

# High spatial heterogeneity of water stress levels in Refošk grapevines cultivated in Classical Karst

Francesco Petruzzellis<sup>a,b,\*</sup>, Sara Natale<sup>a</sup>, Luca Bariviera<sup>a</sup>, Alberto Calderan<sup>a,b</sup>, Alenka Mihelčič<sup>c</sup>, Jan Reščič<sup>d</sup>, Paolo Sivilotti<sup>b</sup>, Katja Šuklje<sup>c</sup>, Klemen Lisjak<sup>c</sup>, Andreja Vanzo<sup>c</sup>, Andrea Nardini<sup>a</sup>

<sup>a</sup> University of Trieste, Department of Life Sciences, via L. Giorgieri 10, 34127 Trieste, Italia

<sup>b</sup> University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, via delle Scienze 206, 33100 Udine, Italy

<sup>c</sup> Agricultural Institute of Slovenia, Department of Fruit Growing, Viticulture and Enology, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

<sup>d</sup> University of Nova Gorica, School for Viticulture and Enology, Dvorec Lanthieri/Lanthieri Mansion Glavni trg 8, 5271 Vipava, Slovenia

## ARTICLE INFO

### Keywords:

Classical Karst  
Drought stress  
Grape quality  
Phenolic profile  
*Vitis vinifera*  
Water status

## ABSTRACT

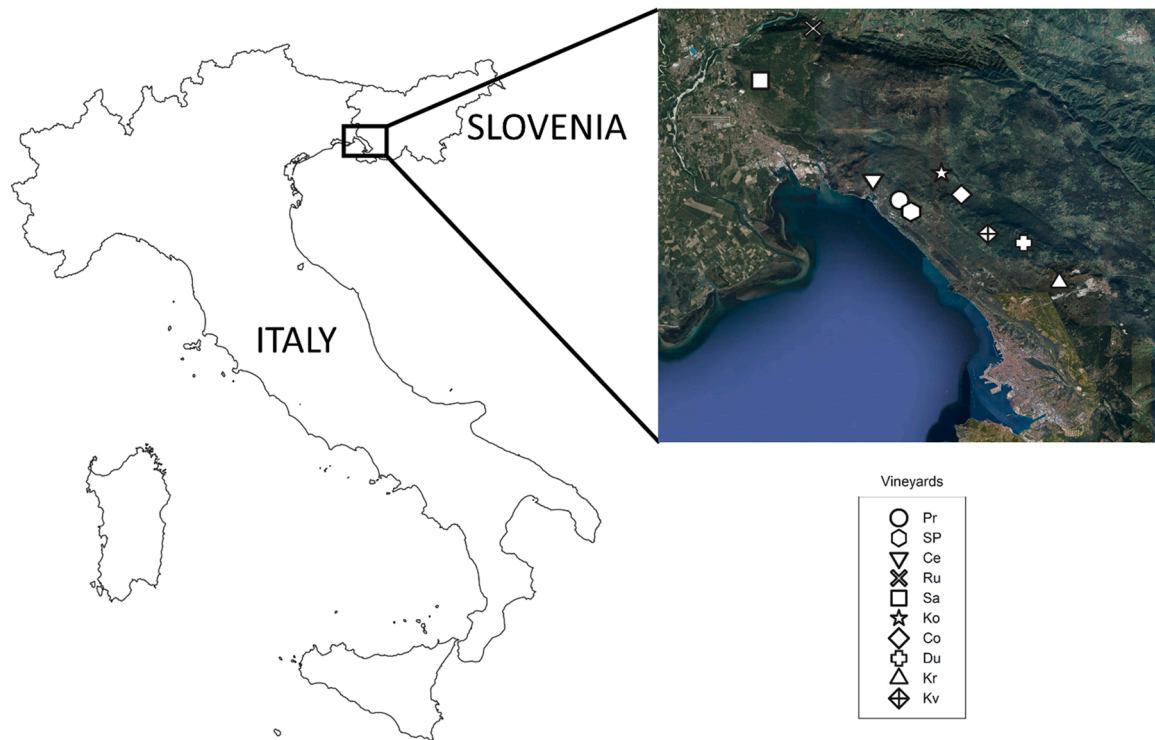
Grapevines are being challenged by climate changes, forcing winemakers to implement irrigation systems to cope with excessive water stress. Previous studies focused on a small set of international varieties, and only few data are available for *terroirs* hosting cultivars with possibly different responses to drought stress. In this light, we monitored grapevine water status and grape's physical and chemical composition, as well as concentration and structural characteristics of grape extractable polyphenols, in ten different Refošk vineyards located in the Classical Karst *terroir* during 2018 and 2019. Grapevines did not suffer severe stress during the two years, but their response to water shortage periods was highly heterogeneous, as pre-dawn ( $\Psi_{pd}$ ) and minimum ( $\Psi_{min}$ ) leaf water potential significantly differed between vineyards, especially during the drier part of the season. Moreover, the timing of maximum water stress differed in the two years, as in 2019 longer water shortage periods and higher temperature occurred at flowering stage and before veraison, while in 2018 they were higher after veraison. These differences influenced berry's quality, as titratable and malic acid concentration in juice, as well as total anthocyanin, total polyphenols and higher high molecular weight proanthocyanidins (HMWP) concentration in skins, were higher in 2019 than in 2018. Regarding seed proanthocyanidins, HMWP concentration, mean degree of polymerisation (mDP) and percentage of galloylation (G) in seeds were higher in 2018 than in 2019. The differences in water status measured in spatially close-related vineyards strongly support the importance of monitoring grapevines' water status dynamics to design adequate and effective water management activities rather than relying on climate data solely. Moreover, the timing of water shortage periods also played a role in determining Refošk grape quality. Our analyses showed that the higher (but still moderate, with  $\Psi_{pd}$  and  $\Psi_{min}$  mean values around  $-0.50$  and  $-1.25$  MPa, respectively) water stress between veraison and harvest occurred in 2018 might reduce Refošk grape acidity and increase concentration, polymerisation and galloylation of seed extractable proanthocyanidins.

## 1. Introduction

Grapevine (*Vitis vinifera* L.) is globally one of the most important fruit crops (Kuhn et al., 2013, Alston and Sambucci, 2019), but wine production is threatened by ongoing climate changes. Over the next decades, the typically suitable areas for grape cultivation might decrease by about 50%, because of decreasing precipitation and increasing air temperature (Hannah et al., 2013). In the Mediterranean region,

temperatures have risen faster than the global average (Giorgi and Lionello, 2008) and this will likely limit wine production in this area within the first half of the 21st century, already (Lionello et al., 2014). Climate is indeed one of the key controlling factors in wine production (Marx et al., 2017). Grape's productivity strictly depends on water availability, which is fundamental for vine's growth and for the development of berries with chemical and physical features assuring high wine quality (Chacón-Vozmediano et al., 2020). According to climatic

\* Correspondence to: Università di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali, via delle Scienze 206, 33100 Udine, Italy.  
E-mail address: francesco.petruzzellis@uniud.it (F. Petruzzellis).



**Fig. 1.** Map of the study area and location of the vineyards Pr = Prepotto, SP = San Pelagio, Ce = Ceroglie, Ru = Rubbia, Sa = Sagrado, Ko = Komen, Co = Coljava, Du = Dutovlje, Kr = Križ, Kv = Krajna vas).

conditions, water consumption of vineyards could range from 300 to 700 mm per year (Jackson, 1994), a value exceeding the annual cumulative rainfall in many viticultural areas. Over the last years, increasing aridity led the winemakers to implement irrigation systems to cope with excessive grape water stress (Ayuda et al., 2020; Costa et al., 2016). In this light, adequate strategies to optimize water use while ensuring production of high-quality wine have been developed, based on knowledge of the ecophysiological responses of grapevines to drought stress (Acevedo-Opazo et al., 2010; Calderan et al., 2021; Chaves et al., 2010; Fernández and Cuevas, 2010; Romić et al., 2020; Tripathi et al., 2016). In general, grapevine responses to drought are influenced by the environment in which plants grow (Hochberg et al., 2018) but are also cultivar-dependent, with some of them displaying relatively high resistance/resilience to environmental stress (Chaves et al., 2010; Tombesi et al., 2014). Hence, the identification of cultivar-specific water use strategies is fundamental to develop adequate water optimization strategies.

Several physiological parameters allow to describe grapevine's water status and many authors have proposed the use of the pressure chamber method as an excellent tool to measure vine water status under irrigated and non-irrigated conditions (Acevedo-Opazo et al., 2010; Calderan et al., 2021; Choné et al., 2001; Girona et al., 2006; Levin, 2019). As a consequence, leaf pre-dawn and minimum water potential ( $\Psi_{pd}$  and  $\Psi_{min}$ , respectively), as well as stem minimum water potential ( $\Psi_{stem}$ ), became the most reliable parameters allowing to assess vine water status quickly and accurately in relation to soil water availability and climatic conditions, soil hydraulic conductivity and the capacity of the vine to transport water from the soil to the canopy (Acevedo-Opazo et al., 2010; Calderan et al., 2021; Gambetta et al., 2020; Savi et al., 2019, 2018). In addition, the turgor loss point ( $\Psi_{tlp}$ , the water potential inducing cell turgor loss) has been recently proposed as a reliable parameter to quantify genotypic and phenotypic plasticity in vine's drought tolerance (Gambetta et al., 2020). Over the last decades, several studies have included these parameters to investigate the response of grapevine to drought stress and the relationships between grapevine water status and

berry chemical composition and wine quality (see Table S1 in Gambetta et al., 2020). In particular,  $\Psi_{min}$  and  $\Psi_{stem}$  have been regularly measured during the growing seasons in a large number of studies as a proxy of vine's water status, and strong correlations have been reported with features such as berry size and yield, as well as sugar and organic acid contents (Gambetta et al., 2020). Consequently, water management strategies based on water potential measurements have been increasingly adopted by winemakers to allow grapevines to withstand water shortage with non-significant decreases of yield, and positive impacts on grape and wine quality (Calderan et al., 2021; Savi et al., 2018). Gambetta et al. (2020) summarized the outputs of studies on four major red grape varieties and reported that moderate water stress levels ( $-0.9 < \Psi_{stem} < -1.1$  MPa) significantly increased sugar concentration and reduce titratable acidity without affecting productivity. However, these results were not consistent among different varieties, and also the timing of water stress was found to significantly affect grape's chemical composition and wine quality (Bucchetti et al., 2011; Castellarin et al., 2007a; Koundouras et al., 2009; Wenter et al., 2018). This is the case of flavonoids, that largely contribute to the grape and wine flavour and quality. In general, water stress results in red wines with higher concentration of anthocyanins, but some studies showed no significant changes across water stress levels applied to vines (Castellarin et al., 2007b; Savoi et al., 2017). In addition to different responses of varieties (Hochberg et al., 2015), pre-veraison water stress seemed to have stronger effects on total skin anthocyanins content (Koundouras et al., 2009). Other flavonoids possibly influenced by water stress are the proanthocyanidins (high and low molecular weight proanthocyanidins or HMWP and LMWP, respectively), also known as tannins, which are known to impact sensory characteristics of red wines, such as the colour and the astringency. Despite their important role, it is still unclear how water stress impacts their synthesis and accumulation in berries (Calderan et al., 2021). Some studies have reported an increase in proanthocyanidin concentration in response to water stress in Merlot (Casassa et al., 2015; Herrera et al., 2015), but others found contrasting results according to the timing of water stress and among different varieties

**Table 1**

List of vineyards (named according to their location), country and associated GPS coordinates.

Vineyard	Abbreviation	Country	Coordinates	
Ceroglie	Ce	Italy	45.78432	13.64022
Coljava	Co	Slovenia	45.80028	13.78566
Dutovlje	Du	Slovenia	45.75454	13.83638
Komen	Ko	Slovenia	45.81647	13.74337
Krajna vas	Kv	Slovenia	45.76477	13.79988
Križ	Kr	Slovenia	45.73687	13.86599
Prepotto	Pr	Italy	45.76059	13.69307
Rubbia	Ru	Italy	45.89803	13.56927
Sagrado	Sa	Italy	45.8723	13.49864
San Pelagio	SP	Italy	45.75684	13.68742

(Gambetta et al., 2020; Pinasseau et al., 2017), as well as when comparing skin and seeds (Kyralou et al., 2017).

