

UV Light Acclimation Capacity of Leaf Photosynthetic and Photochemical Behaviour in Near-isohydric and Anisohydric Grapevines in Hot and Dry Environments

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The photosynthetic and photochemical adaptation of grapevine leaves to high UV radiation, under hot and dry summer conditions, was investigated in near-isohydric Cannonau (syn. Grenache) and near-anisohydric Bovale grande (syn. Carignan). From pea-size stage until harvest, vines with mild to moderate water deficit were subjected to UV-blocking treatment (–UV) and compared to a control exposed to sunlight (C). Canopy light and thermal microclimate, growth and density, maximum leaf gas exchange, primary photochemistry (PSII) and phenols were monitored. Average increments in canopy temperature under –UV tunnels during day-time and night-time were 3.3°C and 0.8°C in Bovale grande and 2.6°C and 1.1°C in Cannonau. Cultivars reached similar leaf area, intrinsic water-use efficiency and stem water potential under C and –UV. Cannonau showed lower stomatal conductance, maximum net assimilation and transpiration rates, but also faster recovery of PSII under heat and moderate water stress. UV radiation induced a stronger and longer impact on leaf assimilation, but the duration of elevated temperatures under –UV induced higher photoinhibition and lower photochemical efficiency. A similar degree of correlation between leaf temperature and gas exchange was found among cultivars and treatments. In Cannonau, leaf anthocyanin decreased due to heat-induced long-lasting PSII photoinactivation under C. Conversely, Bovale grande showed higher phenolic content stability, thus higher photoprotection and recovery of PSII functional units. Agronomical practices affecting leaf phenolic accumulation influence canopy acclimation to heat and high sunlight. Vineyard management must avoid excessive canopy sun exposure and duration of elevated temperatures to favour high assimilation, while reducing photoinactivation and heat damage.

INTRODUCTION

In Mediterranean climate regions, plants are naturally exposed to elevated solar irradiance during their life cycle and, in many grapevine-growing areas, the frequency of extreme events, such as heat waves, has increased in the last decades. In such environments, high temperatures and elevated ultraviolet (UV) and photosynthetically active radiation (PAR) are frequently combined with drought (EEA, 2009). The latest global warming report predicts an average increase in ambient temperature of at least 1.5°C for the low and 2.6°C to 4.8°C for the high greenhouse gas emission scenarios by the end of the 21st century (IPCC, 2018). Although recent projections suggest an ozone recovery by 2100 (Bais *et al.*, 2015), recent studies have indicated that the decreasing trend in cloud cover over the Mediterranean Basin observed since 1970 will continue during the 21st century, together with decreased precipitation (Sanchez-Lorenzo *et al.*, 2017) and increased surface downward solar

radiation (Wild *et al.*, 2015). UV radiation (280 to 400 nm) reaching the Earth's surface is effective in regulating several physiological processes and metabolic pathways of plant development (Morales *et al.*, 2013). The absorption of high UV levels, especially UV-B (280 to 315 nm), can damage biological systems more than the simple heat or sunburn effect of solar radiation by degrading cellular DNA, and reducing plant photosynthesis, chloroplast thylakoid integrity and biomass production (Caldwell *et al.*, 2003; Zlatev *et al.*, 2012).

The biological impacts of UV-B radiation are effective even at low radiation intensities, leading to increased reactive oxygen species production and oxidative stress. Plant sensitivity to UV radiation seems to vary considerably among species and varieties. Some crops show high sensitivity to current natural UV-B levels, while others express great tolerance to enhanced doses (Teramura &

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Sullivan, 1991). For these reasons, and due to the great diversity of physiological and biochemical characteristics among varieties (Zarrouk *et al.*, 2016), as well as the high plasticity and adaptive capacity found under different growing conditions (Lovisolo *et al.*, 2016; Palliotti & Poni, 2016), it is worthwhile to investigate grapevine responses to multiple environmental stresses influencing crop performances under natural conditions.

Several morphogenic mechanisms allow plants to cope with high temperature and sunlight levels during the different phenological stages (Cramer, 2010; Potters *et al.*, 2006) in order to reduce negative impacts on growth and production. Among the plant mechanisms to tolerate abiotic constraints, a decrease in leaf gas exchange can be observed before a reduction in vegetative growth. In effect, photosynthesis is highly sensitive to heat and light stresses, and can be reduced either by the inhibition of photosystem II (PSII) activity and/or by Rubisco inactivation during the primary carbon fixation steps of the Calvin cycle (Wahid *et al.*, 2007; Luo *et al.*, 2011; Feller, 2016). The inhibition of PSII activity leads to a reduction of chlorophyll fluorescence yield of PSII, which means that *in vivo* measurements of the rapid fluorescence kinetic curve (OJIP test) after leaf dark-adaptation (Strasser *et al.*, 2004), together with leaf gas-exchange monitoring, may allow for a better understanding of the photoinhibition processes, as well as for evaluating abiotic stress tolerance, acclimation to high radiation stress, and the recovery capacity of different species and varieties (Luo *et al.*, 2011; Xu *et al.*, 2014). Reductions in grape net leaf CO₂ assimilation by UV-B radiation occur in environments with naturally high sunlight, despite a rapid recovery of photochemical activity from UV photoinhibition induced by UV-screening phenolic substances (Kolb *et al.*, 2001).

As in other important crops, varietal differences in grape physiological behaviour to avoid sunlight and high atmospheric water demand or drought-induced damage in leaves have been revealed in recent decades (Cramer, 2010; Rogiers *et al.*, 2009; Sade *et al.*, 2012). Grapevine varieties are considered drought-tolerant plants (Chaves *et al.*, 2010) and are able to develop different strategies to respond to environmental constraints (Poni *et al.*, 2018). The stomatal control over transpiration is particularly sensitive to abiotic stresses (Düring, 1998; Soar, 2006; Fernandes de Oliveira & Nieddu, 2016b). Due to the combination of varietal differences in hydraulic properties and cell-wall elasticity with hormonal signals, leaf gas exchange performance may vary greatly among cultivars, depending upon the growing conditions of the site (Hugalde & Vila, 2018; Lovisolo *et al.*, 2016). Nevertheless, the differences in stomatal regulation under drought conditions among the varieties studied to date make it possible to classify them as near-isohydric (*pessimistic*) or near-anisohydric (*optimistic*) (Schultz, 2003; Dal Santo *et al.*, 2016; Zarrouk *et al.*, 2016). *Optimistic* performance has been reported in Carignan under Mediterranean climate conditions, in comparison with the *pessimistic* response of varieties from the Grenache family (Chaves *et al.*, 2010; Lovisolo *et al.*, 2010; Keller, 2016; Fernandes de Oliveira & Nieddu 2016b).

Overall, under high evaporative demand and conditions of water scarcity, near-isohydric varieties show tight stomatal

control in order to maintain a constant leaf water potential (Schultz, 2003; Chaves *et al.*, 2010). In near-anisohydric varieties, in contrast, the water potential drops faster until the leaf reaches the turgor-loss point and stomatal closure. Until this point, the leaves present higher stomatal conductance and thus higher CO₂ assimilation (Lovisolo, 2010), but tend to show lower photosynthetic efficiency and photorespiration rate. Furthermore, although being less water-balance conservative, some near-anisohydric varieties may recover rapidly after drought due to higher resistance to cavitation under severe stress conditions, and hence may display higher photosynthetic productivity under mild to moderate water stress (Alsina *et al.*, 2007; Lovisolo *et al.*, 2010; Sade *et al.*, 2012). Many varieties have shown photosynthetic and metabolic tolerance and high acclimation capacity to high insolation and temperature conditions (Young *et al.*, 2016; Castagna *et al.*, 2017). However, decreases in the intensity of leaf gas exchange and distinct behaviours among varieties have been reported in response to UV light, heat and drought conditions (Schultz, 2003; Alsina *et al.*, 2007; Berli *et al.*, 2010), and reductions in net assimilation and water losses during long stress periods are frequently combined with alterations in pigment balance, namely in UV-absorbing pigments (Kolb *et al.*, 2001; Martínez-Lüscher *et al.*, 2013; Del-Castillo-Alonso *et al.*, 2016). By decreasing light absorption, this adjustment mechanism allows plants to protect the photosynthetic apparatus against reactive oxygen species, and thus to tolerate high irradiation levels, avoiding photoinhibition and photooxidation (Liakopoulos *et al.*, 2007; Martínez-Lüscher *et al.*, 2013).

Recent studies have suggested that changes in the photoinhibition dynamics and photoprotection capacity may also depend upon the plant's ability to rapidly synthesise phenolic compounds (Barnes *et al.*, 2015). Besides, diurnal and seasonal variations in the accumulation of phenolics may be linked to changes in environmental irradiance levels (Šebela *et al.*, 2017). We hypothesise that varietal differences in grapevine acclimation responses to UV radiation and heat stresses, so far as leaf photosynthesis and photochemistry performance are concerned, also depend on differences in leaf pigment-accumulation dynamics according to genetic differences in phenolic profiles (Xu *et al.*, 2014; Fernandes de Oliveira *et al.*, 2015). To investigate these relationships, two grapevine varieties that are widespread in Mediterranean viticultural areas (Anderson & Aryal, 2013) were chosen for their different leaf gas exchange performance under water stress and high temperatures (Fernandes de Oliveira & Nieddu, 2016b). During vegetative growth, the effects of UV radiation on grape leaf gas exchange, primary photochemistry and the non-invasive rapid evaluation of leaf pigment balance (Goulas *et al.*, 2004) were analysed to detect alterations in assimilation capacity, photoinhibition and recovery capacity, and to investigate correlations with the accumulation of leaf pigment photoprotection mechanisms.

MATERIALS AND METHODS

Plant material and experimental site

The study was carried out in Oristano, Sardinia, Italy, on the red grape variety collection vineyard of the University of Sassari (39°54'12"N, 8°37'19"E). The climate of the study

area is classified as Mediterranean Pluviseasonal-Oceanic, with upper thermo-Mediterranean, lower dry, euoceanic weak horizons, according to the worldwide bioclimatic classification system (Canu *et al.*, 2015). The altitude of the experimental farm is about 13 m above sea level, and the coastal plain that characterises the Oristano Gulf area gives rise to limited climate variability. Annual precipitation is about 540 mm. The mean, maximum and minimum temperatures are 16.7°C, 21.3°C and 12.1°C respectively (Fick & Hijmans, 2017). Mean, maximum and minimum daily solar exposure is about 15.7 MJ/m², 26.1 MJ/m² (in July) and 6.8 MJ/m² (in January) respectively.

