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## Relationships between grape composition of Tempranillo variety and available soil water and water stress under different weather conditions

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### ABSTRACT

The aim of the research was to analyse the relationship between grape composition of the Tempranillo variety, and available soil water along the growing cycle. The study was conducted in the Rioja DOCa (Qualified Designation of Origin (Spain)). Grape composition (berry weight, acidity and phenolic composition) was analysed between veraison and maturity in non-irrigated vines and related to available soil water along the growing cycle. The available soil water (ASW) was simulated for the period 2008–2018, taking into account soil properties and the weather conditions recorded at each location. Soil water was simulated for each plot and year analysed, after calibration in one plot and the ASW was evaluated under the different conditions. The results showed that soil properties conditioned available soil water, which influenced berry weight, acidity, anthocyanins and total polyphenol index. Under the rainfed conditions in which the vines were cultivated, vines suffered from moderate to high water stress in some periods along the growing cycle. The results showed the most critical periods regarding water availability for grape composition. An increase in available soil water between one and three weeks after bloom and at the end of the ripening period increased acidity and decreased pH while an increase in available soil water content between two and seven weeks after bloom and at the ripening period increased berry weight and decreased anthocyanins and other phenolic compounds.

### 1. Introduction

Berry composition is influenced by factors such as soil characteristics, management, cultivar and climate (van Leeuwen et al., 2004). Among all these factors, climate plays a very important role (Jones and Storchmann, 2001; Robinson et al., 2012). The vine cultivars that can be grown in a given location are conditioned by temperature (Jones et al., 2012) and its variations affect grape development during the growing period and its final composition (Sadras and Moran, 2013; Webb et al., 2012; Greer and Weedon, 2013; Ovadia et al., 2013). However, within a specific climate zone, soil characteristics are important factors controlling vine development and grape composition (Cheng et al., 2014; Zerihun et al., 2015). Soil physical properties, and in particular soil particle distribution and porosity govern the volume of soil that can be explored by roots, and also affect water and nutrient movement and soil water storage (Lanyon et al., 2004; Seguin, 1986; Costantini et

al., 2006; Ramos et al., 2015), as well as water availability, and finally, the vine water status (Costantini et al., 2010; Tramontini et al., 2013). Thus, water availability could play an important role on vine development and the final production, and it could be used to determine vine water stress.

Vines are sensitive to water stress during the growing cycle and both early and late-season deficits decreased yield (Matthews et al., 1987). Berry growth is more sensitive to water deficits after bloom and if the dry periods take place after fruit formation they lead to a decrease in the water used, which has also an impact in grape quality. However, reductions in berry growth caused by water stress during the bud-break-bloom period cannot be reversed by supplemental water inputs during the bloom-veraison and veraison-harvest periods (Grimes and Williams, 1990). These authors indicated that water used by plants decreases when soil water is depleted to the point that plant is stressed, and yields also will be reduced. Peacock (2005) indicates that a water

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deficit up to 50 % of evapotranspiration (ETc) has a minimum effect on yield. However, yield decreases if this threshold is overpassed. In this respect, Ramos and Martínez-Casasnovas (2010) confirmed that the years in which water deficit during the growing period was higher than 50 % ETc, were years of low yield, and that yield was affected by water available during the budbreak-bloom period.

In addition, water deficits can affect also berry composition (Esteban et al., 1999; Deloire et al., 2005; Downey et al., 2006; Gómez-Míguez et al., 2007; van Leeuwen et al., 2009), being the effect dependent on the level of stress and the time period when it appears (Ojeda et al., 2002; Roby et al., 2004; Castellarin et al., 2007). Intrigliolo et al. (2012) found that late water deficits affect berry sugar accumulation due to the effect of water stress on leaf photosynthesis. Cooley et al. (2017) found an increase in total acidity under different irrigations treatments and that the malic acid concentration of grape juice was reduced under both regulated and prolonged deficit irrigation, resulting in an enhancement of the tartaric/malic acid ratio. Bellvert et al. (2016) also indicated that pre-veraison water stress negatively affected aroma quality, titratable acidity, and malic acid.

Regarding the effect on anthocyanins and other phenolic compounds, it has been reported that water deficit changed anthocyanin composition, as well as the composition and the accumulation of flavonols or proanthocyanidins (Torres et al., 2018). In general, water deficit enhanced the accumulation of phenylpropanoids, monoterpenes, and tocopherols, while for other compounds such as carotenoids and flavonoids, the accumulation depend on the grapevine development state (Savoi et al., 2016) and on the stress level. In this respect, Balint and Reynolds (2014) found that total anthocyanin concentration was highly affected by irrigation treatments, finding the lowest anthocyanin and phenol concentrations under the irrigation treatment corresponding to the 100 % of the crop evapotranspiration (ETc). Similarly, Cooley et al. (2017) found wine colour density, anthocyanin, ionised anthocyanin and phenolic substances being affected by the irrigation treatment. Bucchetti et al. (2011) also indicated that water deficits consistently increased anthocyanin concentration by increasing content per berry and reducing fruit growth. Talaverano et al. (2017); Mendez-Costabel et al. (2014) and Ou et al. (2010), among others, indicated the influence of water stress in some alcohols and on different volatile compounds responsible of aroma characteristics, which may have consequences on wine sensory perception.

The research was carried out in the Rioja DOCa, which is the most important producer region of the Tempranillo (*Vitis vinifera* L.) variety worldwide. In previous research carried out in the study area, the effect of weather conditions and the influence of some soil properties in the response of the vine were already analysed (Ramos and Martínez de Toda, 2019). In this research, the aim was to acquire a deep knowledge of the influence of available soil water and the time in which water stress can be more critical for grape composition. To that end, grape composition of the variety Tempranillo, cultivated under rainfed conditions, was analysed in years with different weather characteristics, and related to the stress conditions that the vine suffered along its growing cycle. This variety is adapted to Mediterranean climate and it has been expanded to other countries (among them, Australia, Chile, Greece, USA and South Africa). Thus, the results observed in this specific area related to the influence of available water and water stress on grape quality could be extrapolated to other areas around the world, taken into account the soil characteristic where the vines are planted.

## 2. Material and methods

The research was carried out in the DOCa Rioja (Spain) grapevine growing area. This region is located in central part of northern Spain

and has about 64,000 ha of vines, mostly red varieties. The vines are cultivated from the terraces of the Ebro River to elevations up to about 700 m a.s.l. The climatic conditions recorded along the regions allowed establishing different zones with Atlantic and Mediterranean influence, which are named, respectively, Rioja Alta (about 26,780 ha with Atlantic influence), Rioja Oriental (about 23,870 ha, with Mediterranean influence) and Rioja Alavesa (about 13,000 ha), with intermediate climatic influence (Consejo Regulador DOCa 2017). Tempranillo is the main cultivated variety in the region, which represent about 80 % of the vineyard area. Most vineyards of the Rioja DOCa use tillage for soil-surface management, and the norm of this Appellation limits the red grape yield to 6500 kg/ha.

