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Phenology of Tempranillo and Cabernet-Sauvignon varieties cultivated in the Ribera del Duero DO: observed variability and predictions under climate change scenarios

M. Concepción Ramos[1], Gregory V. Jones[2], and Jesús Yuste[3]

[1] Department of Environment and Soil Science, University of Lleida-Agrotecnio, Lleida, Spain. cramos@macs.udl.es

[2] Center for Wine Education, Linfield College, McMinnville, Oregon, USA. gjones@linfield.edu

[3] Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain. yusbomje@itacyl.es

Abstract

Aim: This research examined relationships between grapevine phenology and climate in the Ribera del Duero DO (Spain). The observed varieties included Tempranillo, the main variety planted in the region, and Cabernet-Sauvignon.

Methods and Results: Phenological events for stages C (budbreak), I (bloom), M (véraison) and N (maturity) were analyzed for 2004-2015. Dormant period chilling and late winter heating requirements to initiate growth were evaluated and accumulated temperature (growing degree days-GDD) prior to each phenological event and in between events were examined for the role they play in influencing growth timing. The results were then used to examine future phenological changes due to climate change using eight models integrated in the Coupled Model Intercomparison Project (CMIP5) and for two Representative Concentration Pathways (RCP) scenarios – RCP4.5 and RCP8.5 – for 2030, 2050, and 2070. Accumulated temperatures after March 20th become important for initiating phenology and are strongly correlated to all growth events. The influence of water availability between budbreak and bloom and between bloom and véraison on phenological timing was also confirmed.

Conclusions: The projections showed that for the RCP4.5 emission scenario, budbreak is predicted earlier by approximately 2 days for 2030, 3 days for 2050 and 5 days for 2070, while bloom is predicted to be 3 to 8 days earlier and véraison 6 to 19 days earlier for the same time periods. For the RCP8.5 emission scenario, budbreak is modeled to take place about 3 days, 5 days and 9 days earlier, respectively for 2030, 2050 and 2070. Bloom is predicted to occur about 5, 10 and 16 days earlier; véraison is predicted earlier by 10 days for 2030, 19 days for 2050, and 28 days for 2070. Maturity and the timing of harvest could be up to 23 days earlier under the RCP4.5 emission scenario and up to 35 days earlier under the RCP8.5 emission scenario. Compared to Cabernet-Sauvignon, Tempranillo exhibited greater phenological sensitivity to temperature changes in the observed time period that is likely to continue into the future with greater changes to earlier growth events projected. This sensitivity could be problematic for the region due to the variety's historic importance and points to the need to examine adaptive measures that can help growers to respond to projected changes in climate.

Significance and impact of the study: The projected climate changes in the future indicate the potential for significant changes in the phenology of Tempranillo in the Ribera del Duero DO, Spain. Given that this variety has the largest contribution and importance in this region, these changes could have impacts on wine quality, indicating the need of establishing strategies to reduce or mitigate the impact from future changes in climate.

Keywords: bloom, budbreak, degree days, RCP4.5, RCP8.5, temperature, véraison

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*Corresponding author :cramos@macs.udl.es

Introduction

Some of the most direct effects of climate variability on grapevines are the changes in the onset and timing of phenology events and changes in the length of the growing season. Increasing temperatures recorded during the last 50 years in numerous wine regions worldwide have resulted in a shortening of the growing season with mostly earlier grapevine phenological stages (Jones, 2005; Duchêne and Schneider, 2005; Sadras and Soar, 2009; Bock *et al.*, 2011; Tomasi *et al.*, 2011; Webb *et al.*, 2012; Ruml *et al.*, 2015). As a result of the shortening of the growing period, ripening occurs under warmer temperatures, which could have a negative impact on grape and wine quality (Duchêne and Schneider 2005; Orlandini et l., 2009; Salazar Parra *et al.*, 2010; Neumann and Matzarakis, 2011; Lorenzo *et al.*, 2012) and yield (Mira de Orduña, 2010).

Each winegrape variety has its own phenological timing due to unique morphological and physiological characteristics, and growing season temperatures define the suitability of each variety to be grown in a given area (Jones *et al.*, 2010). Accumulated temperatures above a base temperature are required to initiate grapevine growth in the spring (Winkler *et al.* 1974) and drive grapevine development and fruit ripening (Sadras and Moran, 2013; Neumann and Matzarakis, 2014; Cook *et al.*, 2015; Webb *et al.*, 2012; Ovadia *et al.*, 2013). Despite the fact that grapevines can be adapted to a small range of climate conditions (Jones *et al.*, 2012), increases in temperatures beyond the limits of the variety may produce modifications in crop suitability and productivity, changes in crop quality, proliferation of weeds and pests and changes in water requirements (Hatfield *et al.*, 2011). Extremely hot temperatures (>35°C) can cause severe grape skin damage in the form of sunburn, which can render the fruit unsuitable for quality wine production. In addition, increasing temperatures and higher solar radiation has been shown to have a direct impact on grape composition and thus flavor development via alteration of secondary metabolites such as flavonoids, amino acids and carotenoids (Greer and Weedon, 2013; Ovadia, *et al.*, 2013). Moreover, higher temperatures would also lead to higher rates of evapotranspiration and increase vine water requirements. In addition to the physical changes brought about by earlier maturity due to higher temperatures, whole vine physiology and metabolism can be modified where sugar ripeness and acid retention is decoupled from phenolic and aromatic maturation in the berries, resulting in lower quality in warm regions like the Ribera del Duero DO (Denomination of Origin) and others worldwide. These temperature impacts, together with changes in the quantity and distribution of precipitation will likely have compounded effects on grapevine growth, productivity, and quality. This may be particularly important in rainfed vineyard regions, where due to the lack of irrigation infrastructure and/or legal framework, rainfall is the only water source. Changes in climatic conditions may force vineyards to be planted in more poleward latitudes, closer to the coast, or higher in elevation with cooler climates, which could be suitable areas for quality wine production (Schultz and Jones, 2010). However, other climate factors may present challenges to suitability in these new regions. The knowledge resulting from modeling of these changes may be a key tool to plan and adapt viticultural management practices or understand where vineyard suitability will likely be in the near future under various climate change scenarios.