The study was conducted on ten-year-old Bovale grande (syn. Carignan) and Cannonau (syn. Grenache) vines grafted onto 779 P rootstock and spaced 2.5 m x 1.0 m, spur-pruned to a unilateral cordon, of 1 m height and a maximum leaf wall height of 1.2 m. The vineyard has a north-south row orientation and a single drip-line irrigation system (4 L/h). In the collection field, grapevine varieties are arranged in three randomised blocks of 20 plants per variety. In each block, the two varieties studied are planted in two contiguous rows. During the 2016 season, two treatments were compared from pea size until harvest: 1) the UV-blocking treatment (-UV), in which the entire canopy of 10 plants of each variety per block was covered with an ultraviolet radiation-blocking filter – polycarbonate panels (Suntuf Plus Clear Embossed, Omega 76/16 profile and 0.8 thickness, Palram Europe Ltd., UK) – with elevated, visible solar radiation transmission ($\geq 80\%$); 2) the control (C), in which ten consecutive vines of each variety were kept directly exposed to ambient solar radiation. The UV-screening panels were mounted in a tunnel structure of 2.5 m in height, 5 m in width and 12 m in length. The north and south sides of the tunnel were left open, while the east and west sides of the vines were completely covered, leaving only 20 cm above the soil surface uncovered to promote natural ventilation. Inside the tunnels, the air temperature increased by about 2°C compared to the outside air temperature inside vine canopy, and reached on average maximum temperatures 5°C higher. Plants were deficit irrigated from pea size until the beginning of ripening, and weekly watering supplies were defined based on stem water potential, imposing a -0.8 MPa lower threshold in order to maintain a mild to moderate water deficit conditions (Myburgh, 2011). The total re-watering amount during the season was 130 mm.

Weather and microclimate data collection

In order to describe the weather conditions during the trial, meteorological data were collected from the closest weather station (about 20 km from the study site) for which a long-term (30-year) series of data was available (Capo Frasca, 39°44'23.59"N, 8°27'34.15"E).

Canopy thermo-hygrometric conditions were monitored continuously using small dataloggers (WatchDog A-Series Loggers, Spectrum Technologies Inc., UK) inside a radiation shield. Single light sensors of global solar radiation (R_g), PAR, Red:Far red, UV-A and UV-B intensity, simultaneously coupled to a portable datalogger (Skye Instruments, Llandrindod Wells, Wales), were used to measure the

incident radiation at the vineyard site, as well as the radiation transmission inside the tunnels. Light microclimate throughout the canopy layers, in PAR wavelengths (400 to 700 nm), was monitored at the fruit zone and in an upper canopy layer (60 cm from the shoot base), as indicated in Fernandes de Oliveira and Nieddu (2016b), using a ceptometer connected to a total and diffuse PAR sunshine sensor (Sunscan SS1 and BF3; Delta T Devices, UK).

Vegetative growth and canopy density

Grape vegetative growth was evaluated during berry growth and ripening stages based on leaf area (LA) estimation models (Lopes & Pinto, 2005) previously calibrated for Cannonau and Carignano. Shoot length, and the LA of the main and lateral shoot and vine, were determined in 12 shoot replicates per variety and treatment. In order to estimate grape leaf area development, the empirical models take into account a few variables that correlate well with the LA of the main and lateral shoot: number of primary and lateral leaves per shoot, and area of the largest and the smallest primary and lateral leaves, which allow for a good estimation of the mean leaf area. Single leaf area was estimated based on the length of the two lateral main veins. Canopy density was analysed using point quadrat analysis (Smart & Robinson, 1991). Average leaf layer number (LLN) and the percentage of internal leaves (PIL) were determined taking into consideration the measurements taken during ripening at six height layers from the vegetative wall base (at 0, 20, 40, 60, 80 and 100 cm from the shoot base) and replicated horizontally along 1 m of vine row at intervals of 20 cm.

Plant physiological status

Plant water status was evaluated using midday stem water potential, Ψ_{Stem} , with a pump-up pressure chamber (PMS Instruments, USA). Weekly measurements were taken at solar midday (13:00) in intact adult leaves, covered with aluminium foil-coated plastic bags one hour prior to the measurement to allow for the equilibration of the leaf with the stem water status. Leaf gas exchange and direct chlorophyll fluorescence of PSII were monitored at midmorning (from 10.00 to 11.00 h) on days with clear skies. This was done *in vivo* on well-exposed adult leaves chosen from the base and the apical parts of the main shoot (leaf position 4 to 6 and 10 to 12 respectively), at three phenological stages (cluster closure, véraison and ripening). Net assimilation (P_n, $\mu\text{mol}/\text{m}^2\text{s CO}_2$), stomatal conductance (g_s , $\text{mmol}/\text{m}^2\text{s}$) and transpiration (T, $\text{mmol}/\text{m}^2\text{s H}_2\text{O}$) rates and leaf temperature (T_{leaf} , °C) data were collected at ambient reference CO₂ concentration (370 $\mu\text{mol}/\text{mol}$), and under the actual PAR, temperature and relative humidity conditions, using a portable infrared gas analyser (Ciras 2, PP systems, UK). Intrinsic water-use efficiency (WUE_i, $\mu\text{mol}/\text{mol}$) was calculated as the ratio of net photosynthesis to stomatal conductance. After a 30-minute dark-adaptation period, the fluorescence transients (OJIP curve) were recorded using a HandyPEA fluorimeter (Hansatech Instruments Ltd., UK) during the application of actinic saturating red light (650 nm wavelength) of 3 000 $\mu\text{mol}/\text{m}^2\text{s}$ single flash, with the signal gain at 1.0 and 30 seconds duration of each replicate. The rapid fluorescence kinetic from minimum (F_o) to maximum

(F_m) fluorescence of the dark-adapted leaves was collected. The fluorescence signals were recorded at a 10 μ s time step, which allowed for determining the OJIP transient and calculating the main fluorescence variables and a range of derived variables for evaluating PSII activity during the photochemical and thermal phases of the fluorescence transient (Strasser, 2004). The maximum yield of primary photochemistry of PSII, ϕ_{Po} , the maximum water-splitting efficiency, F_v/F_o , the quantum yield for electron transport, ϕ_{Eo} , the quantum yield for energy dissipation, ϕ_{Dlo} , and the density of the reaction centres, RC_{QA} , were determined using the equations reported in Table S1. Finally, another chlorophyll fluorescence-derived variable, $1/F_o - 1/F_m$, suggested by Flexas *et al.* (2001) to be a more linear indicator of functional PSII units, was calculated.

Leaf pigment estimation

Leaf chlorophyll, anthocyanin, flavonol and nitrogen balance indexes were estimated in replicates of the basal and apical main shoot leaves during mid-morning on days with clear skies (at the cluster closure, beginning of and full véraison phenological stages), using an optical device, Dualex Scientific+ (FORCE-A, FR) (Cerovic *et al.*, 2012). This portable device is equipped with a leaf clip sensor with a 6 mm diameter measuring surface, and allows for *in situ* and *in vivo* estimation of leaf epidermal phenolics (flavonols and anthocyanins), based on the chlorophyll fluorescence-screening method (Goulas *et al.*, 2004) and leaf chlorophyll contents determined by differential transmittance (Cerovic *et al.*, 2012). Four indexes were derived (Table S2): the chlorophyll index (CHL), the flavonol index (FLA), the anthocyanin index (ANT) and the nitrogen balance index (NBI). The latter is the ratio of leaf CHL to FLA epidermal content (Cartelat *et al.*, 2005) and may give a good estimate of the leaf nitrogen content. In each leaf replicate, five measurements were taken at the adaxial and abaxial leaf sides, avoiding the main veins.

Statistical analysis

Analysis of variance (ANOVA) and the least significant difference (LSD) test were performed to compare means and to investigate significant differences at the 95% confidence level, using the software package SPSS statistics 20 (SPSS, Chicago, USA). Two-way ANOVA was performed in order to evaluate the main influences of cultivar, treatment and leaf level, as well as to detect interaction effects among factors. After scaling and normalising the data of each variable for which significant differences were detected, multi-factor analysis (MFA), followed by a Fisher's least-significant difference (LSD) test ($p \leq 0.05$), was carried out on leaf gas exchange, stem water potential, direct chlorophyll fluorescence variables, and leaf pigment and nitrogen balance indexes. The resulting loading and score plots highlighted common variations and made it possible to summarise the relationships among the variables, and to identify the main factors influencing the physiological patterns in the two cultivars and in the two treatments. Finally, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to discriminate treatment-cultivar groups as functions of the analysed variables and to identify which

variables were the most distinctive in separating the four cultivar x treatment groups.

RESULTS

Weather conditions

The 2016 season was characterised by hot and dry conditions for most of the duration of the trial (Fig. S1). Monthly air temperatures were higher than the average values of the last 30 years in June, July and September, but remained under the average of the 30-year series in August. The environmental conditions of the growth season were aggravated by the limited precipitation during the entire spring/summer period in the study area, which also led to higher potential evapotranspiration throughout the season, especially in June and July. Rain events were practically absent in June and remained lower than the last 30-year average values in July and August.

Light and thermal microclimatic conditions

The daily pattern of Rg, PAR, UV-A and UV-B incident solar radiation at the experimental site, and the average values transmitted by the plastic filter, measured at BBCH stage 74 on a day with clear sky, are presented in Fig. 1. The Rg and PAR intensity at 09:00 averaged 1 937 W/m² and 933 μ mol/m²s respectively, and midday maximum values were about 3 750 W/m² and 1 800 μ mol/m² s respectively. Under the UV-screening filter, incident Rg and PAR were reduced to 1 540 W/m² and 742 μ mol/m² s respectively at 09:00, and similar values were recorded by the end of the afternoon. In the -UV treatment, mid-morning Rg and PAR intensity at the top of the canopy reached 2 200 W/m² and 1 050 μ mol/m² s. The UV filter blocked 99% of UV-B and 87% of UV-A solar radiation, while the Red:Far red varied close to the values of the environmental control treatment. No significant differences within varieties were observed in light transmission inside the canopy at this stage, and only slight differences were detected between -UV Bovale grande and -UV Cannonau plants during ripening (BBCH stage 85) (Fig. S2).

As far as the air temperature inside the canopy is concerned, the UV-screening filter induced an average increase of about 0.8°C and 1.1°C in Bovale grande and Cannonau respectively at night, while the daytime increases averaged 3.3°C and 2.6°C in the Bovale grande and Cannonau respectively (Fig. 2). The differences in canopy temperature were not significant among cultivars, while the higher temperatures recorded under the ultraviolet-blocking filter in the daytime were observed during the entire season of the trial (Fig. S3). Although the percentage duration of temperatures higher than 25°C showed no statistical differences among varieties and treatments, temperatures over 35°C were recorded inside the -UV canopies for 16% and 20% of the trial duration in Cannonau and Bovale grande respectively, compared to the low 2% duration in the Control (Fig. 3). The canopy temperature exceeded 40°C only in the -UV treatments, but this occurred for only 2% of the duration of the trial (ca. 25 hours overall). Nevertheless, on extremely hot days, the canopy temperature experienced the 40°C threshold for as many as two to five consecutive hours (Fig. S3).

