Using forced regrowth to manipulate Chardonnay grapevine (*Vitis vinifera* L.) development to evaluate phenological stage responses to temperature

Maria Teresa Prats-Llinàs[1*], Héctor Nieto[1], Theodore M. DeJong[2], Joan Girona[1], Jordi Marsal[1,†]

[1] Efficient Use of Water in Agriculture Program, Institute of Agrifood Research and Technology (IRTA), Parc de Gardeny (PCiTAL), Fruitcentre, 25003, Lleida, Spain

[2] Department of Plant Sciences, University of California Davis, One Shields Avenue-Mail Stop 2, CA 95616, USA

[†]Passed away in 2017

*Corresponding author

Maria Teresa Prats-Llinàs

Institute of Agrifood Research and Technology (IRTA)

Parc Científic i Tecnològic Agroalimentari de Lleida (PCiTAL)

Parc de Gardeny. Edifici fruitcentre

25003 Lleida (Spain)

maitepratsllinas@gmail.com

Abstract

Time and environmental conditions, such as temperature and photoperiod, are the main drivers governing grapevine development over the growing season. The most obvious growth periods in grapevines are budbreak, bloom, veraison and berry maturity. The aims of this study were to evaluate the environmental and physiological factors influencing the phenological development of Chardonnay grapevines, and to determine the best fit parameters of degree-day calculation methods for the prediction of various phenological stages. Phenological data retrieved from field vines and vines forced to regrow after heavy pruning and defoliation, whose developmental onset conditions were modified, were used to test and parameterize the degree-day calculation methods. An upper temperature threshold (T_U) was optimized for the different developmental stages, and measures of the radiation use efficiency were derived to adjust T_U during berry maturity. According with the candidate methods, the highest T_U value coincided with bloom (29.8ºC), while the lowest was observed at veraison (20.9ºC). The RMSE of the model predictions for specific developmental stages ranged from 2 (fruit set) to 9 days (berry maturity). Modifying vine growth periods by forcing vine regrowth allowed evaluation of temperature and physiological factors that influence grapevine development.

Keywords

Chardonnay grape, Degree-day methods, phenology, physiological factors, radiation use efficiency, upper temperature

- Forcing vine phenology delay allow degree-day methods evaluation under warmer conditions.
- Chardonnay grapevine presented stage-dependent conditions of each phenological stage.
- Bloom to veraison were temperature-driven stages.
- Upper temperature threshold decreased as grapevine development advanced.

1. Introduction

Plant vegetative cycles consist of two processes: growth and development. Growth involves an increase in the size of plants or organs, while development relates to phenology, which is the progression through different phases and implies continuous qualitative changes in plant form, structure and function (Sadras and Moran 2013). Growth is mainly dependent on the ability of plants to acquire chemical energy through photosynthesis, water and nutrients. Development is primarily controlled by temperature if other environmental factors, such as photoperiod and water stress, are satisfied (Pearce and Coombe 2004; Parker et al., 2013; Zapata et al., 2016). The environmental adaptation of crops greatly depends on the timing of key phenological stages, defined as the periods in which important changes take place (Petrie and Sadras 2008). In grapevines (*Vitis vinifera* L.), budbreak, bloom, veraison and berry maturity are the most obvious stages of the growth cycle that are used for timing management practices. However, the time between the different phenological stages may vary considerably depending on grapevine cultivar, climate and geographic location (Jones and Davis 2000, Parker et al., 2011; Fraga et al., 2015). Among white cultivars, Chardonnay is characterized to be one of the most commonly used cultivars for producing sparkling wines (Andrés-Lacueva et al., 1996).

Vineyards are climate-sensitive agricultural systems that may be affected by inter-annual weather variability and global warming (Jones and Web 2010; Fila et al., 2014; Mosedale et al., 2016). In recent decades, several grape-growing areas have reported changes in grapevine phenology, mainly linked to increases in temperature (Jones and Davis 2000; Petrie and Sadras 2008; Duchêne et al., 2010; Tomasi et al., 2011). Earlier phenological development in response to increasing temperatures is one of the expected consequences (Webb et al., 2007; Ramos et al., 2018). Advancements of the phenology of vines may displace berry maturation due to warmer conditions and have a negative impact on the berry composition and the wine quality (Tarara et al., 2008; Bonada et al., 2013). Nevertheless, the responses to these climatic changes may differ according to the grapevine cultivar, specific phenological stage and magnitude of the temperature changes in question (Petrie and Sadras 2008).

Several viticultural practices have been tested to diminish the effect of high temperatures on vine development and berry maturity (Petrie et al., 2017). The most relevant examples are the forcing of vine regrowth (Dry 1987; Gu et al., 2012) and delaying pruning (Friend and Trought 2007; Frioni et al., 2016; Moran et al., 2017). Both of these practices can shift periods of vine growth by delaying their initiation.

The aim of these practices is to modify the conditions under which plant development occurs, altering the usual temperatures that grapevines experience in a given phenophase during the growing season. Thus, these techniques can be used to delay bloom or berry maturity so that they occur under more favourable environmental conditions, where berry composition can be improved while yield can be decreased (Friend and Trought 2007; Gu et al., 2012; Moran et al., 2017; Petrie et al., 2017; Martínez-Moreno et al., 2019). Forcing vine regrowth or delaying pruning allows the evaluation of different phenophase responses, both in terms of timing and speed with which they occur (Moncur et al., 1989; Oliveira 1998).

Phenological models have been developed to predict the appearance and length of different phenological stages in grapevine. These models have mainly depended on temperature as the main driving variable (Jones and Davis 2000; Molitor et al., 2013) and have provided useful information for site and cultivar selection, vineyard management and pest and disease control (Hoogenboom 2000; Caffarra and Eccel 2010; Zapata et al., 2015). The most common phenological models are those based on degree-days, which strongly rely on the relationship between phenology and heat accumulation (Arnold 1959; Chuine et al., 2013). Most of these models assume that temperature has a linear effect throughout phenological development (García de Cortázar-Atauri et al., 2009; Nendel 2010; Parker et al., 2011; Zapata et al., 2015). Others, however, describe the response to temperature during development as non-linear functions (Cafarra and Eccel 2010; Molitor et al., 2013). The calibration of phenological models are typically based on historical phenological data, from single or multiple sites. The use of the phenological data of vines which have been forced to regrow in different times during the growing season, can provide a different approach for developing data to create and test model predictions and approximations. The phenological data obtained with the forced regrowth technique allow to get greater variation in the climate that vines experience. Moreover, the development of the vines take place in real field conditions without the need of heating methods (Sadras and Soar, 2009).

As temperature plays such an important role in plant behaviour, it is important to analyse vine responses to it. However, phenological development has been reported to produce non-linear responses to temperature. This suggests that the observed shifts in phenology may either be governed by resource availability for vine growth and development, or by interactions between the seasonal temperature cycle and the development of vines (Sadras and Moran 2013; Petrie et al., 2017). Measures of growth such as radiation use efficiency (RUE), determined with accumulated biomass in conjunction with intercepted solar radiation (Sinclair et al., 1992) and temperature, may help to elucidate such non-linear responses; and also, the influence of photosynthate availability on grapevine development. This is especially true after veraison, when development is thought to be influenced by temperature, water availability and the source:sink ratio (Petrie and Sadras 2008; Duchêne et al., 2010); and during berry maturation, which has been suggested to be responsive to a combination of temperature and solar radiation (Williams et al., 1985).

Physiologically, the effect of temperature on photosynthesis, respiration and plant development processes are modelled by enzymatic reactions (Bonhomme 2000). The responses of plants to temperature are with base or minimum temperatures and, maximum and optimum temperatures. Their values are obtained with curves relating temperature with the efficiency of enzymatic reactions (Bourdu 1984; Yan and Hunt 1999). Therefore, accurate predictions for phenological models require good estimations of base temperatures (T_B), defined as the threshold temperatures below which plant development ceases, and also the thermal time necessary for the onset of each phenological stage (Zapata et al., 2015). While some authors have taken T_B to be a constant (Williams et al., 1985; Jones and Davis 2000; Parker et al., 2013), Zapata et al. (2016) have found T_B to differ between budbreak, bloom and veraison, as a result of stage-dependent conditions that affect each individual phase. Moreover, Molitor et al. (2013) included an upper temperature (T_U) threshold, above which plant development does not accelerate or can even decrease (see Figure 2 in Molitor et al. 2013), due to the net energy available to the plants as a result of the influence of high temperatures on the rates of photosynthesis and respiration (Taiz and Zeiger 2010). In view of global warming, and the general lack of consideration of high temperatures in degree-day approaches, the incorporation of a T_U threshold into phenological models may help to improve their predictions in such scenarios (Molitor et al., 2013).

Until now, most studies have assumed a single constant T_U threshold for all of the phenological stages. However, the hypothesis in this study is that the T_U threshold may vary over the growing cycle, considering the possible increases in temperature over the whole growing season. Correspondingly, the parameters for calculating degree-days methods may vary according to the stage-dependent conditions of each phenological stage. Thus, the aims of this work were: (a) to evaluate the environmental and physiological factors influencing phenological stage development for Chardonnay grapevines, submitted to treatments that forced vine regrowth at different times; (b) to evaluate the best fit parameters of the distinct degree-day methods and T_U threshold for predicting each phenological stage; and (c) to consider interactions between the effects of high temperatures and RUE on phenological development after veraison.

2. Materials and methods

2.1. Vines and site

Field experiments were conducted in a 16-ha commercial vineyard of Chardonnay grapevines located at Raïmat (41º39’43’’ N – 0º30’16’’ E), Lleida (Catalonia, Spain). The vines (hereafter referred as field vines) were grafted onto SO4 rootstock and planted in 2006 with a spacing of 2.0 x 3.0 m, a north-south row orientation, and a loam soil. The canopies were trained to a vertical shoot positioned, bi-lateral, spur-pruned cordon located 1.0 m above ground level. Vine management followed the production protocol defined by the ‘Costers del Segre’ Denomination of Origin (Catalonia, Spain). The vines were irrigated on a daily basis, according with the crop reference evapotranspiration method (Allen et al., 1998), using a drip irrigation system.

Two different experiments were then performed in the same commercial Chardonnay vineyard. The first involved pruning treatments to force vine regrowth (section *2.2. Forced regrowth methodology*), and the second investigated radiation use efficiency based on measurements of vine growth and canopy light interception (section *2.4.3. Berry maturity method*).

In spring 2015, 172 one-year-old Chardonnay grapevines were grafted onto 1103 Paulsen rootstock at Raïmat (41º39’43’’ N – 0º30’16’’ E), Lleida (Catalonia, Spain). The grapevines were planted in 50-L containers with four holes in their base to allow adequate drainage. The growing media in the containers consisted of loose stones, arranged on the bottom of each container, combined with a substrate mix of equal parts of peat, sand and silty-loam soil. In spring 2016, 90 uniform vines (hereafter referred as container-grown vines) were selected and arranged in two rows, each with 45 vines, with a 3 m separation between rows. Vine management followed the ‘Costers del Segre’ Denomination of Origin (Catalonia, Spain) production protocol. Irrigation was scheduled to satisfy full water requirements of all the vines based on the water balance method (Allen et al., 1998).

2.2. Forced regrowth methodology

Forced regrowth technique was performed as is described in Gu et al. (2012), with the aim of delaying the vegetative cycle of the grapevines. This treatment consisted of cutting the growing shoots to leave just six nodes and then removing all the vegetative organs, including summer lateral shoots, leaves and clusters. This technique stimulated new vegetative growth on the vines in order to start a new growth cycle originating from currently growing shoots.