Previous research has examined how changes in climate might alter where traditionally cultivated varieties will be suitable in the future. To develop spatially predictive models, researchers commonly use different bioclimatic indices such as Winkler index (WI), Huglin index (HI) or other indices that combine temperature and precipitation (e.g., Hydrothermic Index of Branas, Bernon and Levadoux) to assess changes in suitability worldwide (Jones and Goodrich, 2008; Pieri *et al.*, 2012; Fraga *et al.*, 2016; among others). However, due to the differences in baseline climate and variability between different viticultural areas, and the differences in grapevine responses across varieties, more regionally specific analyses are needed to establish suitable viticultural management practices under climate change scenarios.

The Ribera del Duero DO (Spain) is an area with a long tradition in viticulture whose origin dating back to the Roman period. Tempranillo is the dominant variety planted in the DO, accounting for over 95% of the surface area and the region is listed as the world's grape-growing region with largest contribution from and importance of Tempranillo in its wines (Anderson, 2013). Other red varieties authorized for cultivation in the DO are Cabernet-Sauvignon, Grenache, Malbec and Merlot, which together represents only about 2.6% of the cultivated area. These varieties are well adapted to the current climatic conditions in the region. On two of the more commonly used bioclimatic indices, the region is classified as a Region Ib on the WI (1111-1389 GDD) and temperate to warm temperate on the HI indicating that this region is supposed to be suitable for early ripening varieties (Jones *et al.*, 2012). However, the increase in temperatures recorded during the last few decades may change the area to warmer Region II or III, which would imply that changes in the varieties grown and/or their cultivation management under the new conditions would be necessary. To better understand the potential impacts from a changing climate, the aim of this study is the analysis of the phenological timing and variability of Tempranillo associated with the current climatic conditions and compared with Cabernet-Sauvignon. Although Tempranillo can be better suited to cooler climates and Cabernet-Sauvignon to warmer climates, their suitability range overlaps (Jones *et al.*, 2012) indicating that Cabernet-Sauvignon could become more important for the region over time. The comparison of both a traditional regional variety and another more international variety will provide a reference for the potential of other varieties to be cultivated in the region (both those currently approved and not being grown in the region). Based on the analysis of the observed vine phenology and its relationship with climatic variables (temperature, precipitation and bioclimatic indices) changes in phenology and therefore growth suitability, are predicted for different future climate (2030, 2050 and 2070) scenarios (RCP4.5 and RCP8.5).

Materials and Methods

1. Area of study

The study was carried out in the Ribera del Duero DO, located in the north center of Spain (Figure 1). The Ribera del Duero DO extends east-west approximately 115 km along the Duero River, with elevations that range between 720 to more than 1000 m a.s.l. Vineyards occupy about 22319 ha (in 2016), with Tempranillo the dominant variety planted in the DO, accounting for over 95% of the surface area (www.riberadelduero.es). Cabernet-Sauvignon represents about 1.3% of the planted area and is one of the other red varieties authorized in the DO. The climate is temperate with dry winters and hot summers in the western part and temperate with dry winters and temperate summers in the eastern part of the DO area.

Differences in climatic conditions exist between the western and the eastern part of the DO, and between areas located at different elevations in the area. In addition, soil characteristics condition soil water availability, which have further effects on grapevine development and fruit quality (Ramos *et al.*, 2015). This means that within the region, the response may

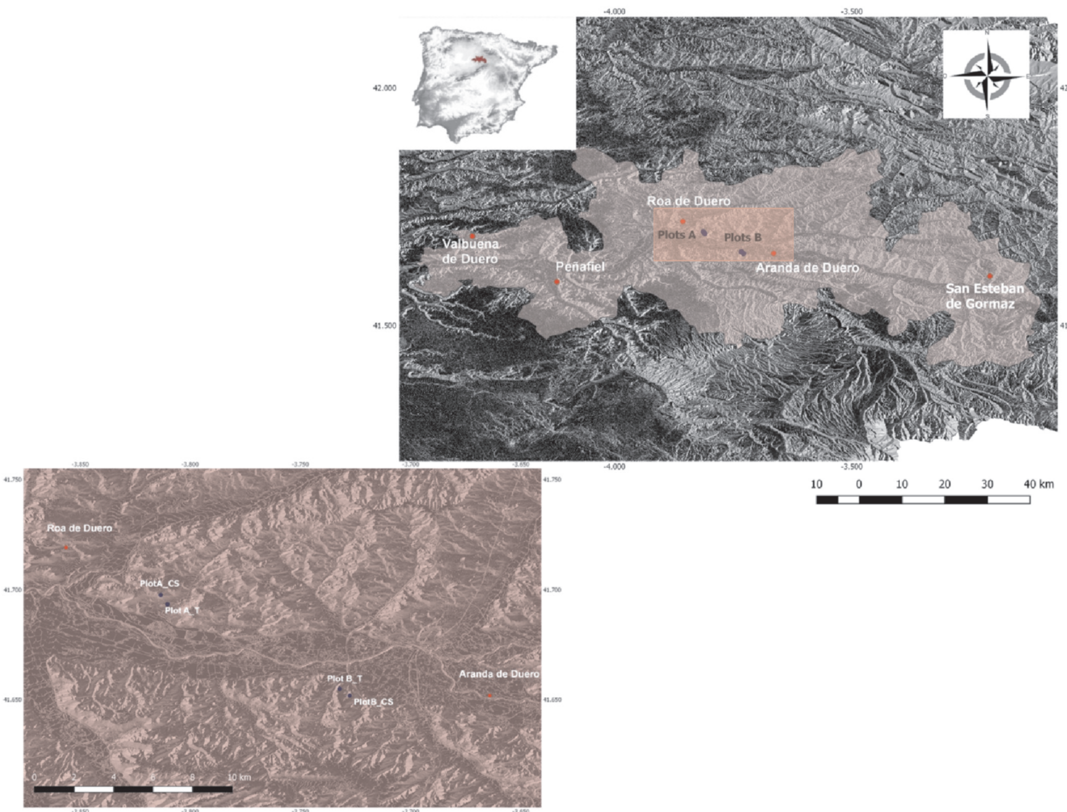

Figure 1. Location of the plots in the study area (PA_CS and PB_CS: Cabernet-Sauvignon; PA_T and PB_T: Tempranillo). The climate data for the study comes from the town of Aranda de Duero.