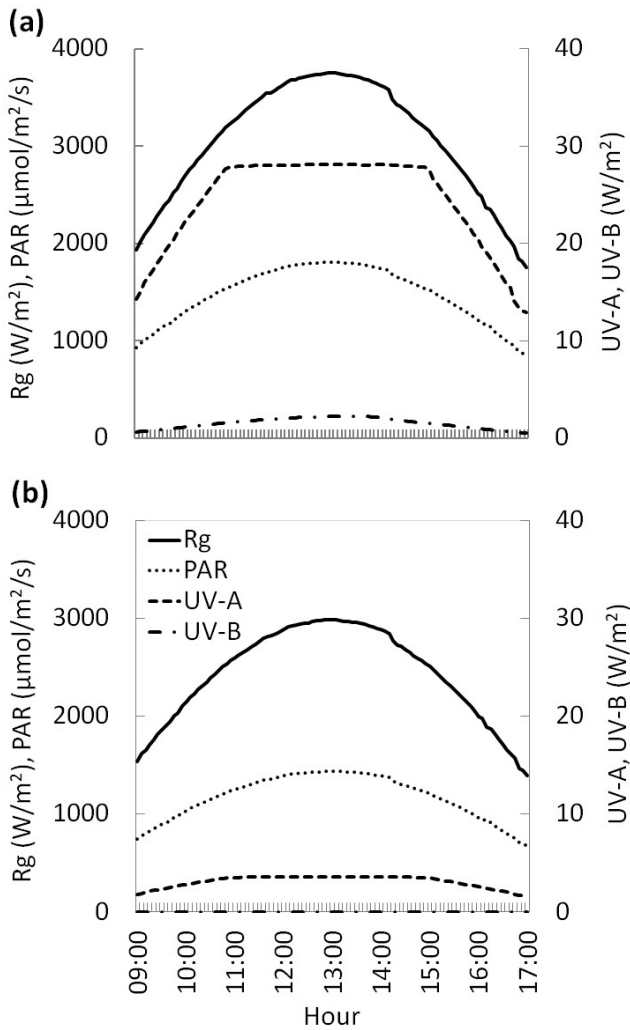


FIGURE 1

Sunlight intensity (a) at the top of the canopy outside (incident radiation) and (b) inside the UV-screening filter (film transmitted radiation). Global solar radiation (Rg, W/m²), photosynthetically active radiation (PAR, μmol/m² s), ultraviolet A (UV-A, W/m²) and ultraviolet B (UV-B, W/m²) radiation, from 9.00 until 17.00 hours at the beginning of the experiment, in a clear sky day (DOY 182).

Vegetative growth

Leaf area development was evaluated at flowering (stage 65), cluster closure (stage 75) and two weeks after véraison (stage 85) (Fig. 4a). The main single LA of Bovale grande was significantly greater than that of Cannonau, averaging about 200 cm² at the maximum development stage (BBCH 75) against the 100 cm² of Cannonau. Also, the lateral leaves were bigger in Bovale grande compared to Cannonau, and the highest average values were measured after véraison, averaging 64 cm² and 43 cm² respectively. No significant effect of the UV-screening treatment was observed in single LA, nor in the main and lateral LA per plant (Table S3). Varietal differences were detected in leaf area development, with Cannonau presenting much lower main LA compared to Bovale grande. During the three stages monitored (flowering,

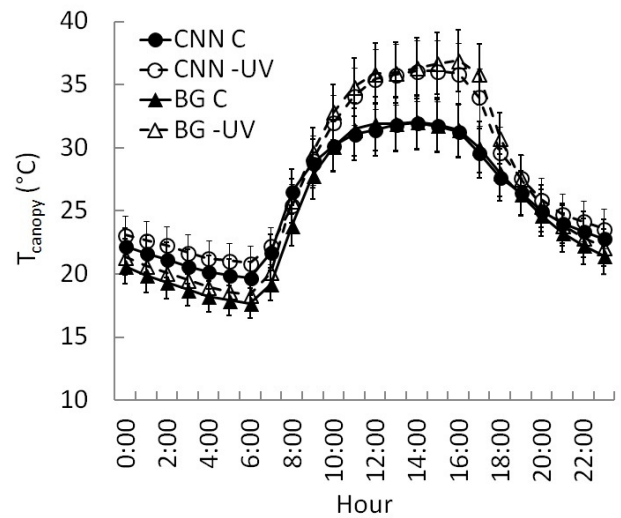


FIGURE 2

Average daily pattern of canopy temperature (T_{canopy}, °C) of Bovale grande (BG) and Cannonau (CNN) plants outside (Control, C) and inside the UV-screening filter (-UV). Mean (n=3) ± SE.

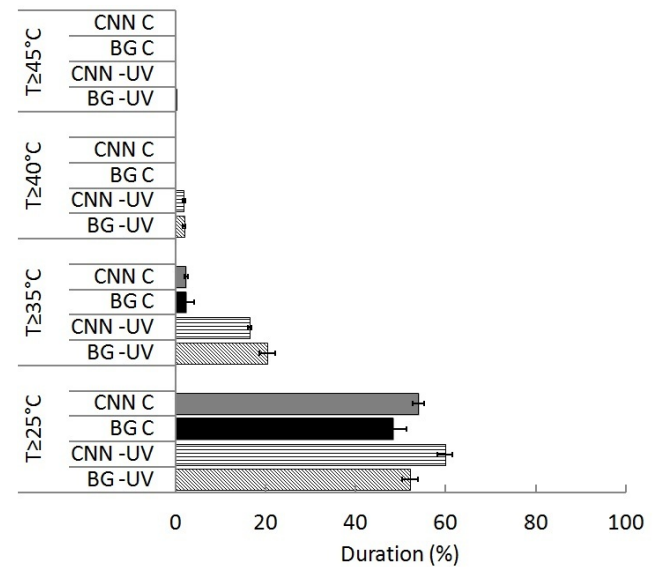


FIGURE 3

Percent duration of canopy temperature above 25, 35, 40 and 45°C thresholds, during the experimental season, in Bovale grande (BG) and Cannonau (CNN) plants outside (Control, C) and inside the UV-screening filter (-UV). Mean (n=3) ± SE.

pea-sized berries and ripening), the main leaves represented about 70%, 46% and 40% of total LA in Cannonau, and 91%, 86% and 75% in Bovale grande. The difference in main LA development was counterbalanced by a significantly higher production of lateral shoots in Cannonau plants (Fig. 4a), allowing the plants to keep a similar vegetative surface as the season proceeded. Only at the first measuring date did Bovale grande present a higher total LA compared to

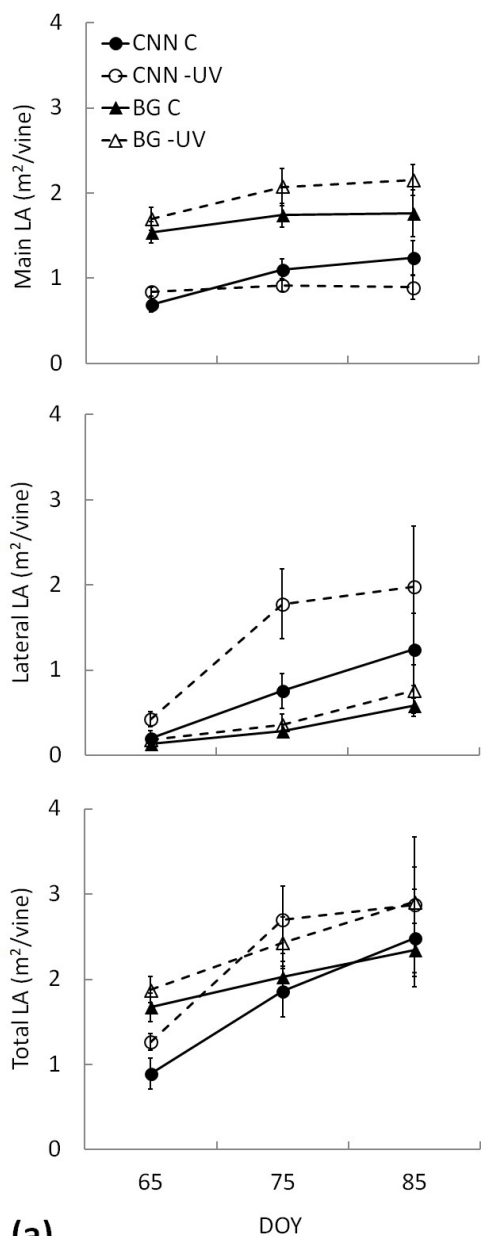
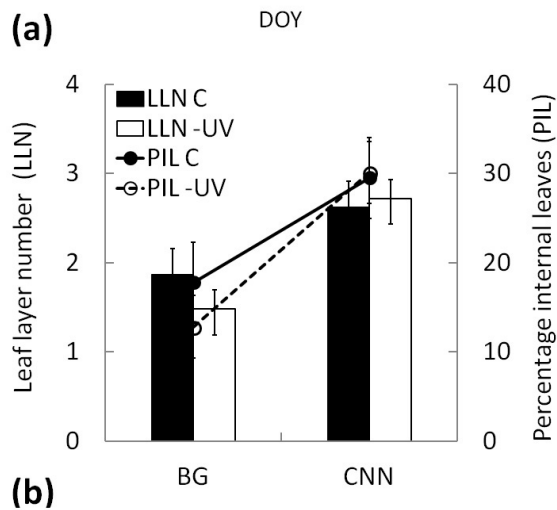


FIGURE 4

Main, lateral and total leaf area (LA, m²/vine) at bloom (BBCH 65), cluster closure (BBCH 75) and two weeks after veraison (BBCH 85) (A); and leaf layer number (LLN) and percentage of internal leaves (PIL) at four weeks after veraison (BBCH 87) in Bovale grande (BG) and Cannonau (CNN) plants outside (Control, C) and inside the UV-screening filter (-UV). Mean (n=12) ± SE.



