf area (SLA) were determined in Experiment II. Leaves were randomly harvested from the intermediate part of the shoots (the 5th–8th leaf counting from the base). Individual leaf area was determined either with a leaf area meter (model WDIGC-2;

WinDIAS, Delta-T Devices, Cambridge, UK) or by digitalisation using the software 'Image J' (<http://rsbweb.nih.gov/ij/>, accessed July 2011). Dry weight was determined after drying leaves in the oven at 70°C, for about 1 week. Chlorophyll (*a* and *b*) content was determined as described by de Souza *et al.* (2005).

Leaf stomatal density was quantified for leaves similar to the ones used in gas-exchange measurements. Here, 4–6 leaves were used per variety. From each leaf we took three small pieces and counted the number of stomata from three different fields of view. Nail-polish inprints were made by brushing the abaxial side of the leaf with nail-polish and further peeling the inprint with the help of a pair of forceps. Peels were mounted on a dry microscopical lamina and viewed under a light microscope. Images were acquired on a Leica (Wetzlar, Germany) DMRD microscope equipped with a metric ocular (10×) and a 20× or 40× objective, using a COHU high performance CCD camera driven by Leica QFISH software.

Statistical design and analyses

The design of Experiment I was a randomised complete block design with three replications and the following irrigation treatments: non-irrigated but rain fed (NI), regulated deficit irrigation (RDI) and well irrigated (WI). Each replicate (plot) had three adjacent rows (two guard rows and a central for measurements) with 20 vines each. Means were subjected to analysis of variance and were separated by least significant difference (l.s.d.) ($P < 0.05$). In Experiment II, vines of five varieties grown in (five) non-randomised plots of the same commercial vineyard were used in 2006 and 2007 to assess for genotype differences in leaf temperature and stomatal conductance to water vapour in response to RDI conditions. Differences between means were assessed by one-way ANOVA ($P < 0.05$) performed using the statistical software SPSS (SPSS Inc., Chicago, IL, USA).

Results

Thermal imaging and plant water status monitoring (Experiment I)

Climate parameters

Meteorological conditions prevailing during the experimental period are presented in Fig. 1 and Table 1. Solar radiation varied between 25.3 and 28.5 MJ m⁻² day⁻¹, indicating conditions of clear sky. VPD calculated from measurements of ambient temperature and RH, (which express the evaporative demand of the atmosphere), showed fluctuations during the period of Experiment I, with the highest value being measured by the end of August (Table 1). Clear differences between the 2 years are apparent, with 2006 being hotter than 2007.

Leaf predawn water potential (Ψ_{pd}), leaf temperature (T_{leaf}) and individual leaf gas exchange

The seasonal course of leaf water potential, leaf temperature assessed by thermal imaging (T_{leaf}) and leaf gas exchange of the varieties ARA and TRI in Experiment I is presented in Fig. 2.

The Ψ_{pd} of the two varieties remained constant under WI conditions until veraison. After arresting irrigation on 2 September (DOY 245), plants of the variety ARA presented