vary according not only to varieties but also to microclimate, specific soils, orientation, elevation, etc. For this research, four plots, two of them planted with Tempranillo and two with Cabernet-Sauvignon, both grafted onto 110 Richter rootstock, were used to analyze the phenological timing. These plots are located in the center of the Ribera del Duero DO area (Figure 1) and the elevations of the plots are respectively 802, 808 m (plots A: PA_T and PA_CS) and 834 and 840 m (plots B: PB_T and PB_CS). The A plots are located on terraces above the river while the B plots are on the hillslopes. The surface area of the A plots are 4.7 and 4.0 ha, while B plots have a surface area of 6.3 and 4.3 ha, respectively for T and CS. The soils are classified, respectively, as *Calcaric fluvisol* and *Calcaric cambisol*. The main differences in soil properties between both areas are the sand and clay contents. While the clay content in the plots located on the river terraces is about 8%, on the hillslopes clay content is about 15%. The river terrace plots have a higher concentration of sand (about 65%) compared to hillslope plots. The organic matter content was quite similar in all plots, ranging between 1.4 and 1.5 %. Thus the water retention capacity of the soil in the A plots are slightly higher than in the B plots. The training system in all plots is a vertical trellis. In addition, the A plots were under deficit irrigation while the B plots were not irrigated. The age of the vines was also similar with 14 and 19 years for Tempranillo and 23 and 24 years for Cabernet-Sauvignon in plots A and B, respectively.

2. Phenology data

Phenology dates referenced to the stages C (budbreak), I (bloom), M (véraison) and N (maturity), according to the Baggiolini classification (Baggiolini, 1952), were analyzed for the period 2004-2015. The information was provided by the Consejo Regulador of Ribera del Duero DO. In each plot, two control areas were taken with 100 vines per area. The phenological date assigned to one stage was defined when it was the most frequent stage in the study area. For each variety the dates corresponding to each phenological stage were averaged for each plot and its timing and variability were analyzed. Comparisons of means were performed using analysis of variance (ANOVA) in order to analyze the significance of the differences among years and among locations. These dates were related to climatic conditions recorded during the period. Grape composition data (pH, titratable acidity, soluble solids, berry weight, total anthocyanins and color intensity) was also collected from the same plots.

3. Climate data and analysis

Daily climate data [maximum and minimum temperature (Tmax, Tmin) and precipitation (P)], recorded at Aranda de Duero (798 m a.s.l), which is located respectively 5.6 and 13.1 km from the A and B plots were analyzed for the same period. Different bioclimatic variables such as the Winkler and Huglin indices were analyzed to characterize the area's viticultural climate. In addition, hourly data of maximum and minimum temperature ($T_h$max and $T_h$min) for the period 2004-2015 were analyzed to evaluate dormant chilling and spring heat accumulation requirements, which can in turn influence the entire phenological cycle. Daily chill accumulation (in Chill Portions) was calculated according to the Dynamic Model (Fishman *et al.*, 1987) using hourly temperature data. Daily heat accumulation (in Growing Degree Hours) was calculated according to Anderson *et al.* (1986), using a base temperature (Tb) of 4°C and an optimum temperature of 26°C. Chilling and Forcing models have been successfully applied to predict grape phenology (Halszny *et al.*, 2011; Caffarra and Eccel, 2010) and GDH is usually proposed to estimate chill units needed for grape growing (http://www.westernfarmpress.com/ grapes/winter-chilling-requirements-grapes). The chill and heat phases were determined by analyzing the relationship between budbreak dates and the means of 10 days of daily chill and heat units from September 15th (of the year preceding the recorded budbreak) to May 10th (date at which budbreak was reached in each of the analyzed years), using a Partial Least Squares (PLS) regression. Negative correlation coefficients were interpreted as periods that produced an earlier budbreak.

Once the chill and heat accumulation phases were delimited, these thermal requirements were expressed in GDD calculated as the sum of the difference between the daily mean temperature and the base temperature (Tb) critical for effective heat accumulation recorded from a given starting date (ti) (Eq. 1).

$$GDD = \sum_{ti}^{tn} \frac{(Tmax+Tmin)}{2} - Tb$$

$$\text{If} \quad \frac{(Tmax+Tmin)}{2} < Tb, \text{ then } \frac{(Tmax+Tmin)}{2} = Tb, \quad (1)$$

In this study, the base temperature for each stage was estimated following the procedure proposed by Zapata *et al.* (2017) (Eq. 2).

$$GDD = \sum_{1}^{n}(Ti - Tb) \cdot n \quad = \sum_{1}^{n}(Ti \cdot n - Tb \cdot n) \quad (2)$$

where Ti is the average daily temperature, Tb is the base temperature and *n* the number of days to reach the corresponding phenological stage.

If If $Ti<Tb$ then $Ti=Tb$ and no GDD were accumulated. Tb was estimated through an iterative process until reaching the temperature that minimized the standard deviation for GDD. The optimization was done using the Generalized Reduced Gradient (GRG) in the SOLVER tool (Microsoft Office Excel 2010). The thresholds were calibrated and the fit of the predicted dates was analyzed using the root mean square (RMSE) calculated as indicated in Eq.3.

$$RMSE = \sqrt{\frac{\sum_1^n (DOYs - DOYo)^2}{n}} \quad (3)$$

where DOYs and DOYo are, respectively, the simulated and observed dates at which the corresponding phenological event occurs. Heat accumulation was estimated from the date at which chill hours were considered fulfilled and considering 26ºC as the maximum optimum temperature as proposed by Parker *et al.* (2011).