(b)

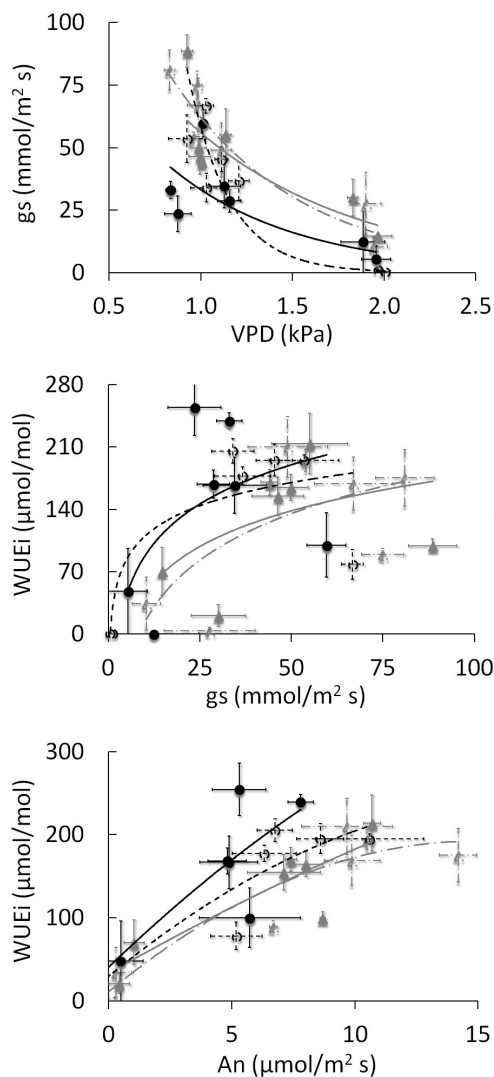


FIGURE 5

Relationship among stomatal conductance (gs), vapour pressure deficit (VPD), intrinsic water use efficiency (WUEi) and net photosynthetic rate (An) in Bovale grande (BG) and Cannonau (CNN) under UV-screening treatment (-UV) and exposed to direct solar radiation (Control, C).

Cannonau. The latter presented slightly but not statistically higher total LA during ripening (stage 85), but also a higher canopy density, with average LLN and PIL values of 2.7% and 30% respectively, against the 1.7% and 15% of Bovale grande (Fig. 4b).

Plant water status

Plant water status ranged from mild to moderate water stress over the entire season of the trial (Table 1). Two re-watering supplies (of about 150 m³/ha each) were applied from fruit set to cluster closure. The lowest Ψ_{Stem} values (ca. -1.2 MPa) were observed at cluster closure stage (BBCH 75) after two weeks of constant increments in maximum and minimum air temperature and a prolonged dry period (60 days without precipitation and only one day of effective rainfall, with 7.9 mm of total rainfall). As the atmospheric evaporative demand increased, a similar decrease in Ψ_{Stem} was observed among varieties and treatments. Thereafter, irrigation was applied weekly (210 m³/ha), allowing for the plants to recover to a mild water stress status (about -0.95 MPa and -0.85 MPa at stages 80 and 83 respectively). The irrigation volume was

reduced by the end of véraison (105 m³/ha/week), and the last irrigation supply was applied two weeks after véraison, totalling about 1 300 m³/ha for the whole season.

Leaf gas exchange and photosynthetic performance

Leaf gas exchange rates were similar in the basal and apical main leaves until véraison (Table 1). Higher rates in young apical leaves were only observed by ripening stage 85 (with average values of Pn, g_s and T of about 7.5 $\mu\text{mol/m}^2 \text{ s}$, 39 mmol/m² s and 1.7 mmol/m² s in the basal leaves respectively, against 10.8 $\mu\text{mol/m}^2 \text{ s}$, 56 mmol/m² s and 2.4 mmol/m² s in the apical leaves). Nevertheless, at this stage the basal leaves were still able to reach maximum values of Pn, g_s and T similar to those of the previous measurements (Table 1). During the first weeks of the trial (stage 75), the treatments did not induce significant differences in mid-morning leaf gas exchange intensity (reaching average Pn, g_s and T values of 5.9 $\mu\text{mol/m}^2 \text{ s}$, 71 mmol/m² s and 2.4 mmol/m² s respectively in the -UV leaves and 7.2 $\mu\text{mol/m}^2 \text{ s}$, 74 mmol/m² s and 1.8 mmol/m² s in the control). However, highly significant differences in stomatal behaviour were observed among

TABLE 1

Effects of leaf level (basal and apical), cultivar (Bovale grande, BG, and Cannonau, CNN) and treatment (UV-screening, -UV, and Control exposed to direct solar radiation, C) on stem water potential (Ψ_{Stem}), net photosynthesis (Pn), stomatal conductance (g_s), transpiration (T) rates, intrinsic water-use efficiency (WUEi), leaf temperature (T_{leaf}), leaf-to-air vapour pressure deficit (VPD), and the chlorophyll (CHL), anthocyanin (ANT), flavonol (FLA) and nitrogen balance (NBI) indexes during the experimental season. Means in columns followed by different letters are significantly different, within main effects on the basis of LSD performed by two-way ANOVA (analysis of variance) at *p* < 0.05 (n = 6).

BBCH stage	Main effect	Ψ_{Stem}	Pn	g _s	T	WUEi	T _{leaf}	VPD	CHL	ANT	FLA	NBI	
75	Leaf	Basal	-	6.6	72	2.1	92	29.5	0.99	29.8	0.20	3.43	8.78
		Apical	-	-	-	-	-	-	-	-	-	-	-
	cv	BG	-0.88 b	7.7	82 a	2.1	94	28.7 b	0.95 b	31.6	0.17 b	3.37	9.44
		CNN	-0.73 a	5.4	63 b	2.1	89	30.3 a	1.02 a	27.9	0.23 a	3.48	8.11
	Treatment	-UV	-0.77	5.9	71	2.4	74	28.5 b	1.00	31.3	0.18	3.42	9.22
		C	-0.85	7.2	74	1.8	100	30.5 a	0.97	28.2	0.22	3.44	8.33
<i>p-value</i>	P _(level)	-	-	-	-	-	-	-	-	-	-	-	
	P _(cv)	0.019	0.095	0.003	0.977	0.806	0.012	0.047	0.174	0.011	0.512	0.184	
	P _(treatment)	0.143	0.311	0.480	0.474	0.458	0.005	0.255	0.257	0.133	0.858	0.358	
	P _(level x cv)	-	-	-	-	-	-	-	-	-	-	-	
	P _(level x treatment)	-	-	-	-	-	-	-	-	-	-	-	
	P _(cv x treatment)	-	0.359	0.048	0.688	0.779	0.375	0.490	0.479	0.106	0.855	0.507	
	P _(level x cv x treatment)	-	-	-	-	-	-	-	-	-	-	-	
80	Leaf	Basal	-	-0.6	13	0.6	14	39.1	1.91	28.2	0.18	3.04 b	9.68
		Apical	-	0.2	12	0.6	30	40.4	1.95	27.3	0.19	3.27 a	8.43
	Cv	BG	-1.18 b	0.4 a	21 a	1.0 a	32	40.5 a	1.91	28.3	0.18	3.18	9.33
		CNN	-1.26 a	-0.9 b	5 b	0.2 b	12	38.9 b	1.95	27.2	0.18	3.13	8.78
	Treatment	-UV	-1.21	-0.6	10	0.4	9	39.1 b	1.95	28.5	0.16	3.01 b	9.88 a
		C	-1.23	0.1	16	0.8	35	40.4 a	1.91	27.0	0.20	3.31 a	8.23 b

TABLE 1 (CONTINUED)

BBCH stage	Main effect	Ψ_{Stem}	Pn	g_s	T	WUEi	T_{leaf}	VPD	CHL	ANT	FLA	NBI	
<i>p-value</i>	P _(level)	-	0.074	0.835	0.931	0.311	0.283	0.525	0.410	0.563	0.043	0.072	
	P _(cv)	0.024	0.005	0.006	0.005	0.224	0.001	0.397	0.321	0.746	0.621	0.425	
	P _(treatment)	-	0.701	0.272	0.126	0.132	0.003	0.399	0.192	0.033	0.011	0.019	
	P _(level x cv)	-	0.701	0.687	0.543	0.658	0.342	0.767	0.009	0.061	0.366	0.645	
	P _(level x treatment)	-	-	0.062	0.044	0.063	0.410	0.180	0.241	0.603	0.171	0.526	
	P _(cv x treatment)	-	0.853	0.642	0.811	0.931	0.861	0.699	0.209	0.400	0.463	0.796	
	P _(level x cv x treatment)	-	0.885	0.233	0.158	0.624	0.200	0.745	0.150	0.055	0.176	0.076	
83	Leaf	Basal	-	7.6	47	1.8	172	34.3	1.06	30.4	0.20	3.46	8.82
		Apical	-	7.2	43	1.6	180	34.6	1.11	31.1	0.17	3.37	9.29
	Cv	BG	-0.88	8.7 a	53 a	1.9 a	175	33.7 b	1.02	31.6	0.19	3.53 a	9.02
		CNN	-0.88	6.2 b	36 b	1.5 b	177	35.3 a	1.15	29.9	0.18	3.31 b	9.09
	Treatment	-UV	-0.85	8.6 a	50	1.8	188	35.2 a	1.10	31.9 a	0.17	3.36	9.57 a
		C	-0.92	6.2 b	40	1.5	164	33.8 b	1.07	29.6 b	0.20	3.47	8.55 b
<i>p-value</i>	P _(level)	-	0.663	0.435	0.535	0.629	0.439	0.278	0.528	0.213	0.327	0.197	
	P _(cv)	1.000	0.006	0.004	0.064	0.882	0.005	0.003	0.131	0.415	0.020	0.855	
	P _(treatment)	0.373	0.008	0.086	0.020	0.150	0.001	0.374	0.054	0.092	0.220	0.008	
	P _(level x cv)	-	0.404	0.599	0.754	0.294	0.935	0.752	0.478	0.508	0.855	0.704	
	P _(level x treatment)	-	-	0.109	0.232	0.819	0.639	0.132	0.498	0.401	0.694	0.724	
	P _(cv x treatment)	-	0.650	0.964	0.799	0.741	0.330	0.629	0.406	0.829	0.231	0.984	
	P _(level x cv x treatment)	-	-	0.752	0.666	0.474	0.639	0.169	0.308	0.304	0.821	0.444	
85	Leaf	Basal	-	7.5 b	39 b	1.7 b	206	36.0	0.68	-	-	-	-
		Apical	-	10.8 a	56 a	2.4 a	206	36.5	0.63	-	-	-	-
	Cv	BG	-1.29	10.7 a	59 a	2.5 a	189 b	36.8 a	0.68	-	-	-	-
		CNN	-1.27	7.6 b	36 b	1.6 b	224 a	35.8 b	0.63	-	-	-	-
	Treatment	-UV	-1.25	10.5 a	56 b	2.4 a	192	36.3	0.67	-	-	-	-
		C	-1.31	7.8 b	39 a	1.79 b	220	36.25	0.64	-	-	-	-
<i>p-value</i>	P _(level)	-	0.005	0.002	0.004	0.976	0.176	0.236	-	-	-	-	
	P _(cv)	0.694	0.005	0.001	0.001	0.019	0.012	0.210	-	-	-	-	
	P _(treatment)	0.369	0.001	0.002	0.011	0.061	1.000	0.414	-	-	-	-	
	P _(level x cv)	-	0.818	0.622	0.750	0.363	1.000	0.873	-	-	-	-	
	P _(level x treatment)	-	-	0.166	0.439	0.296	0.176	0.530	-	-	-	-	
	P _(cv x treatment)	-	0.895	0.784	0.572	0.161	0.001	0.348	-	-	-	-	
	P _(level x cv x treatment)	-	-	0.742	0.955	0.230	1.000	0.304	-	-	-	-	