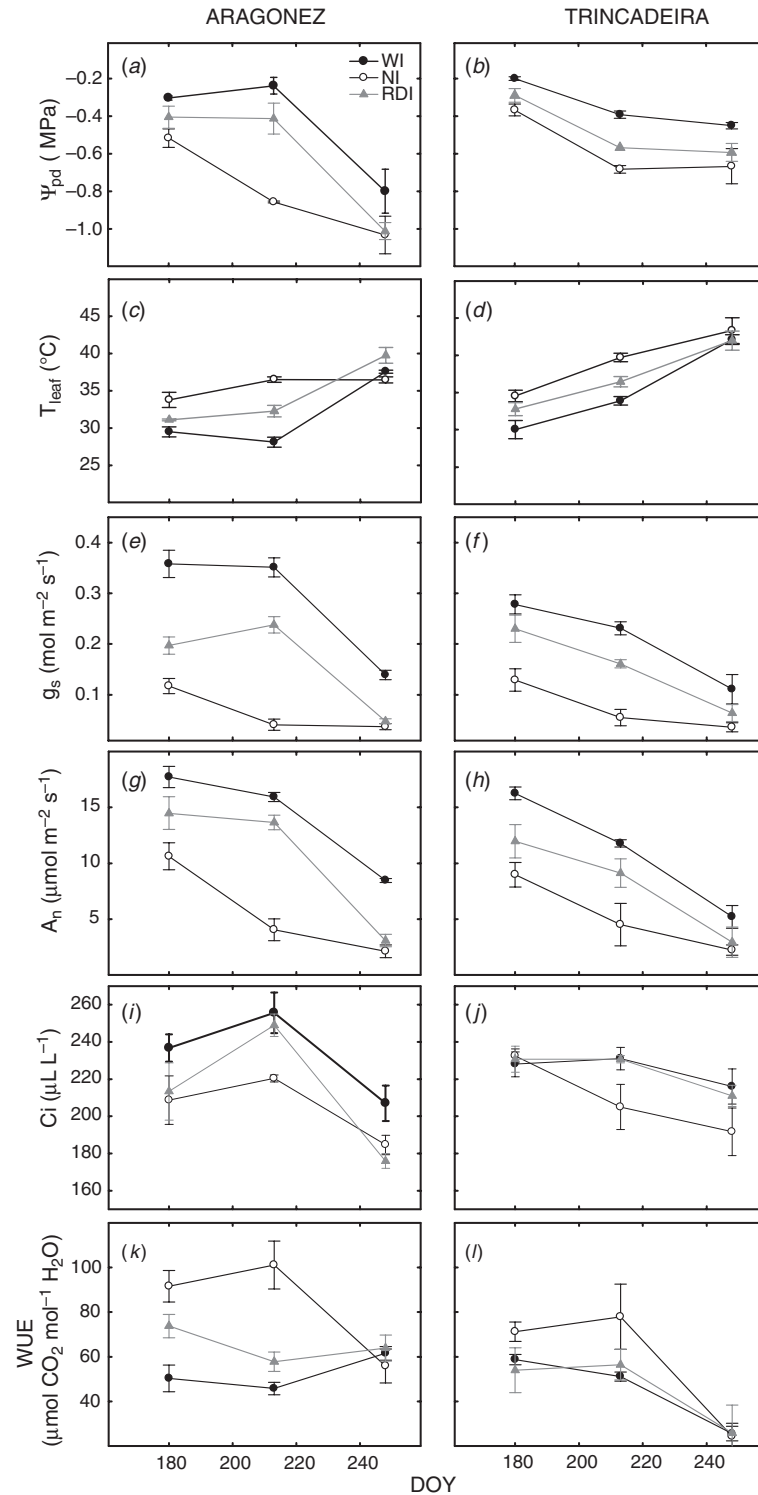


Fig. 2. Seasonal variation of *Vitis vinifera* (a, b) leaf pre-dawn leaf water potential (Ψ_{pd} , MPa), (c, d) leaf temperature (T_{leaf} , °C), (e, f) leaf stomatal conductance to water vapour (g_s , $\text{mol}^{-1} \text{H}_2\text{O m}^{-2} \text{s}^{-1}$), (g, h) net assimilation (A_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), (i, j) internal CO_2 concentration at the substomatal cavity (C_i , $\mu\text{L L}^{-1}$) and (k, l) intrinsic water use efficiency ($\text{WUE}_i = A_n/g_s$, $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$) for the varieties Aragonez (ARA) and Trincadeira (TRI) measured in Experiment I, in 2006. Leaf temperature was determined at early afternoon by using thermal imaging and leaf gas exchange was determined with a portable gas exchange meter Li-Cor 6400 (see ‘Materials and methods’ for details).