Most of the previous studies on the relationship between water stress and berry and wine quality focused on a small set of international varieties. Only few data are available for particular *terroirs*, defined as a “complex interplay of physical factor and cultural influences that interact to define wine quality from a vineyard site or region” (OIV, 2016; Seguin, 1988), hosting cultivars with possibly different responses to drought stress. Teran wines are produced in the *terroir* of Classical Karst from grapes of *Vitis vinifera* L. cv Refošk (syn. Teran), growing in vineyards located in the karstic plateau at the cross-border area between Slovenia and Italy (Kozjak et al., 2003). This *terroir* is characterized by shallow clayish-loamish red soil called ‘terra rossa’ in Italy or ‘jerina’ in Slovenia, lying above highly permeable and fractured carbonate bedrock (Savi et al., 2019). Traditionally, Refošk vineyards are rainfed but due to ongoing climate change Slovenian and Italian winemakers have started irrigation practices to guarantee stable yield production (Savi et al., 2019). However, these practices often do not consider whether vines do actually suffer water stress, and recent studies on Istrian Malvasia grapevines cultivated in Classical Karst demonstrated that vines in this area mainly rely on deep water sources (Savi et al., 2019, 2018) rather than on occasional rainfall or irrigation. This is probably due to the nature of karstic bedrock, which plays a key role in shaping water availability to plants (Nardini et al., 2021; Schwinning, 2010). However, only few studies have focused on the responses of Refošk vineyards to water availability in Classical Karst, and only few data are available to winemakers to optimize their irrigation practices (Calderan et al., 2021).

On these premises, we monitored seasonal changes in water status and grape’s basic physical and chemical composition, as well as concentration and structural characteristics of grape extractable polyphenols in ten different Refošk vineyards located in the Classical Karst area during 2018 and 2019. The aims were to: *i*) assess the water stress levels typically experienced by Refošk grapevines in Classical Karst; *ii*) assess whether different timing of water stress and shortage in the two years affected Refošk grape quality.

## 2. Materials and methods

### 2.1. Study area and sampling design

The study area is located at the cross-border between Slovenia and Italy Karst area (Fig. 1). Ten Refošk vineyards (5 in Slovenia and 5 in Italy) were selected to monitor grapevine water status during the 2018 and 2019 growing seasons, along with grapes basic physico-chemical parameters and the concentration and structural characteristics of grape polyphenols at harvest. The ten vineyards are abbreviated after the corresponding location as summarized in Table 1.

The Karst area is dominated by a limestone plateau extending over ~800 km<sup>2</sup> across southwestern Slovenia and north-eastern Italy (UNESCO, 2016). It stretches between the Vipava Valley, the

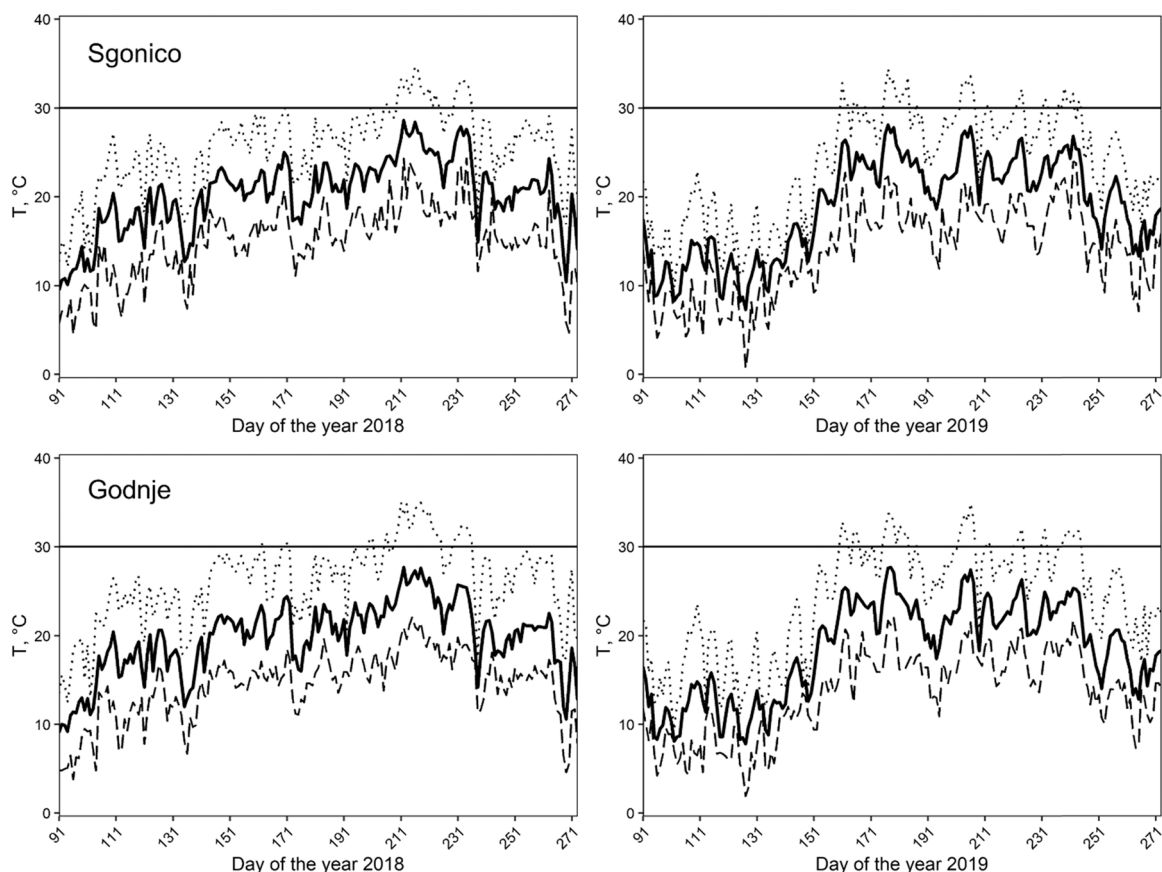
westernmost part of the Brkini hills and the Gulf of Trieste, at altitude ranging between 200 and 400 m above sea level. Karst’s bedrock is composed of relatively soluble rocks (usually limestone) rich in fissures and cracks leading to a pronounced underground water drainage (Gams, 1993). The shallow soil overlying the bedrock is typically red due to the high concentration of silicates and iron (e.g. SiO<sub>2</sub> ~50%, Al<sub>2</sub>O<sub>3</sub> ~20% and FeO ~8%) and it is mainly composed of silt (30–60%) and clay (70–30%) (Cucchi and Piano, 2013). The climate of Karst is heterogeneous, spanning from continental to mild sub-Mediterranean types, and is characterized by high summer temperatures and cold winters, that are often marked by “Bora” cold wind (Mrak and Repe, 2004). The average annual temperature is 13 °C, and yearly cumulative rainfall is 1385 mm, with less than 200 mm falling in July-August (Savi et al., 2018). Throughout the study period, hourly air temperature (T<sub>air</sub>), relative humidity (RH) and daily precipitation data were obtained from Sgonico ([www.osmer.fvg.it](http://www.osmer.fvg.it)) and Godnje weather stations (<https://e-karst.eu/it>). Average midday daily T<sub>air</sub> and RH (12:00–14:00, solar time) were used to calculate midday vapor pressure deficit (VPD), following the equation proposed by Sonntag (1994), using “rh.to.VPD” function in “bigleaf” package (Knauer et al., 2018) for R software (R Core Team, 2020). Daily precipitation data were used to calculate cumulative precipitation, the number of dry days (i.e. days with precipitation < 1 mm) and the maximum number of consecutive dry days (CDD), using “exceedance” function for “RMarineHeatwaves” (Smith et al., 2018) R package.

In each vineyard, we selected 3 plots (each represented by a pair of rows) where water status parameters were measured at regular intervals during the growing season, starting from May until harvest, which occurred in both years between 20th and 25th of September. At harvest, 10 bunches per vineyard were randomly collected and transported to the laboratory for analyses of extractable grape polyphenols (see below for details). The grapes were cooled overnight at 4 °C. Basic physical and chemical analyses were performed on the following day, whereas polyphenols extractions were performed separately for seeds and skin on fresh samples within 48 h from sampling.

### 2.2. Water status, water relations and leaf C isotopic composition

Plant water status was quantified in terms of pre-dawn leaf water potential ( $\Psi_{pd}$ , MPa, proxy of soil moisture) and minimum leaf water potential ( $\Psi_{min}$ , MPa, proxy of the maximum water stress experienced by grapevines on a daily/seasonal basis). For  $\Psi_{pd}$  measurements, 2 leaves from 2 different grapevines in each plot (6 leaves per vineyard) were collected before sunrise (i.e. between 3:30 and 5:30, solar time), while for  $\Psi_{min}$  4 leaves from 4 different grapevines in each plot (12 leaves per vineyard) were collected between 12:00 and 14:00 (solar time). Both parameters were measured every 3 weeks from May to harvest. On each measurement day, mature leaves were detached from shoots and immediately wrapped in cling film, put in plastic bags with a piece of wet paper inside and transported to the laboratory in refrigerated bags. Water potential was measured using a pressure chamber (mod. 1505D, PMS Instrument Company, Albany, OR, USA) within 2 h from sampling.

Water relations parameters were measured at the completion of leaf development (beginning of June) and at the peak of drought stress (late July) in a subset of five vineyards. Eventual osmoregulation during increasing drought was quantified in terms of leaf osmotic potential at full turgor ( $\pi_0$ , MPa). Water potential at turgor loss point ( $\Psi_{tlp}$ , MPa) was also measured as a proxy of vines’ drought resistance and as a reference point for estimating the residual turgor (RT, MPa) of leaves when reaching  $\Psi_{min}$  (Nardini et al., 2003). Specifically, in May and July, two additional leaves for each plot (6 per vineyard) were sampled as described for  $\Psi_{min}$ , and  $\pi_0$  and  $\Psi_{tlp}$  were measured according to Petruzzellis et al. (2019). Once in the laboratory, leaves were rehydrated for two hours to reach the full turgor. Then, leaf dry matter content (LDMC) and  $\pi_0$  were measured on one leaf for each plot. For LDMC measurement, leaf turgid weight (without petioles) was measured with an analytical balance. Then, leaves were oven dried for 24 h at 70 °C and the dry



**Fig. 2.** Daily minimum (dashed lines), mean (bold lines) and maximum (dotted lines) air temperature (T), as recorded from April to September 2018 and 2019 from Sgonico and Godnje weather stations.

weight was obtained as explained above. LDMC was calculated as:

$$\text{LDMC} = \text{leaf dry weight} / \text{leaf fresh weight} \text{ (mg/g)} \quad (1)$$

For  $\pi_0$  measurement, leaves (still sealed in cling film, laminas only) were immersed in liquid nitrogen for two minutes. After that, samples were rapidly crumbled and stored in sealed plastic bottles at  $-20^\circ\text{C}$  until measurements. On the day of measurements, samples were thawed at room temperature for five minutes. Then, measurements of the osmotic potential at full turgor ( $\pi_0$ , MPa) were done with a dew point hygrometer ( $\pi_{0\text{osm}}$ ) (Model WP4, Decagon Devices Inc.). To overcome possible bias due to dilution or enrichment of solutes of symplastic fluids (Bartlett et al., 2012a),  $\pi_0$  and  $\Psi_{\text{tjp}}$  were estimated with the following equations (Petruzzellis et al., 2019):

$$\pi_0 = 0.506 * \pi_{0\text{osm}} - 0.002 * \text{LDMC} \text{ (expressed in mg g}^{-1}\text{)} \quad (2)$$

$$\Psi_{\text{tjp}} = 1.31 * \pi_0 - 0.03 \quad (3)$$

RT was then calculated as the difference between  $\Psi_{\text{min}}$  and  $\Psi_{\text{tjp}}$ . Leaf carbon isotopic composition ( $\delta^{13}\text{C}$ ) was measured as a proxy of water use efficiency (Prieto et al., 2018) on the same leaves sampled in July for LDMC measurements. Leaves were oven dried for 48 h at  $70^\circ$  and then pulverized in a mortar.  $\delta^{13}\text{C}$  was measured by continuous flow isotope ratio mass spectrometry using an IsoPrime 100 mass spectrometer (IsoPrime Ltd, Cheadle, UK). Isotopic analysis was performed by the Center for Stable Isotope Biogeochemistry (University of California, Berkeley). Long-term external precision based on reference material “NIST SMR 1577b” (bovine liver) is 0.10‰ for C isotope analysis.

### 2.3. Basic physical and chemical analyses of grapes

Grapes were sampled at harvest in September 2018 and 2019. A total

100 berries per vineyard were representatively sampled from bunches, weighed and hand squeezed to obtain juice. Total soluble solids, titratable acidity (expressed as g/L of tartaric acid equivalents), malic acid concentration and the pH of the berry juice were determined following International Organisation of Vine and Wine procedures (OIV).