varieties, with the Cannonau g_s averaging 20 mmol/m²/s less than that of Bovale grande. In the following measurements, significant differences in net assimilation and transpiration rates were observed, firstly among varieties (BBCH stage 80), with higher Pn, g_s and T values in Bovale grande (Table 1). A tighter stomatal closure in Cannonau leaves was observed over the whole experiment, and the pattern of the

differences among varieties concerning leaf gas exchange rates was maintained across the season. At stage 80, when plant water status decreased to about -1.2 MPa and the air temperature reached the highest values, stomatal closure was much stronger in the Cannonau leaves, leading to Pn values lower than zero and almost zero transpiration. In Bovale grande, Pn was reduced to values close to zero, yet the higher

stomatal aperture in this cultivar led to higher T compared to that of Cannonau. In addition, the apical Bovale grande leaves under the -UV treatment presented higher T, while in Cannonau those leaves had the lowest average T values.

Also, the leaf temperature was different between cultivars along the season. When lower plant water potential values were reached, Bovale grande evidenced the highest T_{leaf} while under mild water deficit (first and third measurements), a higher T_{leaf} was recorded in Cannonau. At véraison and ripening (stages 80 and 83), the maximum air temperature remained under 30°C, and Pn and T reached values similar to those recorded at the beginning of the experiment (Table 1). Also, g_s recovered, although the values remained lower than that on the first measurement date. At this stage, highly significant differences were observed among varieties and treatments, with higher leaf gas exchange rates being observed in the -UV leaves (Table 1). Despite the significantly higher temperature of the -UV canopies (Fig. S3), these plants were able to keep a lower T_{leaf} at mid-morning compared to the control plants directly exposed to solar radiation until véraison. During the last measurement, the T_{leaf} in the two treatments was similar.

No significant differences in intrinsic water-use efficiency were observed among cultivars, varieties and leaf levels (Table 1). However, Cannonau exhibited higher stomatal control over a range of VPD from 1 to 2 kPa, and a curvilinear relationship was observed between WUEi and g_s , with lower WUEi from 0 to 100 mmol/m² s of g_s in the two varieties (Fig. 5). For the environmental conditions of the season, higher values of WUEi were achieved with lower g_s in Cannonau, but at the expense of significantly lower An compared to Bovale grande, which showed higher increments in assimilation rate.

Primary photochemistry and energy dissipation of PSII

As far as PSII activity is concerned, no significant changes in most of the direct chlorophyll fluorescence variables were observed during the first days of the trial (BBCH 75), except for a decrease in the quantum yield for electron transport (ϕ_{E_0}) in -UV plants (Fig. 6; Table S3). After 20 days of UV-blocking treatment (stage 80), the differences in PSII activity among treatments became highly significant. In the -UV leaves, the heat and water stress conditions (T_{leaf} of 39°C to 40°C, -1.2 MPa of Ψ_{Stem}) induced a lower maximum quantum yield for primary photochemistry (ϕ_{P_0}), and for electron transport (ϕ_{E_0}), water-splitting efficiency (F_v/F_o) and density of the reaction centres (RC_{QA}), which are indicative of photoinhibition. Meanwhile, the control plants did not show increased energy dissipation ($\phi_{D_{lo}}$), and the quantum yield of PSII remained at high values. Nevertheless, when calculating the differences, $1/F_o - 1/F_m$, an opposite pattern was observed among varieties from the second measurement in Fig. 7, in both the basal and apical leaves: Cannonau presented a decreasing trend, in contrast with the increasing trend of Bovale grande.

The PSII in younger adult leaves on the apical part of the shoot was able to perform better than in the basal leaves only at the cluster closure stage, when water deficit and heat stress became stronger (showing higher ϕ_{P_0} , F_v/F_o , and RC_{QA} and lower average $\phi_{D_{lo}}$ values). However, in the following stages,

the differences in the effects of main leaf level became not significant. Despite the tight stomatal closure of Cannonau leaves, a cultivar effect on PSII activity was not statistically evident. In contrast, the effect of treatment remained highly significant and a recovery of ϕ_{P_0} , F_v/F_o , ϕ_{E_0} and RC_{QA} was observed in the average values of the -UV leaves at véraison (BBCH 83). The recovery of ϕ_{E_0} and RC_{QA} was slower in the -UV Bovale grande apical leaves, and was observed only at ripening (Fig. 6). At this stage, no significant differences in the primary photochemistry and energy dissipation parameters of PSII were observed among the varieties, treatments or leaf levels, but an interactive cultivar-treatment effect on ϕ_{E_0} was observed, with higher values in Bovale grande -UV and lower levels in the Cannonau Control (Table S3). A similar trend was observed in RC_{QA} , but it was not statistically significant (Fig. 6).

Leaf chlorophyll, anthocyanin, flavonol and nitrogen balance

Initially, the estimation of leaf pigments at BBCH stage 75 measured with Dualex scientific+ indicated a higher anthocyanin content in Cannonau leaves compared to Bovale grande, while significant differences were observed only among treatments in the following measurements (Table 1; Fig. 7). The control plant presented significantly higher values for the anthocyanin index in both varieties. At véraison (stage 83), a higher anthocyanin index was still observed in the Bovale grande control leaves, but the differences among treatments in Cannonau were reduced (Fig. 7). Regarding the flavonol index, the main differences were detected at stage 80, with lower values being observed in -UV and in the basal leaves of the two cultivars. By véraison, the flavonol index had already increased in -UV to the values measured in the control leaves. As far as the chlorophyll index is concerned, the two varieties presented slightly different trends before véraison, with higher values in the leaves at the basal part of the shoot compared to those at the apex in Cannonau; the opposite behaviour was observed in Bovale grande. At véraison, the -UV leaves presented a higher, yet not statistically significant, chlorophyll index. The treatment effect led to a lower nitrogen balance index in the control plants. This was observed from the first estimation and became increasingly significant as the experiment continued.

Variation of primary leaf photochemistry and gas exchange activity

To determine possible correlations among leaf gas exchange performance, PSII activity, plant water status and the observed variations in the level of key leaf pigments, multi-factor analysis (MFA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were carried out (Fig. 8; Table S5). The focus was on the variables for which significant main effects of treatment and cultivar were detected across the season (Tables 1 and S3). Overall, 64% of the variation in leaf gas exchange, primary photochemistry and pigment level variables were explained by two main factors. The variables derived from chlorophyll fluorescence were highly correlated with the first factor, which can be identified by light conditions and which primarily governs the light-dependent photosynthesis step. In contrast, leaf gas exchange

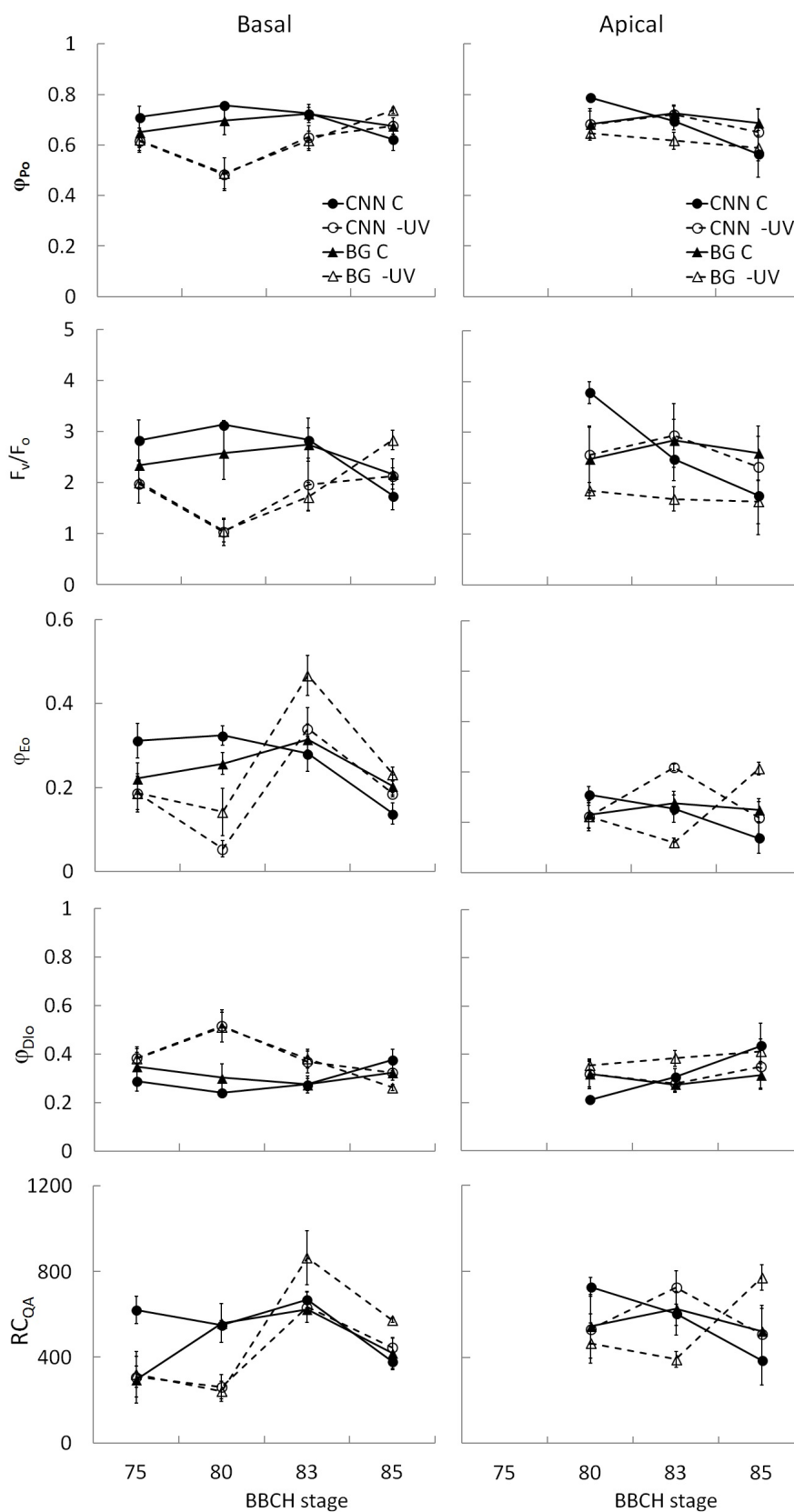


FIGURE 6

Primary photochemical performances of the PSII in Bovale grande (BG) and Cannonau (CNN) basal and apical main adult leaves inside the UV-screening filter (-UV) and outside (Control, C) during midmorning in clear sky days. Maximum yield of primary photochemistry of PSII (ϕ_{Po}) water splitting efficiency (F_v/F_o), quantum yield for electron transport (ϕ_{Eo}) quantum yield for energy dissipation (ϕ_{Dio}) and density of reaction centers (RC_{QA}). Mean ($n=6$) \pm SE.