The average heat accumulation value at which each phenological stage was reached, for each variety, was then considered to determine the changes in phenological dates under future climate scenarios. The analysis was done for each model separately and the average projections are presented for each scenario. Additionally, the effect of water availability on phenological evolution was analyzed. The relationship between the phenological dates and the variable Precipitation - crop evapotranspiration (P-ETc) accumulated in different periods [MA: March 1st – April 15th (before budbreak_stage C); AMy: April 15th - May 30th (period between stages C and F (visible bunches)); J: June 1st - 20th (period between stages F and I); AJ: April 15th - June 20th (period between stages C and I); JAg: June 20th – Aug 3st (period between stages I and M)] were examined and its influence on phenological dates were analyzed using a regression analysis. The crop evapotranspiration (ETc) was evaluated taking into account the reference crop evapotranspiration of, calculated according to the Penman-Monteith equation, and the crop coefficients proposed by Allen *et al.* (1998).

4. Climate change scenarios

The predicted changes in temperature and precipitation for three future time periods 2030, 2050 and 2070, and for two Representative Concentration Pathways (RCP) scenarios – RCP4.5 and RCP8.5 - were taken into account in order to predict the changes in phenology from observed conditions. The RCP4.5 was considered as an intermediate scenario with and average increase in mean temperature of about 1.4ºC (1.1 to 2.6ºC) for the period 2081-2100 while RCP8.5 is the most pessimistic scenario with a projected increase of about 3.7ºC (2.6 to 4.8ºC) for the same time period (Stocker *et al.*, 2013). The information used in the study was based on the results obtained using eight models integrated in the Coupled Model Intercomparison Project (CMIP5) : MIROC5; ACCESS1.0; CNRM_M5; INMCM4; MPI-ESM_MR; CMCC-CM; BCC-CSM1-1; MSI_CGCM1 (http://cmip-pcmdi.llnl.gov/cmip5/guide_to_cmip5.html). Data were downloaded at a daily time scale from the Agencia Estatal de Meteorología (AEMET) for the meteorological station of Aranda de Duero. The data were calibrated and adjusted for both areas using the data recorded through 2015. The periods 2020-2040, 2040-2060 and 2060-2080, denoted as 2030, 2050 and 2070 respectively, were compared with the reference period (1960-2000).

The changes in phenological dates under future climate scenarios were estimated by analyzing the heat accumulation values (GDD) and the thresholds needed to reach each phenological stage. The analysis was done for each model and the results are expressed as the average of changes estimated for the different models.

Results

1. Phenology variability

Table 1 shows the average dates for each phenological stage and their standard deviation in each plot. Budbreak occurs on average in the last week of April, bloom the middle of June, véraison the second to third week in August, and maturity the end of September or early October. Significant differences in the phenological dates were observed among years during the period analyzed due to interannual variability in the climatic characteristics. In addition, despite the small distance between plots, some differences in the average dates for each phenological stage were also found, which are associated with differences in elevation. The plots located at lower elevations showed earlier phenology, although the differences were also seen between varieties. Cabernet-Sauvignon showed greater differences between plots than Tempranillo, in particular in the earlier stages. The differences between plots cultivated with the same variety ranged between 2 and 4 days for Tempranillo and up to 6 days for Cabernet-Sauvignon. The average values of some grape composition parameters at ripening, recorded during the period of analysis, are also shown in Table 1.

2. Climatic conditions

The average climatic characteristics of the years included in the study are shown in Table 2. The average Tmax and Tmin during the growing season (period between budbreak and maturity) ranged between 24.9 and 28.9°C and between 8.0 and 10.4°C, respectively. During the period analyzed (2004-2015), a significant increase in temperature was observed in the Ribera del Duero DO (Spain). On average the warming was greater for Tmax (0.17°C per year) than for Tmin (0.09 °C per year) and temperatures reached higher values than those recorded during the previous period (1980-2000) (Ramos *et al.*, 2015).The WI averaged 1320, ranging between 1090 and 1584 while the HI averaged 2133 and ranged between 1867 and 2434. These values place the area within the Region Ib according to the WI and warm to warm temperate on the HI. WI and HI index values were higher than in the previous period, with growing degree-days increasing 1272±125 and 2076 ±144, respectively for the period 1980-2012 (Ramos *et al.*, 2015). Annual precipitation averaged 389 mm, ranging between 223 and 594 mm, but average precipitation during the growing season (132.8 mm) represented between 25 and 47% of the annual total. About 59% of the growing season rainfall fell between budbreak and bloom; about 23% between bloom and véraison and about 18% between véraison and maturity. This rainfall distribution created significant water deficits during the hottest months within the growing season, although high variability was found between years.

These trends in climatic conditions are in agreement with the warming trends reported in other European viticultural areas (Jones *et al.*, 2005; Duchêne and Schneider, 2005; Orlandini *et al.*, 2009; Neumann and Matzarakis, 2011; Bock *et al.*, 2011; Tomasi *et al.*, 2011; Lorenzo *et al.*, 2012; and others).

3. Relationship between phenological dates and climate parameters

3.1. Relationship between phenological dates and temperature

Figure 2a shows the evolution of the average daily chill and heat units for the period analyzed, 2004-2015. It can be seen that chill units are accumulated until mid-April, while heat units start to increase in the middle of March. The accumulated chilling portion for the years analyzed ranged between 118 and 121. The PLS analysis between budbreak dates and chill units presented negative coefficients in different periods between October 15th and March 20th, with some discontinuities. Figure 2b shows the coefficients of the PLS regression between budbreak and the 10-day average daily chill units for the period between January 1st and April 15th for both varieties. The negative coefficients obtained in the analysis suggest that increases in chilling were correlated to earlier budbreak during that period. From March 20th, higher variability from year to year was observed and correlation coefficients were positive. Regarding the forcing units, heat units presented negative coefficients between mid-November and early June, but with stronger effects after March 20th. Based on the observed results of both, chill and heat phases, heat accumulation was then calculated from March 20th for both varieties.

The Tb values for budbreak ranged between 3.8°C for Tempranillo and 4.7°C for Cabernet-Sauvignon. For bloom, Tb ranged between 6.4°C (for Tempranillo) and 7.2°C (for Cabernet-Sauvignon) and for véraison Tb was 13.4°C for both varieties.

Table 1. Mean date and standard deviation (in days) of the phenological stages during 2004-2015 for each plot (CS: Cabernet-Sauvignon; T: Tempranillo) and average grape quality parameters for the same period at maturity (pH, soluble solids expressed in ºBaumé, weight of 100 berries (BW) and total anthocyanins (AntT). Stages are defined on the Baggiolini classification system.