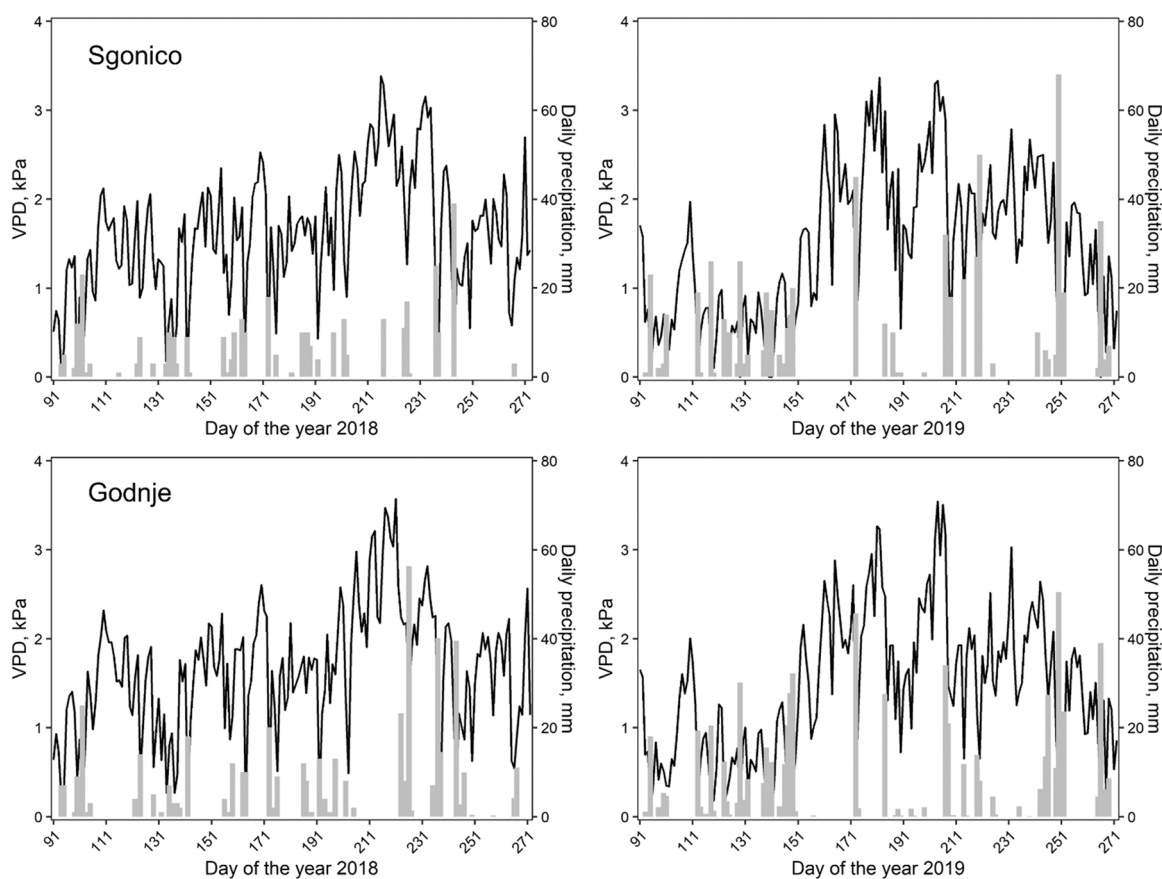
### 2.4. Concentration and structural characteristics of extractable grape polyphenols

During winemaking, only a fraction of the grape polyphenols is extracted into the wine. To investigate the concentration and structural characteristics in wine extractable polyphenols, a selective extraction from the skin and seeds of grape berries in wine-like solution was performed as described in Mattivi et al. (2002). Skins and seeds of 200 g of randomly sampled grape berries were separated and extracted for five days at  $30^\circ\text{C}$  in a 200 mL solution consisting of ethanol:water (12:88 v/v), 100 mg/L of  $\text{SO}_2$ , 5 g/L tartaric acid and a pH value adjusted to 3.2. Extracts were stored in dark glass bottles at  $4^\circ\text{C}$  until spectrophotometric and UHPLC-DAD-MS/MS analyses. All analyses were conducted within one month from preparing skin and seed wine-like extracts.

Spectrophotometric analyses were performed with an Agilent 8453 spectrophotometer (Agilent Technologies, Palo Alto, USA). Skin and seed wine-like extracts were analysed according to the protocols reported by Di Stefano et al. (1989) under optimised conditions of Rigo et al. (2000). Total anthocyanins concentration in grape skin extract was evaluated in mg/kg in grape fresh weight (FW).

Total polyphenols (TP) concentration in grape seed and skin extracts was estimated by a reduction of Folin–Ciocalteu reagent to blue pigments caused by phenols in alkaline solution and expressed as (+)-catechin in mg/kg of grape FW.





**Fig. 3.** Daily midday vapour pressure deficit (VPD, bold lines) and precipitation (grey bars) as recorded from April to September 2018 and 2019 from Sgonico and Godnje weather stations.

High molecular weight proanthocyanidins (HMWP) concentration in grape seed and skin extracts was expressed in mg/kg of cyanidin-chloride of grape FW. This assay provides an evaluation of the total amount of proanthocyanidins, and it is mainly linked to variations in the HMWP corresponding to at least five units of monomers (Vrhovsek et al., 2001).

Low molecular weight proanthocyanidins (LMWP) concentration in grape seed and skin extracts was measured exploiting vanillin reaction and evaluated as (+)-catechin in mg/kg grape FW. The assay provides a good estimation of monomers and a low degree of polymerized flavanols corresponding to two to four units.

Structural characteristics of grape seed and skin extractable proanthocyanidins, namely mean degree of polymerisation (mDP), the percentage of galloylation (G, %) and of prodelfinidines (P, %) were also analysed in both years using UHPLC-DAD-MS/MS technique as described by Lisjak et al. (2020) and Calderan et al. (2021).

## 2.5. Statistical analysis

Generalised Least Square (GLS) models were used to model water status, water relations and water use efficiency parameters variation among the monitored vineyards during 2018 and 2019 (null hypothesis was that mean values of each parameter are equal between the selected vineyards and in the different sampling dates and years). For water status parameters, one GLS was run for each parameter (response variable) separately through “gls” function in “nlme” R package (Pinheiro et al., 2020), and setting vineyards, sampling date, year and their interaction as the explanatory variables. To account for temporal autocorrelation, a corAR1 variance structure was added in the models. After checking for normality and homogeneity of variances and when models were statistically significant ( $p$ -value < 0.05), post-hoc Tukey’s

Honestly Significant Differences comparisons with Bonferroni-Holm correction for the calculation of  $p$ -values were run using “emmeans” function within R package “emmeans” (Lenth, 2020). Differences of  $\pi_0$ ,  $\Psi_{tip}$  and RT values between vineyards were tested as described above. Because a three-way interaction resulted in singular fit of the models, we considered vineyards, sampling dates and their interaction, and years and its interaction with sampling dates, as explanatory variables. For  $\delta^{13}C$ , one gls model was run setting vineyard, year and their interaction as explanatory variables, since leaves were sampled once in each year. Additionally, to account for heterogeneity of variance, a constant variance function structure (varIdent type) was added in the models. When models were statistically significant ( $p$ -value < 0.05), post-hoc Tukey’s Honestly Significant Differences comparisons with Bonferroni-Holm correction for the calculation of  $p$ -values were run as described above.

Student’s  $t$ -tests were run to test differences of basic physico-chemical grape parameters and polyphenols concentration and structural characteristics between the two monitoring years, using “t.test” function in “stats” R package.  $P$ -values were adjusted using “false discovery rate” correction. Statistical analyses were performed with the software R (R Core Team, 2020).

## 3. Results

### 3.1. Meteorological data

Daily minimum ( $T_{min}$ , °C), mean ( $T_{mean}$ , °C) and maximum ( $T_{max}$ , °C) air temperature, cumulative precipitation, and midday VPD, as retrieved from the weather stations of Sgonico (Italy) and Godnje (Slovenia) from April to September in 2018 and 2019 are reported in Figs. 2 and 3, respectively. Mean values of the above parameters along with cumulative precipitation and CDD were calculated for each month (from April

**Table 2**

Mean values and associated standard deviations of minimum ( $T_{\min}$ ), mean ( $T_{\text{mean}}$ ), maximum ( $T_{\max}$ ) temperature and vapour pressure deficit (VPD), along with cumulative precipitation and cumulative number of dry days calculated for each month from April to September in both years and from data retrieved from Sgonico and Godnje weather stations.

	Sgonico		Godnje	
	2018	2019	2018	2019
<b>April</b>				
$T_{\min}$ , °C	9.2 ± 2.7	8.1 ± 2.7	9.1 ± 3.2	7.7 ± 2.3
$T_{\text{mean}}$ , °C	14.8 ± 3.2	12.2 ± 2.6	14.7 ± 3.4	11.8 ± 2.5
$T_{\max}$ , °C	19.9 ± 4.3	16.6 ± 3.5	20.5 ± 4.4	16.7 ± 3.9
VPD, kPa	1.2 ± 0.6	0.8 ± 0.5	1.3 ± 0.6	0.8 ± 0.5
Cumulative precipitation, mm	52	90	55	76
N dry days	21	21	22	17
<b>May</b>				
$T_{\min}$ , °C	14.0 ± 3.0	8.9 ± 3.1	13.1 ± 2.8	8.5 ± 3.1
$T_{\text{mean}}$ , °C	19.0 ± 3.0	12.7 ± 2.5	18.2 ± 2.9	12.5 ± 2.5
$T_{\max}$ , °C	23.7 ± 3.3	16.3 ± 3.1	24.2 ± 3.4	16.8 ± 3.5
VPD, kPa	1.3 ± 0.6	0.5 ± 0.4	1.3 ± 0.6	0.6 ± 0.4
Cumulative precipitation, mm	50	165	60	204
N dry days	22	15	21	14
<b>June</b>				
$T_{\min}$ , °C	15.5 ± 2.1	17.1 ± 3.3	14.8 ± 1.6	16.8 ± 3.0
$T_{\text{mean}}$ , °C	20.9 ± 2.1	23.3 ± 2.8	20.6 ± 2.2	23.0 ± 2.6
$T_{\max}$ , °C	26.2 ± 2.6	28.5 ± 3.1	26.7 ± 2.8	29.2 ± 2.8
VPD, kPa	1.5 ± 0.6	2.0 ± 0.7	1.6 ± 0.6	2.0 ± 0.7
Cumulative precipitation, mm	70	45	68	48
N dry days	22	29	22	27
<b>July</b>				
$T_{\min}$ , °C	17.3 ± 2.3	17.3 ± 2.3	16.8 ± 1.7	16.7 ± 2.3
$T_{\text{mean}}$ , °C	22.8 ± 2.0	23.1 ± 2.4	22.4 ± 1.9	22.4 ± 2.6
$T_{\max}$ , °C	28.0 ± 2.3	28.6 ± 3.2	28.7 ± 2.6	28.7 ± 3.3
VPD, kPa	1.8 ± 0.5	2.2 ± 0.8	1.8 ± 0.7	2.1 ± 0.8
Cumulative precipitation, mm	64	85	79	120
N dry days	22	23	21	20
<b>August</b>				
$T_{\min}$ , °C	18.9 ± 3.1	18.5 ± 2.7	18.1 ± 2.6	17.7 ± 2.2
$T_{\text{mean}}$ , °C	24.4 ± 3.0	23.6 ± 1.8	23.6 ± 3.1	22.3 ± 1.8
$T_{\max}$ , °C	30.1 ± 3.5	28.7 ± 2.1	30.3 ± 3.9	28.9 ± 2.2
VPD, kPa	2.4 ± 0.7	1.9 ± 0.4	2.3 ± 0.8	1.8 ± 0.5
Cumulative precipitation, mm	77	112	150	47
N dry days	24	26	24	22
<b>September</b>				
$T_{\min}$ , °C	13.9 ± 3.2	13.4 ± 3.0	13.5 ± 3.4	12.9 ± 2.6
$T_{\text{mean}}$ , °C	19.3 ± 2.9	18.1 ± 2.9	18.7 ± 3.0	17.7 ± 2.7
$T_{\max}$ , °C	24.9 ± 3.1	23.1 ± 3.4	25.5 ± 3.5	23.3 ± 3.5
VPD, kPa	1.5 ± 0.5	1.3 ± 0.6	1.5 ± 0.6	1.2 ± 0.6
Cumulative precipitation, mm	42	159	70	202
N dry days	28	20	22	19

to August, Table 2), and for different phenological periods, namely 7 days before flowering date (Sgonico 21/05/2018 and 7/06/2019; Godnje 25/05/2018 and 13/06/2019), between June and average veraison dates (27/07/2018 and 11/08/2019) and between veraison and harvest in both years (Table 3). Temperature data had different trends in 2018 and 2019 for both weather stations (Table 2). Specifically,  $T_{\min}$ ,  $T_{\text{mean}}$  and  $T_{\max}$  in April and May 2018 were higher than both the 1992–2020 reference period ([www.osmer.fvg.it](http://www.osmer.fvg.it) and [www.arso.gov.si](http://www.arso.gov.si)) and 2019, by about 2 °C and 3 °C respectively. Conversely, during summer (June–August), 2018 values were close to the reference period and slightly lower than those recorded in 2019. When considering phenological periods, temperature values were higher one week before flowering and between June and veraison in 2019 than in 2018, while the opposite trend occurred between veraison and harvest (Table 3). The spring season (April–May) was quite dry in 2018 (Table 2), as both stations recorded ~110 mm, while in the 1992–2020 reference period

