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Projecting changes in phenology and grape composition of 'Tempranillo' and 'Grenache' varieties under climate warming in Rioja DOCa

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Summary

The aim of this research was to predict the changes in vine phenology under future climate change of two red varieties, cultivated under rainfed conditions, and how grape composition can be affected. The research was conducted in Rioja Designation of Origin (DOCa), a viticultural area located in north central Spain, where red varieties represent near 90 % of the cultivated vineyards. The research focuses on 'Tempranillo' and 'Grenache', which represent about 85 % and 10 %, respectively, of cultivated red varieties in the area. The analysis included data related to vineyards located at different elevations and with different climatic conditions, recorded during the period 2008-2018. Phenological dates related to separated flowers (stage H), veraison (stage M) and maturity of the two varieties as well as the grape composition during the ripening period and at maturity were evaluated. The future scenarios were based on the predicted temperature and precipitation changes under two Representative Concentration Pathway (RCP) scenarios –RCP4.5 and RCP8.5-, which were simulated with an ensemble of models. Projections for 2050 and 2070 were made based on the observed phenological dates and the heat accumulation needed to reach each stage along the growing cycle. An advance of all phenological stages was projected, higher for veraison and maturity than for the earlier stages, and without large differences between both varieties but with differences at different elevations. Veraison is expected to be advanced up to 11 days for 2050 and up to 12 days for 2070 under the RCP4.5 scenario, while under the RCP8.5 scenario, the advance by 2070 could be up to 20 days for both varieties. For maturity, the advance could be up to 27 days for 'Tempranillo' and 25 days for 'Grenache', with differences between the cooler and the warmer areas. These changes resulted in a shortening of the periods between phenological stages, giving rise to maturity under warmer conditions. Based on the variability of grape composition observed in the warmer in relation to cooler years, and on the relationship with climate variables, a decrease in acidity as well as a reduction in the content of total anthocyanins is expected for both varieties, which may be higher for 'Grenache' than for 'Tempranillo'.

Key words: acidity; anthocyanins; climatic change; precipitation; spatial variability; temperature.

Introduction

The suitability of a region to grow some specific varieties is mainly conditioned by climate which is probably the main component of terroir that controls grape production and quality. Nevertheless, soil, plant material and vine management are also important control factors in the vine response (Cheng *et al.* 2014, Zerihun *et al.* 2015, Van Leeuwen *et al.* 2017, Favero *et al.* 2010, Pérez-Bermúdez *et al.* 2015).

The projected changes in temperature and precipitation in mid and long terms present a threat for vineyards that are sensitive crops to temperature as each variety is better adapted to a specific temperature range. During the last decades, evidences on the effect of temperature increases on vines have been observed in different viticultural regions around the world. An advance of phenology has been pointed out in several studies (Duchêne and Schneider 2005, Petrie and Sadras 2007, Bock *et al.* 2011, Webb *et al.* 2011, Cleland *et al.* 2007, Malheiro *et al.* 2013, Ruml *et al.* 2016, Alikadic *et al.* 2019) and in some cases a shortening of the cycle is projected (Tomasi *et al.* 2011, Jones *et al.* 2005, Ramos and Jones 2019). On the other hand, the earlier harvesting, as a consequence of the advance of the phenological phases, may have a negative impact on grape composition (Salazar Parra *et al.* 2010, Duchêne and Schneider 2005, Webb *et al.* 2012, Sadras and Moran 2012, van Leeuwen and Darriet 2016) because harvest will occur at higher temperatures. These changes can have negative impacts on grape composition but they are still dependent on variety, location and characteristics of each region.

'Tempranillo' is the third most cultivated grape wine variety in the world, covering an area of about 232,000 ha and although it may be cultivated in different countries (among them, Australia, Chile, Greece, USA and South Africa), 88 % is located in Spain. It is a variety adapted to Mediterranean climate and its name is associated to an early ripening and a short growth cycle (OIV 2017). 'Grenache' is also a red variety with Spanish origin thought being introduced in France during the Middle Ages. At present, it is the seventh most cultivated grape wine variety in the world, covering about 185,000 ha, predominantly in France (about 55 %) and Spain (about 37 %) of the total surface and in smaller proportion in other countries like Italy, USA, Australia and China. These two varieties are well adapted to a temperature range between 16 and 20 °C (16 to 19 °C for 'Tempranillo' and 17 to 20 °C for 'Grenache') (Jones *et al.* 2012), being the temperature one of the main drivers of the evolution of the

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growing cycle and of the final berry composition (Sadras *et al.* 2007). A few days with temperatures above 30 °C may be beneficial during the ripening period, but its excess may induce plant stress, reductions in photosynthesis (Buttrose and Hale 1973, Greer and Weston 2010) and in secondary metabolites (Wu *et al.* 2019) that finally impact flavonoids, amino acids and carotenoids (Greer and Weedon 2013, Ovadia *et al.* 2013).

Different approaches have been undertaken in order to quantify the potential effects of increasing temperatures on the vine response, both referred to vine phenology and grape composition. Regarding the potential changes in phenology, some projections have been made based on relationships between the phenological dates and the average temperature and precipitation recorded within the growing cycle (Webb *et al.* 2007, Caffarra *et al.* 2011, Parker *et al.* 2011, Mesterházy *et al.* 2014, Fraga *et al*. 2016, Hall *et al.* 2016), while other studies were based on the effect of the accumulation of chilling units followed by heat accumulation (forcing units) up to a critical threshold (Caffarra and Eccel 2011, Parker *et al.* 2011, Hall *et al.* 2016).

Similarly, different models have been also used to predict the effect on grape yield and quality (Acosta *et al.* 2012, Pieri *et al.* 2012, Santos *et al.* 2012, Back *et al.* 2013, Neumann and Matzarakis 2014), for some varieties in different viticultural areas around the world. However, the response may be different depending on each variety and the specific climatic conditions recorded in a given area and few information exists about the response of 'Tempranillo' and 'Grenache'.