The forced regrowth technique was applied in the experiments conducted during the 2015 and 2016 growing seasons. They were run on 40 Chardonnay field vines during the 2015, 20 Chardonnay field vines during 2016, and on 90 container-grown Chardonnay vines during the 2016 growing season. The field vines were forced to regrow 60 and 98 days after budbreak in 2015; and 105 days after budbreak in 2016. Twenty vines were forced on each treatment date. The container-grown vines were forced to regrow 174, 184, 197, 208, 218 and 230 days after budbreak in 2016 (Figure 1, Table 1a). In 2016 the forced regrowth treatment was applied to fifteen container-grown vines on each date (15 vines x 6 forced regrowth dates = 90 vines).

2.3. Phenological and weather data

2.3.1. Bloom, fruit set and veraison

Phenological data recorded from the vines in Raïmat (Figure S1 supplementary material) were used as a calibration data set (Table 1a). The vines studied included: 48 vines from the 16-ha commercial vineyard, monitored during the 2015 and 2016 growing seasons (field vines); 40 forced regrowth field vines in 2015 and 20 forced regrowth field vines in 2016 (forced field vines); and 90 forced regrowth vines grown in containers, in 2016. The phases were registered when 50% of the shoots of the observed vines presented a given development stage according to the BBCH scale, which had the following identification codes: 09 - budbreak, 65 – bloom, 71 – fruit set, 81 – veraison (Lorenz et al., 1995). The phenological stages for the degree-day model calibration data set were: budbreak (n=10), bloom (n=10), fruit set (n=10) and veraison (n=9), and were recorded as days of the year (DOY) based on two observations per week (Figure 1, Table 1a).

Phenological data belonging to wineries and research institutions from several different locations across California (USA) and the Spanish province of Badajoz (Spain) (Figure S1 supplementary material) were used as a validation data set (Table 1b). For these data, the stages were also registered when 50% of the shoots presented the stage, but it was not possible to apply a specific phenological scale. The phenological stages for the validation data set were: budbreak (*n*=27), bloom (*n*=33) and veraison (*n*=30) (Table 1b).

2.3.2. Berry maturity

In this study, two different berry maturity criteria was used depending on the destination of the production of the Chardonnay vines: sparkling base wine berry maturity (*n*=8) and wine berry maturity (*n*=18) (Table 1a and 1b, respectively).The berry maturity for the Chardonnay experiments conducted in the Raïmat vineyards were determined according to sparkling base wine berry maturity criteria (Figure 1, Table 1a). A total berry soluble solids concentration of 16.5ºBrix was used as the berry maturity threshold, in line with the Raïmat winery objectives. To measure the Brix, six berries per vine were collected from each sampled vine (48 field vines in 2015 and 2016; 40 forced field vines in 2015 and 20 forced vines in 2016; and the forced container-grown vines from the treatments which reached the veraison stage in 2016) (Figure 1, Table 1a). Berry analysis measurements were made on a weekly basis from veraison until the threshold value of 16.5ºBrix was reached, using a refractometer (Palette PR-32α; ATAGO, Tokyo, Japan). The berry maturity dates reported by the wineries and research institutions in California (USA) and Badajoz (Spain) were destined for wine production (Table 1b). The berry maturity criteria were decided according to the quality criteria of the winery at each data origin site.

2.3.3. Weather data

Daily maximum and minimum temperatures were retrieved from two different stations at Raïmat (Catalonia, Spain). The weather data for field vineyards throughout 2015 and 2016 were taken from the official Raïmat SMC weather station (SMC, www.ruralcat.net/web/guest/agrometeo.estacions) located 1 km from the study location (Table 1b). Furthermore, the solar irradiance data used in the RUE experiment were also obtained from this station. The meteorological data for forced container-grown vines were retrieved from an automated weather station (Table 2a). The automated weather station was placed in the middle of the container-grown grapevines. It had a Pt100 temperature sensor placed in a shielded protector, at a height of 1.7 m, connected to a data logger (CR800, Campbell Scientific, Inc., Logan, UT, USA). The data acquisition protocols were adjusted to follow those used by the Meteorological Service of the Catalan administration (SMC). In California (USA), the same temperature data were acquired from the California Irrigation and Management Information System (CIMIS, www.cimis.water.ca.gov), whereas for Badajoz (Spain) the data were provided by the Irrigation Advice Network of Extremadura (REDAREX, redarexplus.gobex.es/RedarexPlus/) (Table 2b).

2.4. Method development

2.4.1. Degree-day calculation methods

In this study, four different methods for calculating the degree-days (*DD*) for each growth stage were evaluated. The first method tested, named *UniFORC* only considers a base temperature threshold (Chuine, 2000) (Equations S1-S3, supplementary material). Two of the others methods tested were previously described in Zalom et al. (1983): *Single triangulation* (Equations S4-S10, supplementary material) and *single sine* (Equations S11-S17, supplementary material). The fourth method examined was a modified version of the *single triangle algorithm* method (Zalom et al., 1983; Nendel 2010), in which the sum of degree-days at which a phenophase is likely to occur was calculated as follows (Equations 1-7):

$$thresDD_m = \sum_{i=1}^{m} (DD_{1\,i} - DD_{2\,i}) \quad (1)$$

$T_{max} < T_B$ $\qquad DD_1 = 0 \quad (2)$

$T_{max} > T_B$ and $T_{min} > T_B$ $\qquad DD_1 = \frac{(T_{max}+T_{min})}{2} - T_B \quad (3)$

$T_{max} > T_B$ and $T_{min} < T_B$ $\qquad DD_1 = \left(\frac{T_{max}-T_B}{2}\right) * \left(\frac{T_{max}-T_B}{T_{max}-T_{min}}\right) \quad (4)$

$T_{max} < T_U$ $\qquad DD_2 = 0 \quad (5)$

$T_{max} > T_U$ and $T_{min} > T_U$ $\qquad DD_2 = \frac{(T_{max}+T_{min})}{2} - T_U \quad (6)$

$T_{max} > T_U$ and $T_{min} < T_U$ $\qquad DD_2 = \left(\frac{T_{max}-T_U}{2}\right) * \left(\frac{T_{max}-T_U}{T_{max}-T_{min}}\right) \quad (7)$

Where:

$thresDD_m$, phenological stage degree-day threshold

i, onset of the previous phenological stage

m, phenological stage to be determined

T_B, base temperature (ºC)

T_U, upper temperature (ºC)

T_{max} and T_{min}, daily maximum and minimum temperatures (ºC)

Most of the degree-day calculation methods described above required the definition of a series of parameters in order to predict a change of phenological stage. The T_B and T_U were needed to calculate the DD values, while the DD threshold at which the phenological phase "*m*" was likely to occur (hereinafter $thresDD_m$) was also needed to define the change of stage.

2.4.2. Bloom, fruit set and veraison methods

Based on several previous grapevine studies (Williams et al., 1985; Jones and Davis 2000; Caffarra and Eccel 2010; Parker et al., 2013), and since one of the aims of the study was to determine T_U, we assumed that the T_B would be a constant for all the stages. Two different base temperatures were evaluated: T_B =5ºC and T_B = 10ºC. On the other hand, we assumed that the T_U and $thresDD_m$ values would vary between stages and they were therefore estimated for each of the degree-day methods tested and also for each phenological stage. We used a non-linear optimization with the interior-point algorithm implemented within the MATLAB suite (MATLAB 2014b, The MathWorks, Inc., Natick, Massachusetts, United States). For optimization purposes, both parameters were bound to physical and realistic output values. Thus, T_U ranged from 20ºC to 32ºC, while $thresDD_m$ had to be greater than 10 DD. All four methods were tested with respect to each phenological stage.

2.4.3. Berry maturity method

As with the previous stages, the T_U and $thresDD_m$ thresholds were optimized based on phenological data, but independently for values associated with sparkling base wine berry maturity (Table 1a) and wine criteria (Table 1b). However, in order to simplify the analysis, the assessments of the $thresDD_m$ methods were performed using only one T_B: the one with the best fit value from the previous stages of analysis.

An additional threshold, called the high temperature (T_H), was evaluated after veraison for temperatures above which the degree-days decreased, as described by Molitor et al. (2013). In situations in which the daily maximum temperatures (T_{max}) were above the defined T_H threshold, a new variable named corrected daily maximum temperature (T_{maxC}) was calculated; and then used instead of T_{max} in the degree-day method equations to determine the $thresDD_m$.

The new variable T_{maxC}, was calculated considering the influence of resource availability on Chardonnay vine development in conjunction with the effect of high temperatures. It was determined using a radiation use efficiency (RUE) experiment conducted during the 2015 growing season at the commercial Chardonnay vineyard. Radiation use efficiency was calculated by dividing accumulated dry matter production (DM) by the intercepted solar radiation (f_{IR}) (Sinclair et al., 1992):

$$RUE\ \left(\frac{g}{MJ}\right) = \frac{DM}{f_{IR}} \quad (8)$$

Dry matter production was measured using biomass samples of representative vines of the commercial vineyard at intervals of two weeks, from pre-bloom (May 8) until berry maturity (August 5). Vegetative parts of half of selected vines, including entire shoots with leaves and clusters, were destructively sampled. The dry weights of all those vine organs were recorded after they had been dried to a constant weight in a forced-air oven at 65 ºC. The height and width of the canopy were measured prior to biomass sampling and vegetative biomass data were normalized using canopy height and width dimensions. The total dry matter was obtained by adding together the dry matter values for vegetative and reproductive organs. Rate of dry matter production between two successive measuring dates was calculated as follows:

$$DM\ (g) = \frac{B_{i+1} - B_i}{S_{i+1} - S_i} \quad (9)$$

Where DM is the dry matter production between sampling dates: S_i and S_{i+1} are two consecutive sampling dates expressed in day of the year, and B_i and B_{i+1} are the dry matter production on S_i and S_{i+1} sampling dates, respectively.

The daily integrated fraction of intercepted photosynthetically active radiation (f_{IR} of PAR) was determined using the hourly light interception model of Oyarzun et al. (2007), in which the porosity is estimated. Measurements were made on fifteen representative vines from the commercial Chardonnay vineyard on the same dates that the vines were sampled for biomass. In order to estimate the daily f_{IR}, instantaneous measurements of f_{IR} were made at 11:00 a.m. ± 30 min local time - the time of day when light interception was at its peak - using an 80 cm linear ceptometer probe (Accupar Linear PAR, Decagon Devices, Inc., Pullman, WA, USA). The ceptometer was placed in a horizontal position, at ground level, and perpendicular to the vines. Five equally spaced measurements were then taken on the shaded side of each vine in order to cover the planting grid. Two more measurements were taken at an open space adjacent to each vine in order to determine the incident PAR above the canopy. A canopy porosity parameter was estimated so that the instantaneous value measured in the field could be related to the simulated hourly intercepted value corresponding to local noon. Vine structural parameters such as vine height, and canopy width perpendicular to the row were also measured. The integration of the diurnal course of the f_{IR} simulated from the Oyarzun et al. (2007) model was used to calculate the daily f_{IR} value.

For the calculation of RUE, the intercepted solar radiation values between two successive dates was calculated using Eq. 9. The measures of RUE were related to the maximum daily temperature, which were the average maximum temperatures between biomass sampling dates.

Two combinations of the methods were compared for each berry maturity criteria: using only T_{max} values, and using T_{maxC} values considering T_H = 35ºC (Ferrini et al., 1995).

As we had limited berry maturity criteria data, and given that there were no independent data sets available for berry maturity criteria, a cross-validation technique (MATLAB 2014b, The MathWorks, Inc., Natick, Massachusetts, United States) was used to maintain the testing capacity of the methods.