**Table 3**

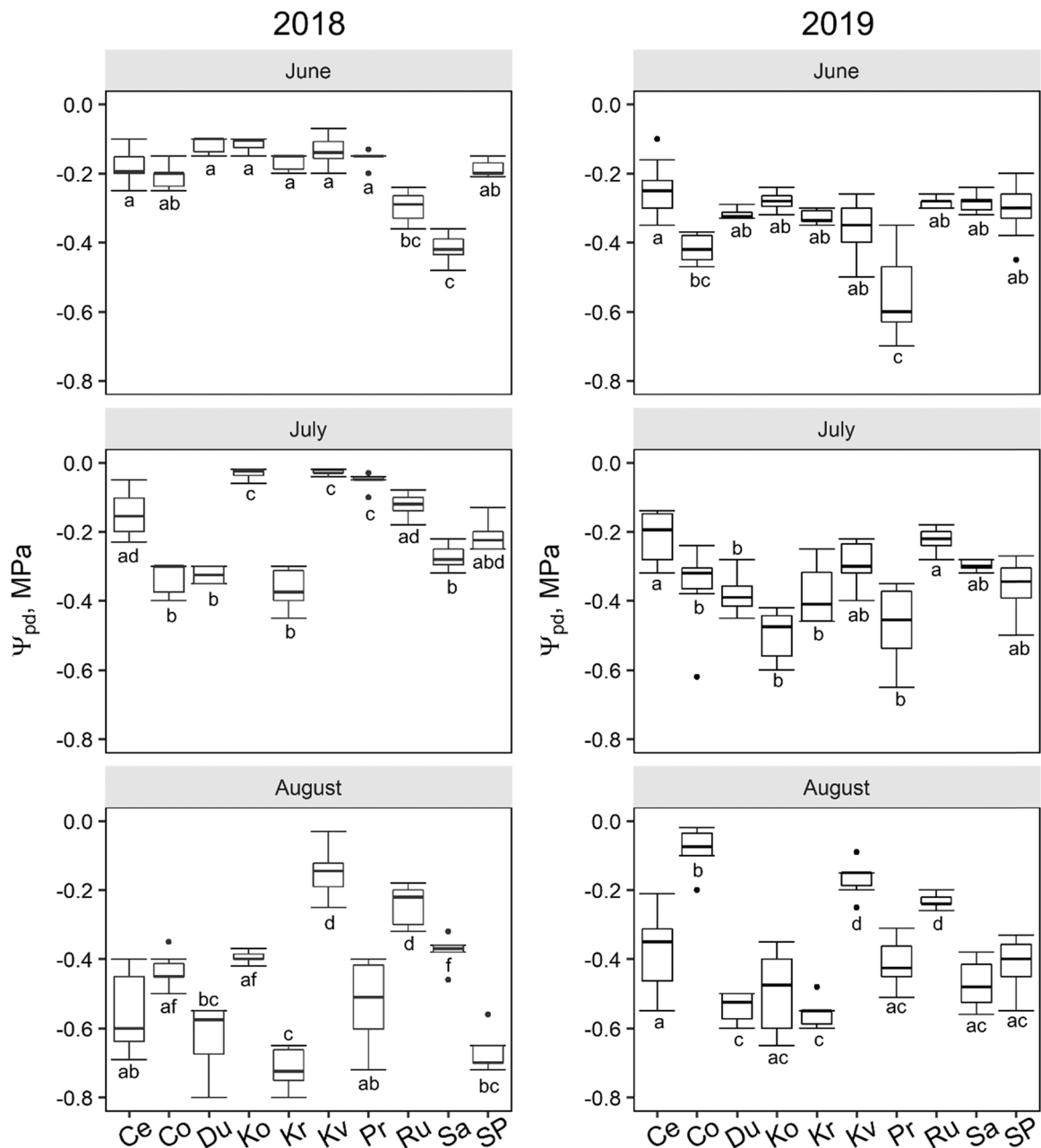
Mean values and associated standard deviations of minimum ( $T_{\min}$ ), mean ( $T_{\text{mean}}$ ), maximum ( $T_{\max}$ ) temperature and vapour pressure deficit (VPD), along with cumulative precipitation, maximum number of consecutive dry days (CDD) and cumulative number of dry days calculated for different phenological periods in both years and from data retrieved from Sgonico and Godnje weather stations.

	Sgonico		Godnje	
	2018	2019	2018	2019
<b>Period: 7 days before flowering</b>				
$T_{\min}$ , °C	11.4 ± 2.9	12.7 ± 2.3	12.6 ± 2.3	16.8 ± 2.9
$T_{\text{mean}}$ , °C	15.9 ± 2.6	18.6 ± 2.4	17.2 ± 2.5	22.6 ± 2.5
$T_{\max}$ , °C	20.5 ± 3.0	23.3 ± 3.3	23.2 ± 2.6	28.7 ± 3.5
VPD, kPa	0.9 ± 0.6	1.2 ± 0.4	1.0 ± 0.7	1.8 ± 0.7
Cumulative precipitation, mm	25	0	26	0
CDD	2	7	2	7
N dry days	3	7	3	7
<b>Period: June–Veraison</b>				
$T_{\min}$ , °C	16.1 ± 2.1	17.2 ± 2.7	15.3 ± 1.7	16.7 ± 2.7
$T_{\text{mean}}$ , °C	21.5 ± 1.9	23.2 ± 2.4	20.8 ± 2.0	22.7 ± 2.6
$T_{\max}$ , °C	26.8 ± 2.4	28.5 ± 2.9	26.7 ± 2.6	28.9 ± 3.1
VPD, kPa	1.6 ± 0.6	2.0 ± 0.7	1.6 ± 0.6	2.0 ± 0.7
Cumulative precipitation, mm	134	229	147	168
CDD	9 (26/06/2018–04/07/2018)	21 (01/06/2019–06/2019)	8 (14/06/2018–21/06/2018)	21 (01/06/2019–21/06/2019)
N dry days	39	59	38	59
<b>Period: Veraison–Harvest</b>				
$T_{\min}$ , °C	17.6 ± 3.1	16.6 ± 3.7	17.1 ± 2.5	15.8 ± 3.2
$T_{\text{mean}}$ , °C	23.2 ± 3.2	21.5 ± 3.4	22.5 ± 3.1	20.9 ± 3.3
$T_{\max}$ , °C	28.8 ± 3.5	26.7 ± 3.8	29.2 ± 3.7	26.9 ± 3.9
VPD, kPa	2.0 ± 0.7	1.7 ± 0.6	2.0 ± 0.8	1.7 ± 0.6
Cumulative precipitation, mm	116	124	205	192
CDD	10 (15/08/2018–24/08/2018)	17 (14/08/2019–30/08/2019)	15 (27/07/2018–10/08/2019)	9 (14/08/2019–22/08/2019)
N dry days	50	33	45	32

mean precipitation was ~200 mm. On the contrary, in 2019 precipitation was double than in 2018 and above average, since both stations recorded a value of about 270 mm. Cumulative precipitation from June to August was similar in the two years (Table 2) and slightly lower than the reference period, which averaged about 300 mm. VPD varied consistently during the growing season (Fig. 3), with highest and lowest values of 3.5 kPa and 0.2 kPa, respectively. Like temperature and precipitation data, VPD had different trends in the two years. In 2018, VPD values were similar in from April to July (1.2–1.5 kPa) and increased in July and August (1.8 and 2.4 kPa, respectively). Otherwise, in 2019, VPD had the lowest values in April and May (0.8 and 0.5 kPa, respectively), while it abruptly increased during the summer season (June to August) with values ranging from 1.9 to 2.2 kPa. Water shortage periods (CDD) were longer in 2019 than in 2018, especially before flowering and between June and veraison date (Table 3). The number of dry days was higher in 2019 than in 2018 one week before flowering and between June and veraison date, while the opposite trend was found between veraison and harvest date (Table 3).

### 3.2. Water status and grape's quality parameters

The trends of  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$  in each vineyard during the two monitoring years and in the different sampling dates are summarized in



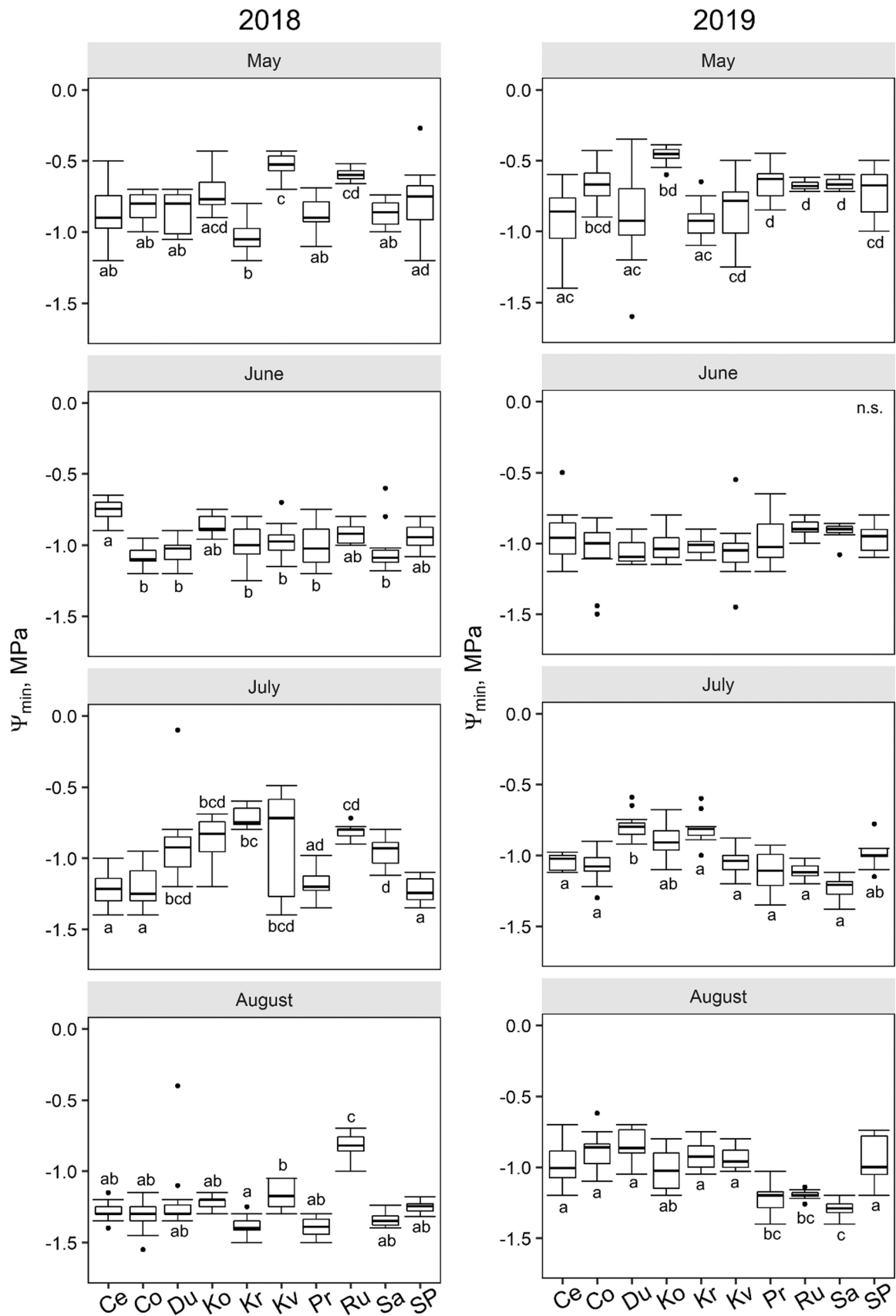
**Fig. 4.** Median values, 25th and 75th percentiles of pre-dawn leaf water potential ( $\Psi_{pd}$ ) measured in the ten vineyards (Ce = Ceroglie, Co = Coljava, Du = Dutovlje, Ko = Komen, Kv = Krajna vas, Kr = Kriz, Pr = Prepotto, Ru = Rubbia, Sa = Sagrado, SP = San Pelagio). Different letters indicate statistically significant differences among vineyards in each sampling date ( $p$ -value < 0.05).