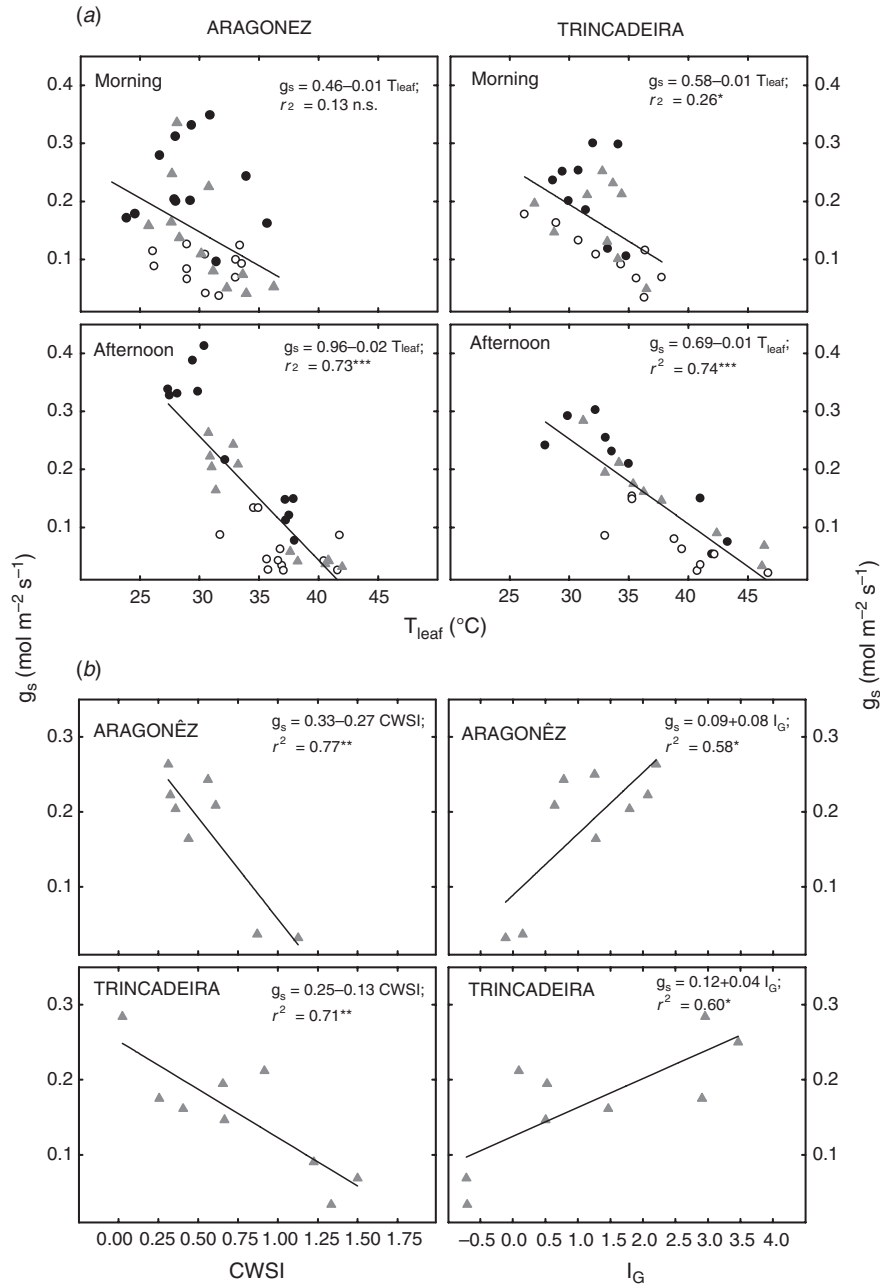


Fig. 3. (a) Relationship between *Vitis vinifera* leaf temperature assessed by thermal imaging (T_{leaf}) at morning and early afternoon, and leaf stomatal conductance to water vapour (g_s , mol H₂O m⁻² s⁻¹), for the varieties Aragonez and Trincadeira, grown under well irrigated conditions (WI) (●), non-irrigated but rain fed (NI) (○) and subjected to regulated deficit irrigation (RDI) (▲). Data was collected during the summer of 2006 (28–29 June, 1–2 August and 29–30 August). The correlation was done for all points for the varieties Aragonez ($n = 12$) and Trincadeira ($n = 9$); (b) Relationship between the two thermal indices (CWSI and I_G) and leaf stomatal conductance to water vapour (g_s , mol H₂O m⁻² s⁻¹) measured at early afternoon (1300–1500 hours) during the summer, for plants of the varieties Aragonez and Trincadeira subjected to regulated deficit irrigation (RDI, 40% ET_c). The indices values derive from thermal imaging measurements conducted along the summer of 2006: 28–29 June, 1–2 August and 29–30 August. Significance levels are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., non-significant.