2.5. Method evaluation

Four indices were evaluated to obtain values for the best fit using degree-day methods. The predicted date for bloom and veraison stages were statistically compared with the observed date for the calibration and validation data sets (Table 1a and 1b, respectively). The goodness-of-fit of the different candidate methods were assessed considering the root mean square error (RMSE), the coefficient of determination (R^2) and the mean bias error (MBE). The akaike information criterion (AIC) (Burham and Anderson, 2002) was also used to select the candidate as the best method for defining each growth stage, according to the lowest AIC value. Because no independent data set was available for the fruit set stage, the best performance of the calibrated method for fruit set was assumed to be that selected to evaluate the veraison stage, and the same statistical indices were used for the evaluation of the method. In the case of the berry maturity stage, the goodness of the cross-validation was evaluated considering RMSE, R^2 and MBE statistics values.

3. Results

3.1. Forced regrowth

All forced regrowth treatments shifted bloom, fruit set, veraison and berry maturity (according to sparkling base wine criteria) phenological stages (Figure 1). Budbreak occurred a few days after the forced regrowth treatment was performed in both the 2015 and 2016 growing seasons. Phenological development of field vines was considered as a control, because their development followed the natural growing conditions of the season. In 2015 the number of days between budbreak and fruit set was less in the forced vines compared with the field vines. Different patterns were observed among fruit set to veraison stages in both regrowth treatments. Forced vines needed more days to reach berry maturity. The same tendencies for the number of days among stages were observed in the experiments in 2016, except for the berry maturity stage, where different trends were observed depending on the forcing treatment (Figure 1).

3.2. Degree-day methods

3.2.1. Bloom, fruit set and veraison

Candidate methods with low RMSE, MBE and AIC values and high R^2 values were selected using the calibration phenological data set (Figure 1, Table 1a). A base temperature of 5ºC produced the best results for the three stages analysed (Table 3) (See Table S1 on supplementary material for all method approaches). From budbreak to bloom development, the *UniFORC* method performed best, with a $thresDD_{BL}$ of 491.2 DD, resulting in an RMSE of 4.3 days, an R^2 of 0.898, an MBE of -0.5 days, and an AIC value of 61.08. For bloom to fruit set, the modified *single triangulation algorithm* method performed best, with a T_U of 25.4ºC and a $thresDD_{FS}$ of 47.6 DD, corresponding to an RMSE of 1.6 days, an R^2 of 0.998, an MBE of -0.1 days and an AIC of 41.51. Finally, for vine development from fruit set to veraison, the *single triangulation* method performed best, with a T_U of 20.9ºC and a $thresDD_V$ of 744.4 DD, with an RMSE of 4.8 days, an R^2 of 0.985, an MBE of -0.1 days and an AIC value of 57.65 (Figure 2a, Table 3).

The best methods for each stage were then applied to the independent data set for method validation (Table 1b). For bloom development, the resulting statistical analysis gave an RMSE of 6.7 days, an R^2 of 0.768 and an MBE of 5.1 days. As there were no available validation data for fruit set, we directly evaluated the veraison stage by sequentially applying the best fit methods for predicting bloom to fruit set and then fruit set to veraison. Then, the values obtained for the veraison prediction were 7.1 days for RMSE, 0.627 for R^2, and -6.1 days for MBE (Figure 2b, Table 3).

3.2.2. Berry maturity

Three different tendencies were observed in the relationship between T_{max} and RUE measurements (Figure 3). There was an increase of RUE with temperature from 5ºC to 25ºC; then, there was a plateau on the curve until 30ºC; and above 30ºC RUE decreased. The equation used to evaluate a decrease of degree-days due to the effect of high temperatures during veraison to berry maturity stages was obtained from this relationship. So that, the calculation of the new variable T_{maxC} from the T_{max} and RUE relationship was done as follows:

$$T_{maxC} = \frac{-0.0001*T_{max}{}^3+0.0043*T_{max}{}^2-0.0368*T_{max}+3.0328}{0.1226} \quad (10)$$

For berry maturity, a base temperature of 5ºC was considered in all the cases analysed (See Table S2 on supplementary material for all method approaches). The method which performed best for predictions of sparkling base wine berry maturity criteria (Table 1a) was the *single sine method* with the T_{max} and RUE relationship described in Eq. (10) with a T_H of 35ºC. The method parameters for sparkling base wine were a T_U of 25.7±0.5ºC and a $thresDD_{BMS}$ of 286.0±15.6 DD (Table 4). The cross-validation statistical analyses were 8.3 days for RMSE, 0.933 for R^2 and 0.1 days for MBE (Figure S2a supplementary material, Table 4).

Applying the same analysis to wine berry maturity, the best approach was the *single triangulation* method, with a T_U of 29.4±1.7ºC and a $thresDD_{BMW}$ of 724.1±16.4 DD (Table 4). Contrary to sparkling base wine, the relationship between T_{max} and RUE did not improve method predictions. The statistics obtained on the cross-validation statistical analyses for wine berry maturity were 8.5 days for RMSE, 0.836 for R^2 and -0.4 days for MBE (Figure S2b supplementary material, Table 4).

3.3. Phenological predictive capacity of the degree-day methods

The seasonal forecasting capacity of the degree-day methods developed in this study, were evaluated for consecutively predicting phenological stages. The best degree-day methods for predicting each stage were implemented sequentially from bloom to the successive phenological stages, until berries met their maturity criteria, using the optimized T_B, T_U, T_H and $thresDD_m$ parameters. The estimated beginning of each stage was taken as the baseline date for predicting the transition to the following stage, as opposed to the previous section, in which the transition between phenological stages was predicted considering the observed stage starting date. The phenological data set from Table 1a was used to evaluate the predictive capacity of the method for sparkling wine berry maturity. The phenological data set from Table 1b was used for doing the same analysis for wine berry maturity. For each stage, the estimated date obtained from each method was compared with the observed date to determine the RMSE, MBE and R^2statistics values.

The statistical values obtained for the different stages, in the evaluation of the predictive capacity of the methods from bloom until sparkling base wine berry maturity, were (Fig 4a): 4.7 days for RMSE and -0.1 days for MBE for the fruit set stage, 3.4 days for RMSE and -1.3 days for MBE in the case of veraison, and an RMSE of 10 days and an MBE of -1.5 days for predicting berry maturity based on sparkling base wine criteria. All of the values of R^2 ranged from 0.926 to 0.993 (Figure 4a). For the seasonal predictions from bloom until the wine berry maturity, the veraison stage prediction was 8.7 days for RMSE and an MBE of 4.5 days, while the wine criteria prediction produced an RMSE of 13.3 days and an MBE of 5.4 days. Lower R^2 values were obtained, with values of 0.497 for veraison prediction and 0.746 for wine berry maturity (Figure 4b).

4. Discussion

4.1. Forced regrowth vines

The observation data set used to calibrate the degree-day methods for the bloom, fruit set and veraison stages were taken from the vine forced regrowth experiment (Figure 1, Table 1a). The annual timing and the climatic time window when these stages normally occur was altered by the forcing treatments. On one hand, doing so it was achieved a variation of climates that vines experience under the same field conditions, reducing the variability on the environmental and soil conditions. But, on the other hand, the environmental factors photoperiod and temperature, which are the signals necessary for vine growth cessation and dormancy induction (Wake et al., 2000; Fennell et al., 2005), were modified. An issue of this study is that photoperiod, which is the duration of light exposure to plants, is one of the key environmental signals that grapevines use to adjust to seasonal changes (George et al., 2018), but this variable was not included in the methods. Furthermore, the pruning to stimulate canopy regrowth on the container-grown vines may have caused a debt on the carbohydrate reserves modifying the growth of those vines. Therefore, the use of phenological data from the forced regrowth vines for the calibration of the degree-days methods may have altered the response of vines to temperature, and influenced the performance of the degree-day methods. Moreover, the observation data to validate the methods may be constrained due to clonal variability and crop management factors, which can also influence the timing of veraison (Parker et al., 2013) and its visual assessment (Fila et al., 2014).

4.2. Physiological basis

4.2.1. Bloom, fruit set and veraison

Bloom and veraison stages were predicted equally well in this study (4 to 7 days) (Table 3). Previous models developed for Chardonnay predicted bloom more accurately than veraison (Caffarra and Eccel 2010; Parker et al., 2013; Zapata et al., 2016). The reason for this may be the high correlation between bloom and temperature (Buttrose and Hale 1973; Tomasi et al., 2011; Fila et al., 2014). Before veraison, vine development involves active cell division (Considine and Knox 1981), which is reflected in an exponential increase in plant growth in response to temperature (Rogiers et al., 2014). On the other hand, predicting veraison is challenging in Chardonnay (Parker et al., 2013; Fila et al., 2014; Zapata et al., 2016) because extreme temperatures and water stress have been reported to influence pigment accumulation in berry skins (Castellarin et al., 2007; Sadras and Moran 2012).

For most phenological studies in grapevines, fruit set was included in the transition between bloom to veraison phenological stages. Apart from temperature, other factors, such as grapevine carbohydrate status and photoassimilate availability, have also been reported to influence fruit set (Caspari et al., 1998; Zapata et al., 2004). Specific studies based on Chardonnay have demonstrated the influence of competition between root and shoot growth, carbohydrate reserve recovery, and soil temperature on fruit set (Rogiers et al., 2011). In view of these factors, the short duration of the fruit set stage (Figure 1), and since it was not evaluated using independent data, the method developed to predict fruit set in this work appeared to be appropriate as an initial approach for predicting the timing of fruit set (2 days) (Table 3).

4.2.2. Berry maturity

The accuracy of the predictions of berry maturity criteria was the lowest of the stages determined in the study, while those for sparkling base wine berries (8 days) were slightly better than for wine berries (9 days) (Table 4). Major changes take place during maturation, when the strongest driver for sugar accumulation in berries is the availability of resources (Sadras and Moran 2013) and when photoassimilation becomes a limiting factor for berry growth as maturation advances (Williams et al., 1985). Other factors, such as crop load (Williams et al., 1985), water availability (Duchêne et al., 2010) and the source:sink ratio (Petrie and Sadras 2008), also influence the maturation rate. On modelling phenology, temperature is the main environmental factor taken into account in the calibration and development of degree-day methods. Apart from temperature, more factors may need to be considered for improvement of predictions of berry maturity development. For instance, using combinations of temperature along with solar radiation, as was tested in this study improved the accuracy of the sparkling base wine maturity (8 days) (Table 4).

4.3. Degree-day calculation parameters

4.3.1. Bloom, fruit set and veraison

When modelling grapevine phenology, it is commonly assumed that the T_B remains constant throughout the growth cycle (Williams et al., 1985; Jones and Davis 2000; Parker et al., 2013). In our study, we evaluated the temperatures thresholds 5 and 10ºC for obtaining a single T_B for the whole growing period. However, various different temperatures have been associated with the timing of the initial and final phenological stages (Sadras and Soar 2009). The best performance was achieved with a T_B of 5ºC in all phenological stages (Table 3). In previous Chardonnay studies, a reported T_B for obtaining bloom was 8.2ºC, and for reaching veraison was 9.7ºC (Zapata et al., 2016); and a range from 7.3 to 7.8 ºC was obtained for bloom, and from 1.4 to 3.6ºC for veraison (Fila et al., 2014). In the development of phenological models on grapevines cultivars under different climatic conditions, several authors have suggested that the T_B might be lower than 10ºC (Moncur et al., 1989; Nendel 2010; Molitor et al., 2013; Parker et al., 2011; Zapata et al., 2015). The weather data used for calibration in this study included the warmest months of the growing season (Table 2a). In a few occasions the minimum temperature could have exceeded 5ºC, which was the T_B threshold providing the best fit. This may indicate that temperatures lower than 10ºC during grapevine development in this study were effective enough to accumulate degree-days to stimulate development, and improved accuracy of the method. These results demonstrate that to model phenology development of grapevines over the growing season, temperatures lower than 10ºC are appropriate to consider as a base or lower temperature threshold for the accumulation of degree-days (Williams et al., 1985, Molitor et al., 2013).