**Figs. 4 and 5.**  $\Psi_{pd}$  significantly differed among years, sampling dates and between vineyards (Table 4). Specifically, Co, Ko and Ru reported the tendentially highest  $\Psi_{pd}$  values, while Kr and SP had the lowest ones in both years. For  $\Psi_{min}$ , statistically significant differences were detected between sampling dates and vineyards, especially in 2018. Kv had the highest  $\Psi_{min}$ , while Pr and Sa had the lowest one in both years. The differences of  $\Psi_{tip}$  and RT values measured in the subset of 5 vineyards are shown in Figs. A1 and A2. While differences between vineyards were not significant,  $\Psi_{tip}$  values were significantly lower in July in both years. Moreover, in July 2019 vines had  $\Psi_{tip}$  values slightly lower than in July 2018 (Table 4). RT did not significantly differ between vineyards in both years (Fig. S2). However, it was  $\sim 0.25$  MPa higher in July 2019 than in June 2019, while no difference was recorded in 2018 and between years (Table 4).  $\delta^{13}C$  values differed between vineyards in each year and were slightly more negative in 2018 than in 2019 and were negatively related

with  $\Psi_{pd}$  mean values between veraison and harvest (Fig. A3).

The global trends of  $\Psi_{pd}$  and  $\Psi_{min}$  during the two monitoring years in the different sampling dates are summarized in Fig. 6. Both values progressively decreased during the growing season, with the lowest values reached in August for both parameters. Specifically,  $\Psi_{pd}$  ranged from  $-0.18$  and  $-0.34$  MPa in June 2018 and 2019, respectively, to  $-0.47$  and  $-0.38$  MPa in August 2018 and 2019, respectively. Regarding  $\Psi_{min}$ , the highest mean values were  $-0.79$  and  $-0.75$  MPa in May 2018 and 2019, respectively, while the lowest mean values were  $-1.25$  and  $-1.05$  MPa in August 2018 and 2019, respectively.  $\Psi_{pd}$  values were significantly lower in June and July 2019 than in the same period in 2018, while the opposite trend was found for August measurements. Moreover, in August 2018  $\Psi_{min}$  values were significantly lower than in August 2019 ( $-1.25$  and  $-1.05$  MPa, respectively, Fig. 6).

Differences of basic physical-chemical grape parameters,



**Fig. 5.** Median values, 25th and 75th percentiles of minimum leaf water potential ( $\Psi_{min}$ ) measured in the ten vineyards (Ce = Ceroglie, Co = Coljava, Du = Dutovlje, Ko = Komen, Kv = Krajna vas, Kr = Križ, Pr = Prepotto, Ru = Rubbia, Sa = Sagrado, SP = San Pelagio). Different letters indicate statistically significant differences among vineyards in each sampling date (p-value < 0.05). n.s. = not significant.



**Table 4**

Summary of GLS models' output. *Df* = degrees of freedom; *Chisq* = Chi squared values.

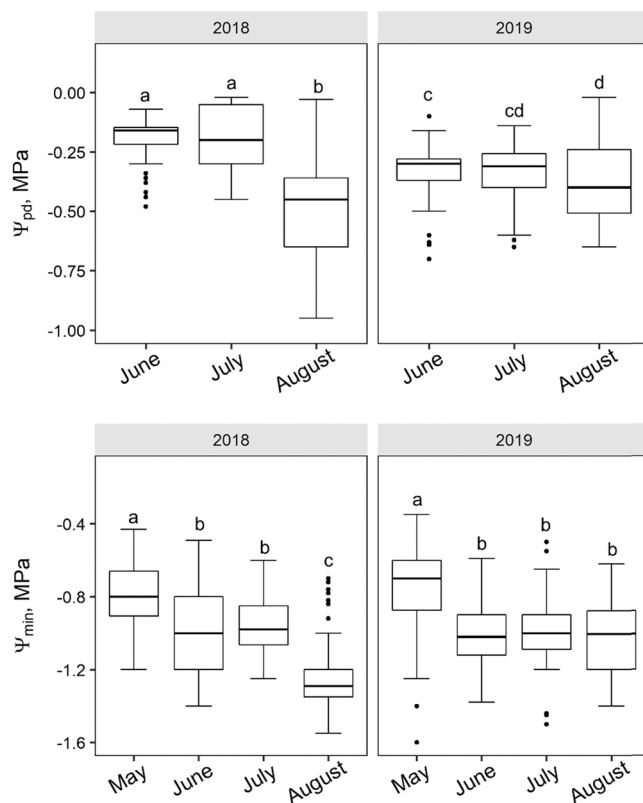
	<i>Df</i>	<i>Chisq</i>	<i>p</i> -value
<b>Response: <math>\Psi_{pd}</math></b>			
Intercept	1	71.2	< 0.001
Vineyard	9	106.7	< 0.001
Date	2	94.9	< 0.001
Year	1	5.5	0.01
Vineyard*date	18	424.7	< 0.001
Vineyard*year	9	139.2	< 0.001
Date*year	2	21.0	< 0.001
Vineyard*Date*year	18	227.3	< 0.001
<b>Response: <math>\Psi_{min}</math></b>			
Intercept	1	303.6	< 0.001
Vineyard	9	71.4	< 0.001
Date	2	91.3	< 0.001
Year	1	1.4	0.24
Vineyard*date	18	199.1	< 0.001
Vineyard*year	9	54.0	< 0.001
Date*year	2	34.0	< 0.001
Vineyard*Date*year	18	166.0	< 0.001
<b>Response: <math>\Psi_{tlp}</math></b>			
Intercept	1	717.7	< 0.001
Vineyard	4	2.6	0.63
Date	1	64.9	< 0.001
Year	1	13.3	< 0.001
Vineyard*date	4	8.8	0.06
Date*year	1	2.6	0.10
<b>Response: RT</b>			
Intercept	1	4.9	0.03
Vineyard	1	2.1	0.71
Date	4	0.6	0.44
Year	1	2.2	0.14
Vineyard*date	4	1.6	0.81
Date*year	1	4.4	0.03
<b>Response: <math>\delta^{13}C</math></b>			
Intercept	1	3449.1	< 0.001
Vineyard	4	11.6	< 0.01
Year	1	4.8	0.03
Vineyard*year	4	16.0	< 0.01

polyphenols concentration and structural characteristics of proanthocyanidins between 2018 and 2019 are shown in Figs. 7, 8, 9 and 10. Specifically, mass of 100 berries did not significantly differ between 2018 and 2019, while titratable and malic acids were higher in 2019 than in 2018, which in turn had lower pH values (Fig. 7). Moreover, total anthocyanin and total polyphenols concentration in skin were higher in 2019 than in 2018, while the opposite trend was found in total polyphenols in seeds (Fig. 8). Regarding proanthocyanidins, HMWP concentration in seeds was higher in 2018 than in 2019, while HMWP in skin, and LWMP in seeds and skin were higher in 2019 (Fig. 9). mDP and G in seed proanthocyanidins were higher in 2018 than in 2019, while no difference was found in mDP, G and P in skin proanthocyanidins (Fig. 10). A complete summary of mean values and associated standard deviation of the above-mentioned parameters, along with Student's *t*-tests results, measured in the ten monitored vineyards in 2018 and 2019 is reported in Table. A1.

#### 4. Discussion

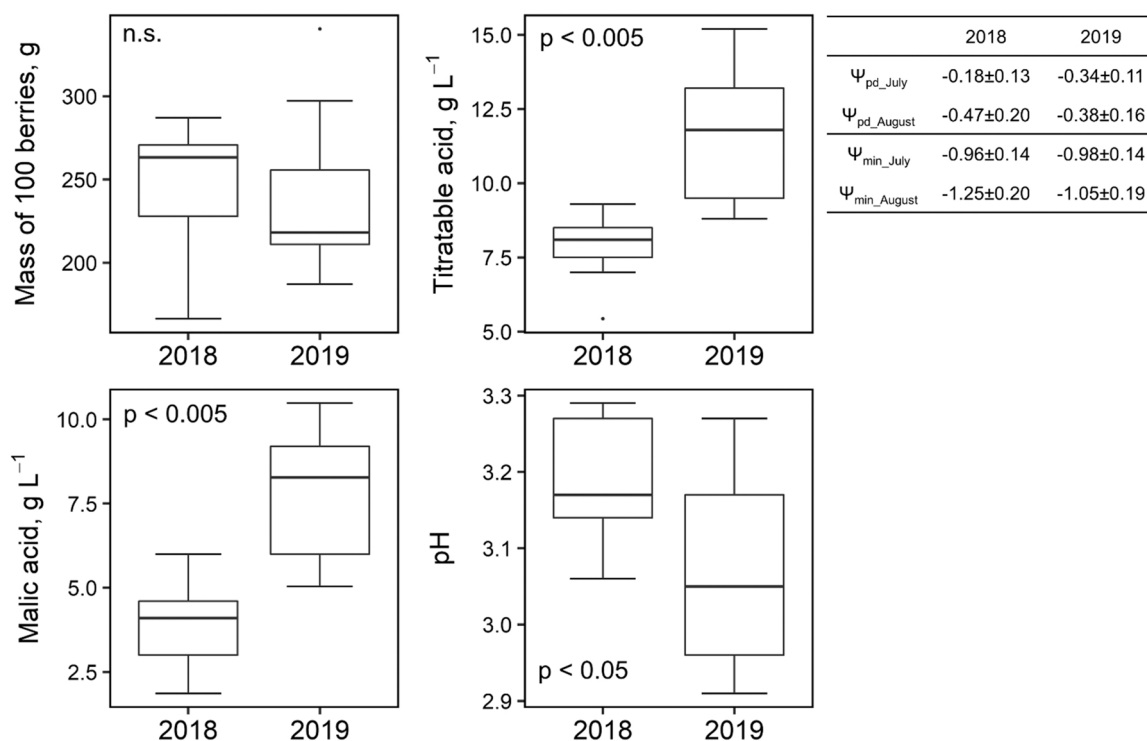
Ongoing climate changes are forcing winemakers to irrigate vineyards during drought periods in Classical Karst (Savi et al., 2018). These practises often do not consider the actual water need of vines, nor the effects of irrigation on wine quality. Our study describes the water stress levels typically experienced by Refošk vineyards in Classical Karst, highlighting its marked spatial heterogeneity and providing useful insight into the relationships between timing of water stress levels and grape's physical and chemical features.

Several studies have identified  $\Psi$  thresholds to quantify water stress levels in *V. vinifera*. According to Carbonneau (1998),  $\Psi_{pd}$  close to

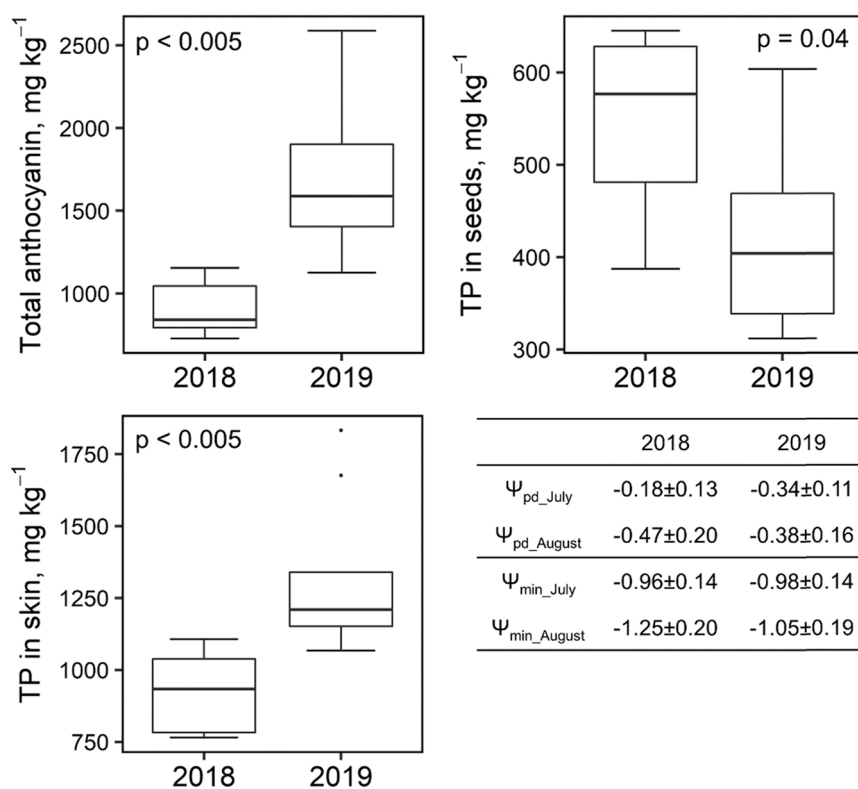


**Fig. 6.** Median values, 25th and 75th percentiles of aggregated pre-dawn and minimum leaf water potential ( $\Psi_{pd}$  and  $\Psi_{min}$ , respectively) measured in the ten vineyards during the growing season (May to August) in 2018 and 2019. Different letters indicate statistically significant differences among vineyards in each sampling date (*p*-value < 0.05).