### 2.1. Study area

This research included the analysis on six plots located at similar elevation (between 428 and 465 m a.s.l.) in the municipalities of Haro (P1 and P2), Cenicero (P3), Fuenmayor (P4), San Vicente de la Sonsierra (P5) and San Asensio (P6) in Rioja Alta zone (Fig. 1). All plots were planted with the Tempranillo variety, between 1987 and 2000 (1993, 1997, 1998, 2000, 1987, 1990 and 1999, respectively). Vines were trained to a vertical shoot positioning with two arms and pruned to six spurs (12 buds) per vine with a planting pattern of 2.5–2.7 m between rows and 1.2–1.3 m between plants, which means 2800 to 3500 vines/ha. The vines were cultivated under rainfed conditions.

An additional plot was considered (P7), in which soil water was measured during four years. This plot was situated in the municipality of Nájera (Fig. 1), and planted with similar pattern (2.7 m between rows and 1.3 m between plants).

### 2.2. Climate data

The weather conditions recorded during the studied period (2008–2018) were analysed using the information from the meteorological stations of Haro, Uruñuela, Logroño, Nájera and San Vicente de la Sonsierra, which were the nearest stations to the analysed plots (Fig. 1). Those stations belong to La Rioja Government. Daily maximum and minimum temperatures, precipitation, solar radiation, relative humidity and wind speed were analysed. Crop evapotranspiration was also evaluated for each meteorological station, which was calculated from the potential evapotranspiration obtained using the FAO Penman Monteith equation and the crop coefficients proposed by Allen et al. (1998). For the plots located between two observatories a weighted average was estimated taking into account the inverse of the distance to each station, and when it was necessary a correction for differences in elevation was applied.

### 2.3. Soil properties

The soils of the selected plots are classified as *Calcixerollic Xerochrept* (IGN, 2006). Soil characteristics of the studied plots related to soil organic carbon, soil particle distribution (clay, sand, silt and coarse elements) and soil water retention corresponding to field capacity and wilting point were obtained from the European Soil data base (ESDAC; esdac.jrc.ec.europa.eu, European Commission, Joint Research Centre). The soils of the studied plots have clay contents that range between 19.7 and 25.5 %; silt contents that range between 34.6 and 45.3 % and sand contents that ranged between 29.2 and 39.3 %. The organic carbon content varied between 0.49 and 1.53 %. The water retention corresponding to field capacity ranged between 25.4 and 30.1 % and that for the wilting point ranged between 10.9 and 15.3 %. The soils characteristics of the plots used in this research are shown in Table 1.

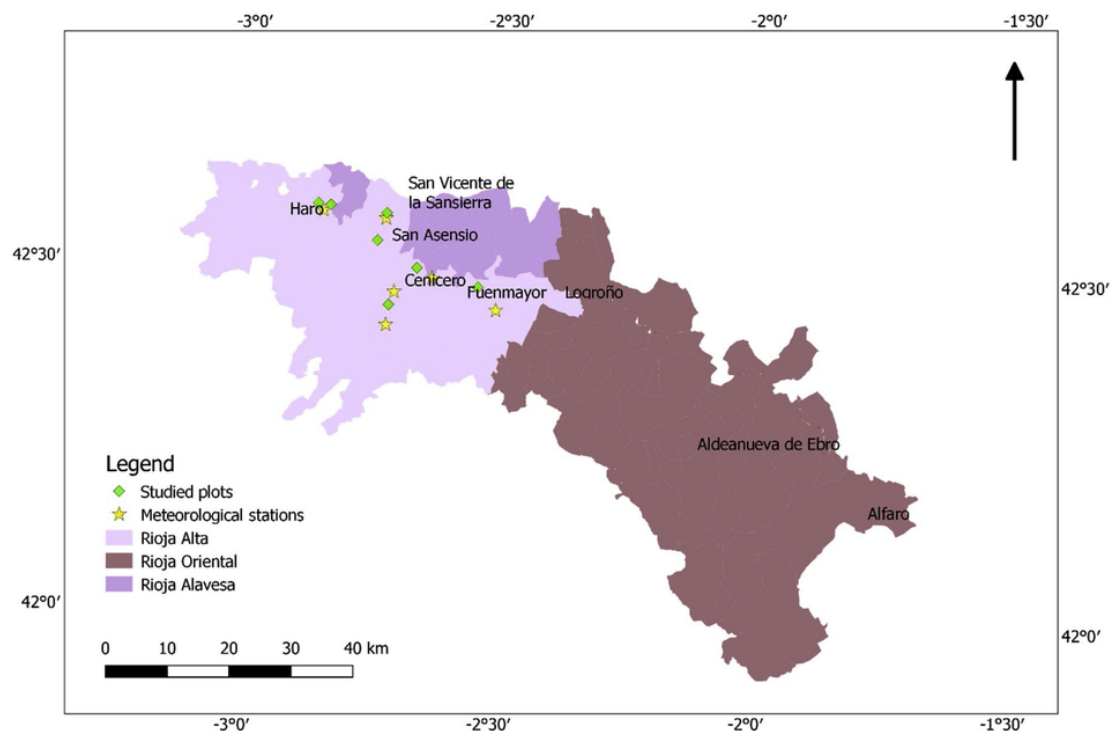


Fig. 1. Map of the three zones in which are divided the Rioja DOCa growing region (Rioja Alta, Rioja Oriental and Rioja Alavesa). Location of the seven plots (P1 to P7) and the six meteorological stations used in this research.

Table 1

Soil properties of the analysed plots (P1–P7). (Elev: plot altitude in m above sea level; OM: organic matter; FC: field capacity and WP: wilting point).

Plot	Elev (m a.s.l.)	Clay (%)	Silt (%)	Sand (%)	Coarse elements (%)	OM (%)	FC (%)	WP (%)
P1	438	22.8	34.6	42.6	13.2	1.10	26.6	12.8
P2	465	22.3	38.4	39.3	14.2	1.00	26.9	12.4
P3	450	25.5	45.3	29.2	13.0	0.75	30.1	13.7
P4	428	19.7	41.7	38.6	18.0	1.53	25.4	11.0
P5	440	22.2	43.2	33.9	17.8	1.40	27.6	15.3
P6	457	25.9	43.4	30.7	14.5	0.49	27.0	10.9
P7	450	18.5	43.2	38.3	12.0	0.96	27.0	15.5

#### 2.4. Available soil water

In order to evaluate the effect of available soil water on grape composition, soil water was simulated for each plot and year, considering soil properties and the weather conditions recorded in each year. The Vineyard-Soil Irrigation Model (VSIM –<https://sites.google.com/a/csumb.edu/vsim/>) was used to simulate soil water. This model allows simulating soil water content at daily time scale for the whole profile, based on a soil water balance, which included water inputs (rainfall and irrigation in case the crop was irrigated), crop evapotranspiration and drainage. The inputs for the model included weather (daily temperature, precipitation and potential evapotranspiration) and soil (soil particle distribution and gravel contents, water retention capacity at –33 kPa and at 1500 kPa and rooting depth) characteristics. Regarding the vines, the model takes into account vine and row spacing and cover crop. The model was calibrated and validated with the data recorded in P7. Soil water content was monitored in that plot from 2009 to 2012 at different soil depths (30, 60 and 100 cm), using TDT (Time Domain Transmissometry) GroGraph Moisture Solution probes (ESI Environ-

mental Sensors Inc, Sidney BC, Canada), which recorded information at 30 min intervals. These data were divided into two series: the periods 2009–2010 and 2011–2012 were used for model calibration and validation, respectively. The model performance for both calibration and validation was analysed using three statistical methods: the ratio of the root mean square error to the standard deviation (RSR); the Nash–Sutcliffe efficiency (NSE; Nash and Sutcliffe, 1970) and the percent bias (PBIAS, %; Gupta et al., 1999). (Eqs. (1, 2 and 3), respectively).