Similar to Zapata et al. (2016) who evaluated T_B, the aim of this work was to evaluate the variations of response to temperature among phenological stages at different ranges of T_U. Moreover, in the work of Molitor et al. (2013) with the Müller-Thurgau grapevine cultivar, the incorporation of a T_U into the degree-day model approach improved their precision. As a result, stage-dependent variations of T_U were developed based on observed decreases in the thresholds corresponding to spring and summer when increases in air temperature occur. A higher T_U value was associated with fruit set (25.4ºC), while a lower was observed for veraison (20.9ºC) (Table 3). In contrast, Zapata et al. (2016) reported that the T_B thresholds tended to increase over the growing cycle. They hypothesized that this was due to the need for an increase in temperature in order to set in motion the biochemical reactions that occur from budbreak to veraison (Johnson and Thornley 1985). In both studies, the stage-dependent variations in each phenological stage were evaluated in a similar way: as phenological stages advanced, the possible range of degree-day accumulation was reduced. In the case of Zapata et al. (2016), there was an increase in the T_B threshold while T_U remained the same, and in our case, while T_B was the same, there was not an initial constraint of T_U threshold for bloom, and then the T_U decreased.

Although the *thresDD* values from the current study cannot be directly compared - since the methods applied performed differently for each stage given that each was governed by different physiological processes -, the veraison requirements were higher (744.4 DD) than those for bloom (491.2 DD) (Table 3). Fruit set was also evaluated independently and had the lowest *thresDD* value (47.6 DD) (Table 3). Similar tendencies have been observed for other regions and cultivars, although in those cases, fruit set was not separately considered but included within the bloom to veraison stage (Duchêne et al., 2010; Parker et al., 2013; Zapata et al., 2016).

4.3.2. Berry maturity

The T_U values obtained for the two kinds of berry maturity criteria differed considerably (25.7±0.5ºC sparkling base wine, 29.4±1.7ºC wine) (Table 4). This was due to the use of a T_H value based on the T_{max} and RUE relationship (Eq 10) for the prediction of the sparkling base wine berry criteria, which reduced the T_U threshold. In both cases, the T_U values were higher than those determined for veraison prediction

(20.9ºC) (Table 3). Moreover, the *thresDD* value for wine berry maturity was noticeably higher than that for sparkling wine berry maturity (286.0±15.6 DD sparkling base wine, 724.1±16.4 DD wine) (Table 4). This can be explained by the fact that berries destined for making wine were harvested later, and therefore accumulated more degree-days. Furthermore, a reduction in the accumulation of degree-days occurred in the case of sparkling wine berry maturity beyond the defined T_H threshold. This is highlighted in the difference between the *thresDD* values. The accuracy of the sparkling base wine berry maturity criteria improved when the T_H reached or exceeded 35ºC (8 days) (Table 4). In contrast, predictions for berries used for wine did not work well, probably because of the high level of variability in the source data, which was provided mainly by growers (Table 1b). The lower performance may have been partially due to subjectivity on the part of the growers making picking decisions when collecting source data (Tomasi et al., 2011). However, the relationship T_{max} and RUE may be capable of improving predictions of wine berry maturity if we could obtain a more controlled data set.

4.4. Applicability of the degree-day methods

The predictive capacity of the different methods over a whole growing season (Figure 4a, Figure 4b) was evaluated considering that the bloom predictions were the same as those used during method development (Figure 2a and Figure 2b). The low level of accuracy, especially for predicting berry maturity, seems to point to the reduced importance of temperature and the increased importance of other factors (such as crop load, the source:sink ratio and water availability), making temperature driven models less accurate. It may be possible to improve model prediction by adding more variables, such as water availability and soil temperature, which have been reported to be strong drivers of phenological development (Ramos and Martínez-Casasnovas 2010; Rogiers et al., 2014), using maximum daily temperatures (Duchêne et al., 2010), or adding source:sink relations. Moreover, although the input data were usually obtained from weather stations located at a given distance from the vineyards, local environmental conditions probably varied across vineyards due to their canopy structure, row orientation and topography (slope and exposure) (Zapata et al., 2016). Studies conducted comparing different cultivars highlight the need to describe the degree-day requirements for each specific phenological stage, and the variability observed between different cultivars, because the temperature threshold definition and accumulated degree-days could help to characterize early and late cultivars (Parker et al., 2013; Zapata et al., 2016).

Although the incorporation of a T_H did not substantially improve the accuracy of the methods, its incorporation into the calibration of phenology models may become important under warmer climatic conditions (Molitor et al., 2013). Increments of temperatures will likely affect quality parameters of the berries, leading to changes in berry composition. A faster rate of maturation is generally associated with higher temperatures throughout maturation and the early onset of ripening (Petrie and Sadras 2008). The biosynthesis of anthocyanins, which is responsible for the coloration on berry skins, can be slowed down by high temperatures (Mori et al., 2007). The same can happen with terpenols: the molecules responsible for aroma (Duchêne et al., 2010). High temperatures can therefore reduce grape quality (Jackson et al., 1993), making it important to develop accurate methods capable of predicting advances in maturity before the desired berry maturity criteria are met.

5. Conclusions

This study showed different responses corresponding to the different phenological stages in the development of Chardonnay grapevines based on an approach that employed different degree-day methods and various T_U thresholds for each stage. The shifts in the vine growth periods, which were manipulated through pruning, delaying its onset to different times, allowed us to evaluate the environmental and physiological factors that influence grapevine development. Using the data obtained from the vine forcing treatments altered the timing and the environmental conditions under which the phenological stages normally occurred. The results obtained accentuated the different factors that drive each phenological stage and contribute to a better understanding of Chardonnay grapevine phenology. During grapevine development from bloom to veraison, the value of T_U progressively decreased, and exhibited a changing pattern at berry maturity. The relationship between maximum air temperature and radiation use efficiency was considered and slightly improved the approach for predicting berry maturity for sparkling wines. The newly developed methods could be useful for improving grapevine phenology models in scenarios of warmer climatic conditions.

Acknowledgements

The work was funded by Spain's National Institute for Agricultural and Food Research and Technology (INIA) [RTA 2012-00059-C02-01], which belongs to the Ministry of Economy and Competitiveness, and by the European Social Fund. The authors would like to acknowledge the collaboration of Mr Antonio Abat from the Codorniu winery, Mr Luis Sanchez from the Gallo winery, and Dr Michael Sipiora and Ms Amanda Chilar from the Treasury Wine Estates for providing the database. We would also like to thank Dr Luis Alberto Mancha and Dr Alexander Levin for providing data from regions of Spain and California. Dr José Manuel Mirás-Avalos for his help on the manuscript, and the staff of the IRTA's Efficient Use of Water in Agriculture Program, particularly Dr Joaquim Bellvert.

References

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and drainage. 56. FAO, Rome.

Andrés-Lacueva C, Gallart M, López-Tamames E, Lamuela-Raventós RM (1996) Influence of variety and aging on foaming properties of sparkling wine (Cava). 1. J Agric Food Chem 44:3826–3829.Arnold CY (1959) The determination and significance of the base temperature in a linear heat unit system. J Am Soc Hortic Sci 74:430–445

Bonada M, Sadras VO, Fuentes S (2013) Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. Aust J Grape Wine Res 19:87–94

Bonhomme R (2000) Bases and limits to using "degree.day" units. Eur J Agron 13:1–10

Bourdu R (1984) Bases physiologiques de l'action des températures. In: Gallais, A. (Ed.), Physiologie du maïs. INRA, Paris, pp. 389–424

Burham KP, Anderson RA (2002) Model Selection and Multimodel Inference: A practical Information-Theoretic Approach. Burnham KP, Anderson DR (ed) Information theory and loglikelihood models: a basis for model selection and inference. Springer, Berlin, pp. 32– 74

Buttrose MS, Hale CR (1973) Effect of temperature on development of the grapevine inflorescence after bud burst. Am J Enol Vitic 24:14-16

Caffarra A, Eccel E (2010) Increasing the robustness of phenological models for *Vitis vinifera* cv. Chardonnay. Int J Biometeorol 54:255–267

Caspari HW, Lang A, Alsbach P (1998) Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Am J Enol Vitic 49:359–366

Castellarin SD, Matthews MA, Di Gaspero G, Gambetta GA (2007) Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta 227:101–112

Chuine I. (2000) A Unified Model for Budburst of Trees. J. Theor. Biol. 207, 337–347.

Chuine I, García de Cortázar-Atauri I, Kramer K, Hänninen H (2013) Plant development models. In Phenology: An Integrative Environmental Science. Springer Dordrecht Heidelberg, New York, London, pp. 275-293

Considine JA, Knox R (1981) Tissue origins, cell lineages and patterns of cell division in the developing dermal system of the fruits of *Vitis vinifera* L. Planta 151:403–412

Dry P (1987) How to grow ‘cool climate´grapes in hot regions. Australian Grape Grower and Winemaker 1987:25-26

Duchêne E, Huard F, Dumas V, Schneider C, Merdinoglu D (2010) The challenge of adapting grapevine varieties to climate change. Climate Res 41:193–204

Fennell A, Mathiason K, Luby J (2005) Genetic segregation for indicators of photoperiod control of dormancy induction in Vitis species. Acta Hort. 689:533–539

Ferrini F, Mattii, GB, Nicese FP (1995) Effect of temperature on key physiological responses of grapevine leaf. Am J Enol Vitic 46:375–379

Fraga H, Costa R, Moutinho-Pereira, CM, Correia CM, Dinis L-T, Gonçalves I, Silvestre J, Eiras-Dias J, Malheiro AC, Santos JA (2015) Modeling Phenology, Water Status, and Yield Components of Three Portuguese Grapevines Using the STICS Crop Model. American Journal of Enology and Viticulture, 66:482-491Fila G, Gardiman M, Belvini P, Meggio F, Pitacco A (2014) A comparison of different modelling solutions for studying grapevine phenology under present and future climate scenarios. Agric For Meteorol 195–196:192–205

Friend AP, Trought MCT (2007) Delayed winter spur-pruning in New Zealand can alter yield components of Merlot grapevines. Aust J Grape Wine Res 13:157–164

Frioni T, Tombes S, Silvestroni O, Lanari V, Bellincontro A, Sabbatini P, Mateo G, Lanari V, Poni S. (2016) Post-Budburst Spur-Pruning Reduces Yield and Delays Fruit Sugar Accumulation in cv. Sangiovese in Central Italy. Am J Enol Vitic 67:419–425

García de Cortázar-Atauri I, Brisson N, Gaudillere JP (2009) Performance of several models for predicting budburst date of grapevine (*Vitis vinifera* L.). Int J Biometeorol 53:317-326

George IS, Fennell AY, Haynes PA (2018) Shotgun proteomic analysis of photoperiod regulated dormancy induction in grapevine. J Proteomics 187:13-24

Gu S, Jacobs, SD, McCarthy BS, Gohil HL (2012) Forcing vine regrowth and shifting fruit ripening in a warm region to enhance fruit quality in "Cabernet Sauvignon" grapevine (*Vitis vinifera* L.). J Hortic Sci Biotechnol 87:287–292

Hoogenboom G (2000) Contribution of agrometeorolgy to simulation of crop production and its applications. Agric For Meteorol 103:137–157

Jackson DI, Lombard PB, Kabinett LQ (1993) Environmental and management practices affecting grape composition and wine quality - A review. Am J Enol Vitic 4:409–430