The accumulation of evidences about the responses of each variety under a range of conditions may help in the design of alternatives to mitigate the impacts of climate change on the wine sector. This research focus on the analysis of the two red varieties ('Tempranillo' and 'Grenache') cultivated in the Rioja DOCa, which is one of the most representative areas of these varieties in Spain, in particular for 'Tempranillo', one of the main producing areas in the world. The research tries to contribute to the knowledge of the response of these two varieties at present, and to evaluate the differences between them under scenarios of predictable climate changes. The analysis focuses on altered phenology under warmer conditions that might happen under different climate scenarios as well as its potential effects on grape composition in both varieties cultivated at different elevations. The information extracted from this research could help to define strategies in the selection of varieties best adapted to warmer conditions and to extract information of the zones that could suffer lower impacts under potential climate changes.

Material and Methods

Study area: The research was conducted in the Rioja DOCa (Suppl. Fig. S1), which is a viticultural region of Spain with long tradition and, at present, with the highest qualification (DOCa) within the viticultural designation of origin (DOs) of Spain. The total Rioja DOCa includes about 65000 ha of vineyards, mostly cultivated with red varieties at elevations that range from about 300 to 700 m above the sea level (m.a.s.l.) Within the Rioja DOCa, differences in climatic conditions allow the definition of different areas that are recognized as Rioja Alta (about 27,000 ha) with Atlantic influence, Rioja Oriental (about 24,000 ha) with Mediterranean influence and Rioja Alavesa (about 13,000 ha) with intermediate climatic conditions. 'Tempranillo' is the main variety cultivated in the area and represents about 85 % of the red varieties cultivated in the region, while 'Grenache' ranks second and occupies about 10 % of the total (Consejo Regulador Denominación de Origen Rioja, 2017). The response of the two varieties were analysed in plots located in different areas covering the conditions recorded in Rioja Alta (RA) and Rioja Oriental (RO) (Suppl. Fig. S1) and at different elevations (325 m, 397 m, 460 m, 525 m, 560 m and 600/635 m.a.s.l.). Vineyards planted with the two varieties were compared for each elevation. The soils of the selected plots are classified as *Calcixerollic Xerochrept* (IGN 2006) with loam and silty loam textures. Soil characteristics of the selected plots were obtained from the European Soil data base (ESDAC). The soils of the selected plots have organic carbon contents (SOC) that varied between 1.1 and 1.9 %, clay ranges between 19.0 and 27.1 %, silt between 33.4 and 48.5 % and sand between 30.2 and 46.2 %, while the coarse fraction varies between 8.4 and 15.4 % (Suppl. Tab. S1).

Climate data: present and future scenarios: The weather conditions during the study period were recorded in meteorological stations located near the plots. Data from the meteorological stations of Villar de Torre, Uruñuela, Nájera, Logroño, Albelda de Iregua, Ausejo, Quel, Aldeanueva de Ebro and Alfaro (Suppl. Fig. S1), that belong to La Rioja Government, were considered in this research. The information included hourly and daily temperatures and daily precipitations. When the plots were located between two meteorological stations, the data were interpolated taking into account the inverse of the distance to the meteorological stations.

For each plot, maximum and minimum temperature and precipitation related were averaged for the growing season, considering for each year the beginning and the end of the cycle according to the vine information recorded for the area (from the Consejo Regulador Rioja DOCa, personal communication). The daily temperature was used to calculate the Winkler Index (WI) (Winkler 1974) and the accumulated growing degree days (GDD) needed to reach the different phenological stages. The average GDDs accumulated to reach each phenological stage was used later to predict the changes in phenology under the future climate change scenarios.

In order to establish the initial day from which to accumulate the GDD, the chilling units were accumulated during the dormant period up to a critical chilling threshold, followed by the accumulation of heat units up to a threshold, simulating bud break. The analysis was done for each variety using the information recorded in plots located in RA. Daily chill accumulation (in Chill Portions) was calculated according to the Dynamic Model specified by Fishman *et al.* (1987) using hourly temperature data. Daily heat accumulation (in Growing Degree Hours (GDD)) was calculated according to Anderson *et al.* (1986). The chill and heat phases were determined by analysing the relationship between bud break dates and the means of 10 d of daily chill and heat units from 15th September (of the year preceding the recorded bud break) to 15th May using a Partial Least Squares (PLS) regression. Negative correlation coefficients are interpreted as periods that produced an earlier bud break. Once the chill and heat phases were delimited, the thermal requirements to reach each phenological stage were calculated. For each variety and stage, a base temperature threshold was determined following the process described in Ramos and Jones (2018), and the maximum temperature in the accumulation of heat units was limited to 26 °C, following the same criteria that were used in the heat accumulation previously (Anderson *et al.* 1986). The agreement between the observed and predicted dates was evaluated using the root mean square error (RMSE) and the d index (Willmott *et al.* 2011) calculated as indicated in Eq.1 and Eq. 2, respectively:

$$RMSE = \sqrt{\frac{\sum_1^n (DOYs - DOYo)^2}{n}} \quad (1)$$

$$d = 1 - \frac{\sum_1^n (DOYs - DOYo)^2}{\sum_1^n [(DOYs - \overline{DOY}o) + (DOYo - \overline{DOY}o)]^2} \quad (2)$$

where DOYs and DOYo are, respectively, the simulated and observed dates occurring in the corresponding phenological stages.

Daily maximum and minimum temperatures and precipitation were projected under two emission scenarios (RCP4.5 and RCP8.5) by 2050 and 2070, using an ensemble of models (BCC_CSMI_1M; CSIRO_MRk3-6-0;GFDL_ESM2M; GISS_E2H; HADGEM_ES; IPSL_CMJA_MR; MIROC_ESM_CHEM; MIROC5; MRI_CGCM3; NorESMI_M). The average values for the ensemble of models were obtained using the MarkSim™ DSSAT weather file generator (http://gismap.ciat.cgiar.org/MarkSimGCM/docs/doc.html).This application works with a 30 arc-second climate surface derived from WorldClim. The average of 20 replications was considered for each scenario.