lower Ψ_{pd} than TRI (-0.8 MPa and -0.4 MPa for ARA and TRI respectively) (Fig. 2a, b). NI vines presented a gradual decrease of Ψ_{pd} since the beginning of the observations on DOY 180 (Fig. 2a, b). Regulated deficit irrigation resulted in intermediate values of Ψ_{pd} and T_{leaf} compared with NI and WI vines in both varieties (Fig. 2a–d). Leaf temperature of NI plants was ~ 2 – 4°C higher than in RDI and 4 – 8°C higher than in WI vines, in both ARA and TRI. This corresponds to a more pronounced reduction of g_s observed in NI plants (3–3.5 times lower than in WI plants and ~ 2 – 3 times lower than in RDI plants, until mid season) (Fig. 2e, f). The reduced g_s resulted in lower internal carbon dioxide concentration (C_i) values and net photosynthetic rates in NI plants and intermediate values in RDI vines than WI ones (Fig. 2e–j).

By the end of the season (DOY=245), Ψ_{pd} of plants from NI and RDI treatments were lower than those from WI vines. Similarly, g_s and A_n of NI and RDI vines were similar by the end of the season, but significantly lower than WI vines (Fig. 2e–h). The decrease observed in leaf water potential of WI plants in the last date of observation may be related with a short interruption of irrigation that occurred before mid August (Fig. 2a, b). RDI plants showed lower WUE_i than NI plants until mid season, in particular in ARA (Fig. 2k, l).

In both varieties, the negative relationship between T_{leaf} and g_s was significant in the afternoon ($r^2=0.73$ and $r^2=0.74$, $P<0.001$, for ARA and TRI respectively) (Fig. 3a). In the morning, only TRI showed a statistically significant relationship between T_{leaf} and g_s ($r^2=0.26$, $P<0.05$) (Fig. 3a).

The relationship between the thermal indexes, CWSI and I_G , derived from thermal imaging measurements and leaf stomatal conductance to water vapour for the entire set of data is shown in Fig. 3b. There was an inverse relationship between the CWSI and g_s ($r^2=0.77$, $P<0.01$, for ARA and $r^2=0.71$, $P<0.01$ for TRI) and a positive relationship between g_s and the index I_G ($r^2=0.58$, $P<0.05$, for ARA and $r^2=0.60$, $P<0.05$ for TRI) (Fig. 3b).

Leaf temperature and characterisation of grapevine varieties (Experiment II)

Climate parameters

Meteorological conditions for Experiment II are shown in Table 1. Solar radiation measured in August 2006 and 2007 corresponded to conditions of clear sky and the average air temperature was $\sim 1.5^\circ\text{C}$ higher in 2006 than in 2007 (Table 1). VPD values were higher in 2006 as well. Wind speed by the time of measurements was low in both years.

Plant and soil water status, leaf temperature and individual leaf gas exchange

The lowest values of Ψ_{pd} measured at veraison were observed in 2006 (Table 3), which is attributed to the combined effect of a dryer spring and consequently less water available in the soil, or to higher average air temperatures and VPD at the time of measurements (Table 1). No significant differences among genotypes were found for the Ψ_{pd} in 2006. In 2007, there were differences between varieties but Ψ_{pd} values corresponded to the absence of water stress (Table 3).

Regarding leaf temperature, the highest values were measured in 2006 (more severe stress than in 2007) (Fig. 4; Table 3). In both years TOU vines had significantly lower leaf temperatures ($33.9 \pm 1.0^\circ\text{C}$ in 2006 and $29.5 \pm 1.2^\circ\text{C}$ in 2007) than SYR ($36.9 \pm 0.2^\circ\text{C}$ in 2006 and $34.4 \pm 1.5^\circ\text{C}$ in 2007) (Fig. 4). Leaf temperature of TRI was also significantly higher than TOU in 2006 but not in 2007. In turn, T_{leaf} of ARA and CAB did not differ from TRI, SYR and TOU (Fig. 4).

SYR generally presented the lowest g_s values, whereas TOU showed the highest (Table 3), which is in line with the thermal imaging measurements.