$$RSR = \frac{\sqrt{\sum_{i=1}^n (Y_m - Y_s)^2}}{\sqrt{\sum_{i=1}^n (Y_m - \bar{Y})^2}} \quad (1)$$

$$NSE = 1 - \frac{\sum_{i=1}^n (Y_m - Y_s)^2}{\sum_{i=1}^n (Y_m - \bar{Y})^2} \quad (2)$$

$$PBIAS = \frac{\sum_{i=1}^n (Y_m - Y_s) * 100}{\sum_{i=1}^n (Y_m)} \quad (3)$$

Where  $Y_m$  is the measured value,  $Y_s$  is the simulated value and  $\bar{Y}$  is the mean of the measured values of the parameter analysed.

Then, the model was run for each plot and year. Taking into account the maximum soil water content at field capacity (FC) and the wilting point (WP), the maximum available soil water (water held in the soil between its field capacity and permanent wilting point) of each soil was calculated (AWC). The available soil water (differences between soil water and the wilting point) (ASW) was evaluated along the growing cycle for each plot and year. Particular attention was paid to the periods between flowers separated and veraison and after veraison. The information about phenology, according to the Baggliolini scale, was obtained from the Consejo Regulador of Rioja DOCa (personal

communication). The water stress conditions that the existing ASW implied for the vine were also analysed. Vine water stress was quantified considering the fraction of the ASW, estimated considering the ASW and the AWC. The levels of water deficit (weak, moderate and severe) were established from the results observed by Pellegrino et al. (2005) and van Leeuwen et al. (2009). The thresholds were established in terms of the percentage of the AWC as follow: weak water deficit: 32-20 % of AWC; weak to moderate water deficit: 20–8 % of AWC; moderate to severe 8–2 % of AWC and severe water deficit for values < 2 % of AWC. Those thresholds were considered to estimate the vine water deficit.

## 2.5. Grape composition

Grape composition was analysed weekly during ripening (from veraison to maturity) for the period of analysis (2008–2018). Berry weight (weight of 100 berries-WB), sugar content (expressed as probable volumetric alcoholic degree-PVAD), total acidity (AcT), malic acid (AcM), pH, total anthocyanins (AntT), total polyphenol index (TPI) and colour intensity (CI) were evaluated for each plot and year. The information was provided by the Consejo Regulador of Rioja DOCa and it was obtained following the methods recommended by the OIV (OIV, 2012). The differences between plots were evaluated taking into account the soil water content and the ASW in each plot. A partial Least Squares (PLS) regression between grape parameters and the ASW was performed in order to analyse its influence and the timing when it occurred. Weekly values of ASW from the stage H until ripening (defined when PVAD = 13° was reached) were included in the analysis. The data corresponding to two plots with different characteristics (P2 and P6) regarding the AWC were used to create the model while the rest of plots were used in the cross validation. The grape parameters were analysed by groups: acidity (AcT, AcM and pH), anthocyanin and phenolic characteristics (AntT, TPI and CI and the ratio AntT/PVAD) and BW, considering the values of these variables when the PVAD reached 13°.

## 3. Results

### 3.1. Weather conditions recorded during the period of study

Years with different characteristics were recorded during the analysed period. The average maximum, minimum and mean temperature (TmaxGS, TminGS and TmGS) and the precipitation (PGS) recorded during the growing season (April-October) in the observatories included in this research are shown in Table 2. The TmGS ranged between 16.9 and 17.6 °C. The TmaxGS varied between 23.8 and 24.5 °C, while the TminGS ranged between 10.6 and 12.5 °C. The differences among meteorological stations were slightly higher for TminGS than for TmaxGS. The average PGS ranged between 179 and 257 mm (Table 2), but with high variability from year to year. Water inputs during the growing season were smaller than crop evapotranspiration in most of years, giving rise to important water deficits, which

were not covered by the water reserves accumulated in the soil during the dormant period. Within the analysed period, years 2008, 2013 and 2018 were within the wettest years in the series, while 2009, 2011 were the driest. In addition, both mentioned years were within the warmest years of the series analysed, with 2017 as the warmest one. In year 2016, despite annual precipitation was above the average, precipitation recorded in the growing season was very low, even lower than in the mentioned dry years. Year 2014 presented intermediate conditions regarding both temperature and precipitation, which was more homogeneously distributed along the year, with about 50 % of annual precipitation recorded during the growing season. According to the weather conditions observed, some years were selected to analyse the vine response: 2009, 2011 and 2016 as dry years; 2008, 2013 and 2018 as wet years; 2017 as a warm year and 2014 as a year with intermediate conditions.

### 3.2. Differences in phenology among years

The average phenological dates of the analysed years were 26<sup>th</sup> May ± 8 days for stage H, 12<sup>th</sup> August ± 7 days for stage M (veraison) and ripening was reached on average on 30<sup>th</sup> September ± 11 days. In the wet years, the dates of stages H and M were on average 10 days later than in the dry years, while ripening took place up to 18 days later. In the warmest year, veraison and ripening occurred 8 and 9 days earlier than in the coolest years, respectively.

### 3.3. Soil water measured and simulated under different climatic conditions

#### 3.3.1. Model calibration

Fig. 2 shows the measured and simulated soil water in the profile for P7, for the calibration (2009 and 2010) and validation periods (2011 and 2012). Despite the different characteristics of the years, the model was good fitted. Validation is presented separately for both years. The statistics are shown in Fig.2. For the calibration period, PBIAS was about 5 %, the NSE was 0.825 and the RMSE was 0.42, which could be considered as very good (according to the criteria proposed by Moriasi et al., 2007). For the two years used in the model validation, the PBIAS was 0.75 and -1.12 %, the NSE was 0.66 and 0.78, and the RSR was 0.48 and 0.58, respectively. This means satisfactory results (between good and very good).

#### 3.3.2. Simulated soil water contents and available soil water for the selected plots and years

Using the model, soil water was simulated for each selected plot and year. Due to soil characteristics, the maximum AWC that each soil can accumulate in the profile varied between 139.5 mm in P1 and 165.1 mm in P3. In the rest of the plots, the values were 145.6, 144.5, 155.6 and 158.7 mm, respectively for P2, P4, P5 and P6. The ASW along the growing cycle in each plot in the analysed years is shown in Fig. 3. It can be observed that the ASW reached the maximum capacity in almost all plots and years at the beginning of the growing cycle. However, after stage H, the ASW decreased very fast reaching the 50 %

**Table 2**

Average mean, maximum and minimum temperature (TmGS, TmaxGS, TminGS), precipitation (PGS) and crop evapotranspiration (ETcGS) recorded during the growing season (April-October) and precipitation recorded in the hydrological year (1<sup>st</sup> - Oct- 30<sup>th</sup> Sep) (PHY) in the studied period (2008–2018), in the meteorological stations used in this research.