Vine phenology and grape composition: Information of vine phenology and grape composition for the two varieties, recorded in plots located in the selected zones, which was supplied by the Consejo Regulador of Rioja DOCa, were considered in this analysis. The information included the dates at which the phenological stages H (separated flowers) and M (veraison) defined according to Baillod and Bagiollini (1993) for at least two plots per variety at each location for the period 2008-2018. Maturity was defined based on the date at which the probable volumetric alcoholic degree (PVAD) = 13° was reached. Regarding grape composition, the information included pH, titratable acidity (AcT), malic acid (AcM), total anthocyanins (AntT), total polyphenols index (TPI) and colour intensity (CI) from veraison to harvest every year on each 14 plots indicated in Suppl. Fig. S1. All analysis followed the methods recommended by the OIV (OIV 2012).

The Mann Kendall test (Libiseller and Grimvall 2002) was applied to analyse significant trends at 95 % level in the climate data. The different responses between plots were compared with a mean test (Tukey's test) and ANOVA, with temperature, precipitation and elevation as factors. The plots that presented significant differences were considered to stablish zones for which analyse the response of future responses under climate change scenarios. A stepwise linear multiple regression analysis (forward selection) was done to evaluate the relationship of grape composition with climate variables. The variance accounted by each variable was analysed. The differences in grape composition among years with different characteristics were also taken into account in the interpretation of the potential changes under climate change scenarios.

Results

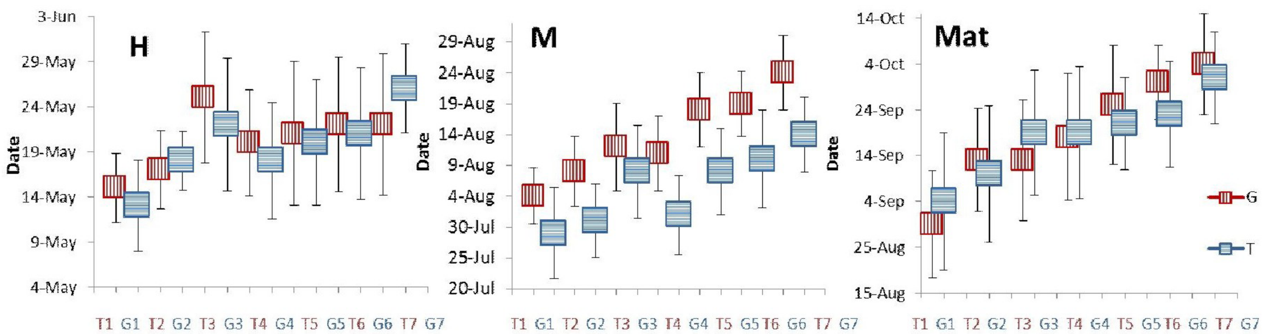
Climatic characteristics of the studied zones: Years with different climatic characteristics were recorded during the period under study, with some differences within the DOCa. Tab. 1 summarizes the average growing season maximum and minimum temperatures and precipitation recorded during the period 2008-2018 for each station). Mean growing season temperatures (TmGS) ranged between 15.7 and 18.9 °C, with higher values in the weather stations located in RO than in RA. The growing season maximum temperature (TmaxGS) varied between 22.4 and 26.1 °C while the minimum temperature (TminGS) ranged between 9.3 and 12.9 °C. Differences in the mean temperatures higher than 1 °C exist between zones within the DO, with higher values in RO than in RA. Accordingly, higher WI values were recorded in zones located in RO than in RA (maximum value of 1842 GDD in RO and minimum value of 1224 GDD in RA). High variability in average temperatures was recorded as denote the standard deviations of the variables. Regarding precipitation during the growing season (PGS), the mean value varied between 178 and 261 mm which represents about 50 % of the precipitation recorded in the hydrological year (PHY). The amount of precipitation recorded during the ripening period was usually very low (about 10 % of the PGS). Despite being a short time series, during the analysed period there was a significant decreasing trend ($p < 0.005$) in the number of d with T < 0 °C and an increasing trend ($p < 0.05$) in the number of extreme temperatures (T > 30 °C) (in all weather stations) with a consequent increase in TmaxGS.

Phenology: The average phenological dates for 'Grenache' and 'Tempranillo' recorded in each plot during the period under study are shown in the Figure. For 'Grenache', the stage H was reached between May 15 and 28; the stage M (veraison) occurred between July 31 and August 24, and maturity was reached between September 2 and October 3. For 'Tempranillo', the stage H took place 2 to 4 d earlier than in 'Grenache', while the stage M was 9 d earlier. The maturity date (PVAD = 13°) varied between plots but on average, it was earlier for 'Tempranillo' than for 'Grenache'. High variability was observed among plots, with significant differences($p < 0.05$) between the cooler and warmer areas for all three analysed phenophases, with earlier phenology in the plots located at lower elevation in RO than in the areas located at higher elevation in RA. The duration of the period between separated flowers and

Table 1

Mean values of the climatic variables related to temperature and precipitation recorded in different meteorological stations located in the study area during the period of study (2008-2018). TminGS: mean minimum growing season temperature; TmaxGS: mean maximum growing season temperature; TaverGS: mean average growing season temperature; WI: Winkler index; NdT < 0: number of d with temperature < 0 °C; NdT > 30: number of d with temperature > 30 °C; PHY: mean annual precipitation (referred to the hydrological year: 1st Oct-30th Sep); PGS: mean growing season precipitation; P BB-BL: mean precipitation recorded during the period budbreak to bloom; P BL-V: mean precipitation recorded during the period bloom-veraison; P V-M: mean precipitation recorded during the period veraison to maturity