Johnson IR, Thornley JHM (1985) Temperature dependence of plant and crop processes. Ann Bot 55:1–24

Jones GV, Davis RE (2000) Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am J Enol Vitic 51:249–261

Jones GV, Webb LB (2010) Climate change, viticulture, and wine: challenges and opportunities. J Wine Res 21:103–106

Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U, Weber E (1995) Phenological growth stages of the grapevine, *Vitis vinifera* L. ssp. vinifera. Codes and 515 descriptions according to the extended BBCH scale. Aust J Grape Wine Res 1:100-103

Martínez-Moreno A, Sanz F, Yeves A, Gil-Muñoz R, Martínez V, Intrigliolo DS, Buesa I (2019) Forcing bud growth by double-pruning as a technique to improve grape composition of *Vitis vinifera* L . cv . Tempranillo in a semi-arid Mediterranean climate. Sci Hortic 256:108614

Molitor D, Junk J, Evers D, Hoffmann L, Beyer M (2013) A high-resolution cumulative degree day-based model to simulate phenological development of grapevine. Am J Enol Vitic 65:72–80

Moncur MW, Rattigan K, Mackenzie DH, McIntyre GN (1989) Base temperatures for budbreak and leaf appearance of grapevines. Am J Enol Vitic 40:21–27

Moran MA, Sadras VO, Petrie PR (2017) Late pruning and carry-over effects on phenology, yield components and berry traits in Shiraz. Aust J Grape Wine Res 23:390–398

Mori K, Goto-Yamamoto N, Kitayama M, Hashizume K (2007) Loss of anthocyanins in red-wine grape under high temperature. J Exp Bot 58:1935–1945

Mosedale JR, Abernethy KE, Smart RE, Wilson RJ, ad Maclean IMD (2016) Climate change impacts and adaptive strategies: lessons from the grapevine. Glob Chang Biol 22:3814–3828

Nendel C (2010) Grapevine bud break prediction for cool winter climates. Int J Biometeorol 54:231–241

Oliveira M (1998) Calculation of budbreak and flowering base temperatures for *Vitis vinifera* cv. Touriga Francesa in the Douro region of Portugal. Am J Enol Vitic 49:74–78

Oyarzun RA, Stöckle CO, Whiting MD (2007) A simple approach to modeling radiation interception by fruit-tree orchards. Agric For Meteorol 142:12–24

Parker AK, De Cortázar-Atauri IG, Van Leeuwen C, Chuine I (2011) General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. Aust J Grape Wine Res 17:206–216

Parker A, García de Cortázar-Atauri I, Chuine I, Barbeau G, Bois B, Boursiquot JM, … van Leeuwen C (2013) Classification of varieties for their timing of flowering and veraison using a modelling approach: A case study for the grapevine species *Vitis vinifera* L. Agric For Meteorol 180:249–264

Pearce I, Coombe BG (2004) Grapevine Phenology. In: P. Dry and B.G. Coombe (Eds), Viticulture Volume 1 – Resources. (Winetitles: Adelaide, South Australia) pp. 150–166.

Petrie PR, Sadras VO (2008) Advancement of grapevine maturity in Australia between 1993 and 2006: Putative causes, magnitude of trends and viticultural consequences. Aust J Grape Wine Res 14:33–45

Petrie, PR, Brooke, SJ, Moran, MA and Sadras, VO (2017) Pruning after budburst to delay and spread grape maturity. Aust J Grape Wine Res 23:378–389

Ramos MC, Jones GV, Yuste J (2018) Phenology of Tempranillo and Cabernet-Sauvignon varieties cultivated in the Ribera del Duero DO : observed variability and predictions under climate change scenarios. Oeno One, 52: 31–44

Ramos MC, Martínez-Casasnovas JA (2010) Effects of precipitation patterns and temperature trends on soil water available for vineyards in a Mediterranean climate area. Agric Water Manag 97:1495–1505

Rogiers SY, Clarke SJ, Schmidtke LM (2014) Elevated root-zone temperature hastens vegetative and reproductive development in Shiraz grapevines. Aust J Grape Wine Res 20:123–133

Rogiers SY, Smith JP, Holzapfel BP, Hardie WJ (2011) Soil temperature moderates grapevine carbohydrate reserves after bud break and conditions fruit set responses to photoassimilatory stress. Funct Plant Biol 38:899–909

Sadras VO, Moran MA (2012) Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. Aust J Grape Wine Res 18:115–122

Sadras VO, Moran MA (2013) Nonlinear effects of elevated temperature on grapevine phenology. Agric For Meteorol 173:107–115

Sadras VO, Soar CJ (2009) Shiraz vines maintain yield in response to a 2-4 ºC increase in maximum temperature using an open-top heating system at key phenostages. Eur J Agron 31:250–258

Sinclair TR, Shiraiwa T, Hammer GL. (1992) Variation in Crop Radiation-Use Efficiency with Increased Diffuse Radiation. Crop Science 32:1281–1284

Taiz L, Zeiger E (2010) Plant physiology. Fifth edition. Palgrave Macmillan.

Tarara JM, Lee J, Spayd SE, Scagel CF (2008) Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. Am J Enol Vitic 59:235–247

Tomasi D, Jones GV, Giust M, Lovat L, Gaiotti F (2011) Grapevine Phenology and Climate Change: Relationships and Trends in the Veneto Region of Italy for 1964-2009. Am J Enol Vitic 62:329–339

Wake CFM, Fennell A (2000) Morphological, physiological and dormancy responses of three *Vitis* genotypes to short photoperiod. Physiol. Plant. 109:201–210

Webb L, Whetton P, Barlow EWR (2007) Modelled impact of future climate change on phenology of wine grapes in Australia. Aust J Grape Wine Res 13: 165–175

Williams DW, Andris HL, Beede RH, Luvisi DA, Norton MVK, Williams LE (1985) Validation of a Model for the Growth and Development of the Thompson Seedless Grapevine. II. Phenology. Am J Enol Vitic 36:283–289

Yan W, Hunt LA (1999) An Equation for Modelling the Temperature Response of Plants using only the Cardinal Temperatures. Ann Bot 84:607–614

Zalom FG, Goodell PB, Wilson LT, Barnett WW, Bentley WJ (1983) Degree-Days: The Calculation and Use of Heat Units in Pest Management. Cooperative Extension. Educational Agency of the University of California

Zapata C, Deléens E, Chaillou S, Magné, C (2004) Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). Journal of Plant Physiology 161:1031–1040

Zapata D, Salazar M, Chaves B, Keller M, Hoogenboom G (2015) Estimation of the base temperature and growth phase duration in terms of thermal time for four grapevine cultivars. Int J Biometeorol 59:1771–1781

Zapata D, Salazar-Gutierrez M, Chaves B, Keller M, Hoogenboom G (2016) Predicting key phenological stages for 17 grapevine cultivars (*Vitis vinifera* L.). Am J Enol Vitic 68:60–72

Table 1a. Description of the calibration data set used for bloom, fruit set and veraison stages; and the cross-validation for sparkling base wine berry maturity. For each vine condition is provided the type of weather station, distance from the observation site and the weather station, years of observations, and the number of phenological observations from the phenological stages.

Vine conditions	Weather data Raïmat (Catalonia, Spain)	Mean distance from observation sites (km)	Observation years	Phenological stage observations				
				Budbreak	Bloom	Fruit set	Veraison	Sparkling base wine berry maturity
				n	*n*	*n*	*n*	*n*
Control	Raïmat weather station	1	2015, 2016	2	2	2	2	2
Forced		1.1		3	3	3	3	3
Forced container-grown	Automatic weather station	0	2016	5	5	5	4	3

Table 1b. Description of the validation data set used for bloom, fruit set and veraison stages; and the cross-validation for wine berry maturity. For each location site (CA, means California, USA) is provided the weather station, number of observation sites associated with each weather station, mean distance between them, years of observations, the number and the descriptive statistics of phenological stages mean, maximum and minimum in day of the year.

Location	Weather station name	Number of observation sites	Mean distance from observation sites (km)	Observation years	Phenological stage observations (day of the year)															
					Budbreak				Bloom				Veraison				Wine berry maturity			
					n	mean	max	min	*n*	mean	max	min	*n*	mean	max	min	*n*	mean	max	min
North Coast (CA)	Carneros	2	1.5	2004-2010, 2014, 2015	12	76	91	62	14	140	164	123	14	208	229	194	9	265	285	148
	Oakville	1	1.5	2010-2014	5	85	92	72	5	141	153	128	5	210	227	198	-	-	-	-
Central Coast (CA)	San Benito	1	2.5	2014	1	66	-	-	1	125	-	-	1	196	-	-	1	252	-	-
	King City-Oasis rd.	1	7	2014-2015	-	-	-	-	2	117	122	111	2	200	202	197	1	247	-	-
South Central Coast (CA)	Nipomo	1	16	2010-2013, 2015	3	73	81	62	5	130	140	106	5	209	219	191	1	242	-	-
Badajoz (Spain)	La Orden	1	0.5	2008, 2012-2016	6	77	87	65	6	134	147	125	3	198	207	190	6	228	254	208

Table 2a. Monthly mean maximum (T_{max}) and minimum (T_{min}) air temperature (ºC) from the nearest weather station from the weather station located in Raïmat (Spain) (Raïmat, www.ruralcat.net/web/guest/agrometeo.estacions), and automatic weather station placed in the middle of the container-grown forced vines.

Weather data	Observation years	Average temperature (ºC)	Month								
			Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Raïmat weather station	2015	T_{max}	17.4	20.9	25.9	30.0	33.3	30.5	24.7	21.3	14.3
		T_{min}	4.9	7.0	10.5	15.0	19.1	17.1	12.1	8.5	5.4
	2016	T_{max}	15.2	19.0	23.0	28.8	32.1	31.5	28.1	20.8	13.9
		T_{min}	3.1	6.1	9.4	14.2	16.8	15.8	13.9	10.1	3.2
Automatic weather station	2016	T_{max}	15.2	19.0	23.0	29.1	33.4	32.5	29.1	21.8	14.3
		T_{min}	3.1	6.1	9.4	14.8	18.4	17.5	15.9	12.1	5.1

Table 2b. Monthly mean maximum (T_{max}) minimum (T_{min}) air temperature (ºC) weather data retrieved from the Californian Irrigation and Management Information System (CIMIS, www.cimis.water.ca.gov) for the California (CA) region (USA), and the Irrigation Advice Network of Extremadura (REDAREX, redarexplus.gobex.es/RedarexPlus/) for Badajoz (Spain) location.

Location	Station name	Average temperature (ºC)	Month								
			Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
North Coast (CA)	Carneros	T_{max}	14.3	16.5	19.4	20.4	22.7	25.9	27.0	27.2	27.5
		T_{min}	2.6	4.5	4.8	5.2	7.4	9.0	10.8	10.4	8.7
	Oakville	T_{max}	16.6	17.1	18.6	22.0	24.9	27.7	28.7	28.6	29.3
		T_{min}	2.2	3.4	5.0	6.3	7.3	9.9	11.0	10.6	9.1
Central Coast (CA)	San Benito	T_{max}	21.3	18.5	21.4	22.6	25.8	26.3	28.3	27.1	27.4
		T_{min}	3.4	6.3	7.4	7.7	10.2	10.4	13.7	13.0	13.1
	King City-Oasis rd.	T_{max}	21.3	20.5	24.3	24.2	25.2	29.7	30.5	31.0	31.1
		T_{min}	2.5	4.6	5.6	5.6	7.4	8.9	11.8	12.1	10.8
South Central Coast (CA)	Nipomo	T_{max}	18.6	17.5	18.2	17.9	17.7	17.3	18.9	19.5	20.9
		T_{min}	5.6	5.8	6.4	7.0	7.7	8.5	11.3	11.5	10.9
Badajoz (Spain)	La Orden	T_{max}	13.3	14.5	17.7	20.6	25.1	30.1	33.3	32.7	28.7
		T_{min}	2.8	2.5	4.6	8.0	10.4	14.2	16.6	16.0	14.0

Table 3. Best fit degree-day methods with a base temperature (T_B) of 5ºC for the bloom, fruit set and veraison stages. Parameters of the methods of each phenological stage, the statistics descriptors RMSE, R^2, MBE and AIC for method calibration and the statistics descriptors RMSE, R^2, MBE for method validation. Methods fits were significant (p-value < 0.05).