Meteorological station	TmGS(°C)	TmaxGS (°C)	TminGS(°C)	PHY 1 <sup>st</sup> Oct-30 <sup>th</sup> Sep (mm)	PGS(mm)	ETcGS (mm)
Logroño	17.6 ± 0.8	24.1 ± 1.0	12.1 ± 0.5	472 ± 122	241 ± 109	495 ± 42
Uruñuela	16.9 ± 0.8	24.4 ± 1.1	10.6 ± 0.6	477 ± 108	246 ± 91	403 ± 100
Haro	17.4 ± 0.7	24.4 ± 1.0	12.1 ± 0.5	464 ± 113	190 ± 93	426 ± 26
Nájera	17.5 ± 0.9	24.5 ± 1.2	11.6 ± 0.5	369 ± 105	179 ± 58	437 ± 26
San Vicente de la Sonsierra	16.9 ± 0.6	23.8 ± 0.9	10.9 ± 0.5	573 ± 121	257 ± 84	488 ± 43

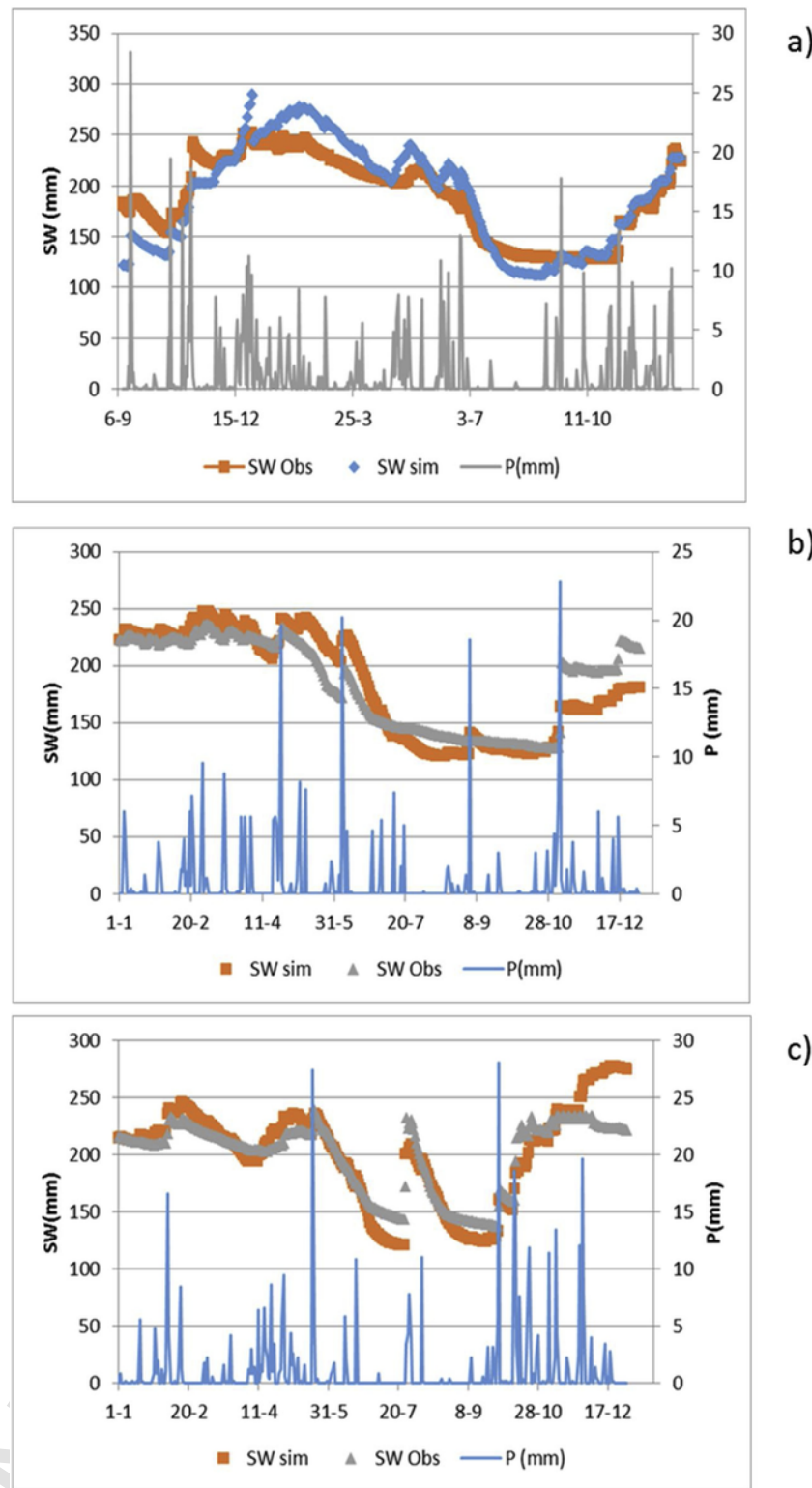


Fig. 2. Simulated (SW sim) and measured (SW Obs) soil water in P7 during a) the calibration period (2009–2010) and b) and c) validation period (2011 and 2012), respectively.

of the maximum holding capacity after few weeks, although it varied among plots and years.

In dry years, like 2009, the 50 % of the maximum ASW was reached after two weeks from stage H in P6 and after five weeks in P2 or P3 (Fig. 3). In addition, in the following weeks, there were differences between these two plots, not only in the minimum level of water

reached but when it took place. While in P3, ASW was decreasing, reaching the level below 20 % of the AWC (considered as the threshold to define moderate to weak water deficit) after eight weeks, in the case of P2 it happened after six weeks, with the minimum level reached at veraison, which was about 7 % of the maximum capacity. In that situation vine stress was much higher in P2 than in P3. In other plots, inter-