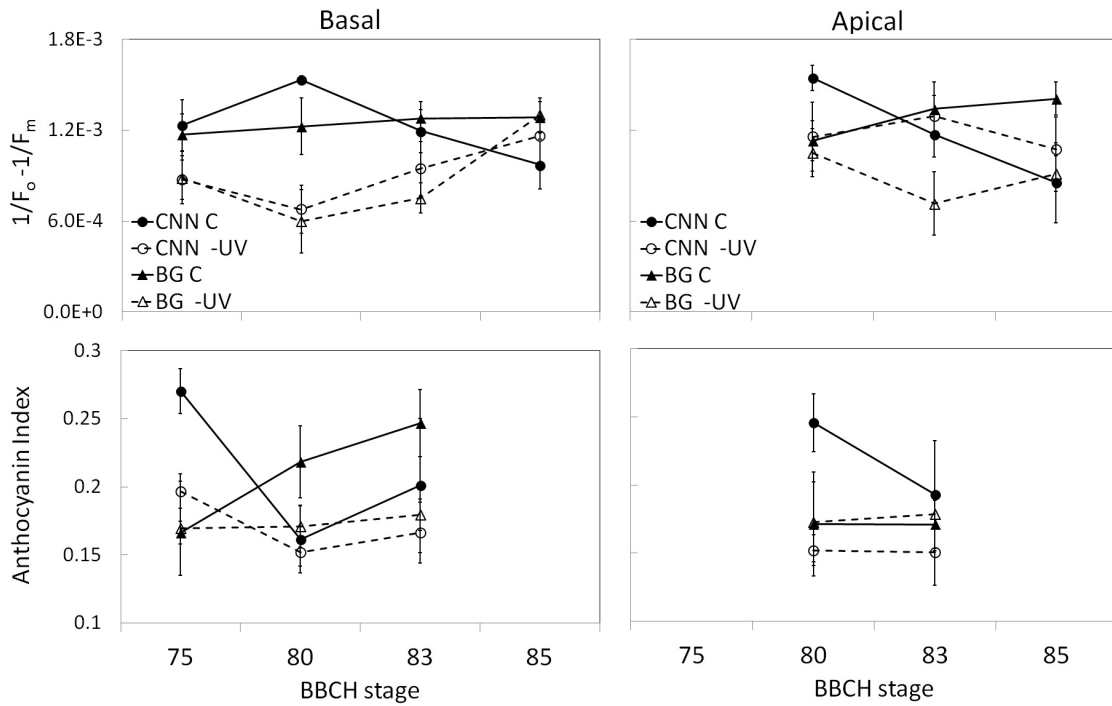


FIGURE 7

Functional PSII units parameter ($1/F_o - 1/F_m$) (a) and Anthocyanin Index (b) in Bovale grande (BG) and Cannonau (CNN) basal and apical main adult leaves outside (Control, C) and inside the UV-screening filter (-UV) during midmorning in clear sky days. Mean (n=6) ± SE.

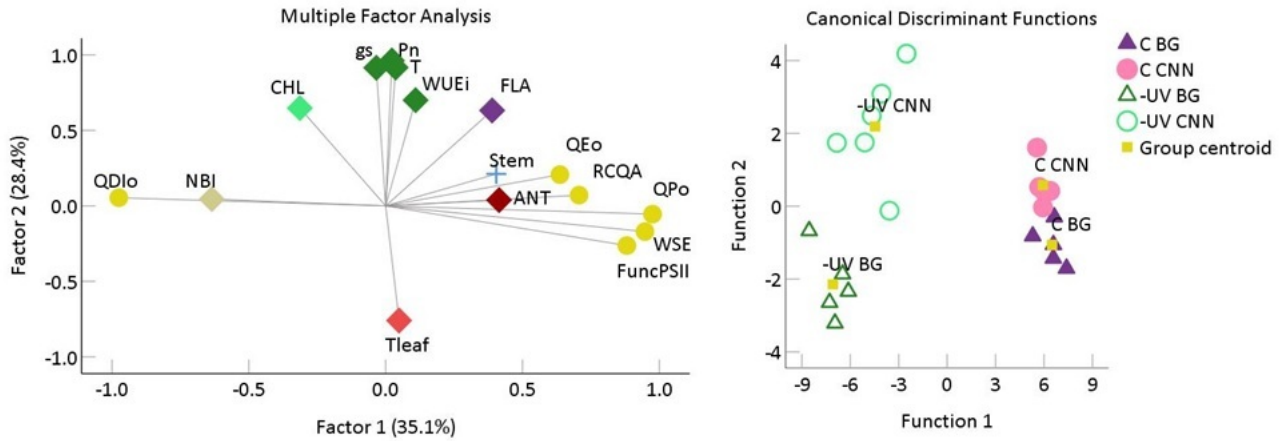


FIGURE 8

Multi-factor analysis (a) and orthogonal partial least square discriminant analysis (b) score plots of leaf gas exchange, stem water potential, primary photochemical performances of the PSII and leaf pigment indexes during the experimental season (from BBCH stage 75 to 85) taking into account the whole leaf level, cultivar and treatments. The analyses were performed after scaling and normalizing the input variables: stem water potential (Stem), leaf temperature (Tleaf), net photosynthesis (Pn), stomatal conductance (gs), transpiration (T) rates, intrinsic water use efficiency (WUEi), maximum yield of primary photochemistry of PSII (QPo) water splitting efficiency (WSE), quantum yield for electron transport (QEo) quantum yield for energy dissipation (QDIo), PSII functional units (funcPSII), density of reaction centers (RCQA), anthocyanin (ANT), flavonol (FLA), chlorophyll (CHL) and nitrogen balance (NBI) indexes. Primary photochemical variables, that represent the performances of light-dependent photosynthetic step, are presented in yellow coloured circles. Green rhombus symbolize leaf gas exchange variables. Leaf temperature is represented by red rhombus and violet, red, green and grey squares correspond respectively to flavonol, anthocyanin, chlorophyll and nitrogen balance indexes.

and leaf temperature varied according to the second factor, which is associated with temperature, specifically with the well known temperature-dependent regulation of maximum leaf gas exchange performance. Furthermore, leaf pigment levels were influenced, to a lesser extent, by the two factors extracted. The OPLS-DA separated the four treatment x cultivar groups according to two significant functions. The independent variable that firstly fitted to parsimoniously classify the four groups was Ψ_{Stem} . The second function subsequently discriminated the groups according to the following variables: PSII functional units, CHL, NBI, gs, T and T_{leaf} . A third discriminant function included Pn, ANT and FLA and other PSII photochemistry variables, adding even further distinction among groups when compared to the first two functions (Fig. 8, Table S4). When MFA was applied exclusively to control the leaves, both the photochemical variables and leaf gas exchange parameters were correlated the most with factor 1 (Fig. 9). The ANT, NBI and CHL indexes and Ψ_{Stem} correlated the most with factor 2, and ϕ_{Eo} and T also correlated with factor 2 to a significant degree. Under the -UV treatment, gas exchange in the leaf and plant water status showed a strong correlation with factor 2, along with T_{leaf} (in the opposite direction), while the primary photochemistry of the leaf, NBI and ANT were mainly related to factor 1. In this case, FLA correlated similarly

with the two main factors. When MFA was performed considering the two varieties separately, the variance in primary photochemistry of the leaf again was explained by factor 1, and the performance of gas exchange in the leaf and the temperature were related to factor 2. In Bovale grande, the influence of factor 1 was higher and related more closely to ANT, while FLA correlated with factor 2. In Cannonau, the influence of factor 2 on the gas exchange performance and T_{leaf} was slightly higher; a lower correlation of factor 1 was found with ANT and Ψ_{Stem} . Overall, as expected, the quantum yield for energy dissipation varied in the opposite direction of the maximum quantum yield for primary photochemistry, the density of the reaction centres in PSII, electron transport and water-splitting efficiency, while the maximum gas exchange in the leaf correlated negatively with leaf temperature (Fig. 9; Table S5).

DISCUSSION

Treatment-dependent responses

During the study season, air and canopy temperature reached very high values ($> 35^{\circ}\text{C}$) from midmorning until afternoon on several days. Under such conditions, photosynthetic assimilation is reduced or even shut down and, consequently, vegetative growth is limited (Kadir *et al.*, 2007). Some studies have also reported decreases in canopy growth