Based on measurements of g_s and A_n SYR did not show a significantly higher WUE_i compared with the other varieties (Table 3). However, data from the ‘long-term WUE’, given by $\delta^{13}\text{C}$ measurements, showed that in 2006 the varieties ARA, TRI

Table 3. Leaf area (LA, cm^2), specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), leaf stomatal density at abaxial side (SD abaxial, stomata mm^{-2}), chlorophyll content (total Chl, mg m^{-2}), pre-dawn leaf water potential (Ψ_{pd} , MPa), net assimilation rate (A_n , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance to water vapour (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), intrinsic water use efficiency ($\text{WUE}_i = A_n/g_s$, $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) and maximum quantum yield of the PSII system (F_v/F_m) measured under dark conditions, at dawn (0300–0400 hours solar time) measured for individual leaves of the varieties Aragonez (ARA), Trincadeira (TRI), Syrah (SYR), Cabernet (CAB) and Touriga Nacional (TOU)

Measurements were taken in August 2006 and 2007. Values are means \pm s.e. ($n=6$ – 8 leaves). Significance levels are indicated: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; n.s., non-significant. Different letters within the same column indicate significant differences for each year of observation

Year	<i>Vitis vinifera</i> variety	LA	SLA	SD abaxial	Total Chl	Ψ_{pd}	A_n	g_s	WUE _i	F_v/F_m
2006	ARA	249 \pm 15a	113 \pm 7	124 \pm 5ab	244 \pm 19a	-0.58 ± 0.05	9.9 \pm 1.3ab	0.086 \pm 0.008ab	123 \pm 22	0.819 \pm 0.005
	TRI	218 \pm 16a	113 \pm 9	116 \pm 5ab	222 \pm 12a	-0.56 ± 0.04	8.6 \pm 1.3ab	0.073 \pm 0.007b	116 \pm 14	0.827 \pm 0.004
	SYR	183 \pm 10ab	114 \pm 7	106 \pm 6b	225 \pm 17ab	-0.55 ± 0.02	5.6 \pm 1.2b	0.046 \pm 0.006c	123 \pm 26	0.817 \pm 0.004
	CAB	154 \pm 6b	118 \pm 10	131 \pm 6a	189 \pm 8b	-0.54 ± 0.02	10.3 \pm 1.1ab	0.132 \pm 0.019a	91 \pm 18	0.803 \pm 0.003
	TOU	140 \pm 12b	112 \pm 4	120 \pm 6ab	232 \pm 9ab	-0.48 ± 0.03	12.3 \pm 0.8a	0.145 \pm 0.017a	92 \pm 14	0.811 \pm 0.005
		***	n.s.	*	*	n.s.	***	***	n.s.	n.s.
2007	ARA	297 \pm 12a	126 \pm 4b	–	–	$-0.25 \pm 0.01b$	10.7 \pm 0.9	0.160 \pm 0.008ab	68 \pm 4ab	–
	TRI	249 \pm 20b	135 \pm 7ab	–	–	$-0.10 \pm 0.06a$	10.3 \pm 1.8	0.208 \pm 0.065ab	57 \pm 10ab	–
	SYR	231 \pm 10b	125 \pm 3b	–	–	$-0.19 \pm 0.02ab$	9.3 \pm 0.3	0.125 \pm 0.015b	71 \pm 4a	–
	CAB	189 \pm 8c	142 \pm 4a	–	–	$-0.21 \pm 0.07ab$	11.9 \pm 0.6	0.216 \pm 0.008ab	56 \pm 1ab	–
	TOU	171 \pm 9c	140 \pm 5a	–	–	$-0.10 \pm 0.02a$	12.4 \pm 0.5	0.258 \pm 0.043a	51 \pm 6b	–
		***	*	–	–	*	n.s.	*	*	–