Meteo. Station	Elev (m.a.s.l)	TminGS (°C)	TmaxGS (°C)	TaverGS (°C)	WI (GDD)	NdT < 0 (d)	NdT > 30 (d)
Alfaro	315	11.6 ± 0.4	26.1 ± 0.8	18.4 ± 0.9	1752 ± 150	6.5 ± 4.4	42.7 ± 7.0
Aldeanueva de Ebro	343	12.9 ± 1.3	25.8 ± 1.0	18.9 ± 0.8	1842 ± 136	24.5 ± 6.0	45.2 ± 6.0
Ausejo	563	12.3 ± 0.4	23.8 ± 1.2	17.4 ± 0.9	1558 ± 164	13.9 ± 6.6	34.0 ± 10.0
Quel	430	11.4 ± 0.7	25.1 ± 1.1	18.0 ± 0.9	1697 ± 168	26.1 ± 6.4	46.1 ± 9.0
Albelda de Iregua	450	10.6 ± 0.4	24.6 ± 0.7	17.3 ± 0.5	1364 ± 467	23.7 ± 7.1	42.2 ± 8.1
Logroño LG	408	12.1 ± 0.5	24.2 ± 1.0	17.6 ± 0.7	1601 ± 129	20.5 ± 11.5	12.1 ± 6.8
Uruñuela	450	10.5 ± 0.5	24.4 ± 1.2	16.9 ± 0.8	1436 ± 138	24.9 ± 7.1	37.4 ± 7.3
Nájera	510	9.3 ± 0.7	23.4 ± 0.9	16.0 ± 0.7	1538 ± 171	29.1 ± 8.9	36.4 ± 9.2
Villar de Torre	735	10.1 ± 0.5	22.4 ± 0.8	15.7 ± 0.5	1224 ± 75	27.7 ± 9.9	22.6 ± 7.3
Meteo. Station	Elev (m.a.s.l)	PHY (mm)	PGS (mm)	P BB-BL (mm)	P BL-V (mm)	P V-M (mm)	
Alfaro	315	384.0 ± 94.0	195.9 ± 57.1	73.9 ± 48.6	67.7 ± 35.6	17.2 ± 14.6	
Aldeanueva de Ebro	343	432.0 ± 94.1	227.4 ± 99.2	84.5 ± 45.6	71.5 ± 34.6	20.3 ± 17.5	
Ausejo	563	461.9 ± 111.2	242.5 ± 70.4	90.7 ± 47.2	50.5 ± 34.2	28.7 ± 17.1	
Quel	430	424.1 ± 89.1	240.0 ± 80.7	79.6 ± 27.8	89.1 ± 56.1	23.1 ± 21.5	
Albelda de Iregua	450	455.2 ± 91.0	228.7 ± 75.2	76.4 ± 23.1	72.0 ± 40.4	18.5 ± 17.0	
Logroño LG	408	469.7 ± 95.4	236.2 ± 91.6	241.7 ± 93.6	92.4 ± 43.4	57.5 ± 35.4	
Uruñuela	450	474.2 ± 102.0	242.3 ± 93.9	94.3 ± 72.9	50.0 ± 21.9	41.7 ± 23.7	
Nájera	510	369.4 ± 112.1	178.9 ± 87.0	75.4 ± 63.0	39.5 ± 28.3	30.7 ± 19.3	
Villar de Torre	735	530.2 ± 88.2	261.2 ± 74.5	121.2 ± 73.5	63.1 ± 23.1	42.5 ± 25.0	



ANOVA Factor	Date (stage H) (Sep.arated flowers)		Date (stage M) (Veraison)		Date (stage Mat) (Maturity)	
Elevation (Z)	0.0045***	0.0007 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0001 ***
Temperature (T)	NS	NS	NS	NS	NS	NS
Precipitation (P)	NS	NS	NS	NS	NS	NS
Means	G	T	G	T	G	T
Elevation						
325	15-May a	13-May a	4-Aug. a	29-Jul.. a	5-Sep. a	4-Sep. a
395	17-May a	18-May ab	8-Aug. Ab	31-Jul. A	13-Sep. Ab	10-Sep. Ab
460	20-May b	18-May bc	11-Aug. b	1-Aug. b	18-Sep. ab	19- Sep. b
525	22-May b	21-May b	19-Aug. c	9-Aug. b	26-Sep. bc	22-Sep. bc
565	22-May b	26-May b	19-Aug. C	10-Aug. b	30-Sep. C	23-Sep. bc
530/560	25-May ab	22-May ab	12-Aug. b	8-Aug. b	13-Sep. b	19-Sep. b
635	25-May b	26-May c	24-Aug. c	14-Aug. b	4-Oct. c	1-Oct. c

Figure: Results of the phenology ANOVA for the studied plots planted with 'Tempranillo' and 'Grenache' in the period (2008-2018). *** $p < 0.001$: significant at 99.9 % NS: no significant. Different letter indicates significant differences between plots in phenology.

veraison was, on average, 85 and 90 d for 'Grenache' and 78 and 81 d for 'Tempranillo' in RO and RA, respectively. The period between veraison and maturity was on average 28 and 42 d for 'Grenache' and 41 and 48 d for 'Tempranillo', in RO and RA, respectively. The ANOVA results showed that the elevation had a significant effect on the phenological dates for veraison and maturity (Figure), which allowed establishment of three zones: zone 1 located at the lowest elevation in RO (< 400 m), zone 2 located at the highest elevation in RA (> 630 m.a.s.l) and zone 3 located between 460 and 560 m.a.s.l. These three zones were considered in the analyses of the projected changes under climate change.

Thermal requirements: The PLS analysis between bud break dates and chill units presented negative coefficients in different periods between October 15 and April 30, with some discontinuities, and with positive values after March 23 for the 'Tempranillo' variety and after March 11 for the 'Grenache'. Regarding the forcing units, heat units presented negative coefficient between mid-November and April 3, but with higher values after March 7 for 'Tempranillo' and after April 10 for 'Grenache'. Based on these results, heat accumulation was started on March 15 for both varieties. Using the above mentioned criteria, the phenological dates were forecasted. Starting heat accumulation on that date, the threshold temperature to reach the considered phenological stages were 1.2, 0.5 and 3.0 for 'Tempranillo' and 5.3, 1 and 3.1 for 'Grenache', respectively for separated flowers, veraison and maturity. Evaluating the accuracy of the predictions, it was found that the RMSE for the stages H, M and maturity were 6.8, 5.5 and 8.4 for 'Tempranillo' and 7.0, 6.5 and 9.7 for 'Grenache', respectively. The d-Willmott index values were 0.56, 0.77 and 0.88 for 'Tempranillo' and 0.81, 0.82 and 0.82 for 'Grenache' for the stages H, M and maturity, respectively. According to the criteria given by Moriasi *et al.* (2007) for the RMSE and by Willmott (1992) for the d index, these results indicate moderate to good agreement between simulated and observed dates. The average GDD values needed to reach the stages H, M and maturity, taken into account the corresponding base temperatures, were 730 ± 102, 2235 ± 114 and 2590 ± 113 GDD for 'Tempranillo', and 475 ± 15, 1760 ± 113 and 1700 ± 178 GDD for 'Grenache', respectively for the three stages.