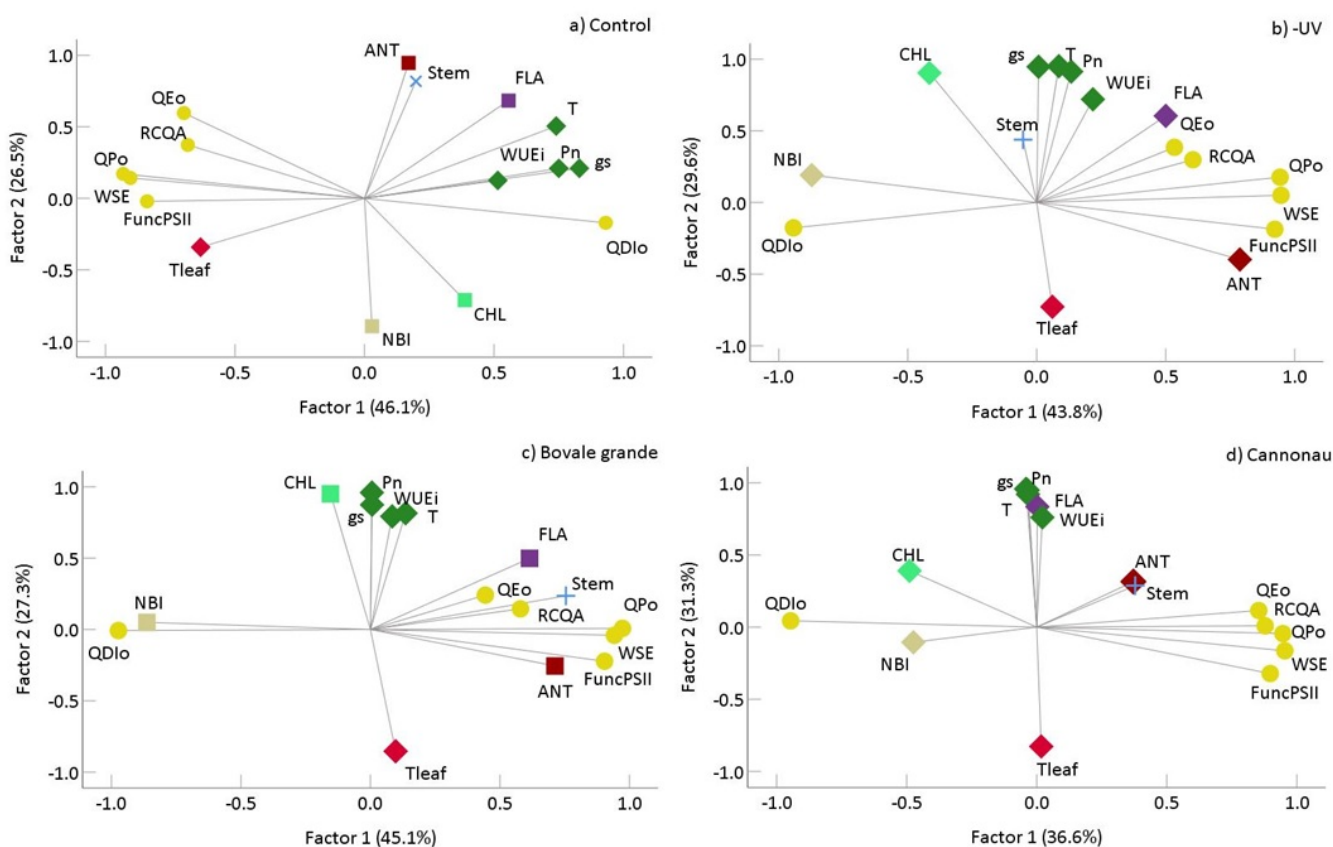


FIGURE 9

Multi-factor analysis score plots of scaled and normalized data of leaf gas exchange, stem water potential, primary photochemical performances of PSII and leaf pigment indexes during the experimental season (from BBCH stage 75 to 85) taking separately into account treatment (a and b) and cultivar (c and d) effects.

caused by high UV-B radiation levels (Berli *et al.*, 2010; Doupis *et al.*, 2011). In our study, light treatment did not induce significant differences in the development of total leaf area, and mild to moderate water stress imposed by deficit irrigation allowed the leaf area to keep growing, also in the –UV plants, although subjected to higher heat stress.

The main differences between the two treatments were found in maximum leaf gas exchange and PSII performance. Despite the higher temperature inside the tunnels, the UV-blocking treatment resulted in a higher maximum photosynthetic rate but lower primary photochemical efficiency. These plants invested in efficient heat dissipation, avoiding further increases in temperature. Though photoinhibition, the limitations of PSII were evident under –UV, and the plants were able to restore high Pn, T and gs rates (Lovisolo *et al.*, 2010). Although the –UV plants were subjected to significantly higher heat stress compared to the control, sufficient PAR light ($\geq 1\ 000\ \mu\text{mol}/\text{m}^2\ \text{s}$) conditions and the moderate water status imposed on both varieties enabled a complete recovery of the PSII apparatus, and even higher PSII activity and maximum net assimilation by the end of the season. In contrast, gas exchange rates in the leaves of plants exposed directly to solar UV radiation became more limited as the season advanced, also showing a higher correlation with the light conditions (factor 1 in Fig. 9a) and with the primary photochemical variables compared to the –UV plants. The high ϕ_{po} in the control leaves during the first stages of the trial is in agreement with a lower photoinactivation of PSII grapevines acclimated to environments with high levels of light. Nevertheless, the decreases in F_v/F_o , ϕ_{Eo} and RC_{QA} from véraison to harvest suggest photoinhibition and a reduced recovery capacity of PSII in UV-exposed plants. In fact, previous works by Flexas *et al.* (2001) indicates that, in grapevines grown in Mediterranean areas that are acclimated to high levels of light, the values of ϕ_{po} may give the impression of a high quantum yield when in fact a large portion of the functional PSII units may be inactivated.

Moreover, the fact that the –UV plants were able to recover PSII activity, and reach even higher midmorning leaf gas exchange rates during ripening compared to the control plants, is consistent with a higher acclimation capacity to heat than to UV light stress. High recovery capacity under heat stress was also demonstrated by the sharp increase in the maximum quantum yield for electron transport (ϕ_{Eo}) and the density of the reaction centres (RC_{QA}) observed in the –UV leaves at véraison, when temperature decreased and mild plant water status was restored by an increase in re-watering supply.

Martínez-Lüscher *et al.* (2013) reported a high acclimation to UV-B radiation in Tempranillo grapevines under optimal thermal conditions (25°C/15°C day/night temperatures). These authors did not observe a significant reduction in maximum PSII efficiency, despite the reduction in gas exchange rates in the leaves. In fact, small reductions in photosynthesis may be counterbalanced by the accumulation of phenolic compounds in the leaves (Del-Castillo-Alonso *et al.*, 2016). The multi-factor analysis applied to the whole data separated variations in leaf gas exchange from direct primary photochemistry; however, under the high direct

solar radiation conditions of the control plants, gas exchange performance in the leaves was affected the most by sunlight and was linked to the performance of the light-dependent photosynthesis step. Generally, leaf temperature of the –UV plants was highly correlated with chlorophyll fluorescence parameters, namely PSII activity, photoinhibition and recovery, as well as with the chlorophyll index. In the control plants, the weak relationship among the temperature, net assimilation and primary photochemistry variables and chlorophyll can be ascribed to the interposition of the UV-screening photoprotection effect of leaf phenolics.

Higher values of the two phenolic compounds in the UV-exposed leaves were statistically significant at the beginning of véraison, while chlorophyll and nitrogen balance indexes became higher under the UV-blocking filter. Grifoni *et al.* (2016), using a similar optical instrument in grapevine leaves, also observed decreases in the accumulation of flavonols in the absence of UV radiation, along with increased accumulation when the leaves were re-exposed to direct solar radiation. In our experiment, we found a reduction in the UV-screening photoprotection capacity due to the decrease in anthocyanin content in the leaf epidermis, which is consistent with the decrease in PSII activity in the control plants during the season and helps to explain the loss of PSII functional units under conditions of high insolation. This was particularly evident in the cultivar Cannonau, in which the total anthocyanin contents are known to be highly sensitive to elevated temperature (Fernandes de Oliveira *et al.*, 2017).

Variety-dependent responses

Although Bovale grande and Cannonau showed different balances in primary and lateral leaf area growth, the two varieties reached similar total leaf area under the control and –UV treatments. The differences in canopy density did not induce significant differences between varieties in light transmission into the canopy, probably because the higher lateral leaf area of Cannonau was composed of small leaves that allowed sun-flecks to penetrate into the inner canopy layers.

Bovale grande and Cannonau plants exhibited differences in maximum leaf gas exchange rates, but similar primary photochemistry efficiency, as atmospheric demand and water deficit increased. As expected, the Cannonau plants exhibited typical isohydric behaviour (Fernandes de Oliveira *et al.* 2013), characterised by tighter stomatal control over transpiration (gs remaining about 20 mmol/m² s lower, ranging from 63 mmol/m² s in the first measurement to 5 mmol/m² s when the lowest Ψ_{Stem} was recorded) compared to that of Bovale grande (with an average gs of 82 mmol/m² s on the first date and 21 mmol/m² s when the vines reached a lower Ψ_{Stem}). However, the leaves of both varieties balanced the daily maximum gas exchange in the leaves according to the environmental conditions, being able to display similar intrinsic water-use efficiency and stem water content. They also adjusted their maximum photosynthetic performance to the environmental stresses (high UV light, heat and moderate water deficit), following the same pattern and recovering similarly during the season. In Cannonau, this was achieved at the expense of a lower maximum assimilation rate (about

2 to 3 $\mu\text{mol}/\text{m}^2 \text{ s}$ less under mild water and heat stress to 1.3 $\mu\text{mol}/\text{m}^2/\text{s}$ less with moderate water and heat stress compared to Bovale grande), indicating that, under mild to moderate water deficit and elevated temperatures, the near-anisohydric behaviour of Bovale grande can result in a more efficient strategy from a crop-productivity perspective, as also suggested by other authors (Sade *et al.*, 2012).

The long duration of heat stress, combined with moderate water deficit, led to a substantial decrease of the maximum leaf photosynthetic and transpiration rates in both varieties. Whenever the temperature reached 40°C, the assimilation rate was promptly reduced, and further transpiration losses and damage to the photosynthetic apparatus were avoided by stricter stomatal control, a coordinated reduction in PSII activity and an increase in energy dissipation. Luo *et al.* (2011), who studied heat stress effects on the primary photochemistry of young potted Zuoyouhong grapevines (*Vitis amurensis* L.) grown under controlled day/night temperature cycles and about 1 000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ maximum PAR, observed a significant inhibition in net assimilation rate and stomatal conductance only when the temperature reached 40°C and 45°C.

Regardless of the light treatment, the reduction of stomatal aperture under tougher temperature and water stress conditions in our experiment (T_{leaf} of 40°C and moderate water deficit) differed in the two varieties. The lower g_s of Cannonau led to lower evaporative cooling (from 0.4 to 0.9 $\text{mmol}/\text{m}^2/\text{s}$ lower T). However, this was not followed by a significantly different level of primary photochemistry quantum yield and energy dissipation efficiency among the varieties. Nevertheless, a higher water-splitting efficiency of PSII and a faster recovery of the PSII reaction centre was observed in Cannonau when thermal and water stress increased, which led to stomatal closure. Kadir *et al.* (2007), on the other hand, when studying the effect of sudden versus gradual heat stress on the primary photochemistry of photosynthesis in the leaf in two varieties, namely Vignoles and Cynthiana, observed that a different grade of damage to PSII, expressed by the percentage decrease in ϕ_{po} , was caused by heat stress, regardless of the duration of the heat shock. These authors also reported greater recovery of PSII in one of the two varieties. Such varietal difference was not observed in our study, only a slower increase in the ϕ_{Eo} and RC_{QA} values in the Bovale grande –UV apical leaves by véraison, and this had already recovered at the beginning of ripening.