Phenological stage	Method parameters			Method calibration				Method validation		
	Method	T_U (ºC)	*thresDD* (DD)	RMSE (days)	R^2	MBE (days)	AIC	RMSE (days)	R^2	MBE (days)
Bloom	*UniFORC*	-	491.2	4.3	0.988	-0.5	61.08	6.7	0.768	5.1
Fruit set	*Single triangle algorithm*	25.4	47.6	1.6	0.998	-0.1	41.51			
Veraison	*Single triangulation*	20.9	744.4	4.8	0.985	-0.8	57.65	7.1	0.627	-6.1

T_U, upper temperature; *thresDD*; degree-day threshold at which phenological stage occur

RMSE, root mean square error; R^2, coefficient of determination; MBE, mean bias error; AIC, akaike information criterion

Table 4. Best fit degree-day methods with a base temperature (T_B) of 5ºC for berry maturity. Parameters of the methods for each berry maturity criteria, and the statistics descriptors RMSE, R^2 and MBE resulting from the cross-validation. The phenological data set used for sparkling base wine were described in Table 1a, and for wine in Table 1b. Methods fits were significant (p-value < 0.05).

Berry maturity	Method parameters				Cross-validation		
	Method		T_U (ºC)	*thresDD* (DD)	RMSE (days)	R^2	MBE (days)
Sparkling base wine	*Single sine* with T_H=35ºC	Mean	25.7	286.0	8.3	0.933	0.1
		SD	±0.5	±15.6			
Wine	*Single triangulation*	Mean	29.4	724.1	8.5	0.836	-0.4
		SD	±1.7	±16.4			

T_U, upper temperature; *thresDD*; degree-day threshold at which phenological stage occur; T_H, high temperature

RMSE, root mean square error; R^2, coefficient of determination; MBE, mean bias error

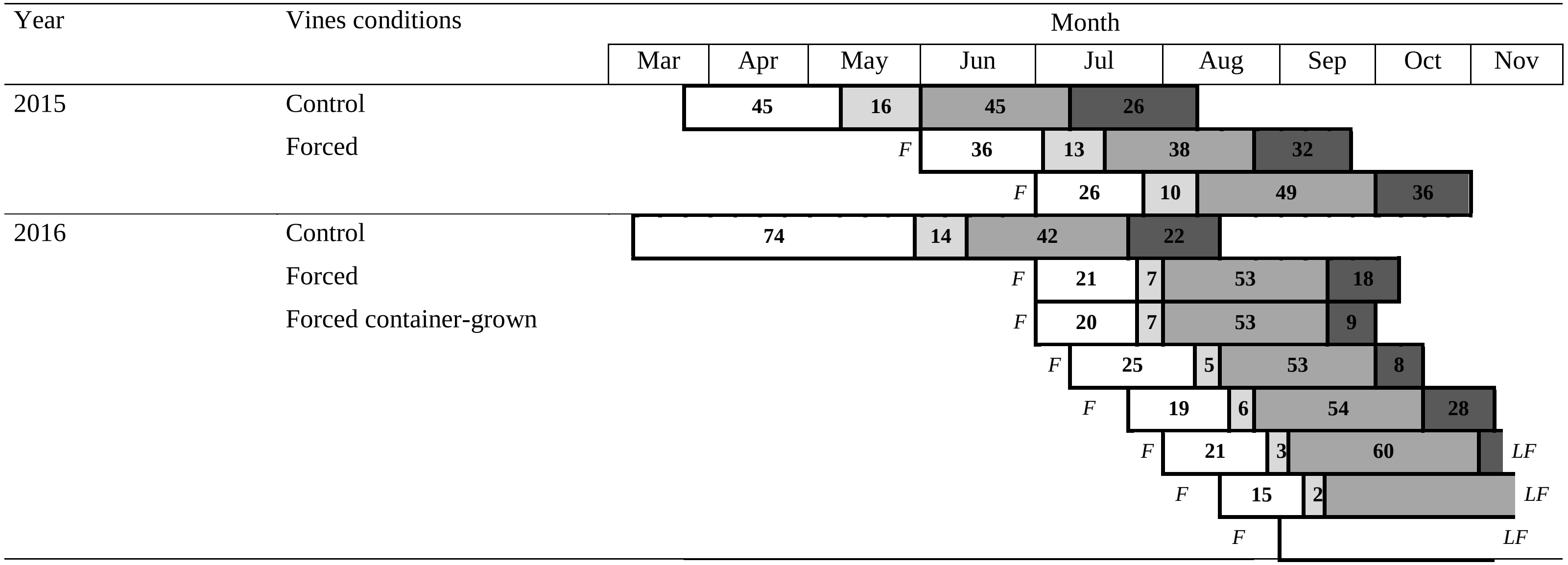


Figure 1. Phenological data used for the calibration of the degree-day methods for bloom, fruit set and veraison stages, and the cross-validation of the method for berry maturity according with sparkling base wine. The letter *F* indicates when the forced regrowth treatments was performed, and *LF* indicates the timing of leaf fall in the vines that did not reach berry maturity stage. The vegetative cycle is shown by phenological stages: budbreak to bloom (white), bloom to fruit set (clear grey), fruit set to veraison (grey), veraison to sparkling base wine berry maturity (black). Numbers indicate the duration of each stage in days.

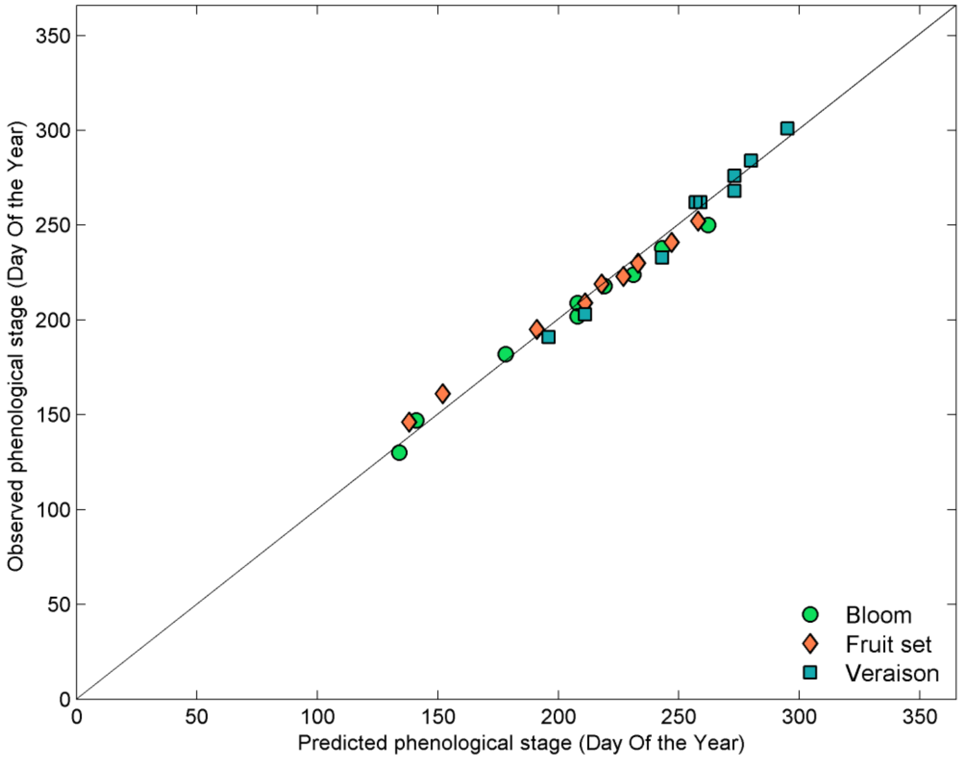


Figure 2a. Comparison between predicted and observed day of the year for bloom, fruit set and veraison for the best fit values on the calibration of the degree-day methods, with the data set shown in Table 1a. All the stages reached their best fit values with T_B =5ºC. Solid line is 1:1 line.

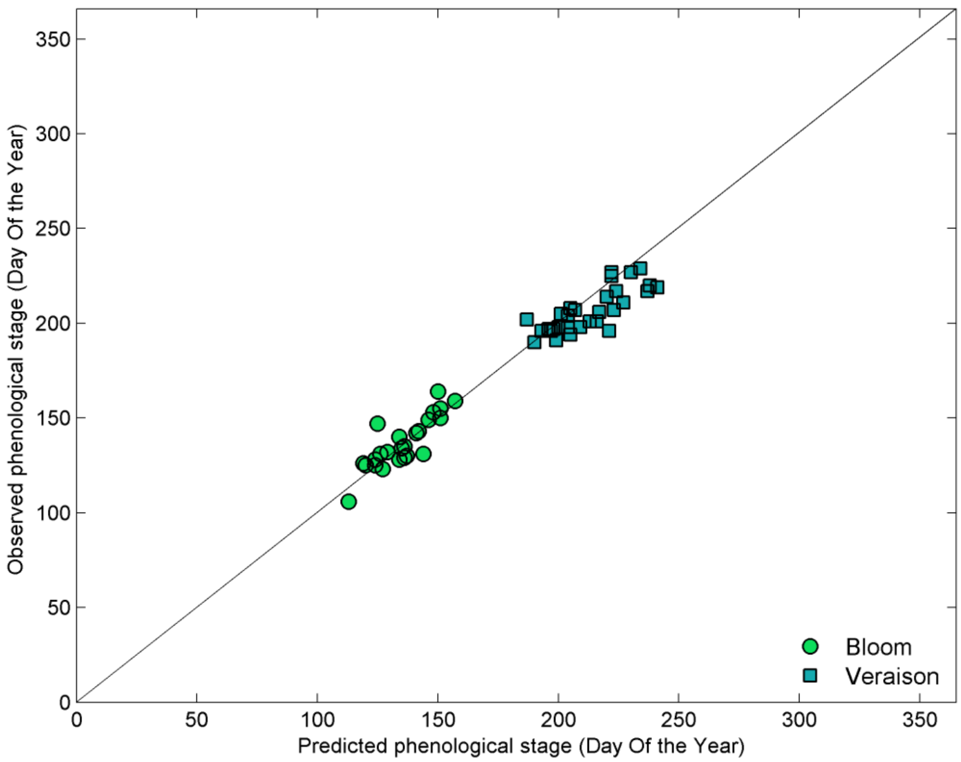


Figure 2b. Comparison between predicted and observed day of the year for bloom and veraison on the validation of the best fit methods with the data set shown in Table 1b. Solid line is 1:1 line.