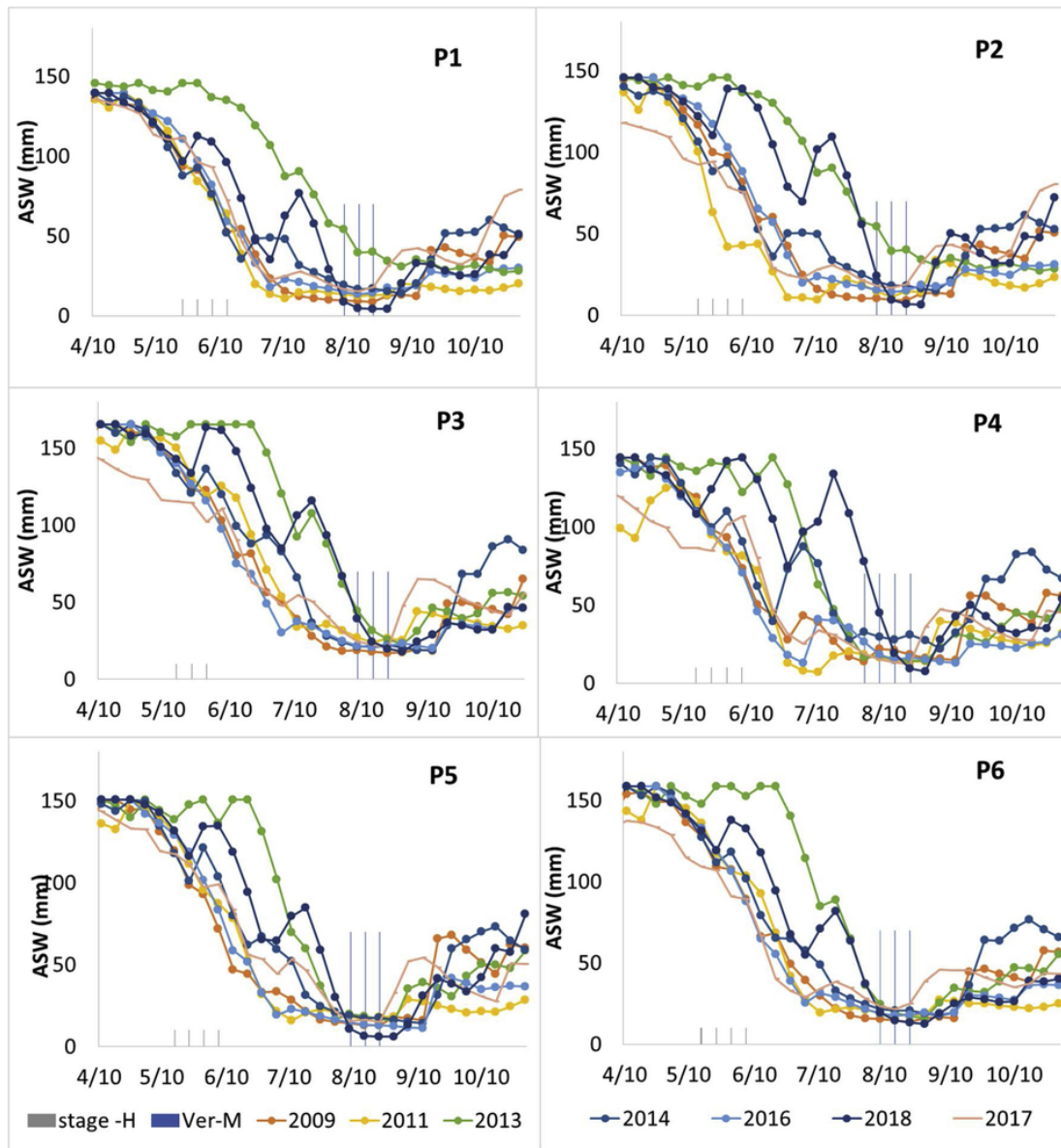


Fig. 3. ASW along the growing cycle in the selected plots (P1-P6), in years with different weather conditions (2009, 2011 and 2016: dry years; 2013 and 2018: wet years; 2017: very high temperatures; and 2014: intermediate characteristics). The periods in which the stage H (stage -H) and veraison (Ver-M) took place are also indicated.

mediate situations were given. For example, in P6, with an AWC relatively high, the ASW reached the value corresponding to 50 % of the maximum two weeks after stage H, but the decrease was more moderate and the minimum values were higher than in P2 (Fig. 3). The differences among plots in other dry years were quite similar. In 2011, for example, the value corresponded to the 50 % of the maximum ASW was reached after two and five weeks after stage H in P3 and P2, respectively, while the value corresponding to 20 % of the AWC was reached after five and seven weeks, respectively, and the minimum values reached in P3 was near double than in P2. In 2016, despite being the annual precipitation higher than the average in the area, the precipitation recorded during the growing season was scarce and vines started to suffer stress two or three weeks after stage H (Fig. 3).

For wet years like 2013, ASW was higher than 50 % of the maximum capacity during up to nine weeks after the stage H in P3 and P6, and about during up to five or six weeks in the plots with lower AWC

(like P1 and P2). The ASW was above 20 % until one week before veraison in P3, and about five weeks before veraison in the plots with lower AWC (Fig. 3).

In years with high temperatures, like 2011 and in particular 2017, the higher evapotranspiration made that soil water decreased earlier than in the above mentioned years and the 50 % of the AWC was reached just one or two weeks after stage H, depending on the plots. Although the minimum values of ASW reached in all plots were higher than in drier years, water deficits although weak (according to the established thresholds) were maintained during longer time, between six and eight weeks before veraison, which could also have negative effects for grape development.

At veraison, ASW usually reached very low values, around 10 % of the maximum capacity, except in the very wet years (e.g. year 2013) (Fig. 3). However, even in the driest years (e.g. 2009 or 2011), those minimum values were reached before in the plots with lower AWC.

### 3.4. Differences in grape composition among plots and years

Due to the different climatic conditions recorded during the years of the analysed period, differences in the grape composition during maturation were observed, both in timing and in the final characteristics. Maturity was defined in relation to a given probable alcoholic degree (PVAD). Thus, the grape characteristics were analysed during ripening (from veraison to harvest) and in particular when the PVAD equal to 13° were reached, in order to compare the differences among plots. Figs. 4–6 show the variation among years of the berry weight, acidity and phenolic compounds in the analysed years, respectively. In these figures it is also shown the average water content between budbreak

(stage C) and stage H and between stage H and veraison (stage M) recorded in each year.

Regarding berry weight, although it was clear that in all plots the lowest values were recorded in 2011 (one of the driest and warmest years) and the highest values in 2018 (one of the wettest year), the differences among dry and wet years were not clear when all analysed years were evaluated as a whole (Fig. 4). Thus, water content may be not the main, or the only factor that affects berry weight, or it should be also considered the time when the highest and the lowest water levels were recorded.

Total acidity was, in general, higher in the wet than in the dry years, with some differences between plots (Fig. 5). In wet years, the lowest value was recorded in P3, while in dry years it was recorded in P4. However, in the warmest year analysed (2017), the differences

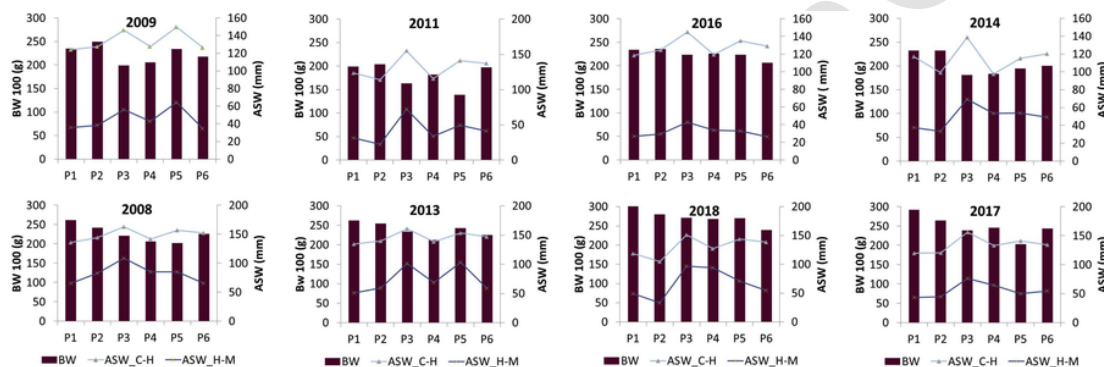


Fig. 4. Average values of the 100-berry weight (BW) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when PVAD = 13° were reached. ASW during the period between stage C and H (ASW\_C-H), and between stage H and M (ASW\_H-M) are also shown.