– 0.2 MPa is typical of well-irrigated vines, – 0.4 MPa of mild stressed vines and – 0.8 MPa of severely stressed plants. On the other hand, the  $\Psi_{min}$  threshold of – 0.8 MPa was identified as proxy for well-irrigated vines, – 1.2 MPa for mild stressed vines and – 1.5 MPa for severe stress conditions (Girona et al., 2006). Generally, Karst grapevines experienced only mild water stress during the two monitoring years, with lowest values of  $\Psi_{pd}$  and  $\Psi_{min}$  being recorded in August (Figs. 4 and 5). This is confirmed by  $\Psi_{tlp}$  and RT measured in the subset of five vineyards (Figs. A1 and A2).  $\Psi_{tlp}$  decreased in summer in both years because of osmoregulation, which is a common response of plants to increasing drought conditions (Bartlett et al., 2014, 2012b). However, RT values were similar between seasons and years, with higher values in summer 2019, and ranged between 0.1 and 0.4 MPa, indicating that leaf turgor was maintained throughout the growing season. Since vineyards never reached severe water stress according to  $\Psi_{pd}$  and  $\Psi_{min}$  values, it was not deemed as necessary to apply irrigation systems in the two monitored years. Nevertheless, the vineyards had heterogeneous levels of water stress, with marked differences during the driest and warmest period (August). The variables underlying these differences might be due to multiple factors, spanning from management of vines (e.g. leaf removal and trimming) and vineyards (e.g. presence/absence of floor cover vegetation), to micro-climatic conditions in the vineyards and soil/bedrock characteristics (Abad et al., 2019; Bavougian and Read, 2018; Hochberg et al., 2018; Lovisollo et al., 2016; Nardini et al., 2021). Even though the identification of the sources of heterogeneity among vineyards was not the aim of the present study, we hypothesized that rooting depth and soil characteristics might play a key role in determining different water status between vineyards. In fact, the highest differences between vineyards were found in  $\Psi_{pd}$  (0.5 MPa in August in both years), while  $\Psi_{min}$  values were more homogeneous.  $\Psi_{pd}$  is a



**Fig. 7.** Median values, 25th and 75th percentiles of mass of 100 berries, and titratable and malic acid and pH measured in berries' juice in 2018 and 2019. p = p-values calculated through Student's *t*-test. n.s. = not significant. The table shows mean values ± standard deviations of Ψ<sub>pd</sub> and Ψ<sub>min</sub> averaged among the monitored vineyards in July and August 2018 and 2019.



**Fig. 8.** Median values, 25th and 75th percentiles of total anthocyanin, and total polyphenols (TP) concentration in seeds and skin measured in 2018 and 2019. p = p-values calculated through Student's *t*-test. n.s. = not significant. The table shows mean values ± standard deviations of Ψ<sub>pd</sub> and Ψ<sub>min</sub> averaged among the monitored vineyards in July and August 2018 and 2019.

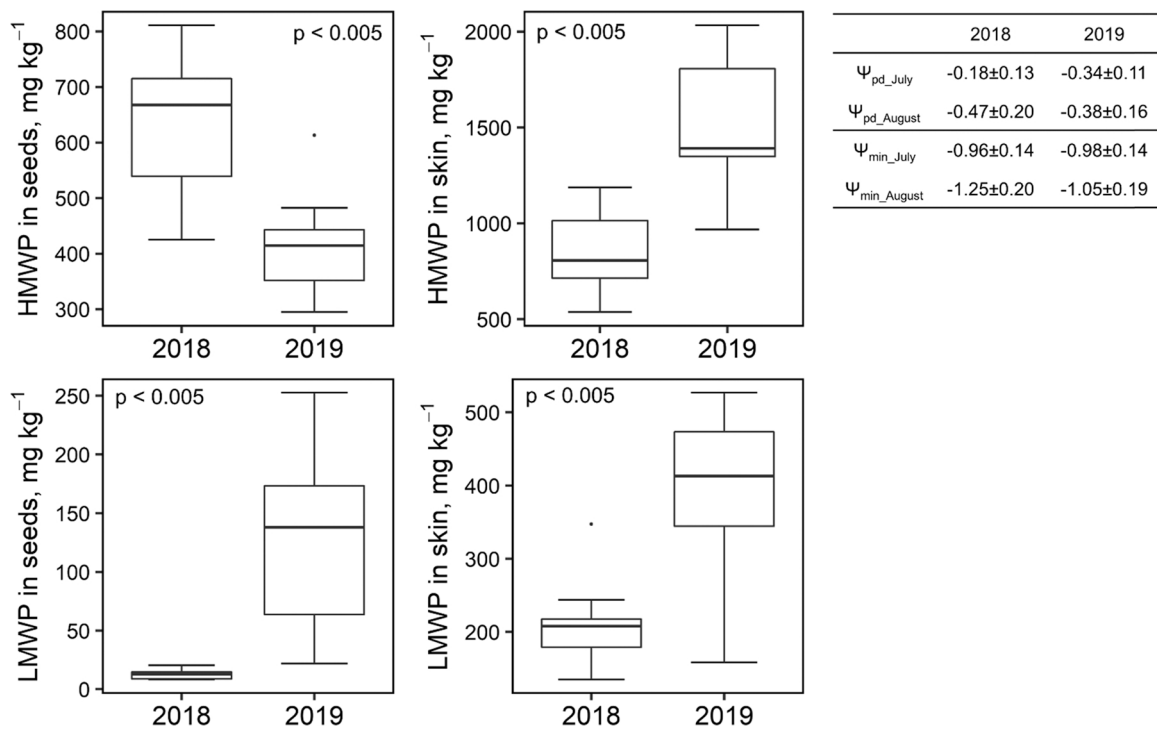


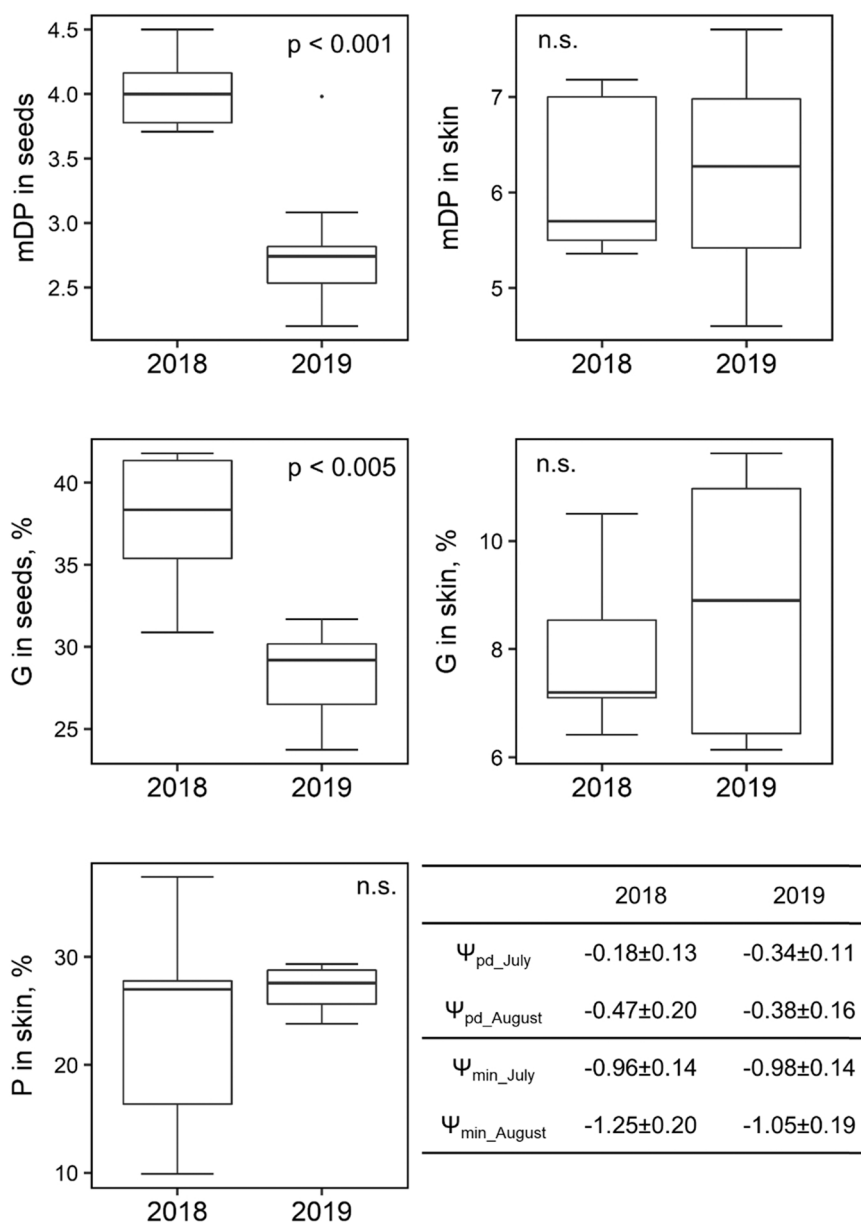
Fig. 9. Median values, 25th and 75th percentiles of HMWP and LMWP concentration in seeds and skin measured in 2018 and 2019.  $p$  =  $p$ -values calculated through Student's  $t$ -test. n.s. = not significant. The table shows mean values  $\pm$  standard deviations of  $\Psi_{pd}$  and  $\Psi_{min}$  averaged among the monitored vineyards in July and August 2018 and 2019.

commonly used proxy to estimate the water potential of soil volumes explored and exploited by the root system (Sellin, 1999) and was recently proposed as an easily and quickly measurable proxy for monitoring grapevines water status in karstic areas (Savi et al., 2019). Plants with higher  $\Psi_{pd}$  usually have higher below-ground water availability, which could be due either to a higher available water content in the soil or in rocks (Nardini et al., 2021) or to the ability of plants to access deeper water reservoirs (Nardini et al., 2016). Savi et al., (2018, 2019) have shown that Istrian Malvasia grapevines in Classical Karst can access water sources down to 6–7 m depths, possibly exploring the complex system of cracks and fissures typical of carbonate bedrocks in the area. Grapevines might have different rooting depths among the monitored vineyards, possibly explaining their heterogeneous water status. Grapevines with higher  $\Psi_{pd}$  also had lower  $\delta^{13}C$  (Fig. A3), indicating that plants accessing more stable water sources could sustain higher stomatal aperture. In addition,  $\delta^{13}C$  values ranged from  $-26.2\text{‰}$  to  $-28.3\text{‰}$ , indicating that vineyards had heterogeneous water use efficiency, spanning from high ( $< -27\text{‰}$ ) to low ( $> -28\text{‰}$ ) levels (Bota et al., 2016).

Besides heterogeneity between vineyards, water status parameters were slightly different between the two monitoring years. In fact, in July 2019  $\Psi_{pd}$  values were more negative than in July 2018, while  $\Psi_{min}$  values measured in August were lower in 2018 than in 2019 (Fig. 6). This suggests that vines experienced a (still moderate) water stress in different periods in the two years, likely as a result of the different timing of water shortage in the two years (Tables 2 and 3). We analysed meteorological data both on a monthly basis and by grouping dates on the basis of phenological periods, since several studies reported that the water stress conditions experienced by vines in relation to their phenology might be correlated with berry and wine quality (e.g.: Koundouras et al., 2009; Kuhn et al., 2014; Casassa et al., 2015; Gambetta et al., 2020). In this light, meteorological data suggest that vines suffered a longer period of water shortage after veraison in 2018, while in 2019 the longer drought period occurred in June, and only a relatively short dry spell occurred after veraison (Table 3). This was

probably due to the timing of maximum drought (in terms of CDD), which was different in the two years (Table 2). In 2019, highest CDD were recorded during June, indicating that the lack of precipitation might have impacted deep water reservoirs in early summer, thus influencing  $\Psi_{pd}$  values. On the contrary, cumulative rainfalls between the end of July and the beginning of August were much higher in 2019 (Table 2), and in fact  $\Psi_{pd}$  were higher in August 2019 than in 2018 (Fig. 6). Generally,  $\Psi_{min}$  values were similar between the two years, but they differed in August. Specifically,  $\Psi_{min}$  values were higher in August 2019, and this was probably due to precipitation regime as well. In fact, in 2018 highest CDD were recorded in the 2nd half of July. Conversely, in 2019 CDD were highest in June, and only one relatively short drought period (CDD = 9) was recorded in the 2nd half of August. Consequently, the longest drought period occurred closer to the measurement date in 2018 than in 2019, possibly leading to higher  $\Psi_{min}$  values in August 2019.