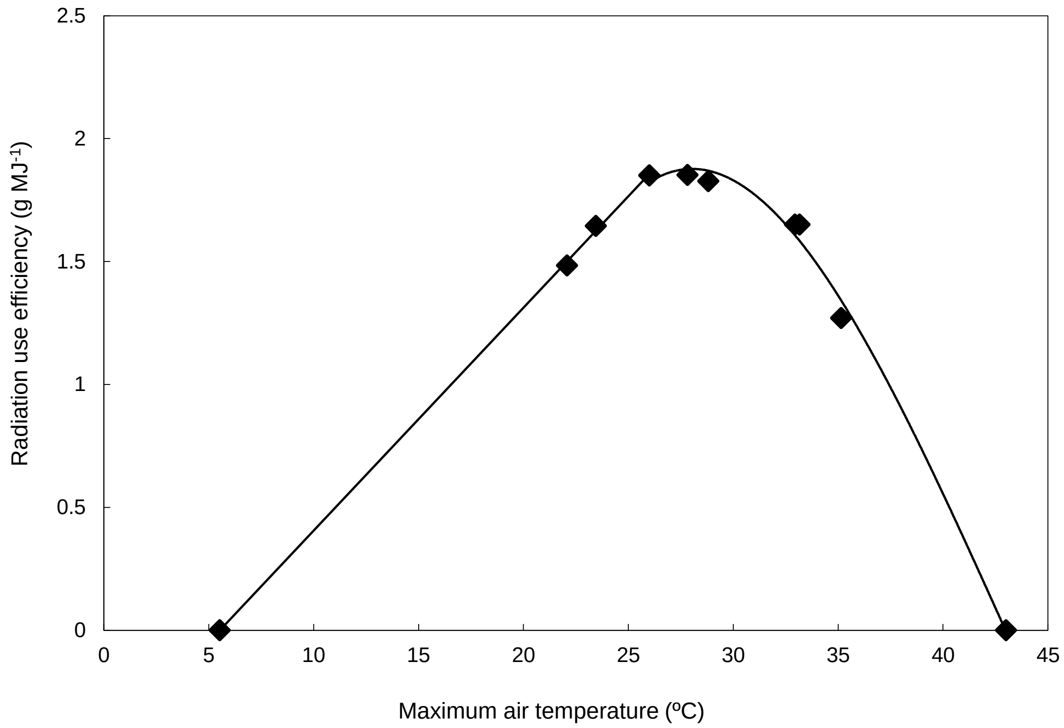


Figure 3 Influence of resource availability on Chardonnay vine development in conjunction with the effect of high temperatures. Represented by the relationship between the maximum air temperature and the radiation use efficiency for a Chardonnay cultivar from the post bloom to the berry maturity phenological stage.

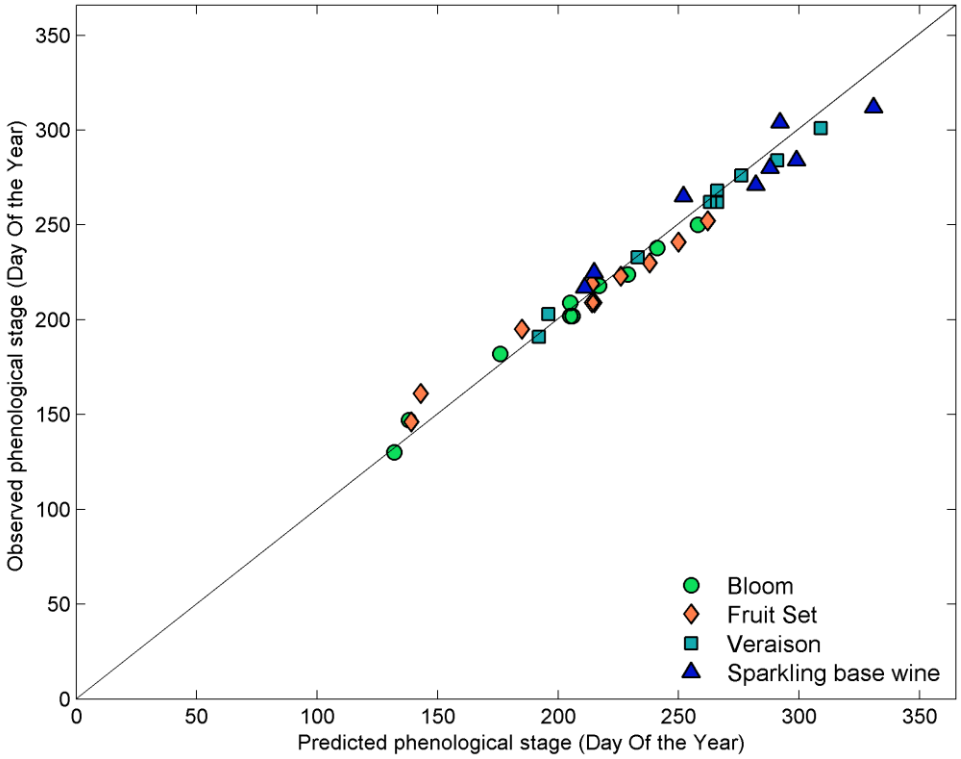


Figure 4a. Phenological prediction from bloom to sparkling base wine berry maturity with the methods selected for each stage. The RMSE statistics for the best methods for each stage were 4.7 (days) for fruit set, 3.4 (days) for veraison and 10 (days) for sparkling base wine berry maturity. Solid line is 1:1 line.

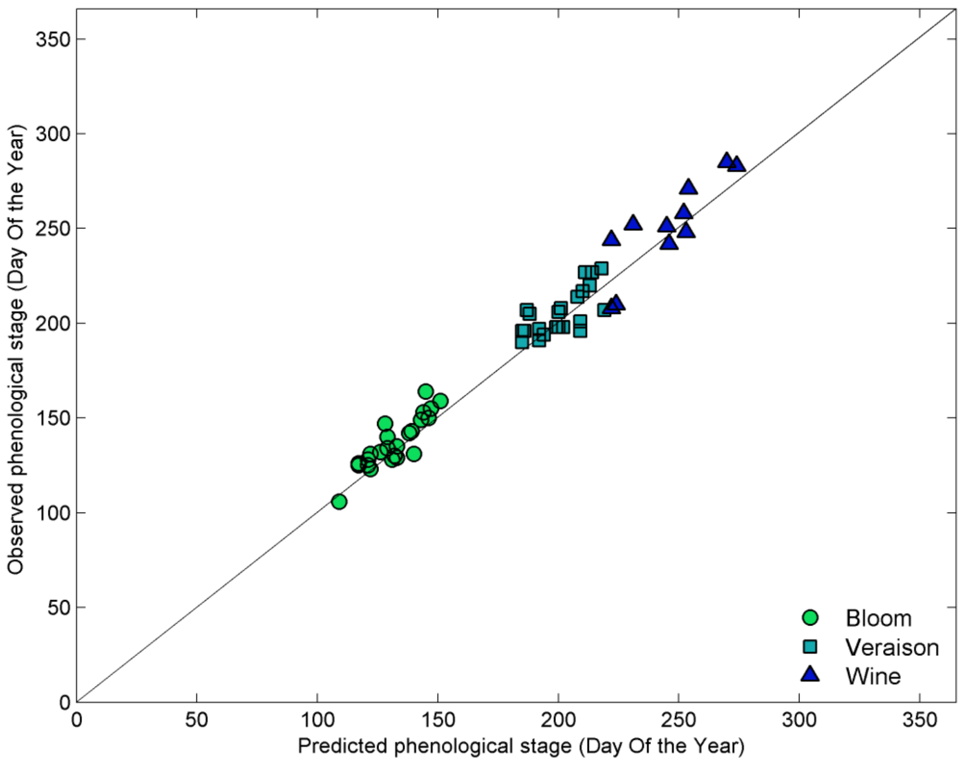


Figure 4b. Phenological prediction from bloom to wine berry maturity with the methods selected for each stage. The RMSE statistics for the best methods for each stage were 8.7 (days) for veraison and 13.3 (days) for wine berry maturity. Solid line is 1:1 line.

Supplementary material

This document contains supporting information belonging to “Using forced regrowth to manipulate Chardonnay grapevine (*Vitis vinifera* L.) development to evaluate phenological stage responses to temperature” by Maria Teresa Prats-Llinàs, Héctor Nieto, Theodore M. DeJong, Joan Girona and Jordi Marsal.

The information provided is the following:

Supplementary figures

The figures are referred through the main text.

Supplementary equations

The equations of the degree-days methods used on methods development are provided in this section. The three methods described are:

- *UniFORC model* (Chuine, 2000)
- *Single triangulation method* (Zalom et al., 1983)
- *Single sine method* (Zalom et al., 1983)

Supplementary tables

All the methods approaches with the description of method parameters, and the statistics for method development and validation are described in the supplementary tables, considering a base temperature (T_B) of 5ºC in all cases.

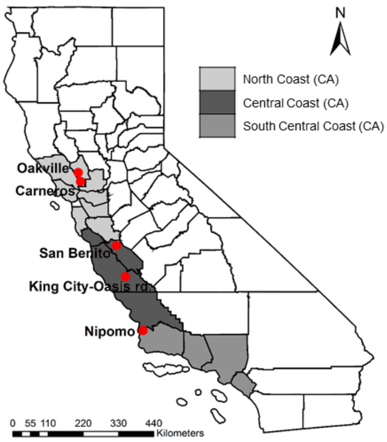
References

Chuine I. (2000) A Unified Model for Budburst of Trees. J. theor. Biol. 207, 337–347.

Zalom FG, Goodell PB, Wilson LT, Barnett WW, Bentley WJ (1983) Degree-Days: The Calculation and Use of Heat Units in Pest Management. Cooperative Extension. Educational Agency of the University of California

Supplementary figures

(a)



(b)

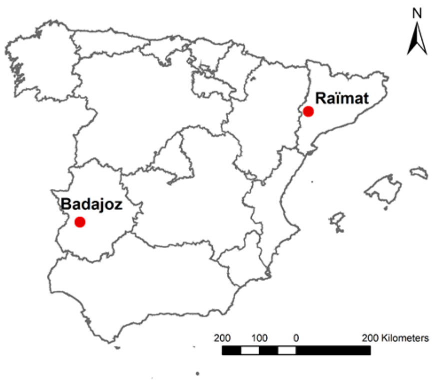


Figure S1. Location of the weather stations (red dots) used in the study across (a) the California (USA) region and (b) Spain.

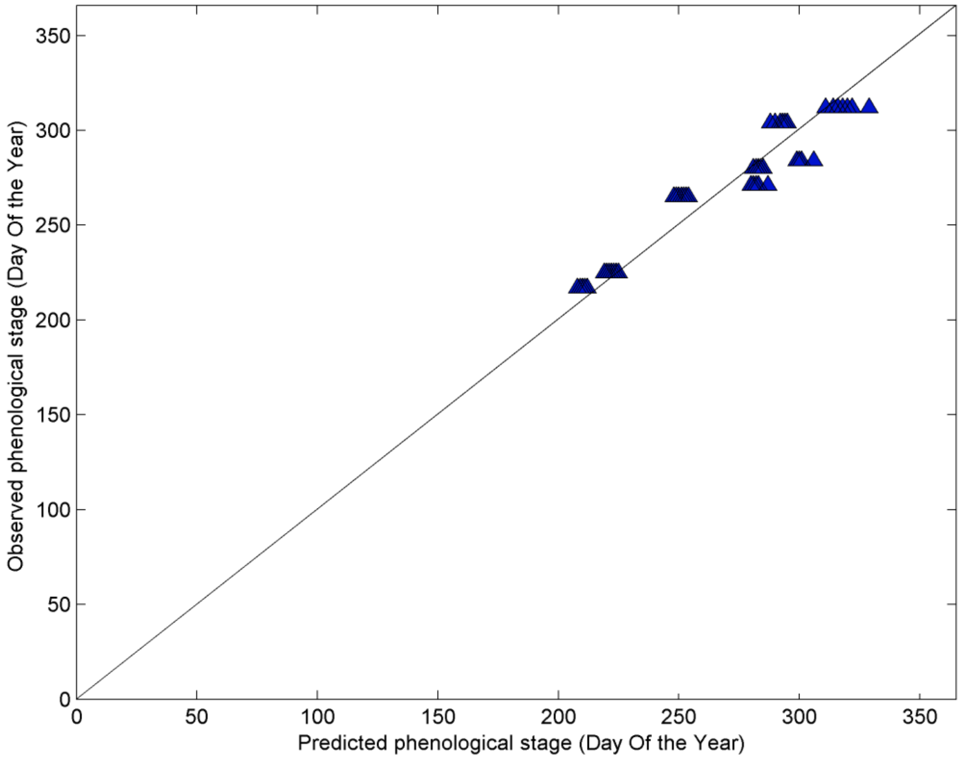


Figure S2a. Predicted and observed day of the year references for sparkling base wine berry maturity (Table 1, dataset) with the best methods based on the cross-validation technique. The statistics for the methods are shown in Table 4. Solid line is 1:1 line.