Plot	Elev. a.s.l. (m)	Stage C Budbreak (BB)	Stage I Bloom (BL)	Stage M Véraison (V)	Stage N Maturity (M)	pH (at maturity)	ºBaumé (at maturity)	BW (at maturity)	AntT (at maturity)
PA_CS	802	24-Apr±5	15-Jun±9	16-Aug±9	2-Sep±6	3.70±0.09	12.9±0.7	206±25	600±159
PA_T	808	26-Apr±8	15-Jun±9	14-Aug±9	30-Aug±8	3.59±0.09	12.6±0.6	167±26	600±134
PB_CS	840	30-Apr±8	18-Jun±9	19-Aug±8	4-Sep±6	3.55±0.09	12.7±0.9	186±29	638±138
PB_T	820	26-Apr±6	18-Jun±9	12-Aug±8	2-Sep±8	3.59±0.13	12.5±1.3	173±22	629±178

Table 2. Average and standard deviation of the climatic variables recorded at Aranda de Duero (Figure 1) for the period analyzed (2004-2015): TmaxGS (maximum temperature during the period between budbreak and maturity), TminGS (minimum temperature during the period between budbreak and maturity), P (precipitation during the period: GS -between budbreak and maturity-; HY -hydrological year Oct 1st - Sep 30th; BB-BL- between budbreak and bloom; BL-V- between bloom and véraison; V- M: between véraison and maturity);WI (Winkler index - April 1st - October 31th), HI (Huglin index April 1st - September 30th)

Year	TMaxGS (°C)	TMinGS (°C)	TmGS (°C)	WI (C°)	HI (C°)	PGS (mm)	PHY (mm)	PBB-BL (mm)	PBL-V (mm)	PV-M (mm)
Avg	26.6	9.3	17.9	1320	2133	132.8	395.5	75.9	29.1	28
Std	1.3	0.5	0.9	176	189	70.5	97.6	52.4	21.5	20

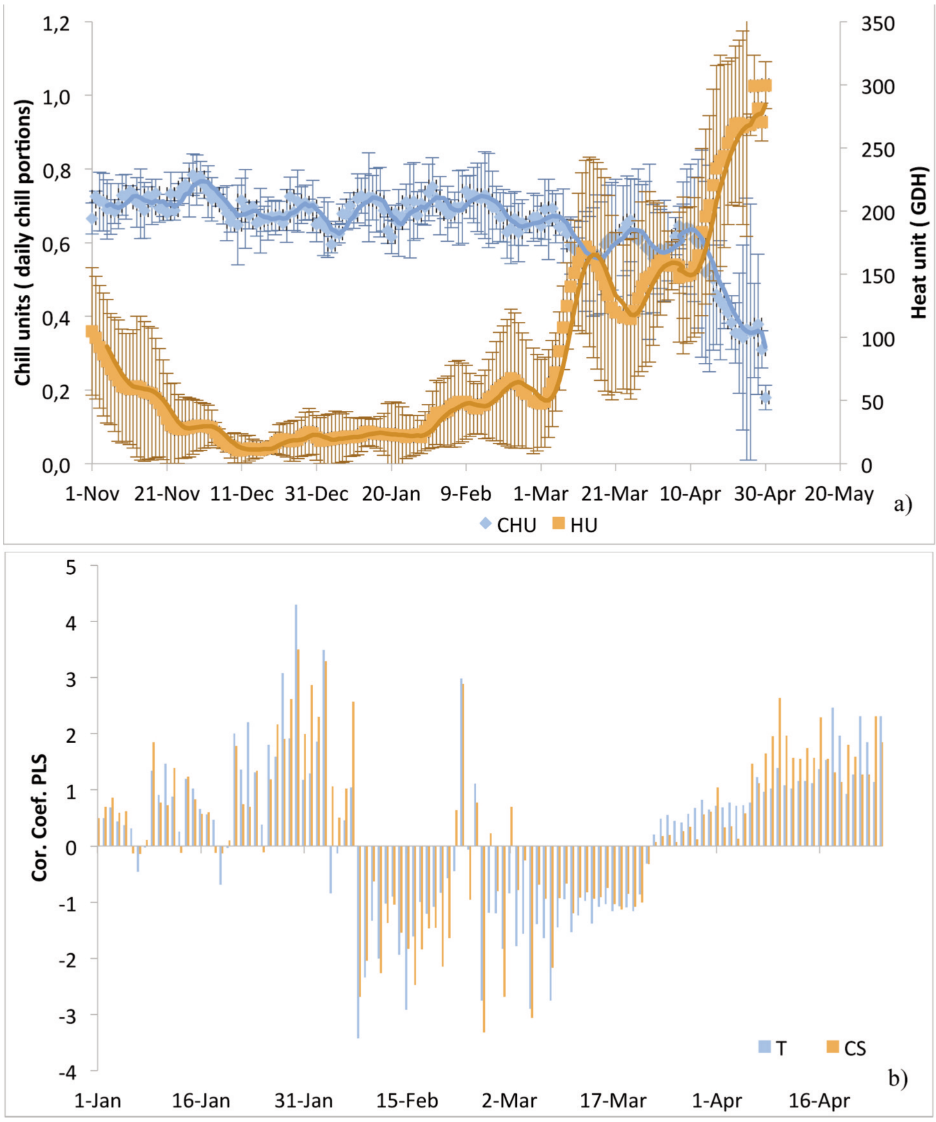

Figure 2. a) Average daily chill (CHU) and heat units (HU), estimated from hourly data from 2004-2015, for the period between 1st November to 15th May. b) Correlation coefficient of the PLS analysis for both varieties obtained using 10-day averages between January 1st and April 30th (T: Tempranillo; CS: Cabernet-Sauvignon).

Table 3. Statistical relationships between phenological dates and the P-ETc variable for each plot (precipitation minus evapotranspiration during the period -AMy: between April 15th and May 30th; AJ: between April 1st and June 30th). Slopes and R^2 given from multiple stepwise regressions. NS is not significant.