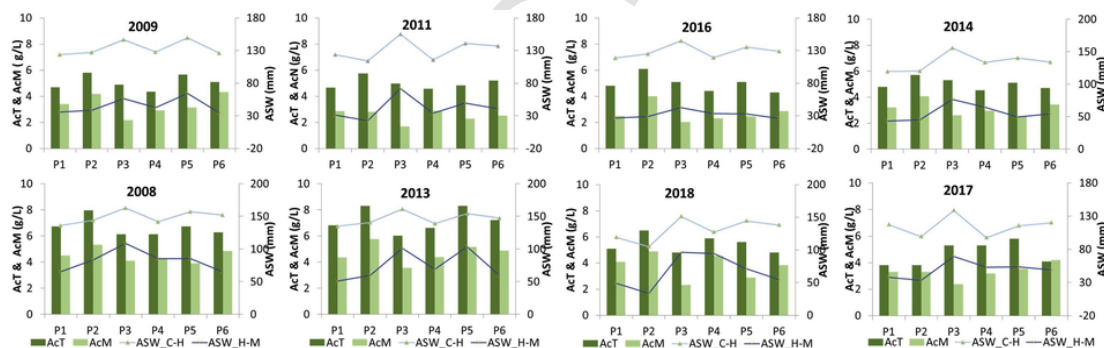


Fig. 5. Average values of total acidity (AcT) and malic acid concentration (AcM) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when PVAD = 13° were reached. ASW during the period between stage C and H (ASW\_C-H), and between stage H and M (ASW\_H-M) are also shown.

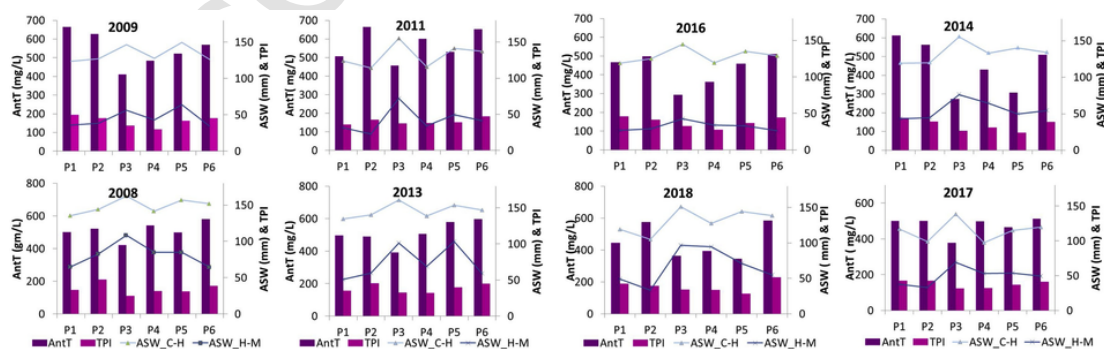


Fig. 6. Average values of total anthocyanins (AntT) and total polyphenol index (TPI) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when PVAD = 13° were reached. ASW during the period between stage C and H (ASW\_C-H), and between stage H and M are also shown.

among plots followed the same pattern than in the wet years until reaching a PVAG = 12°, but the lowest values were recorded in P1 and P2 when the PVAD = 13° was reached. In that year, ASW between stage H and veraison (stage M) was higher in P3, followed by P5, in which total acidity recorded the highest values at the end of ripening (Fig. 5). Similarly, for the malic acid, the lowest values were also recorded in P3, however, there were not a clear pattern among the rest of plots regarding water available (Fig. 5). Thus, water available seems to affect total acidity but it is necessary to investigate the time in which this water is available to confirm in what stage water can have higher effect. In addition, the effect of temperature on acidity was also confirmed. The lowest acidity values at ripening were observed in the warmest year (2017).

For the total anthocyanin concentrations, the highest values were observed in the dry years like 2009 or 2011. However, in 2016, which was also a dry year during the growing season, total anthocyanin concentrations were lower than in the other dry years (Fig. 6). As it was already said, 2016 recorded higher annual precipitation than the average, but precipitation recorded during the growing season was very low and ASW was lower during the whole growing season than in the other years, even in the first stages. This was one of the differences between 2016 and the years 2009 and 2011. Another difference was the

temperature, which was lower in 2016 than in the other two mentioned years. In 2017, which was the warmest year of the analysed series, the differences in total anthocyanin content among plots were lower. Nevertheless, it was confirmed that P3 recorded always the lowest levels, being the plot in which ASW during the period between stage H and veraison was the highest (Fig. 6). Thus, it is necessary to take into account not only water available in the whole growing season but in different stages.

The TPI index was smaller in the wet than in the dry years, but similarly to that commented for the anthocyanin concentrations, the TPI showed lower values in 2016 than in 2009 and 2011. The highest differences among plots were found in years in which there were differences in ASW not only during ripening but also during the previous stages (Fig. 6)

### 3.5. Relationship between water deficit and grape composition

The PLS regression analysis performed for the ensemble of analysed plots and years, between the grape parameters and ASW along the growing cycle, allowed confirming the time for which water availability plays an important role for grape quality. The fit coefficients for each variable are shown in Fig. 7, in which the coefficients for each