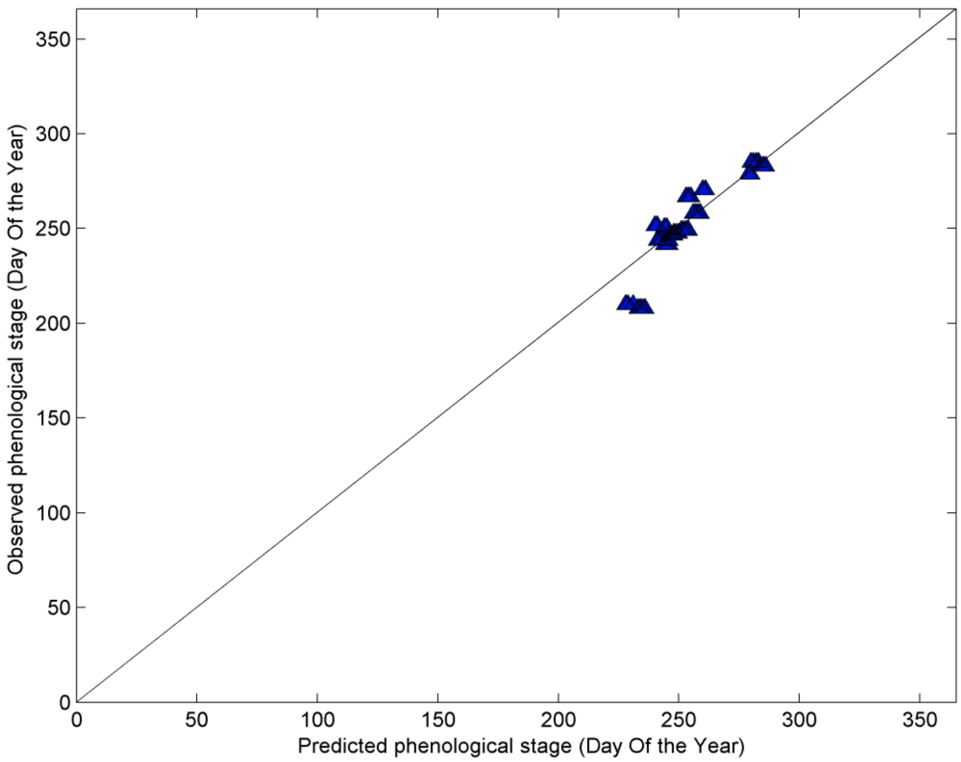


Figure S2b. Predicted and observed day of the year references for the best wine berry maturity (Table 2, dataset) performance using the cross-validation technique. The statistics for the methods are shown in Table 4. Solid line is 1:1 line.

Supplementary equations

thresDD, phenological stage degree-day threshold
i, onset of the previous phenological stage

m, phenological stage to be determined

T_B, base temperature (ºC)

T_U, upper temperature (ºC)

T_{mean}, daily mean temperature (ºC)

T_{min}, daily minimum temperature (ºC)

T_{max}, daily maximum temperature (ºC)

- *UniFORC model* (Chuine, 2000)

$$treshDD = \sum_{i=1}^{m} DD_{UF} \quad \text{(S1)}$$

$T_{mean} < T_B$ $\quad DD = 0$ (S2)

$T_{mean} > T_B$ $\quad DD = T_{mean} - T_B$ (S3)

- *Single triangulation method* (Zalom et al., 1983)

$$treshDD_{ST} = \sum_{i=1}^{m} DD_{ST} \quad \text{(S4)}$$

$T_{min} > T_U$ $\quad DD_{ST} = T_U - T_B$ (S5)

$T_{max} < T_B$ $\quad DD_{ST} = 0$ (S6)

$T_{max} < T_U$ and $T_{min} > T_B$ $\quad DD_{ST} = \frac{6 * (T_{max} + T_{min} - 2*T_B)}{12}$ (S7)

$T_{max} < T_U$ and $T_{min} < T_B$ $\quad DD_{ST} = \left(\frac{6 * (T_{max} - T_B)^2}{T_{max} - T_{min}}\right)/12$ (S8)

$T_{max} > T_U$ and $T_{min} > T_B$ $\quad DD_{ST} = \frac{6*(T_{max} + T_{min} - 2* T_B)}{12} - \left[\left(\frac{6*(T_{max} - T_U)^2}{T_{max} - T_{min}}\right)/12\right]$ (S9)

$T_{max} > T_U$ and $T_{min} < T_B$ $\quad DD_{ST} = \left[\frac{6 * (T_{max} - T_B)^2}{T_{max} - T_{min}} - \frac{6*(T_{max} - T_U)^2}{T_{max} - T_{min}}\right]/12$ (S10)

- *Single sine method* (Zalom et al., 1983)

$$\alpha = \frac{T_{max} - T_{min}}{2}$$

$$\theta_1 = sin^{-1} * \left[\left(T_B - \frac{T_{max} + T_{min}}{2}\right)/\alpha\right]$$

$$\theta_2 = sin^{-1} * \left[\left(T_U - \frac{T_{max} + T_{min}}{2}\right)/\alpha\right]$$

$$thresDD_{SS} = \sum_{i=1}^{m} DD_{SS} \quad \text{(S11)}$$

$T_{min} > T_U$ $\quad DD_{SS} = T_U - T_B$ (S12)

$T_{max} < T_B$ $\quad DD_{SS} = 0$ (S13)

$T_{max} < T_U$ and $T_{min} > T_B$ $\quad DD_{SS} = \frac{T_{max} + T_{min}}{2} - T_B$ (S14)

$T_{max} < T_U$ and $T_{min} < T_B$

$$DD_{SS} = \frac{1}{\pi} * \left[\left(\frac{T_{max} + T_{min}}{2} - T_b\right) * \left(\frac{\pi}{2} - \theta_1\right) + \alpha \cos(\theta_1)\right] \quad \text{(S15)}$$

$T_{max} > T_U$ and $T_{min} > T_B$

$$DD_{SS} = \frac{1}{\pi} * \left[\left(\frac{T_{max} + T_{min}}{2} - T_b\right) * \left(\theta_2 + \frac{\pi}{2}\right) + (T_U - T_B) * \left(\frac{\pi}{2} - \theta_2\right) - [\alpha \cos(\theta_2)]\right] \quad \text{(S16)}$$

$T_{max} > T_U$ and $T_{min} < T_B$

$$DD_{SS} = \frac{1}{\pi} * \left[\left(\frac{T_{max} + T_{min}}{2} - T_b\right) * (\theta_2 - \theta_1) + \alpha\,[\cos(\theta_1) - \cos(\theta_2)] + (T_U - T_B) * \left(\frac{\pi}{2} - \theta_2\right)\right]$$

(S17)

Supplementary tables

Table S1 Degree-day methods with a base temperature (T_B) of 5ºC for the bloom, fruit set and veraison stages. Parameters of the methods of each phenological stage, the statistics descriptors RMSE, R^2, MBE and AIC for method development using the calibration data set, and the statistics descriptors RMSE, R^2, MBE for the evaluation of the methods using the validation data set. Methods fits were significant (p-value < 0.05).

Phenological	Method parameters			Method development				Method evaluation		
stage	Method	T_U (ºC)	*thresDD* (DD)	RMSE (days)	R^2	MBE (days)	AIC	RMSE (days)	R^2	MBE (days)
Bloom	**UniFORC**	**-**	**491.2**	**4.3**	**0.898**	**-0.5**	**61.08**	**6.7**	**0.768**	**5.1**
	Single triangulation	28.9	508.9	7.9	0.966	0.8	71.92	12.9	0.133	19.4
	Single sine	22.5	417.5	4.8	0.986	-0.3	63.05	7.4	0.718	11.0
	Single triangle algorithm	21.0	154.9	7.0	0.970	1.2	70.70	10.2	0.389	34.6
Fruit set	UniFORC	-	160.6	2.3	0.996	0.1	48.91			
	Single triangulation	29.9	166.3	2.2	0.996	-0.3	47.91			
	Single sine	31.0	159.2	2.1	0.996	0.1	46.70			
	Single triangle algorithm	**25.4**	**47.6**	**1.6**	**0.998**	**-0.1**	**41.51**			
Veraison	UniFORC	-	900.1	6.7	0.971	0.2	63.52	6.1	0.725	-66.1
	Single triangulation	**20.9**	**744.4**	**4.8**	**0.985**	**-0.1**	**57.65**	**7.1**	**0.627**	**-6.1**
	Single sine	23.2	776.7	5.2	0.983	0.2	58.85	8.2	0.509	-9.3
	Single triangle algorithm	21.0	254.2	5.3	0.982	0.1	59.45	9.1	0.389	20.2

T_U, upper temperature; *thresDD*; degree-day threshold at which phenological stage occur

RMSE, root mean square error; R^2, coefficient of determination; MBE, mean bias error; AIC, akaike information criterion

Table S2 Degree-day methods with a base temperature (T_B) of 5ºC for berry maturity Parameters of the methods for each berry maturity criteria, and the statistics descriptors RMSE, R^2 and MBE resulting from the cross-validation. The data set used for sparkling base wine was in Table 1, and for wine in Table 2 of the main manuscript. Methods fits were significant (p-value < 0.05).

Berry maturity	Method parameters				Cross-validation		
	Method		T_U (ºC)	*thresDD* (DD)	RMSE (days)	R^2	MBE (days)
Sparkling base wine	UniFORC	Mean	-	295.9	9.0	0.922	1.8
		SD	-	± 24.9			
	Single triangulation	Mean	25.7	299.9	9.4	0.915	0.2
	with T_H=35ºC	SD	± 0.3	± 22.7			
	Single sine	**Mean**	**25.7**	**286.0**	**8.3**	**0.933**	**0.1**
	with T_H=35ºC	**SD**	**± 0.5**	**± 15.6**			
	Single triangle algorithm	Mean	23.6	199.0	11.4	0.877	1.0
	with T_H=35ºC	SD	± 2.6	± 64.8			
Wine	UniFORC	Mean	-	715.2	9.6	0.788	-0.1
		SD	-	± 15.8			
	Single triangulation	**Mean**	**29.4**	**724.1**	**8.5**	**0.836**	**-0.4**
		SD	**± 1.7**	**± 16.4**			
	Single sine	Mean	28.1	691.8	10.3	0.791	-1.0
		SD	± 2.4	± 20.1			
	Single triangle algorithm	Mean	20.3	246.9	15.1	0.537	0.3
		SD	± 0.1	± 7.1			

T_U, upper temperature; *thresDD*; degree-day threshold at which phenological stage occur; T_H, high temperature

RMSE, root mean square error; R^2, coefficient of determination; MBE, mean bias error