Plot	Stage C (Budbreak)	Stage I (Bloom)	Stage M (Véraison)	Stage N (Maturity)
PA_CS	NS	$+0.18\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.81$	$+0.16\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.84$	$+0.095\ (P\text{-}ETc)_{AJ}$ $R^2= 0.34$
PA_T	NS	$+0.17\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.81$	$+0.16\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.74$	$+0.097\ (P\text{-}ETc)_{AJ}$ $R^2= 0.35$
PB_CS	NS	$+0.14\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.66$	$+0.09\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.50$	$+0.096\ (P\text{-}ETc)_{AJ}$ $R^2= 0.34$
PB_T	NS	$+0.18\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.78$	$+0.12\cdot(P\text{-}ETc)_{AMy}$ $R^2= 0.46$	$+0.097\ (P\text{-}ETc)_{AJ}$ $R^2= 0.35$

For maturity, Tb ranged between 9.2ºC for Tempranillo and 10.5 ºC for Cabernet-Sauvignon. The RMSE values ranged between 5.2 and 7.3 days for budbreak, between 3.8 and 7.6 days for bloom and between 5.1 and 7 days for véraison. For maturity, the RSME of the fits based on heat accumulation were poorer, ranging between 8.9 and 12.2 days. It was observed that the worst fits corresponded to the years in which the highest temperatures were recorded (2009 and 2015, followed by 2014). In those very warm years, the predicted date was up to 20 days before the real dates. An opposite result was found in years 2005 and 2006, for which dates were predicted to be later by up to 19 days. The temperatures recorded in these two years were around the average values, however, they were very dry years. The effect of temperatures was not as exacerbated in the rest of the phenological periods. Differences between simulated and observed dates ranged between 0 and 8 days, with the poorest results seen under the extreme conditions (very dry or very wet years), but with differences of 2-3 days for the rest of years. Thus, the changes in phenology may be affected by both temperature and water availability, which may be indirectly modified by increasing temperatures.

The average GDD value needed to reach each phenological stage (BB, BL, V and M), using the derived base temperatures, were respectively 250±45, 425±51, 398±53 and 410±76 for Tempranillo and 205±43, 395±73, 386±42 and 372±88 for Cabernet-Sauvignon. The average values were also considered to predict the changes under the different climate change scenarios analyzed.

3.2. Relationship between phenology dates and available water

The water available during the growing cycle also exhibited some influence on phenology (Table 3). The results show that the most critical period for water availability was between April 15th and May 30th. While water deficits were insignificant for earlier events, the bloom and véraison dates were related to water availability (P-ETc) recorded during the period April 15th – May 30th. The effect was similar for both varieties for bloom, but differed between plots for véraison, without any clear pattern. Water deficits during this period produced earlier bloom and véraison events with the effect averaging 1-2 days per 10 mm. Water availability in the period budbreak to bloom affects also maturity, although in a lower proportion.

3.3. Projections for climate change scenarios

The changes in temperature (maximum and minimum) based on the ensemble of models integrated in the Coupled Model Intercomparison Project (CMIP5) were analyzed for the growing season, and for each phenological period (see Table 1), and for two Representative Concentration Pathways (RCP) scenarios – RCP4·5 and RCP8·5. For the period 1960-2000, during the growing season the average Tmax was 22.6±2.0º and Tmin was 7.9±0.8ºC. The results showed that under the RCP4.5 scenario increases in maximum temperature (Tmax) during the growing season are projected to range between 1.2°C for 2030 to 3.0°C for 2070. For the RCP 8.5 scenario, the predicted Tmax increase ranged between 1.4°C for 2030 to 4.3°C for 2070. The predicted changes of minimum temperature (Tmin) in the study area are smaller than those for Tmax, ranging between 0.9°C for 2030 to 1.9°C for 2070, under the scenario RCP4.5. Under the RCP8.5 scenario, the predicted changes in Tmin ranged between 1.2°C for 2030 to 3.4°C for 2070. During the reference period (1960-2000), the average Tmax in the periods from budbreak to bloom, bloom to véraison and véraison to harvest were respectively 19.7±1.9ºC; 25.6±2.7ºC and 23.9±2.6ºC. For the same periods, the average Tmin were 5.8±1.8ºC; 9.7±1.1ºC and 7.9±1.2ºC. Warming is projected to occur during each phenological period with bloom to véraison and véraison to maturity period likely to see the highest increases. By 2070 warming of about 3.0°C (RCP4.5) and 5.0°C (RCP8.5) during ripening is projected to occur.

The predicted changes in precipitation and for each phenological period (included the dormant period = period since leave fall to budbreak) were relatively small, without a clear trend and with high variability among models, which made it difficult to extract conclusions about trends and their potential effects. In addition to lower precipitation values during the time period (2004-2015), higher variability from year to year was also observed: 132.7 ±70 mm in the period 1960- 2000 *vs.* 217 ±22 mm in the period 2004-2015. Similarly during the periods between phenological events, precipitation was also lower (85.3±10.1 *vs.* 75.9±52.4 mm for the period budbreak to bloom; 62.2±10.1 *vs.* 29.1 ±21.5 mm for the period bloom to véraison and 56±11.4 *vs.*28±20 mm for véraison to maturity). For that reason this information was not taken into account in the prediction of changes in phenology.

3.4. Phenology changes under climate change scenarios

The changes in each phenological stage were estimated based on the predicted changes of the accumulated heat units (GDD calculated from Tmax and Tmin) during the growing season. The predicted changes in phenology based on the predicted changes of the accumulated heat units (GDD) are shown in Table 4.