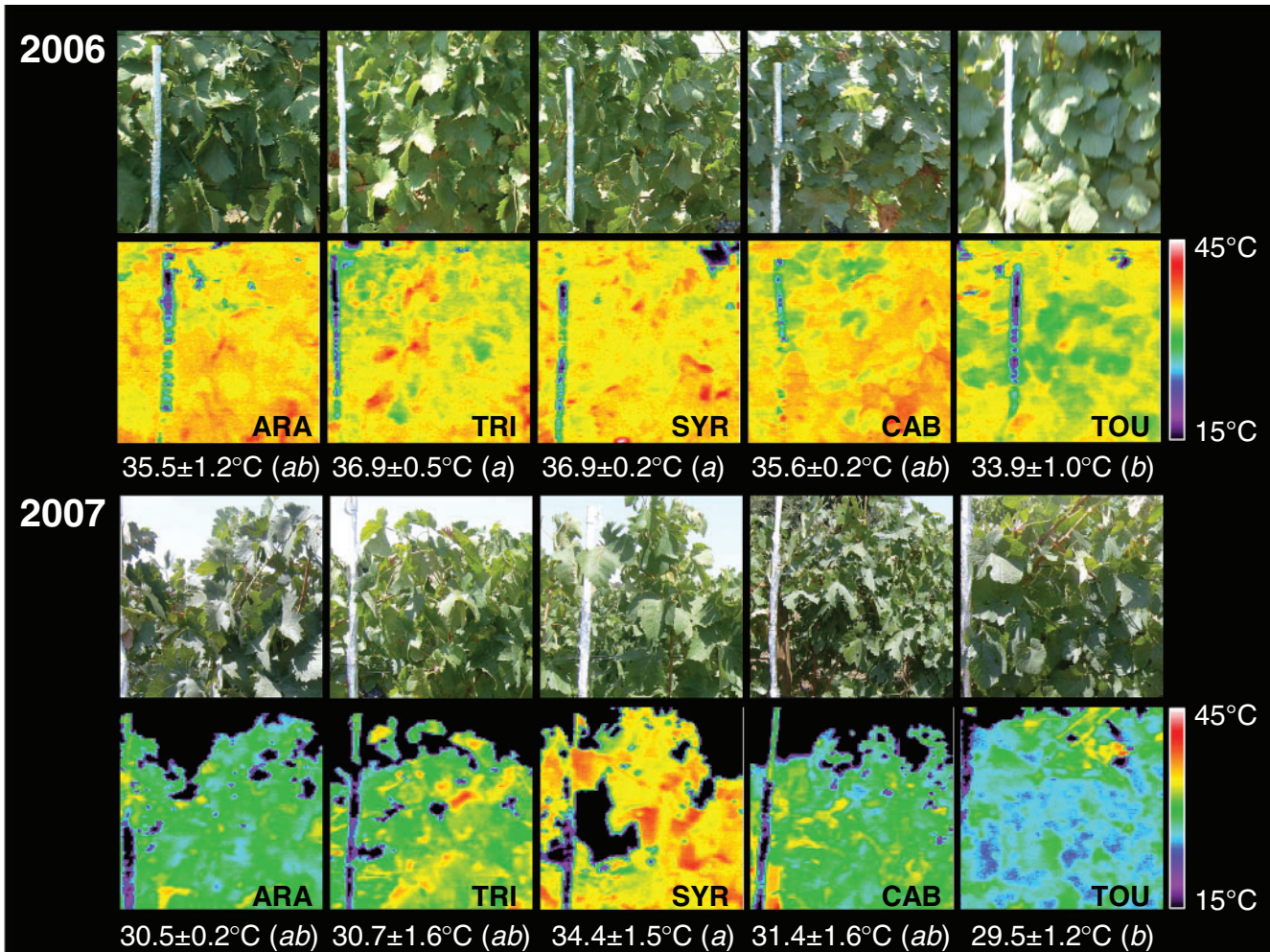


Fig. 4. Visible and false coloured infrared images of the canopies of *Vitis vinifera* Aragonez (ARA), Trincadeira (TRI), Syrah (SYR), Cabernet (CAB) and Touriga Nacional (TOU), measured at early afternoon (1300–1530 hours) at beginning of August of 2006 and 2007. Images were taken on 8 August 2006 (air T_{\max} = 38.2°C, air RH_{min} = 18.2%) and on 9 August 2007 (air T_{\max} = 36.8°C, air RH_{min} = 13%). Values are averages \pm s.e. calculated for the sunlit side of the canopy (n = 3–4 plants). Aluminium markers were used to assist selection of leaves of interest in the infrared images. Different letters indicate significant differences between varieties (P < 0.05), within the same year.

and SYR were more efficient in water use than TOU and CAB (Fig. 5). In 2007, with less stress, such differences were less apparent. Nevertheless, TOU had again a significantly lower $\delta^{13}\text{C}$ than the remaining varieties (Fig. 5). SYR, in turn, showed one of the highest values of $\delta^{13}\text{C}$, although not significantly different from ARA, TRI or CAB.