When there was a higher intensity of solar radiation and evaporative demand, the two varieties were exposed to high natural and gradual heat, light and water stresses. The accumulated exposure of the canopy to temperatures over 35°C was about 10 days in –UV, and did not exceed one day in the control plants. Furthermore, the T_{canopy} reached 40°C only in the –UV-treated plants and for a maximum duration of one day. Taking into consideration previous work on photosynthetic regulation in grapevines subjected to increasing water stress and high evaporative demand (Flexas & Medrano, 2002; Bertamini & Nedunchezian, 2003; Luo *et al.*, 2011), we can affirm that both Cannonau and Bovale grande were able to respond efficiently to heat stress and water deficit.

The results also show that the high leaf gas exchange performance was closely related to leaf temperature, while the photochemical performance was related less to the temperature condition of the leaves in both the near-isohydric and the near-anisohydric varieties. Also, the relationship between plant water status and the light-dependent photosynthetic step was much weaker in the Cannonau leaves than in Bovale grande (Fig. 9c and 9d). These findings are in accordance with the tighter stomatal control and the higher efficiency of hydraulic regulation mechanisms governing leaf conductance and water losses in Cannonau, namely leaf architecture and anatomy, hydraulic conductance and cell wall elasticity (Chouzouri & Schultz, 2005; Hugalde & Vila, 2018). Nevertheless, the two varieties were able to maintain similar intrinsic water-use efficiency and plant water status under both UV and heat stress conditions. The estimation of leaf metabolites showed evidence of seasonal differences among the varieties in the accumulation of UV-screening pigments during growth, firstly in anthocyanins and then in flavonols. Nevertheless, these differences did not induce changes in the nitrogen balance index. This result also suggests an important plasticity of the two cultivars in adapting to conditions of thermal and light stress without suffering a significant alteration in leaf nitrogen metabolism or without it affecting the integrity of the photosynthetic apparatus. The variation in both indexes of phenolics closely and positively followed the photochemical performance in Bovale grande, while in Cannonau the anthocyanin content in the leaves was somewhat related to primary photochemistry efficiency. Moreover, the contrasting trends between the varieties, both in PSII functional units and the anthocyanin index of the basal and apical Cannonau control leaves (Fig. 7), together with the increasing photoinhibition trend over the season, indicate that UV light exposure induced long-lasting photoinactivation of PSII in this variety. This result suggests that phenolic accumulation in the leaf epidermis may have functioned efficiently as a photoprotection mechanism in both varieties before véraison, but the higher sensitivity to anthocyanin degradation under elevated temperatures, previously observed in Cannonau berry skin (Fernandes de Oliveira & Nieddu, 2016a), led to a loss in leaf photoprotection and photochemical reaction capacity under high solar UV light conditions. This finding was also supported by the multifactor analysis of variety effects (Fig. 9c and 9d), which showed that, in Bovale grande, the leaf primary photochemical performance varied positively with the anthocyanin index, while the variation in gas exchange was weakly related to this index. Besides, a weaker correlation between the anthocyanin index and leaf primary photochemistry and leaf gas exchange-related variables was observed in Cannonau. The flavonol index was affected less by temperature, showed lower variation between treatments and correlated with both photosynthetic and photochemical performance in Bovale grande, but only with the variation in leaf gas exchange in Cannonau. This result is consistent with the higher stability of flavonols under high sunlight and heat compared to anthocyanin. Such differences between the two phenols can also be associated with the predominant role of anthocyanins in red grape metabolism, since in white grape varieties flavol contents

have been reported to increase under moderate water deficit and high sunlight conditions compared to well watered vines (Deluc *et al.*, 2009; Fernandes de Oliveira *et al.*, 2019).

CONCLUSIONS

The near-isohydric and near-anisohydric grapevines differed significantly in their acclimation responses to UV radiation under high evaporative demand, and mild to moderate water stress. Both varieties showed good leaf physiological performance and photochemical efficiency, together with a high recovery capacity of the photosynthetic apparatus upon intense solar radiation. Thus, no significant reductions in vegetative growth were observed, although Cannonau invested in lateral shoots, while Bovale grande was able to enhance its main shoot leaf area during the fruit-development stages. Natural UV radiation induced a stronger and longer negative impact on leaf physiological performance in both varieties. In fact, other than being distinguished by their water loss regulation mechanisms, the two varieties on trial were also separated according to the effects that UV exposure and heat stress induced in the regulation of

photosynthetic and photochemical performance, as well as in leaf phenolics and the chlorophyll balance (Fig. 10). Besides for lower transpiration rates, lower net assimilation and recovery capacity after high heat stress were also detected in the near-isohydric Cannonau exposed to high natural UV levels. Among the photoprotective mechanisms underlying the high acclimation capacity, the accumulation of phenolic compounds plays a crucial role in grapevines. Varietal differences in the seasonal dynamics of pigment accumulation were evident in the main leaves of both the basal and apical shoots. In addition, Cannonau showed higher sensitivity to the thermal degradation of anthocyanins, which are major UV-screening phenolic compounds in red grapes. Despite these differences, both varieties were able to efficiently balance leaf water use and plant water status.

It is for these reasons that we can conclude that, when mild to moderate water stress thresholds are maintained, both Bovale grande and Cannonau show high acclimation to heat and UV light stresses. Furthermore, the impact of high UV radiation and elevated temperatures on photosynthetic capacity and photochemical performance of near-isohydric

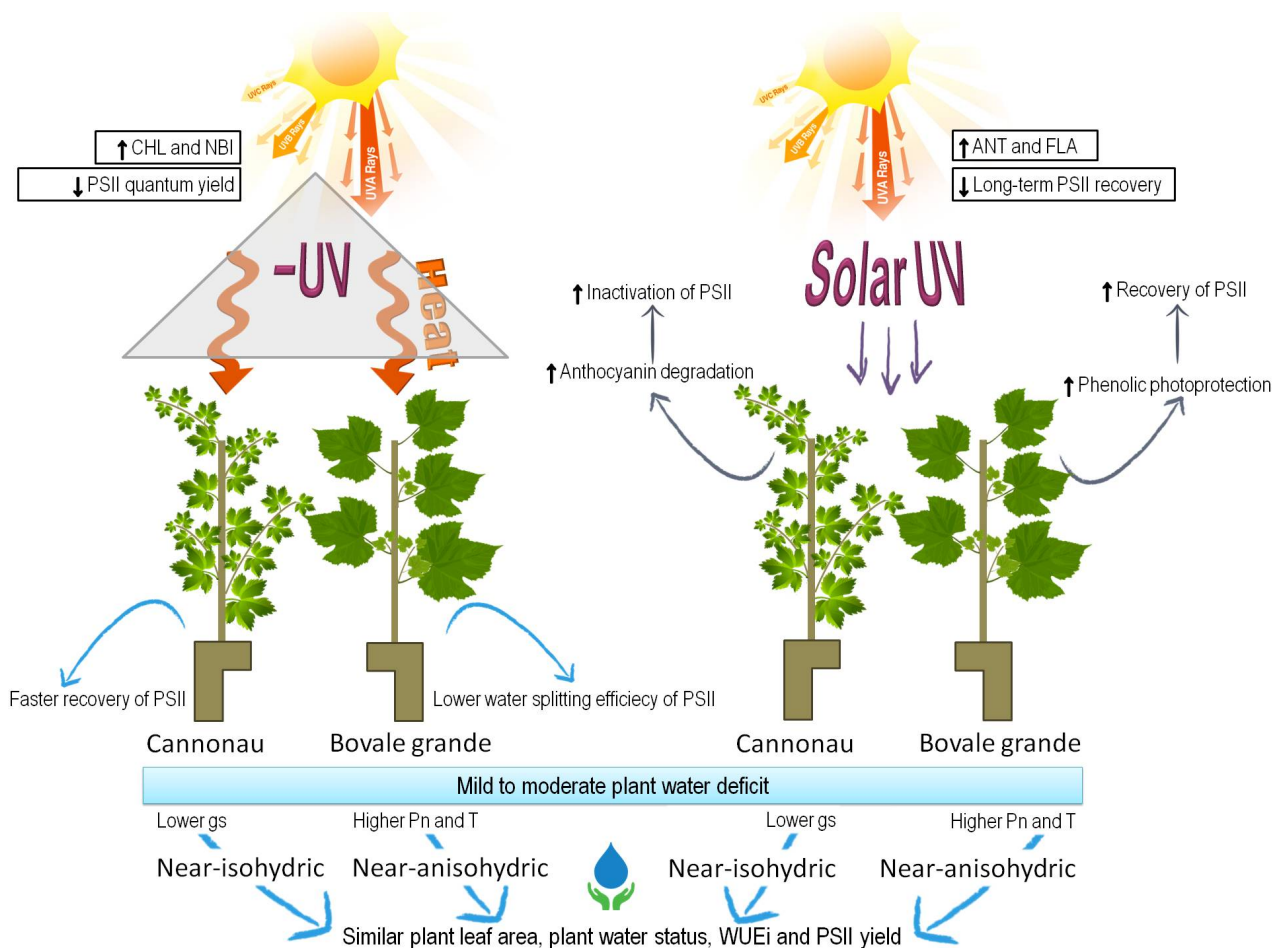


FIGURE 10

Conceptual representation of cultivar and treatment related responses concerning plant leaf area balance and water status, maximum leaf gas exchange and primary photochemistry efficiency and leaf pigment variation upon high solar UV and heat stress conditions of field-grown Bovale grande and Cannonau plants kept under mild to moderate water deficit.

and anisohydric varieties depends on the perception and regulation mechanisms of each variety, including differences in leaf hydraulic properties and anatomic characteristics. However, it can also be affected by differences in phenolic accumulation dynamics and phenolic profiles. In this sense, agronomical practices that affect the synthesis and accumulation of leaf pigments can influence canopy acclimation to heat and conditions of high sunlight. Under Mediterranean conditions, the decisions concerning vineyard management, like the pruning system, the timing, frequency and intensity of canopy management operations, as well as the best irrigation system and scheduling, must take into consideration the need to avoid excessive sunlight exposure and long duration of elevated temperatures during the hot and dry season. Moreover, in such conditions, monitoring the canopy temperature may give an accurate indication of plant physiological status and water requirements for both near-isohydric and near-anisohydric varieties, since it is closely related to plant water status and, on the whole, to photo-assimilation performance during the growing season. Finally, using the new optical tools that allow for rapid and non-destructive estimation of leaf pigments, further research could be conducted, both *in situ* and *in vivo*, to provide new information on how and how fast grapevines can balance these key metabolites in their leaves in order to recover their photosynthetic and photochemical functionality. Such new knowledge will allow for a deeper understanding of short- and long-term response mechanisms, as well as of the degree of plasticity that different varieties may achieve under multiple stress conditions.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version.

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