The results showed that for the RCP4.5 scenario all events were earlier with budbreak ranging about 2 days for 2030, between 3.3 and 3.4 days for 2050 and between 4.9 and 5.1 days for 2070 respectively for Tempranillo and Cabernet-Sauvignon. Bloom was also earlier in all cases, ranging between 3.0 and 3.3 days for 2030, between 5.3 and 6.0 days for 2050 and between 7.9 and 9.4 days for 2070. For véraison, Tempranillo was predicted to occur slightly earlier than Cabernet-Sauvignon, with 13.7 and 13.3 days and 18.6 and 17.3 days, respectively for 2050 and 2070. For the RCP8.5 scenario the predicted budbreak dates are also earlier than at present, ranging between about 3 days on average for 2030 and about 9 days for 2070. Earlier dates for bloom were also predicted ranging between about 5 days for 2030 to more than 16 days for 2070; while véraison was also predicted to be earlier ranging between 10 days for 2030 and between 28 and 25 days for 2070, respectively for Tempranillo and Cabernet-Sauvignon. The projections show that maturity is likely to be between 10 and 23 days earlier under the scenario RCP4.5 and up to 35 days earlier for the scenario RCP8.5. The differences in prediction among models ranges between up to 2 days for the budbreak and bloom stages and up to 5 days for véraison and maturity under the RCPC4.5 scenario

Table 4. Predicted advances in phenological dates (in days) under climate change scenarios RCP4.5 and RCP8.5 and for the 2030, 2050 and 2070 time periods.

stage	RCP4.5			RCP8.5		
	2030	2050	2070	2030	2050	2070
Tempranillo						
C-budbreak	-2.1±0.7	-3.3±1.3	-4.9±1.9	-3.3±0.5	-4.9±0.8	-8.9±1.9
I-bloom	-3.3±1.0	-6.0±2.0	-7.9±2.2	-5.4±1.6	-9.9±2.7	-16.3±3.8
M-véraison	-6.4±2.1	-13.7±5.0	-18.6±5.7	-10.4±3.4	-18.7±3.7	-28.1±7.0
N-maturity	-10.6±3.0	-18.4±3.9	-23.9±4.9	-11.5±3.8	-21.7±3.1	-35.0±7.9
Cabernet-Sauvignon						
C-budbreak	-2.3±0.8	-3.4±1.1	-5.1±1.5	-3.0±0.6	-5.3±2.1	-9.4±2.4
I-bloom	-3.0±1.4	-5.3±3.1	-9.4±3.7	-5.4±1.9	-10.1±2.5	-16.9±3.1
M-véraison	-7.6±3.5	-13.3±5.5	-17.3±4.5	-10.0±2.8	-17.0±3.3	-25.4±6.1
N-maturity	-9.4±3.6	-18.7±6.0	-22.7±4.8	-13.2±4.9	-21.8±5.0	-33.5±7.8

and between 3 and 8 days under the RCP8.5 scenario.

Discussion

The plots located at lower elevation showed earlier phenology, while Cabernet-Sauvignon showed higher differences between plots compared to Tempranillo, in particular in the earlier stages. The differences between plots cultivated with the same variety ranged between 2 and 4 days for Tempranillo and up to 6 days for Cabernet-Sauvignon. These differences may be due to differences in elevation (approximately 30 m) and to differences in soil properties. Falcao *et al.* (2010) indicated differences in grapevine growth associated with elevation, a fact that has been considered as a potential adaptation strategy in order to decrease the impacts of climate change on grapevines (Caffarra and Eccel, 2011). However, Verdugo-Vasquez *et al.* (2016) found significant spatial variability in the phenological development and maturation at the field scale, with small differences in elevations. The higher clay and lower sand contents and coarse elements in the A plots compared to the B plots may imply higher water retention capacity and available soil water, which could explain the differences in phenology.

The chilling and heat accumulation phases were in agreement with those found by other authors (Martínez-Lüscher *et al.*, 2016), who indicated the period between the end of September and the end of February as the chilling phase, and that heat accumulation can occur during the chilling phase. In this study, however, the chilling phase was considered through the middle of March. From the end of the chilling phase to budbreak, the model identified base temperatures that ranged between 3.3 and 3.7°C, which were close to the 4°C proposed by Moncur *et al.* (1989) for the earlier phases, although the accumulation period was slightly different in their study. Zapata *et al.* (2017), however, found higher Tb values for Cabernet-Sauvignon (8.4°C) when temperatures were accumulated from January until budbreak. For the period between budbreak and bloom, the base temperature in this study was also found to be lower than those found by Zapata *et al.* (2017) for different red varieties (from 8.2 to 9.6°C). However, for the period between bloom and véraison the estimated Tb was slightly higher in this study (13.4°C vs. 12.5°C). These observations and those in the research referenced above indicate the need of using a base temperature different for each stage instead of using a general Tb = 10ºC, which has been used extensively. The use of different base temperatures for each phase may improve the modeling of phenological dates, in particular for the earlier stages for which a smaller Tb is required (Ramos, 2017).

As a result of the expected warming trends in the future, grapevine phenology was earlier for both varieties (Tempranillo and Cabernet-Sauvignon) examined in this study. The predicted earlier grapevine growth timing was higher for bloom and véraison than for budbreak, with the greatest change predicted for maturity. In numerous wine regions in Portugal, Fraga *et al.* (2016) indicated that the greatest influence of the thermal conditions on phenology was particularly significant during flowering, which in turn influenced the following phases. These authors showed strong links between budbreak and winter temperature, between bloom and spring temperatures and between véraison and spring-summer temperatures. Urhausen *et al.*, (2011), for cooler areas in the Upper Moselle Valley of Germany, found that the best predictions were based on accumulated degree days: the accumulated degree days in March for budbreak; the accumulated degree days in May and April, the mean daily maximum temperature in June, and the date of the budburst event for bloom.

The prediction of the earlier phenological events was similar for the two varieties analyzed, although they were slightly higher for Tempranillo than for Cabernet-Sauvignon in the first stages of the growing season under the scenario RCP4.5 and for almost all stages under the scenario RCP8.5. This result reveals that the main variety cultivated in the area of study – Tempranillo - may suffer greater phenological changes, which could produce negative effects for the viticultural economy in the area. The results are in line with analyses and predictions made in other areas (Webb *et al.*, 2007; Fraga *et al.*, 2016). Webb et al (2007) found that budburst for Cabernet-Sauvignon in Coonawarra (Australia) was projected to occur earlier by four to eight days in the year 2030, and by six to 11 days in 2050. Fraga *et al.* (2016), examining red varieties cultivated in various areas of Portugal, predicted earlier budbreak between 1 and 5 days and earlier bloom between 2 and 6 days, but they projected the greatest changes (between 6 and 14 days) for véraison. In the Ribera del Duero DO the greatest changes to earlier events was predicted under the scenario RCP8.5, not only for véraison but also for bloom. For maturity, despite the higher variability in the projections, our results predict smaller changes in dates of maturity than found for other wine regions. In this respect, in Australia and under the highest emission scenario and warmest future conditions, Webb *et al.* (2007) indicated that harvest could be 45 days earlier by 2050. While harvest dates could be controlled by additional factors, such as picking decisions based upon wine style differences, the results in this study and others show that maturity and therefore harvests in the future will likely occur both earlier in the year and in a warmer part of the year and may ultimately affect grape quality attributes.