Projected changes in temperature and precipitation: Suppl. Fig. S2 shows the projected average changes in temperatures (Tmax and Tmin) and precipitation (P) (ratio Ppredicted/Ppresent), for the actual growing season that occurs from April to October, according to the RCP4.5 and RCP8.5 emission scenarios. Higher changes in temperature are projected in the areas located in RO than in RA, particularly in zones at lower elevation. The increase in Tmax ranged between 2.7 and 2.8 °C by 2050 under the emission scenario RCP4.5 and up to 3.6 °C under the scenario RCP8.5. For 2070, the increase in Tmax may be up to 3.3 °C in RO and up to 3 °C in RA under the RCP4.5 scenario, and up to 5.1 °C and 4.4 °C, respectively, in RO and in RA, under the RCP8.5 scenario. A reduction of 20 % in precipitation during the growing season might occur according to the RCP4.5 scenario and up to 30 % considering the RCP8.5 scenario.

Projected changes in the phenological dates for both varieties under different scenarios: The projected changes in the phenological dates corresponding to the stages H and M and the date at which the PVAD = 13° can be reached under different climate change scenarios, based on the predicted changes of the accumulated heat units, are shown in Tab. 2.

Table 2

Predicted advances in phenological dates (in days) of stages H, M and maturity (PVAD = 13°) under climate change scenarios RCP 4.5 and RCP 8.5 and for the time periods of 2050 and 2070

	RCP 4.5	stage	T1	T2	T3	T4	T5	T6	T7
Tempranillo	2050	H	4	5	5	3	3	3	3
		M	7	7	9	9	7	6	6
		Mat PVAD13	10	12	14	13	13	12	8
	2070	H	6	4	6	4	6	4	6
		M	9	9	11	9	9	8	8
		Mat PVAD13	14	15	17	17	16	15	13
	RCP 8.5								
	2050	H	6	5	7	5	7	5	6
		M	11	10	11	10	11	10	10
		Mat PVAD13	17	18	19	20	14	18	16
	2070	H	9	8	10	9	9	9	10
		M	16	15	16	16	17	16	17
		Mat PVAD13	22	24	26	28	22	26	27
	RCP 4.5		G1	G2	G3	G4	G5	G6	G7
Grenache	2050	H	4	5	5	5	5	5	4
		M	7	7	7	7	7	8	5
		Mat PVAD13	10	9	13	12	13	12	8
	2070	H	6	7	7	6	7	7	5
		M	9	9	10	9	10	11	7
		Mat PVAD13	12	12	19	15	22	16	13
	RCP 8.5								
	2050	H	7	8	9	8	9	7	7
		M	10	11	12	10	12	12	8
		Mat PVAD13	13	14	20	17	20	18	14
	2070	H	11	12	13	13	13	13	11
		M	16	16	17	16	17	18	17
		Mat PVAD13	21	20	28	24	28	25	26

The results showed that for the RCP4.5 scenario, all events can be reached earlier, more pronounced for veraison than for separated flowers, and a significant advance of maturity date. The advance of the stage H for 2050 varied for both varieties between 4 and 7 d and between 8 and 10 d under the RCP4.5 and RCP8.5 scenarios, respectively. In 2070, the advance ranged between 6 and 9 d under the RCP4.5 scenario and up to 13 d under the RCP8.5. In 2050, the projected advance of veraison was between 6 and 12 d and between 10 and 18 d under the RCP4.5 and RCP8.5 scenarios, respectively, and up to 18 d earlier in 2070. Larger advances were projected for maturity that could occur up to 28 d earlier under the most unfavourable scenario, especially at higher elevations in RO.

The projected changes in the phenological dates suggest a shortening of the periods between phenological stages. In 2070, 'Tempranillo' might experience a 5-7 d shorter period between stages H and M and up to 11 d for the ripening period. The earlier period might be shorter in the warmer than in the cooler area, while the later period might be longer in the cooler than in the warmer zones. For 'Grenache', the shortening could be up to 5 and 7 d for the period between stages H and M by 2070, and up to 9 and 12 d for the ripening period under the RCP4.5 and RCP8.5 scenarios, respectively.

Potential effect of the changes in temperature and precipitation on grape composition: The average values and standard variation of the grape composition at maturity are shown in Tab. 3. There were significant differences in acidity and phenolic compounds among years and among plots. Acidity was higher in 'Grenache' than in 'Tempranillo' and the highest values were recorded in RA at higher elevations. The concentrations of anthocyanins at maturity, for the same PVAD, were higher in 'Tempranillo' than in 'Grenache'. 'Tempranillo' showed the highest concentrations of anthocyanin in RO at low elevation and the lowest values at higher elevation. The differences among 'Grenache' plots at that PVAD (13°) were smaller, with the highest values recorded at the highest elevation but with large variability among years. TPI and CI were also larger in 'Tempranillo' than in 'Grenache', following a similar pattern to anthocyanins among plots.

The relationship between grape composition and climatic variables showed that temperature had significant effect on acidity and on phenolic compounds. The best fit was found to maximum temperatures (average growing season maximum temperature and number of days with extreme temperatures). The regression results are given in Tab. 4. For both varieties, an increase in temperature implied an increase in pH and a decrease in acidity (both titratable acidity and malic acid decreased). Total precipitation also showed influence on acidity, in particular for 'Tempranillo', in agreement with the higher values reached in the wetter years. The effect of temperature on total anthocyanins and on polyphenols was not so clear, but the sign of the relationship indicated that anthocyanins and TPI decreased with increasing temperatures.