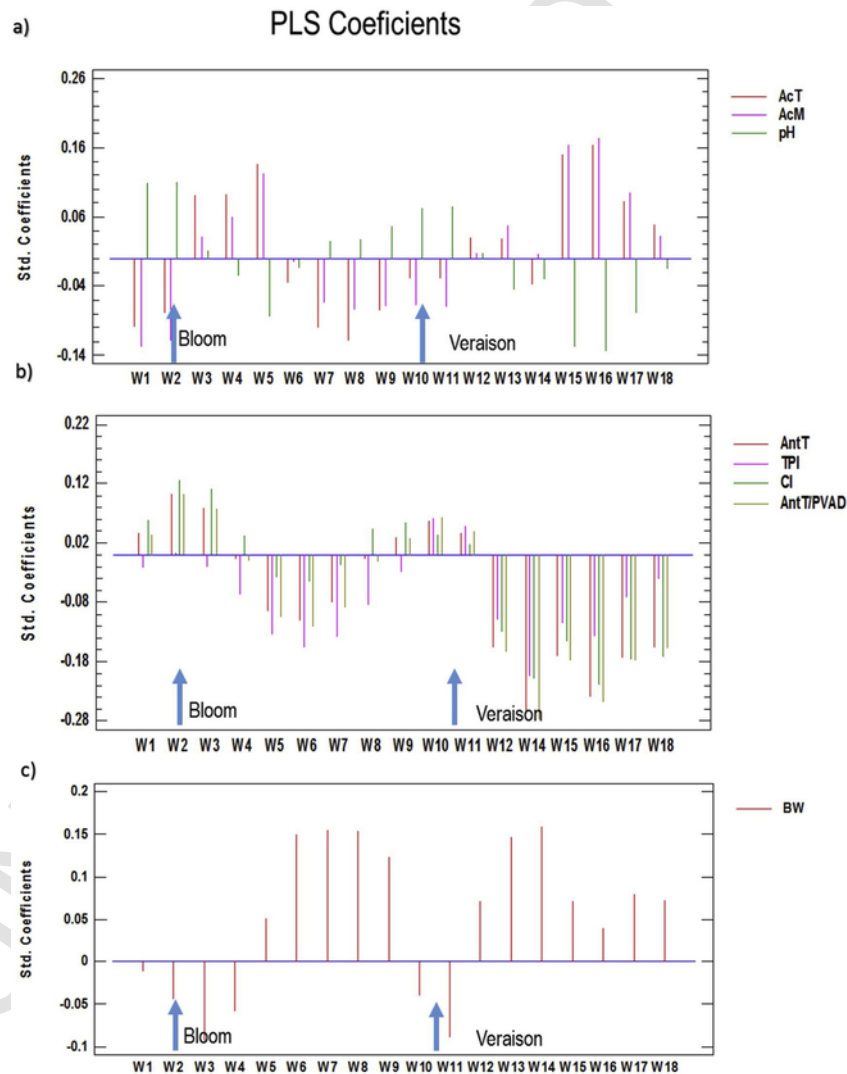


Fig. 7. Coefficients of the PLS regression analysis performed for the ensemble of analysed plots and years, between the grape parameters and ASW from stage H until reaching ripening (13°) analysed weekly (week 1–18; W1-W18): a) acidity; b) polyphenols, c) berry weight.

week starting after the stage H and ending when the maturity was reached (weeks from 1 to 18 are presented). The results shown in the figures corresponds to the number of components which gave the best fit and lower error, being in all cases the variables significantly fitted at 95 % ( $p < 0.05$ ). For the variables related to acidity, two components were retained, and the percentage of the variance explained was 65.2, 52.0 and 61.7 %, respectively for AcT, AcM and pH. For the variables associated with the phenolic compounds, one component was retained, and the percentage of the variance explained was 55.3, 65.1, 46.1 and 54.4 %, respectively for AntT, TPI, CI and AntT/PVAD ratio. Finally, BW was fitted using only one component and the percentage of the variance explained was 27.5 %. During the analysed years, bloom was reached two weeks after stage H, veraison took place between nine and 12 weeks after the stage H, and maturity was reached between 17 and 20 weeks after stage H.

For the variables related with acidity, it was observed that ASW in the period between four and seven weeks after the stage H and at the end of the ripening period increased acidity and decreased pH, but there was a period in between in which the effect was negative or had not significant effect (Fig. 7a). The correlations were significant at 99 % for the three parameters (AcT, AcM and pH). Regarding the phenolic characteristics, it was observed that soil water content in different periods within the growing cycle had influence on the final value of anthocyanins as well as on the TPI and CI. The results were significant at 99 % level for the three parameters and it showed to be important before veraison and in particular during ripening. It can be observed that the three parameters presented an inverse relationship with ASW, which means that they increase when ASW decreases. As it was commented before, ASW decreased very fast before veraison, reaching the conditions of moderate stress in five to eight weeks after stage H, on average in the dry years. In wet years, the situations of moderate stress were reached between seven to 11 weeks after stage H. According to the results, the lowest ASW in the weeks after veraison, when water stress already existed, favoured anthocyanin concentrations and increased the TPI (Fig. 7b). The ratio anthocyanin/PVAD presented similar relationship with ASW, thus is, it increased with water stress (Fig. 7b). Finally, it was observed that ASW had influence on berry weight in different time periods along the growing cycle, in particular in the period after bloom in which the berry was growing and then during ripening (Fig. 7c).

#### 4. Discussion

This research covered years with different rainfall amounts and distribution and also with differences in temperature, which allowed extracting information about soil water content and water availability under different weather conditions. During the period analysed (2008–2018), vines suffered different levels of stress during the growing season, and differences in temperature were observed, which allowed also confirming the effect of high temperatures on grape composition.

It was observed that the soils usually maintained the maximum ASW up to stage H and from that time, ASW decreased very fast reaching values corresponding to 20 % (considered as the threshold to define moderate to weak water deficit of the maximum capacity) between five and eight weeks after that stage H. In all plots, levels below 50 % of the maximum holding capacity were reached during the growing season, usually between bloom and veraison stages. For example, for 2014, soil water reached the values corresponding to 50 % of the maximum capacity in early July in P3, while it was reached in the middle of June in P1. In wet years, like 2013, in which rainfall was about 20 % greater than the average, the soils with the maximum water holding capacity maintained soil water levels higher for longer time period. Besides, the ASW below the 50 % of the maximum was reached at the

end of July or at the beginning of August. However, in the plots with lower holding capacity, this occurred in the third early part of July. On the other hand, in dry years like 2009, 2011 or 2016, which received about 40 % less of precipitation than in the first case (2014), with about 30 % of it recorded during the growing season, the differences between plots were smaller, and the 50 % of ASC in the soil profile was reached in all cases around the middle of June. At veraison, the ASW usually reached very low values, around 10 % of the AWC, except in the very wet years (e.g. 2013) (Fig. 4). However, that minimum values were reached before in the plots with lower maximum available water content, even in the driest years (e.g. 2009 or 2011). Nevertheless, for a given year it happened up to 15 days earlier in some plots in relation to the rest, depending on soil characteristics.

The evolution of ASW and the minimum values observed were similar to that found by other authors (Pellegrino et al., 2005; Ramos and Martínez-Casasnovas, 2014). These authors expressed the ASW in relation to the AWC and they found values below 0.1 in some of the analysed years and locations, by the end of the growing cycle. Under these low values of ASW, the vines suffered from weak to moderate stress at veraison and during most time of the ripening period and it was confirmed that the reduced ASW at the end of the growing cycle influenced not only berry weight but also its composition.

Lakso and Pool, (2006) indicated that vines are sensible to water stress in different moments along the growing cycle. Water stress reduces shoot growth under any stress level, which could be beneficial in grapes for wine production, in particular when the vines have excessive foliar area. However, in the study case, no water stress was shown during the first stages of the growing cycle. More important could be the effect on berry growth during the first weeks after bloom, which could affect the cellular division and can affect the rest of the cycle.

As it was commented before, in the study case the water deficit started in some plots just two or three weeks after stage H. The situations of moderate stress, however, were recorded after five weeks of stage H, even in the dryer years, and the highest water stress were observed at veraison, in which soil water content was very low and ASW was below 10 % in most years and plots. According to Lakso and Pool (2006), water stress in the following weeks to veraison is not as critical as it is at the end of the cycle, when it can produce both a decrease in berry size and in sugar accumulation. However, Zulini et al. (2007) indicated that water stress may affect the photosynthesis and lower yields may be produced because not all berries achieve the full ripeness. Berry weight was mainly related to ASW as was previously stated (Ojeda et al., 2001; Reynard et al., 2011; Wenter et al., 2018; Ramos and Martínez de Toda, 2019), with higher values in the wettest than in the drier years, but it could be seen that there were some specific periods in which the effect on berry growth was higher. Water status had positive effects not only during the pre-harvest period but also in some periods before veraison. In this study case, ASW had positive influence in almost all cycle. The coefficients of the PLS were significantly high during berry growth, since two or three weeks after bloom, and then during the first weeks of ripening. The effect of this last period is consistent with that reported by van Leeuwen et al. (2009), who indicated smaller berry size under water restrictions on the maturation.