Given the different timing of maximum drought periods and of the water stress experienced by grapevines in the two years, we hypothesized that grape's physical and chemical composition and polyphenols concentration and structural characteristics might differ between the two monitored years. Indeed, several studies have demonstrated that water status directly affects berry's growth (Mirás-Avalos and Intrigliolo, 2017) and biochemical properties, for example influencing the total anthocyanins and polyphenols content (Chaves et al., 2010; Pajovic et al., 2014). Moreover, timing of water stress as well as temperature have been reported to play a key role in berries ripening, with effects also on berry's physical and chemical characteristics (Kuhn et al., 2014). In our study, berry fresh mass did not differ between the two years, indicating that the different timing of water stress did not significantly impact productivity. However, in 2018 grapevines suffered a higher water stress (in terms of  $\Psi_{min}$ ) between veraison and harvest than in 2019, and displayed lower titratable acidity and lower malic acid concentration and higher pH (Fig. 7), supporting previous results on different *Vitis* varieties (Gambetta et al., 2020). Different timing of water stress between the two years also impacted grape anthocyanins and



**Fig. 10.** Median values, 25th and 75th percentiles of mDP and G in seeds and skin, as well as of G in skin, measured in 2018 and 2019. p = p-values calculated through Student's *t*-test. n.s. = not significant. The table shows mean values  $\pm$  standard deviations of  $\Psi_{pd}$  and  $\Psi_{min}$  averaged among the monitored vineyards in July and August 2018 and 2019.

polyphenols concentration. Generally, a higher water stress in red grapes is associated with higher total anthocyanin concentration (Calderan et al., 2021; Herrera et al., 2015; Sivilotti et al., 2005), but there are studies showing no correlation (Brillante et al., 2018; Herrera et al., 2017). In our study, total anthocyanin concentration was higher in 2019 than in 2018. Despite  $\Psi_{min}$  values between veraison and harvest were lower in 2018 than in 2019,  $\Psi_{pd}$  in July was lower in 2019, probably because of the higher VPD and CDD recorded between June and veraison (Table 3). This may have affected total anthocyanins concentration, as pre-veraison water stress seemed to have stronger effects on total skin anthocyanins content (Koundouras et al., 2009). Regarding polyphenols, the higher water stress between veraison and harvest in 2018 resulted in higher TP in seeds, but lower TP in skin (Fig. 8). The effects of water stress on anthocyanin and proanthocyanidin concentration are still debated or poorly understood (Casassa et al., 2015; Castellarin et al., 2007a; Gambetta et al., 2020; Savoi et al., 2017). Both pre- and post-veraison water deficit increased proanthocyanidin concentration in

Cabernet Sauvignon (Casassa et al., 2015, but see Castellarin et al., 2007a), while no effects were detected in Merlot berries (Yu et al., 2016). In addition, Lorrain et al. (2011) reported that water shortage close to flowering stage could be correlated with an increase in ABA levels, which in turn activates the flavonoid pathway leading to an increase in proanthocyanidins concentration in Cabernet-Sauvignon and Merlot. In our study, HMWP in seeds were higher in 2018, while HMWP in skin and both LMWP in seeds and skin were higher in 2019 (Fig. 9), when longer water shortage periods and higher temperatures were observed at pre-veraison and at flowering stages. These results suggest that different timing and magnitude of water shortage might have an effect on polyphenols concentration and structural characteristics. In addition, in 2019 phenology and grape's maturation were delayed compared to 2018, probably because of lower temperature and higher cumulative precipitation (Kuhn et al., 2014), and this may have affected polyphenols concentration and structural characteristics as well. Proanthocyanidins could interact with membranes and cell walls,

resulting in a poor extractability in weak solvents as those used here (Calderan et al., 2021). Bucchetti et al. (2011) reported that the concentration of proanthocyanidins decreases during maturation and this could explain why higher values were recorded during 2019, when grapes' maturation was delayed. Moreover, in non-mature grapes the extractability of proanthocyanidins from seeds is limited by the consistency of the flesh and by the presence of a thick layer of polysaccharides covering the seeds. In 2019, the lower extractability in seeds privileged the less polymerised proanthocyanidins and with the lowest G, possibly explaining why these two parameters were significantly higher in 2018 (Fig. 10). Thus, more challenging water stress conditions in some of the vineyards could have accounted for lower extractability of proanthocyanidins in seeds. Differently, mDP and G did not differ in skin (Fig. 10). Calderan et al. (2021) reported a wider discussion on the role of water stress on grape proanthocyanidins of Refošk grapes. Proanthocyanidins affect wine quality, since HMWP are important contributors to the colour stability of red wines (Somers, 1971) and are responsible for astringency (Chira et al., 2011; Vidal et al., 2003), as well as mDP and G (Lisjak et al., 2020), while LMWPs are more responsible for bitterness (Robichaud and Noble, 1990). In this light, our results suggest that the higher water stress levels between veraison and harvest in 2018 reduced the astringency of Teran wine.

## 5. Conclusions

Karst vineyards never suffered severe water stress in both 2018 and 2019 vintages, but they displayed spatially heterogeneous responses to dry and hot spells. These differences occurred between spatially close-related vineyards, strongly supporting the importance of small-scale monitoring of grapevines' water status dynamics to design adequate and effective water management activities rather than relying on climate data solely, especially in karstic areas. Moreover, these results suggest that ongoing climate change might have different effects in the monitored vineyards, highlighting the need to better understand the mechanisms at the base of different responses to water stress. Given the differences in  $\Psi_{pd}$  values between vineyards and among years, we hypothesized that soil characteristics and rooting depth might determine water status heterogeneity between vineyards, and that these variables should be taken into account when planning water management activities (Savi et al., 2019, 2018).

Differences between the two monitoring years suggested that timing of water shortage also played a role in determining Teran wine quality, as it affected grape's physical and chemical composition. Our analyses showed that the higher (while still moderate, with  $\Psi_{pd}$  and  $\Psi_{min}$  mean values around  $-0.50$  and  $-1.25$  MPa, respectively) water stress occurred in 2018 between veraison and harvest could improve Teran wine quality, mainly by reducing acidity and by improving concentration and structural characteristics of proanthocyanidins in seeds.

## Funding

This work was supported by Agrotur II and Acquavitis projects within the Programme Interreg V-A Italy-Slovenia 2014–2020 funded by the European Regional Development Fund, and of research programme no. P4-0133 funded by Slovenian Research Agency.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agwat.2021.107288](https://doi.org/10.1016/j.agwat.2021.107288).

## References

- Abad, F.J., Marín, D., Loidi, M., Miranda, C., Royo, J.B., Urrestarazu, J., Santesteban, L. G., 2019. Evaluation of the incidence of severe trimming on grapevine (*Vitis vinifera* L.) water consumption. *Agric. Water Manag.* 213, 646–653. <https://doi.org/10.1016/j.agwat.2018.10.015>.
- Acevedo-Opazo, C., Ortega-Farías, S., Fuentes, S., 2010. Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: an irrigation scheduling application to achieve regulated deficit irrigation. *Agric. Water Manag.* 97, 956–964. <https://doi.org/10.1016/j.agwat.2010.01.025>.
- Alston, J.M., Sambucci, O., 2019. Grapes in the world economy. In: Cantu, D., Walker, M. A. (Eds.), *The grape genome*. Springer, Cham, pp. 1–24.
- Ayuda, M.I., Esteban, E., Martín-Retortillo, M., Pinilla, V., 2020. The blue water footprint of the Spanish wine industry: 1930–2015. Working paper no. 248. American Association of Wine Economists, New York.
- Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012a. Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. *Methods Ecol. Evol.* 3, 880–888. <https://doi.org/10.1111/j.2041-210X.2012.00230.x>.
- Bartlett, M.K., Scoffoni, C., Sack, L., 2012b. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol. Lett.* 15, 393–405. <https://doi.org/10.1111/j.1461-0248.2012.01751.x>.
- Bartlett, M.K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K., Sack, L., 2014. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecol. Lett.* 17, 1580–1590. <https://doi.org/10.1111/ele.12374>.
- Bavougián, C.M., Read, P.E., 2018. Mulch and groundcover effects on soil temperature and moisture, surface reflectance, grapevine water potential, and vineyard weed management. *PeerJ* 6, e5082. <https://doi.org/10.7717/peerj.5082>.
- Bota, J., Tomás, M., Flexas, J., Medrano, H., Escalona, J.M., 2016. Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. *Agric. Water Manag.* 164, 91–99. <https://doi.org/10.1016/j.agwat.2015.07.016>.
- Brillante, L., Martínez-Lüscher, J., Kurtural, S.K., 2018. Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (*Vitis vinifera* L., cv. Syrah) in Central California. *Sci. Hortic.* 227, 261–271. <https://doi.org/10.1016/j.scienta.2017.09.048>.
- Bucchetti, B., Matthews, M.A., Falginella, L., Peterlunger, E., Castellarin, S.D., 2011. Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Sci. Hortic.* 128, 297–305. <https://doi.org/10.1016/j.scienta.2011.02.003>.
- Calderan, A., Sivilotti, P., Braidotti, R., Mihelčić, A., Lisjak, K., Vanzo, A., 2021. Managing moderate water deficit increased anthocyanin concentration and proanthocyanidin galloylation in “Refošk” grapes in Northeast Italy. *Agric. Water Manag.* 246, 106684. <https://doi.org/10.1016/j.agwat.2020.106684>.
- Carbonneau, A., 1998. Aspects qualitatifs, 258–276. *Traite d'irrigation*. Tec&Doc. Lavosier Ed., Paris, 1011.
- Casassa, L.F., Keller, M., Harbertson, J.F., 2015. Regulated deficit irrigation alters anthocyanins, tannins and sensory properties of cabernet Sauvignon grapes and wines. *Mol.* 20, 7820–7844. <https://doi.org/10.3390/molecules20057820>.
- Castellarin, S.D., Matthews, M.A., Di Gaspero, G., Gambetta, G.A., 2007a. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227, 101–112. <https://doi.org/10.1007/s00425-007-0598-8>.
- Castellarin, S.D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., Di Gaspero, G., 2007b. Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30, 1381–1399. <https://doi.org/10.1111/j.1365-3040.2007.01716.x>.
- Chacón-Vozmediano, J.L., Martínez-Gascuña, J., García-Navarro, F.J., Jiménez-Ballesta, R., 2020. Effects of water stress on vegetative growth and ‘Merlot’ grapevine yield in a semi-arid mediterranean climate. *Hortic.* 6, 95. <https://doi.org/10.3390/horticulturae6040095>.
- Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676. <https://doi.org/10.1093/aob/mcq030>.
- Chira, K., Pacella, N., Jourdes, M., Teissedre, P.-L., 2011. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chem.* 126, 1971–1977. <https://doi.org/10.1016/j.foodchem.2010.12.056>.
- Choné, X., van Leeuwen, C., Dubourdieu, D., Gaudillère, J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* 87, 477–483. <https://doi.org/10.1006/anbo.2000.1361>.
- Costa, J.M., Vaz, M., Escalona, J., Egipto, R., Lopes, C., Medrano, H., Chaves, M.M., 2016. Modern viticulture in southern Europe: vulnerabilities and strategies for adaptation to water scarcity. *Agric. Water Manag.* 164, 5–18. <https://doi.org/10.1016/j.agwat.2015.08.021>.
- Cucchi, F., Piano, C., 2013. Brevi note illustrative della carta geologica del Carso Classico Italiano. *Progetto GEO-CGT-Cartografia Geologica di sintesi in scala, 1 (10.000)*, 41.
- Di Stefano, R., Cravero, M.C., Gentilini, N., 1989. Methods for the study of wine polyphenols. *L'Enotecnico* 5, 83–89.
- Fernández, J.E., Cuevas, M.V., 2010. Irrigation scheduling from stem diameter variations: a review. *Agric. Meteorol.* 150, 135–151. <https://doi.org/10.1016/j.agrformet.2009.11.006>.
- Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S.D., 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *J. Exp. Bot.* 71, 4658–4676. <https://doi.org/10.1093/jxb/eraa245>.