Discussion

During the growing seasons from 2008 to 2018, differences of 2 °C in average and 3 °C in maximum temperatures were observed and they were about the same or even higher than those projected for 2050 (Figure). As a consequence, differences in grapevines responses were observed, which may give useful information about the potential changes that climate warming may produce in the vines and wines.

Table 3

Average values and standard deviation of the parameters related to grape composition recorded in each plot in the period 2008-2018. (Elev: elevation above sea level; titratable acidity (AcT); malic acid (AcM); potasium concentration (K); total anthocyanins (AntT); total polyphenol index (TPI); color intensity (CI)). factor Z(elevation); factors temperature and precipitation were not significant

		Elev (m.a.s.l.)	BW (100 berries (g)	AcT ($g·L^{-1}$)	pH	AcM ($g·L^{-1}$)	K ($mg·L^{-1}$)	TPI	AntT ($mg·L^{-1}$)	CI
Tempranillo	T1	325	212 ± 16 a	5.1 ± 0.5 a	4.0 ± 0.3 a	3.1 ± 0.5 b	2552 ± 331 a	39.2 ± 5.9 a	479 ± 113 a	10.4 ± 3.8 ab
	T2	375	179 ± 26 b	5.0 ± 0.5 a	3.8 ± 0.1 b	2.1 ± 0.6 b	2155 ± 218 b	39.3 ± 6.6 a	494 ± 83 a	11.7 ± 2.5 a
	T3	460	201 ± 41 ab	5.2 ± 0.5 a	3.7 ± 0.1 b	2.4 ± 0.8 b	2143 ± 323 b	39.4 ± 6.6 a	512 ± 86 a	13.01 ± 2.6 a
	T4	525	214 ± 33 a	6.2 ± 2.6 b	3.7 ± 0.1 b	3.4 ± 1.5 b	2550 ± 255 a	41.4 ± 8.9 b	467 ± 135 a	12.0 ± 4.3 a
	T5	550	236 ± 42 a	5.8 ± 0.8 b	3.7 ± 0.1 b	2.8 ± 1.4 b	2295 ± 313 ab	42.7 ± 8.9b	425 ± 132 ab	10.1 ± 3.6 ab
	T6	560	210 ± 32 a	5.2 ± 0.7 a	3.7 ± 0.1 b	2.6 ± 0.7 ab	2176 ± 194 a	33.7 ± 4.3 b	389 ± 60 b	8.8 ± 1.5 ab
	T7	635	219 ± 28 a	6.2 ± 0.8 b	3.7 ± 0.1 b	4.2 ± 0.9 c	2604 ± 199 a	33.0 ± 4.7 c	477 ± 57 a	11.4 ± 2.3 a
	Mean		210 ± 17	5.5 ± 0.5	3.7 ± 0.1	3.0 ± 0.7	2359 ± 202	38.4 ± 3.9	463 ± 42	11.0 ± 1.4
	ANOVA factor Z		0.0067***	0.0014***	0.0006***	0.0000***	0.0001***	0.0065***	0.0294**	0.0403**
Grenache	G1	325	186 ± 13 ab	6.0 ± 0.6 a	3.5 ± 0.1	1.8 ± 0.6 a	2101 ± 194	38.1 ± 6.8 a	217 ± 37 a	5.9 ± 0.9 a
	G2	375	180 ± 15 ab	5.4 ± 0.5 a	3.5 ± 0.1	1.0 ± 0.4 b	1872 ± 184	29.4 ± 4.7 b	215 ± 52 a	5.2 ± 1.2 a
	G3	460	164 ± 22 a	6.1 ± 0.9 a	3.6 ± 0.6	2.3 ± 1.0 bc	2172 ± 247	36.5 ± 3.7 a	248 ± 73 ab	6.9 ± 1.9 ab
	G4	525	207 ± 20 b	7.0 ± 1.8b	3.4 ± 0.1	2.3 ± 0.5 bc	1903 ± 132	37.6 ± 6.0 a	204 ± 42 a	6.1 ± 1.2 ab
	G5	550	198 ± 28 b	6.5 ± 0.9 b	3.4 ± 0.1	2.1 ± 0.9 c	1995 ± 205	38.6 ± 5.6 b	286 ± 74 ab	8.1 ± 2.1 ab
	G6	530	194 ± 19 b	6.6 ± 0.9 b	3.4 ± 0.1	2.7 ± 0.8 bc	1789 ± 194	29.1 ± 2.8 b	228 ± 70 b	6.4 ± 2.0 b
	G7	635	186 ± 17 b	6.5 ± 0.8 b	3.4 ± 0.1	2.0 ± 0.8 bc	1977 ± 294	39.7 ± 6.9 b	253 ± 72 b	7.6 ± 2.1 b
	Mean		189 ± 14	6.4 ± 0.6	3.5 ± 0.1	2.2 ± 0.6	1973 ± 132	35.6 ± 4.4	240 ± 31	6.7 ± 1.0
	ANOVA factor Z		0.002***	0.0188 ***	0.0001 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.000 ***

Table 4

Regression coefficients between grape parameters and climate variables (TmaxGS: average growing season maximum temperature; TminGS: average growing season minimum temperature; PHY: mean annual precipitation (referred to the hydrological year: 1st Oct-30th Sep); P-ETc BBLBL: Precipitation minus evapotranspiration in the period BB-BL; P-ETc BL-V: Precipitation minus evapotranspiration in the period BL-V; P-ETc V-Mat: Precipitation minus evapotranspiration in the period V-Mat; Titratable acidity (AcT); malic acid (AcM); Total anthocyanins (AntT); Total polyphenol index (TPI); Color intensity (CI)