While earlier phenological event dates are evident in this study and many others worldwide, there is growing evidence for differential changes that are shortening the phases between events. Jones *et al.* (2005) found shorter intervals between the main phenological events for 17 varieties across nine countries in Europe that ranged from 4 to 14 days. A study of 18 varieties in Conegliano, Italy, also found that intervals between events had shortened from 6 to 15 days (Tomasi *et al.*, 2011). In this research the relationships between temperature and phenology during 2004-2015 guide predictions that indicate a potential shortening of the period between budbreak and bloom of up to 3 days for Tempranillo and 5 days for Cabernet-Sauvignon and up to 10 days for the interval between bloom and véraison under the scenario RCP4.5, and up to 7 days and 11 days, respectively under the scenario RCP8.5. In Portugal, Fraga *et al.* (2016) indicated projected reductions of 1–2 days for budbreak-bloom and between 4 and 8 days for the bloom-véraison interval. In this respect, Moriondo and Bindi (2007) found that higher temperatures did not decrease the length of budbreak-anthesis phase (in many cases even a longer duration of the period was simulated) whereas the duration of anthesis-maturity phase was shortened in the future compared to the present period. However, Ruml *et al.* (2015) indicated that the change in the timing of phenological events did not significantly affect the duration of the growth intervals due to significant inter-correlation between the onset of each phenological stage.

Earlier phenological timing will mean that ripening will take place in summer under higher temperatures than at present, which may affect grape quality. In particular the development of polyphenols may be negatively affected (Sadras and Moran, 2013). However, increasing temperatures is not the only negative factor. The increase in temperature may have additional impacts due to influences on increasing evapotranspiration. The variability observed in the predicted changes in precipitation did not allow for the assessment of additional changes in phenology associated with changes in precipitation. However, the observed earliness of bloom and véraison with increasing water deficits indicates potentially even greater changes to earlier phenology if water stress increases. The seasonality of rainfall in the area usually results in enough water accumulated during the dormant period (period between leaf fall and budbreak) that ultimately provides enough for vine growth during the first stages of the growing season. However, significant water deficits were observed in other periods (mainly budbreak to bloom and bloom to véraison) that are particularly important on grapevine growth. Our data shows that observed water deficits produced an advance of bloom, véraison and maturity. While the results from the models exhibited too much variability to establish clear trends, it was evident that during the period analyzed precipitation was lower than in previous periods. Present trends show that bloom and véraison may shift earlier by about 1-2 days per 10 mm of water deficit. Thus, the observed earliness of bloom and véraison with increasing water deficits indicates potentially even earlier phenology. However, the existence of available water can modulate the effect of increasing temperature on phenology. This observation indicates that both temperature and water availability should be included in the models to predict the changes derived from climate change, otherwise erroneous predictions could be made for extreme wet or dry conditions.

Higher water deficits could have additional effects on vine development and on grape composition. Matthews *et al.* (1987) found that differences in water status did not affect the timing of bloom, véraison and harvest, but other factors such as shoot elongation and node production were affected with early water deficits. During the period analyzed, a significant decreasing trend was observed in P-ETc during the phase between bloom and véraison (about 3.8 mm per year on average) and it was observed that those deficits were correlated with an increase in the titratable acidity (about 0.18 g l^{-1} per 10 mm deficit increase), and a decrease of total anthocyanins (ranging between 25.5 and 34 mg l^{-1} per 10 mm deficit increase) in the Tempranillo plots. Color intensity, however, presented better relationships with the available water between budbreak and bloom and between véraison and harvest, but in both stages higher water deficits gave rise to lower color intensity (about 0.38 and 0.27 units of CI less per 10 mm deficit increase). Chaves *et al.* (2010) indicated that the timing and intensity of water deficits influence the extent of alterations occurring in berry metabolism and therefore in wine color and flavor. van Leeuwen *et al.* (2009) confirmed that temperature is a less decisive factor concerning vintage quality than water deficit stress in Bordeaux.

Kizildeniz *et al.* (2015) found that Tempranillo berry growth is very sensitive to water deficit at the beginning of berry development and that these negative effects cannot be reversed by supplemental water supply during the following stages. In addition, Ojeda *et al.* (2001) confirmed that early water deficits modify the structural properties of the cell components and consequently limit the subsequent enlargement of pericarp cells, which results in a reduction of berry size and berry weight along with affecting polyphenol and acid concentrations. Thus, the decrease in water availability due to increasing temperatures and changes in rainfall amounts and distributions may have additional effects on grape yield and quality and should be considered when climate change effects on vine development are examined.

Conclusion

This study contributes to the knowledge of climate change effects on grapevines and in particular with red varieties cultivated in the Ribera del Duero DO (Spain). The observations in this research agree with other research in Europe and worldwide, helping to further understand the roles that temperature plays in phenological development in grapevines. The results show that increasing temperatures may produce significant change to earlier phenological events for Tempranillo and Cabernet-Sauvignon in the Ribera del Duero DO (Spain), which will likely be additionally conditioned by water availability. Using the observed relationships between GDD and grapevine phenology, scenarios for future climate conditions indicate that phenology will likely continue to be earlier with greater effects seen in bloom and véraison than in budbreak. In addition to the predicted earlier events, differential changes in the future will likely result in a continued decrease in the duration of the period between different phenological events. The projections used in this research suggest a more pronounced effect on Tempranillo compared to Cabernet-Sauvignon. Given that Tempranillo is the dominant variety in the region, the results would indicate the need to examine adaptive strategies for managing the variety in warmer and potentially drier climates projected for the future. The projections on plant growth also need to be examined in terms of both fruit and wine quality and production. Together these relationships will ultimately drive adaptive actions in both vineyard and winery that can potentially address issues related to a warming climate.

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