F_v/F_m values were around 0.8 in all varieties indicating the absence of photoinhibition by the time of measurements (August). However, we have found differences in leaf Chl content among the five varieties in 2006, the more stressful year of Experiment II (Table 3).

Leaf morphology

The varieties TOU and CAB presented the lowest individual leaf area but no differences were observed in SLA (Table 3). The individual leaf area measured in 2006 (severe stress) was significantly smaller than that of 2007 (mild water stress), in

particular for varieties with larger individual leaf area like ARA and TRI (Table 3), which suggests an adaptation response to water stress. In turn, SLA was higher in 2007 than in 2006 (Table 3). The number of stomata on the abaxial side of leaves was highest in CAB but with statistically significant differences only in relation to SYR. ARA, TOU and TRI did not differ from SYR or CAB (Table 3). However, the variation observed in stomatal density did not parallel the variation observed for stomatal conductance (Table 3).

Discussion

Thermal imaging appropriately monitored plant water status and therefore drought stress, in field conditions. Significant temperature differences measured with thermal imaging were detected between plants under different irrigation regimes and consequently different water status. This is in line with previous findings for grapevine (Jones *et al.* 2002; Grant *et al.* 2007; Möller

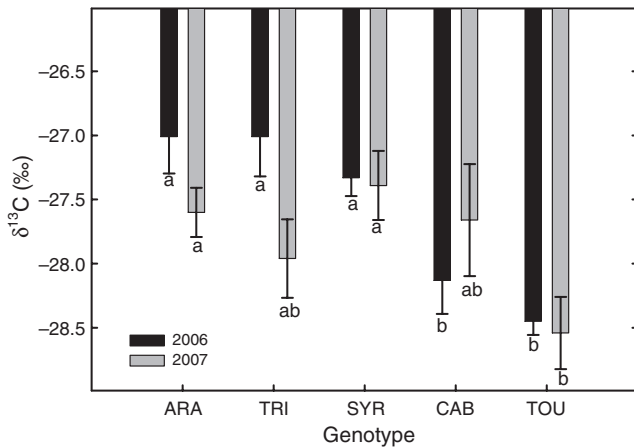


Fig. 5. Carbon isotope composition ($\delta^{13}\text{C}\text{‰}$) measured for leaves of the five *Vitis vinifera* varieties Aragonz (ARA), Trincadeira (TRI), Syrah (SYR), Cabernet (CAB) and Touriga Nacional (TOU). Measurements were done on the summer season of 2006 (high stress, black bars) and 2007 (moderate stress, grey bars). Values are means \pm s.e. ($n = 5\text{--}6$ leaves). Different letters indicate significant differences between varieties ($P < 0.05$), within the same year.

et al. 2007). Plants of the varieties ARA and TRI subjected to severe and mild water stress by application of NI and RDI strategies respectively, showed leaf temperatures $\sim 2.5\text{--}5^\circ\text{C}$ higher than well irrigated vines. This was directly related to moderate to severe reduction of stomatal conductance to water vapour in RDI and NI vines and consequently to decreased transpiration and thus decreased 'evaporative cooling'. However, the negative relationship between leaf temperature and g_s was only significant at early afternoon (Fig. 3), suggesting that thermal imaging measurements are more sensitive at that time of the day as maximum differences between stressed and unstressed plants will be seen. This is in line with previous literature stating that sensitivity of thermal imaging to estimate leaf transpiration increases with higher air temperature, leaf-to-air vapour pressure deficit and radiation (Jones 1999).