Grape variety	Grape composition variable	Variables	Sign	r^2	Partial r^2	*p*
Tempranillo	AcT	TmaxGS	-	0.4846	0.3436	0.0009
		PHY	+		0.141	0.0030
	AcM	TmaxGS	-	0.3206	0.2681	0.0029
		P HY	+		0.0525	0.0500
	pH	P-Etc BB-BL	-	0.2548	0.2022	0.0000
		P-Etc BL-V	+		0.0526	0.0272
	AntT	ndT > 30	-	0.1400		***
	TPI	TminGS	-	0.1619	0.1619	0.0003
	CI	ndT > 30		0.1661		***
	AcT	TmaxGS	-	0.3375	0.3375	0.0000
Grenache	AcM	TmaxGS	-	0.1297		0.0014
	pH	TmaxGS	+	0.4252	0.3451	0.000
		P-ETc BL-V	-		0.0380	0.0246
		P-ETc V_Mat	-		0.0721	0.0112
	AntT	TmaxGS	-	0.1200		0.0023
	TPI			NS		
	CI	TmaxGS	-	0.2002		0.0001

NS: no significant $P < 0.05$: significant at 95 %; $p < 0.01$: significant at 99 %: $p < 0.001$: significant at 99.9 %.

From year to year in that period, differences in stage H of up to 14 and 19 d for 'Tempranillo' and 14 to 22 d for 'Grenache' ocurred, repectively in RO and RA. For the stage M, differences of 19 and 22 d for 'Grenache' and 24 and 21 d for 'Tempranillo' were found, respectively in plots located in RA and RO, while the date at which maturity was reached differed for more than 1 month among years (between 35 and 37 d for 'Tempranillo' and between 33 and 36 d for 'Grenache', respectively in RA and RO). The differences between the earliest and the latest phenological dates were up to 10, 12 and 21 d, for the stages H, M and maturity, respectively. The earliest ripening took place in years like 2017, or in 2011 in which the highest temperatures were recorded, and also in 2009, which was a very dry year. On the contrary, the latest phenological dates were recorded in years 2013 and 2008, which were very wet and cooler years, and in 2018, which was also wet (besides being the year of highest yield in the entire history of Rioja).

The differences in the phenological dates among years were smaller in the plots located at lower elevation in RO and at the highest elevation in RA.

Climate change scenarios projected increased temperatures that have the potential for occurrences of earlier phenological stages. Based on the accumulated degree days needed to reach each phenological stage, and considering the projected increase in temperature, an advance in all phenological events were predicted (Tab. 2), confirming that the earlier stages will suffer smaller advances than the later stages under higher temperatures. Thus, veraison and maturity are projected to be more advanced than flowering. This means that ripening and harvesting will take place under warmer conditions that strongly affect the grape composition and an amplified effect in warmer conditions in relations to cooler ones. Significant differences were observed at present between the plots located at the lowest elevation and the rest, for all three stages and for both varieties. In addition, for maturity, higher discrimination between location occurred, in particular for 'Grenache', with higher advance in the intermediate elevation (450-560 m.a.s.l) (Tab. 2). Maturity at those elevations could be advanced more than 15 and 25 d under the RCP4.5 and RCP8.5 scenarios, respectively, while in the areas located at lower elevation the advance could be up to 3-4 d less for 'Tempranillo' and 3-5 d less for 'Grenache', respectively under both scenarios. Despite the smaller advance predicted in warmer areas and the earliest phenology timing currently recorded in those areas, the predicted changes imply ripening under even warmer conditions. Caffarra and Eccel (2011) and Schleip *et al.* (2006) indicated more prominent growth changes in areas with suboptimal temperatures than in ones in which at present the temperature was already optimal.

The projected advance in phenology with increasing temperatures agrees with results found in the study area in warmer years and also with the results found in different viticultural areas around the world for other varieties. Ramos *et al.* (2018) projected for 'Tempranillo' in another Spanish viticultural area, located at about 800 m.a.s.l., an advance of bloom of about 6 d by 2050 and up to 7.9 d by 2070, and an advance of veraison of about 13 and 18 d for 2050 and 2070, respectively. Pieri *et al.* (2012) projected advances larger than 10 d for flowering and harvest by 2050 and nearly a doubling by 2070 for 'Merlot' in different areas of France. Fraga *et al.* (2016) indicated that bloom is expected to advance between 2 and 6 d and veraison between 6 and 14 d. Webb *et al.* (2007) found that the 'Cabernet Sauvignon' harvest could be 45 d earlier by 2050 under a warmer scenario. Ruml *et al.* (2016) indicates average advances in the beginning of bloom, veraison and harvest of 3.1, 5.2 and 7.4 d for each increase of 1 °C. Hall *et al.* (2016) projected advances in bud break and harvest for 'Shiraz' in Australian viticultural zones, which varied depending on the climatic conditions of each area. These authors indicated that the harvest date can advance 24 d for warmer areas to 62 d in coolest areas. In the area of study, although the changes in the phenological dates differed between zones and changes could increase with elevation within a zone, the projections show that the all phases will continue being earlier in warmer area of RO, as at present. Despite de smaller advance projected in the warmer areas, the earliest phenology timing currently recorded in those areas (more than 15 d for 'Tempranillo' and more than

20 d for 'Grenache' in relation to the areas with the highest predicted advance), the changes will imply ripening under even warmer conditions. The results suggest that under the RCP8.5 scenario, the 'Tempranillo' harvest in the lowest area of RO could take place in mid-August, and about 12 to 14 d later in the areas located at higher elevation, while in the coolest area located at higher elevation harvesting could occur between early to mid-September. For 'Grenache', the PVAD = 13° could be reached between 15 and 26 August in the warmer area in RO and in the first half of September (between 7 and 13 September) in the coolest area.