- Gams, I., 1993. Origin of the term "karst," and the transformation of the classical karst (kras). *Environ. Geol.* 21 (3), 110–114. <https://doi.org/10.1007/BF00775293>.
- Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. *Glob. Planet. Chang.* 63, 90–104. <https://doi.org/10.1016/j.gloplacha.2007.09.005>.
- Girona, J., Mata, M., del Campo, J., Arbonés, A., Marsal, J., 2006. The use of midday leaf water potential for scheduling deficit irrigation in vineyards. *Irrig. Sci.* 24, 115–127. <https://doi.org/10.1007/s00271-005-0015-7>.
- Hannah, L., Roehrdanz, P.R., Ikegami, M., Shepard, A.V., Shaw, M.R., Tabor, G., Zhi, L., Marquet, P.A., Hijmans, R.J., 2013. Climate change, wine, and conservation. *Proc. Nat. Acad. Sci.* 110, 6907–6912. <https://doi.org/10.1073/pnas.1210127110>.
- Herrera, J.C., Bucchetti, B., Sabbatini, P., Comuzzo, P., Zulini, L., Vecchione, A., Peterlunger, E., Castellarin, S.D., 2015. Effect of water deficit and severe shoot trimming on the composition of *Vitis vinifera* L. Merlot grapes and wines. *Aust. J. Grape Wine Res.* 21, 254–265. <https://doi.org/10.1111/ajgw.12143>.
- Herrera, J.C., Hochberg, U., Degu, A., Sabbatini, P., Lazarovitch, N., Castellarin, S.D., Fait, A., Alberti, G., Peterlunger, E., 2017. Grape metabolic response to postveraison water deficit is affected by interseason weather variability. *J. Agric. Food Chem.* 65, 5868–5878. <https://doi.org/10.1021/acs.jafc.7b01466>.
- Hochberg, U., Degu, A., Cramer, G.R., Rachmilevitch, S., Fait, A., 2015. Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior. *Plant Physiol. Biochem.* 88, 42–52. <https://doi.org/10.1016/j.plaphy.2015.01.006>.
- Hochberg, U., Rockwell, F.E., Holbrook, N.M., Cochard, H., 2018. Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. *Trends Plant Sci.* 23, 112–120. <https://doi.org/10.1016/j.tplants.2017.11.002>.
- Jackson, R.S., 1994. *Wine Science: Principles and Applications*. Academic Press.
- Knauer, J., El-Madany, T.S., Zaehle, S., Migliavacca, M., 2018. Bigleaf—An R package for the calculation of physical and physiological ecosystem properties from eddy covariance data. *PLoS One* 13, e0201114. <https://doi.org/10.1371/journal.pone.0201114>.
- Koundouras, S., Hatzidimitriou, E., Karamolegkou, M., Dimopoulou, E., Kallithraka, S., Tsialtas, J.T., Zioziou, E., Nikolaou, N., Kotsieridis, Y., 2009. Irrigation and rootstock effects on the phenolic concentration and aroma potential of *Vitis vinifera* L. cv. cabernet sauvignon grapes. *J. Agric. Food Chem.* 57, 7805–7813. <https://doi.org/10.1021/jf901063a>.
- Kozjak, P., Korošec-Koruz, Z., Javornik, B., 2003. Characterisation of cv. Refošk (*Vitis vinifera* L.) by SSR markers. *Vitis* 42, 83–86.
- Kuhn, N., Guan, L., Dai, Z.W., Wu, B.-H., Lauvergeat, V., Gomès, E., Li, S.-H., Godoy, F., Arce-Johnson, P., Delrot, S., 2014. Berry ripening: recently heard through the grapevine. *J. Exp. Bot.* 65, 4543–4559. <https://doi.org/10.1093/jxb/ert395>.
- Kyraleou, M., Kallithraka, S., Theodorou, N., Teissedre, P.-L., Kotsieridis, Y., Koundouras, S., 2017. Changes in tannin composition of syrah grape skins and seeds during fruit ripening under contrasting water conditions. *Mol* 22, 1453. <https://doi.org/10.3390/molecules22091453>.
- Lenth, R.V., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package Version 1.5.0. (<https://CRAN.R-project.org/package=emmeans>).
- Levin, A.D., 2019. Re-evaluating pressure chamber methods of water status determination in field-grown grapevine (*Vitis* spp.). *Agric. Water Manag.* 221, 422–429. <https://doi.org/10.1016/j.agwat.2019.03.026>.
- Lionello, P., Abrantes, F., Gacic, M., Planton, S., Trigo, R., Ulbrich, U., 2014. The climate of the Mediterranean region: research progress and climate change impacts. *Reg. Environ. Change* 14, 1679–1684. <https://doi.org/10.1007/s10113-014-0666-0>.
- Lisjak, K., Lelova, Z., Žigon, U., Bolta, S.V., Teissedre, P.-L., Vanzo, A., 2020. Effect of extraction time on content, composition and sensory perception of proanthocyanidins in wine-like medium and during industrial fermentation of Cabernet Sauvignon. *J. Sci. Food Agric.* 100, 1887–1896. <https://doi.org/10.1002/jsfa.10189>.
- Lorrain, B., Chira, K., Teissedre, P.-L., 2011. Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: comparison to 2006, 2007 and 2008 vintages. *Food Chem.* 126, 1991–1999. <https://doi.org/10.1016/j.foodchem.2010.12.062>.
- Lovisol, C., Lavoie-Lamoureux, A., Tramontini, S., Ferrandino, A., 2016. Grapevine adaptations to water stress: new perspectives about soil/plant interactions. *Theor. Exp. Plant Physiol.* 28, 53–66. <https://doi.org/10.1007/s40626-016-0057-7>.
- Marx, W., Haunschild, R., Bornmann, L., 2017. Climate change and viticulture - a quantitative analysis of a highly dynamic research field. *VITIS* 56, 35–43. <https://doi.org/10.5073/vitis.2017.56.35-43>.
- Mattivi, F., Prast, A., Nicolini, G., Valentini, L., 2002. Validazione di un nuovo metodo per la misura del potenziale polifenolico delle uve rosse e discussione del suo campo di applicazione in enologia. *Riv. di Vitic. e di Enol.* 55, 55–74.
- Mirás-Avalos, J.M., Intrigliolo, D.S., 2017. Grape composition under abiotic constraints: water stress and salinity. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.00851>.
- Mrak, I., Repe, B., 2004. Vine and the vine growing in the area of Kras (Slovenia). *Geodria* 9, 223–242.
- Nardini, A., Salleo, S., Trifilò, P., Gullo, M.A.L., 2003. Water relations and hydraulic characteristics of three woody species co-occurring in the same habitat. *Ann. Sci.* 60, 297–305. <https://doi.org/10.1051/forest:2003021>.
- Nardini, A., Casolo, V., Dal Borgo, A., Savi, T., Stenni, B., Bertoncin, P., Zini, L., McDowell, N.G., 2016. Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms differentially affected by an extreme summer drought. *Plant Cell Environ.* 39, 618–627. <https://doi.org/10.1111/pce.12646>.
- Nardini, A., Petruzzellis, F., Marusig, D., Tomasella, M., Natale, S., Altobelli, A., Calligaris, C., Floriddia, G., Cucchi, F., Forte, E., Zini, L., 2021. Water 'on the rocks': a summer drink for thirsty trees? *N. Phytol.* 229, 199–212. <https://doi.org/10.1111/nph.16859>.
- OIV, 2016. OIV Rep. World Vitivinic. Situat. 2000, 3. (<http://www.oiv.int/public/medias/5029/world-vitiviniculture-situation-2016.pdf>).
- Pajovic, R., Raicevic, D., Popovic, T., Sivilotti, P., Lisjak, K., Vanzo, A., 2014. Polyphenolic characterisation of Vranac, Kratosija and Cabernet Sauvignon (*Vitis vinifera* L. cv.) grapes and wines from different vineyard locations in Montenegro. *South Afr. J. Enol. Vitic.* 35, 139–148.
- Petruzzellis, F., Savi, T., Bacaro, G., Nardini, A., 2019. A simplified framework for fast and reliable measurement of leaf turgor loss point. *Plant Physiol. Biochem.* 139, 395–399. <https://doi.org/10.1016/j.plaphy.2019.03.043>.
- Pinasseau, L., Vallverdú-Queralt, A., Verbaere, A., Roques, M., Meudec, E., Le Cunff, L., Péros, J.-P., Ageorges, A., Sommerer, N., Boulet, J.-C., Terrier, N., Cheyner, V., 2017. Cultivar diversity of grape skin polyphenol composition and changes in response to drought investigated by LC-MS based metabolomics. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.01826>.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R., 2020. nlme: Linear and nonlinear mixed effects models. R. Package Version 3, 1–149. (<https://CRAN.R-project.org/package=nlme>).
- Prieto, I., Querejeta, J.I., Segrestin, J., Volaire, F., Roumet, C., 2018. Leaf carbon and oxygen isotopes are coordinated with the leaf economics spectrum in Mediterranean rangeland species. *Funct. Ecol.* 32, 612–625. <https://doi.org/10.1111/1365-2435.13025>.
- Rigo, A., Vianello, F., Clementi, G., Rossetto, M., Scarpa, M., Vrhovšek, U., Mattivi, F., 2000. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *J. Agric. Food Chem.* 48, 1996–2002. <https://doi.org/10.1021/jf991203d>.
- Robichaud, J.L., Noble, A.C., 1990. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* 53, 343–353. <https://doi.org/10.1002/jsfa.2740530307>.
- Romić, D., Karoglan Kantić, J., Preiner, D., Romić, M., Lazarević, B., Maletić, E., Ondrašek, G., Andabaka, Z., Bakić Begić, H., Bubalo Kovačić, M., Filipović, L., Husnjak, S., Marković, Z., Stupić, D., Tomaz, I., Zovko, M., 2020. Performance of grapevine grown on reclaimed Mediterranean karst land: Appearance and duration of high temperature events and effects of irrigation. *Agric. Water Manag.* 236, 106166. <https://doi.org/10.1016/j.agwat.2020.106166>.
- Savi, T., Petruzzellis, F., Martellos, S., Stenni, B., Dal Borgo, A., Zini, L., Lisjak, K., Nardini, A., 2018. Vineyard water relations in a karstic area: deep roots and irrigation management. *Agric. Ecosyst. Environ.* 263, 53–59. <https://doi.org/10.1016/j.agee.2018.05.009>.
- Savi, T., Petruzzellis, F., Moretti, E., Stenni, B., Zini, L., Martellos, S., Lisjak, K., Nardini, A., 2019. Grapevine water relations and rooting depth in karstic soils. *Sci. Total Environ.* 692, 669–675. <https://doi.org/10.1016/j.scitotenv.2019.07.096>.
- Savoi, S., Wong, D.C.J., Degu, A., Herrera, J.C., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F., Castellarin, S.D., 2017. Multi-Omics and Integrated Network Analyses Reveal New Insights into the Systems Relationships between Metabolites, Structural Genes, and Transcriptional Regulators in Developing Grape Berries (*Vitis vinifera* L.) Exposed to Water Deficit. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.01124>.
- Schwinning, S., 2010. The ecohydrology of roots in rocks. *Ecohydrol* 3, 238–245. <https://doi.org/10.1002/eco.134>.
- Seguin, G., 1988. Ecosystems of the great red wines produced in the maritime climate of Bordeaux. In *Proceedings of the Symposium on Maritime Climate Winegrowing*. L. Fuller-Perrine, 36–53.
- Sellin, A., 1999. Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? *Acta Oecol* 20, 51–59. [https://doi.org/10.1016/S1146-609X\(99\)80015-0](https://doi.org/10.1016/S1146-609X(99)80015-0).
- Sivilotti, P., Bonetto, C., Paladin, M., Peterlunger, E., 2005. Effect of Soil Moisture Availability on Merlot: From Leaf Water Potential to Grape Composition. *Am. J. Enol. Vitic.* 56, 9–18.
- Smith, A.J., et al., 2018. RmarineHeatWaves: Package for the calculation of marine heat waves. Version 0.16.1. University of the Western Cape, Bellville, Cape Town, South Africa. (<https://github.com/ajsmiit/RmarineHeatWaves>).
- Somers, T.C., 1971. The polymeric nature of wine pigments. *Phytochem* 10, 2175–2186. [https://doi.org/10.1016/S0031-9422\(00\)97215-7](https://doi.org/10.1016/S0031-9422(00)97215-7).
- Sonntag, D., 1994. Advancements in the field of hygrometry. *Meteorol. Z.*, N. F. 3, 51–66.
- Tombesi, S., Nardini, A., Farinelli, D., Palliotti, A., 2014. Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of *Vitis vinifera*. *Physiol. Plant.* 152, 453–464. <https://doi.org/10.1111/ppl.12180>.
- Tripathi, A., Tripathi, D.K., Chauhan, D.K., Kumar, N., Singh, G.S., 2016. Paradigms of climate change impacts on some major food sources of the world: a review on current knowledge and future prospects. *Agric. Ecosyst. Environ.* 216, 356–373. <https://doi.org/10.1016/j.agee.2015.09.034>.
- Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheyner, V., Waters, E.J., 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.* 83, 564–573. <https://doi.org/10.1002/jsfa.1394>.
- Vrhovšek, U., Mattivi, F., Waterhouse, A.L., 2001. Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. *VITIS* 40, 87–91. <https://doi.org/10.5073/vitis.2001.40.87-91>.
- Wenter, A., Zanotelli, D., Montagnani, L., Tagliavini, M., Andreotti, C., 2018. Effect of different timings and intensities of water stress on yield and berry composition of grapevine (cv. Sauvignon blanc) in a mountain environment. *Sci. Hortic.* 236, 137–145. <https://doi.org/10.1016/j.scienta.2018.03.037>.
- Yu, R., Cook, M.G., Yacco, R.S., Watrelot, A.A., Gambetta, G., Kennedy, J.A., Kurtural, S. K., 2016. Effects of Leaf Removal and Applied Water on Flavonoid Accumulation in

Grapevine (*Vitis vinifera* L. cv. Merlot) Berry in a Hot Climate. J. Agric. Food Chem. 64, 8118–8127. <https://doi.org/10.1021/acs.jafc.6b03748>.