The comparison of the five varieties in Experiment II (Fig. 4; Table 3) on the basis of thermal imaging and leaf gas-exchange measurements showed that for identical, or very close leaf water potential at pre-dawn (Table 3), leaf temperature varied with the variety (Fig. 4), which was related to different regulation of transpiration rates by stomata.

This shows that in addition to the monitoring of plant water status the use of thermal imaging to characterise canopy temperature has fundamental importance to properly assess plant condition, namely the regulation of stomata in different varieties.

The relationship between g_s and CWSI in our study confirms that this index is linked to stomatal regulation via its effect on crop energy balance. In general, CWSI values should be within a range from 0 to 1 (Möller *et al.* 2007). However, our results include values slightly above 1 such as reported by other authors for grapevine (Grant *et al.* 2007; Zia *et al.* 2009). This may be due to the fact that the plants used to determine T_{wet} were not fully irrigated but solely well irrigated. Consequently, we may expect

that the T_{wet} values measured on a well irrigated plant will be higher than those of a fully irrigated vine, resulting in higher values of CWSI (larger than 1).

Measurements of leaf stomatal density showed that differences in g_s between varieties are not correlated with the number of stomata at the abaxial side of leaves. CAB showed a significant higher number at the abaxial side of leaves but no correlation with g_s seems to exist. The absence of such correlation has also been reported in other varieties of grapevine grown in field conditions (Rogiers *et al.* 2009).

The values of maximum quantum yield (F_v/F_m) were ~ 0.8 , in Experiment I (data not shown) and Experiment II and even in 2006 when stress was more severe (Table 3). This supports the idea that the photochemical apparatus of grapevine is highly resistant to water stress (Flexas *et al.* 1999; Maroco *et al.* 2002) and suggests that the differences in net assimilation rates among varieties are not attributed to photoinhibition but rather to diffusive limitations, in particular stomatal related. Further, g_s has been considered the major determining factor of leaf photosynthesis under mild and moderate water stress (Chaves 1991; Cornic 2000; Flexas *et al.* 2004). This is also in line with the seasonal courses of leaf gas exchange observed in Experiment I (Fig. 2e–h) where g_s and A_n varied in parallel, emphasising the role of stomatal regulation in grapevine photosynthesis (Chaves *et al.* 1987, 2010; Flexas *et al.* 1999; Moutinho-Pereira *et al.* 2004).

In both years of Experiment II (2006 and 2007) SYR presented the lowest g_s and A_n at different irradiances under similar leaf water potential values (data not shown).

Differences among genotypes may be related to different hormonal regulation of stomata and eventually to hydraulic differences (Schultz 2003; Lovisolo *et al.* 2010). Moreover, the size of the canopy leading to different velocities in dehydration may also play a role in explaining differences between the varieties (Rogiers *et al.* 2009).

Our results from leaf temperature and gas exchange suggest that the variety TOU, due to its higher capacity of heat dissipation via evaporative cooling (larger g_s and lower T_{leaf}) could be well adapted to warmer climate conditions when water is not dramatically reduced or under irrigated viticulture. However, if we consider WUE as selective criterion, which remains disputable, this could be a hindrance for TOU. In contrast, SYR although more efficient in terms of water use showed limitations in heat dissipation due to its reduced g_s , which can favour the occurrence of leaf sun burn under conditions of heat stress and drought.

Irrigation is an efficient way to improve plant water status and decrease leaf temperature of grapevine plants under hot and dry conditions and is being used in Mediterranean vineyards. Nevertheless, genotypes show variation in their response to soil water deficits and in their capacity transpire and loose heat via evaporative cooling that has to be taken into account.

Therefore, the full characterisation of grapevine genotypes in terms of leaf gas-exchange characteristics, stomatal regulation and leaf temperature in response to mild to severe water stress is very important for breeding against drought. This becomes especially relevant in the context of the ongoing climate changes that may increase the incidence of drought and high temperature stresses in the Mediterranean area (Chaves *et al.*

2010; Schultz and Stoll 2010). Our results demonstrate the possibility of using thermal imaging as a means to characterise transpirational behaviour of grapevine genotypes, which opens up the possibility of using the technique in the selection and management of varieties under different conditions of water availability.

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