There are only two periods in which the berry weight showed a negative relationship with the ASW, two or three weeks after bloom and two weeks before veraison. The explanation could be linked to the fact that, in these two periods, the balance between vegetative and reproductive growth is more critical than in other periods, and the greater availability of water would favour vegetative growth compared to the development of the berry. In the case of the post-bloom period, it could also be influenced by the fact that there is a greater fruit set at higher ASW so, as the number of berries is greater, its unit weight would decrease. In the case of veraison, a higher ASW would favour

the vegetative growth, delaying the development of the berries at the beginning of the ripening period.

In addition, several studies have pointed out the influence of water deficit on grape quality. Water stress affects nitrogen metabolism and assimilation (Bertamini et al., 2004). In this respect, Hochberg et al. (2015) indicated that water deficits increased amino acid content and Valdés et al. (2019) pointed out the effect of pre-veraison vine water status on amino acid concentrations. Other parameter that can be directly affected by water levels is the acidity. The highest acidity values were observed in the wettest analysed years. Cheng et al. (2014) linked higher acidity to the wetter soils and higher acidity has been reported under excessive soil moisture (Jackson and Lombard, 1993) and under well-watered grapes (Girona et al., 2009; Reynolds et al., 2007), while Lopes et al. (2011) indicated that in treatments where soil water content during spring was reduced, must titratable acidity experience a significant reduction. Similarly, Peyrot des Gachons et al. (2005); de Souza et al. (2005) and dos Santos et al. (2007) indicated lower total acidity due to water deficit and/or to changes in temperature and sun-exposed berries in non-irrigated vines. Regarding the time along the cycle in which some effects were recorded on both total acidity and malic acid, the reason may vary. Higher water available in the first stages of the fruit development, in which the berry is very small and when acids are being produced, can have positive effect on the final acidity, which could explain the observed positive PLS coefficients in that period. The negative PLS coefficients in the following period could be due to a dilution effect within the berry that increases in size quickly. It was also found that higher ASW during ripening favours the increase in acidity. This result agrees with that found by Ramos and Martínez de Toda (2019), in a study which covered almost the whole Rioja vine growing area. They found the highest acidity values in the wettest years, in which an important amount of water was accumulated not only in the period bloom to veraison but also in the period veraison to maturity. In that case, in addition, the lower temperatures recorded in the wetter years could have also slow malic acid combustion in particular during ripening.

The positive effect of water stress on anthocyanins and total phenols observed in this study case are in agreement with observations from other authors (Hochberg et al., 2015; Cáceres-Mella et al., 2017; Ferrer et al., 2014; Ramos and Martínez de Toda, 2019), although the different compounds may be affected in different ways depending the cultivars and rootstocks (Berdeja et al., 2014; Hochberg et al., 2015). However, Niculcea et al. (2015) found that water deficit decreased anthocyanins due to decreasing glucoside derivatives and increasing acetyl and coumaroyl derivatives but increased flavonols in Tempranillo and decreased flavonols and catechins in Graciano. Basile et al. (2011) indicated that anthocyanin and polyphenol concentrations improved when no water stress occurred from bloom to fruit set, with mild water stress between fruit set and veraison, and with moderate to severe water stress in post-veraison. This is in agreement with the positive coefficients observed in the first weeks, and with the negative coefficients observed after veraison. In this respect, Ferrer et al. (2014) pointed out higher anthocyanin concentrations under mild to moderate water deficit during maturation. The only periods in which there were a discrete positive relationship between water availability and phenolic compounds coincide exactly with the two periods, in which the ASW reduces the berry size. Therefore, we can say that the highest concentration of phenolic compounds is due to the effect of ASW on berry size. In the 18 weeks studied, from stage H to maturity, there were only two short periods, of two or three weeks each (with the exception of one of five weeks in the case of acidity), in which the effect of the water availability was contrary, although less intense, to the general effect during the rest of the vegetative period. That is to say, the greater ASW in those two concrete periods decreased berry weight and acidity and increased anthocyanins and other phenolic compounds. These two

periods correspond, in all cases, with bloom and with veraison, and the explanation could be related to the general physiological, and especially hormonal, changes that occurs in the vine in those two stages: in both, the vegetative development it slows down in concurrence with reproductive development; in one case to form the fruit and in the other case to start the ripening period (Martínez de Toda, 1991).

Water deficit also affected positively the anthocyanin /sugar balance. Under high temperatures in summer, it can be observed a decoupling between anthocyanin and sugar content (Martínez de Toda and Balda, 2015). Nevertheless, the effect of temperature should be considered in addition to that of water availability. Sadras and Moran (2012), indicated that a moderate water deficit before veraison could partially restore the anthocyanin/sugar balance and Mori et al. (2005) indicated the effect of the night temperatures on anthocyanin concentrations, with increasing values under cool nights. Similarly, Torres et al. (2017) indicated that both factors, water deficit and high temperature, contribute to modify metabolite profiles of amino acids, anthocyanins and flavonols. In this study case, the PVAD was used to define ripening, but the levels of anthocyanin varied from year to year, and it can also be confirmed the effect of ASW on this ratio: the lowest the ASW the highest the anthocyanin/sugar ratio was, having influence on it the ASW in different periods along the growing cycle. Regarding the effects of temperature on acidity, the lowest acidity found in the warmest year, confirmed its effect, and the result agreed with those found in other studies (Sadras et al., 2013; Martínez de Toda et al., 2019), in which it is indicated that water deficits and high temperatures give rise to acidity lower than the average.

## 5. Conclusions

From this research we can conclude that the level of water stress and the time when it appears under similar weather conditions varied between plots, due to differences in soils characteristics and it has influence on grape composition. An increase in the available soil water in most of the period between bloom and maturity increases berry size and acidity and decreases the concentration of anthocyanins and other polyphenolic compounds. In particular, an increase in available soil water between one and three weeks after bloom and at the end of the ripening period increased acidity and decreased pH; an increase in available soil water between two and seven weeks after bloom and at the ripening period increased berry weight and decreased anthocyanins and other phenolic compounds. Under climate change scenarios, in which temperature is predicted to increase, soil water available could decrease due to higher evaporative demands, whatever the changes in precipitation can be. In this respect, the zones located at higher elevation, which could have lower temperatures, has been indicated as potential areas to mitigate the effects of climate change on the vines. But additionally, the selection of the soil that can offer more favourable soil water reserves and with higher availability should be considered.

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Declaration of Competing Interest

None.

## Uncited references

Bucchetti et al. (2011).

CRediT authorship contribution statement

**María Concepción Ramos:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Writing -

Review and editing. **Eva Pilar Pérez-Álvarez:** Resources, Investigation, Writing - Review and editing. **Fernando Peregrina:** Resources, Funding acquisition, Investigation, Writing - Review and editing. **Fernando Martínez de Toda:** Conceptualization, Resources, Investigation, Formal analysis, Writing - Review and editing. **CRedit** authorship contribution statement **María Concepción Ramos:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Writing - Review and editing. **Eva Pilar Pérez-Álvarez, Fernando Peregrina, Fernando Martínez de Toda** does not match the list of acceptable roles. Please choose a role from the below list for this author: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

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