The advance in the phenological dates will shorten the periods between phenological events than can also be found in other areas, and that seems to be larger in the ripening period. In this respect, Fraga *et al.* (2016) indicated projected reductions of 1-2 d for bud break-bloom and between 4 and 8 d for the bloom-veraison interval in Portugal. Hall *et al.* (2016) projected in Australia a reduction of the length of the growing season for 'Shiraz' up to 9 and 21 d, for scenarios of 1.26 and 2.61 °C increases in the mean temperature. Ramos *et al.* (2018) indicated a potential shortening of the period between bud break and bloom of up to 3 d for 'Tempranillo' and 5 d for 'Cabernet Sauvignon' and up to 10 d for the interval between bloom and veraison under the scenario RCP4.5, and up to 7 d and 11 d, respectively, under the scenario RCP8.5. However, Ruml *et al.* (2015) did not find significant shortening of growing season and growth intervals, which they explained due to the interdependency of onset of each phenological phase. The increase in temperature associated to climate change can also affect grape quality. It was observed that higher acidity levels were recorded in the coolest and wettest years and it agrees with the relationship found between acidity and climatic variables. The results indicated that an increase of temperature under climate change scenarios will produce less acidic grapes. Acidity in 'Tempranillo' was also significantly affected by precipitation recorded during the whole year, in agreement with the observed higher acidity in the wet than in the dry years. Thus, the projected reductions in predictions (between 20 % to 30 % lower than at present) also will contribute to decrease acidity, when vines continue being cultivated under rainfed conditions. Taking into account the changes in temperature projected for the area of study, titratable acidity could decrease by 2050 between 0.77 and 1.2 $g \cdot L^{-1}$ for 'Tempranillo' and between 1 and 1.3 $g \cdot L^{-1}$ in 'Grenache', respectively under the RCP4.5 and RCP8.5 scenarios. By 2070, the decrease could be of up to 1.7 $g \cdot L^{-1}$, under the warmer scenario. These projections may represent a decrease of about 21.8 and 19 %, for 'Tempranillo' and 'Grenache', respectively, by 2050, given the most unfavourable scenario. Nevertheless, the decrease may be smaller the cooler than in the warmer areas, due to differences in the projected changes in temperature. Malic acidity may also decrease, eventually even higher in (up to 1.3 $g \cdot L^{-1}$ in 'Tempranillo' and up to 1.75 $g \cdot L^{-1}$ in 'Grenache' under the RCP8.5 emission scenario by 2070). During the last decades, a decrease in titratable acidity has been already found in some areas (Vršič and Vodovnik 2012, van Leeuwen and Darriet 2016) in association to increased temperature and solar radiation, and to changes in management techniques (van Leeuwen and Darriet 2016). Barnuud *et al.* (2014) in Australia projected, by 2070 under the A2 climate change scenario (high emission scenario), a decrease in titratable acidity of about 15 % and 12 % for 'Shiraz' and 'Cabernet Sauvignon', respectively, and Neuman and Matzarakis (2014) in Germany projected a decrease in titratable acidity between 0.5 and 2 $g \cdot L^{-1}$ in a 30-year period under the A1B and A2 emission scenarios.

Regarding the concentration of anthocyanins, it was observed that total anthocyanins and colour intensity decreased with increasing TmaxGS in 'Grenache'. The found relationships for this variety suggest a decrease in anthocyanins of up to 26 and 50 $mg \cdot L^{-1}$ by 2050, under the RCP4.5 and RCP8.5 scenarios, respectively, which means a reduction of up to 21 %. For 'Tempranillo', the best fit was found with the increasing number of extreme temperatures (days with temperatures > 30 °C). Based on the observed relationships between the concentration of anthocyanins with the number of warm extremes, anthocyanins are expected to decrease between 22 and 30 $mg \cdot L^{-1}$ by 2050, under RCP4.5 and RCP8.5 scenario, respectively, which means a reduction of up to 10 %. Regarding the relationships of anthocyanin concentration with temperature and precipitation, Cheng *et al.* (2014) attributed the increases in anthocyanin concentrations in one given year to a smaller number of days with extreme temperature (> 35 °C) and rainfall. These projections are in agreement with the observed values recorded in years with different weather conditions. Thus, in 2017, the warmest year of the series, the concentration of anthocyanins was, on average, 26 % lower than the average in 'Grenache' and 18 % lower in 'Tempranillo', with smaller differences in the cooler than in the warmer areas. The concentration of anthocyanins are actually lower in 'Grenache' than in 'Tempranillo', but the projected decrease is even higher for 'Grenache'. This reduction might imply a negative impact on the quality of the vines from 'Grenache'. Mori *et al.* (2007) indicated that high temperature increases the anthocyanin degradation on grape skins. The projected decrease of anthocyanins with increasing temperatures is corroborated by Barnuud *et al.* (2014) who projected that, under climate change, anthocyanin accumulation will be reduced up to 12 % by 2030 and up to 33 % by 2070 in the northern wine regions of Australia, while in the southern wine regions the reductions might be smaller (up to 2 and 18 % lower, respectively, in the same periods). The effect of temperature changes on colour intensity follows the anthocyanin trend, thus, a negative impact on colour can be expected. According to the observed relationships, CI may also decrease in up to 12 %, in 'Tempranillo' and near double in 'Grenache' by 2050, under the warmer scenario.

Given the projected advance in grape ripening, which will take place under warmer conditions, one of the best ways to mitigate the effects of climate change may be establishing measurements to delay phenological timing. Among the various viticultural techniques to delay ripening, the canopy management techniques are especially interesting because they can be performed on already installed vineyards without need to establish new vineyards, like relocation of the vineyards to cooler areas or the use of new plant material better adapted to future conditions. It is worth mentioning that various canopy management techniques have been proposed in Rioja, for delaying grape ripening, such as late winter pruning, shoot trimming and minimal pruning among others (Martínez de Toda 2019). Each of these techniques allows delaying the ripening of the grape between 15 and 20 d. Any of these techniques can delay ripening and practically counteract the predicted advance due to climate warming.

Conclusions

An earlier onset of all phenological stages is projected under the two climate change scenarios (RCP4.5 and RCP8.5), with larger advance for veraison and ripening than for earlier stages. The predicted changes may be similar for the two red varieties although it seems that they could be slightly larger for 'Tempranillo' in acidity and in anthocyanins in 'Grenache', although with differences between the warmest and the coolest areas located at different elevation. The advances in phenology and the shortening of the growing cycle might place the harvest in mid-August in warmer areas of RO and during the first half of September in the cooler areas of RA, which may produce an unbalanced ripening. Both acidity and phenolic composition may be negatively affected, with a decrease in acidity and in anthocyanins.

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