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Phenological development in warmer temperatures and water stress during post-harvest affects Chardonnay grapevines (Vitis vinifera L.)

Maria Teresa Prats Llinàs

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Universitat de Lleida

Ph.D. THESIS

Phenological development in warmer temperatures and water stress during post-harvest affects Chardonnay grapevines (*Vitis vinifera* L.)

Thesis submitted for the degree of doctor in
Agricultural Food Science and Technology

by

Maria Teresa Prats Llinàs

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In memoriam

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"Οι ρίζες της εκπαίδευσης είναι πικρές,
ο δε καρπός της γλυκός."

Αριστοτέλης

“Be a free thinker and don’t accept everything you hear as truth.
Be critical and evaluate what you believe in.”

Aristotle

Que tot està per fer.
Pots anar-te'n tranquil, estarem bé.
Buscar i descobrir,
tota la vida que deixes viu en mi.
Hem de caminar
malgrat que avui ens faci mal,
sobrevolar les pors i mirar endavant.

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Index

Introduction

Methodology

Chapters

Annexes

Resum

Una de les principals preocupacions en l'agricultura és la incertesa deguda als efectes del canvi climàtic. En aquest aspecte, l'estudi i adopció de noves tècniques de maneig dels cultius i la gestió de l'aigua són elements essencials per tal de garantir la productivitat dels cultius. En la regió mediterrània, la vinya (*Vitis vinifera* L.) representa un dels cultius més importants, el qual es podria veure afectat especialment en aquelles zones on no hi ha disponibilitat d'aigua. A més, en els últims anys s'ha observat un avançament en la fenologia de la vinya, provocant que, entre d'altres, la fase de la post-verema esdevingui una fase crítica degut a la seva major durada i sota unes condicions més càlides i amb menor precipitació. Degut a que en els propers anys es preveu un increment en la freqüència i intensitat dels esdeveniments de sequera, a més d'un augment significatiu de les temperatures, els elements crítics per combatre els efectes del canvi climàtic són: la disponibilitat de models fenològics per predir els patrons estacionals de les fases fenològiques; i la selecció del moment més idoni per l'adopció d'estratègies de reg deficitari, per tal d'estalviar aigua i poder garantir uns bons rendiments productius de la verema amb la composició dels raïms adequada per a l'elaboració de vins i caves.

En aquest sentit, l'ús de models fenològics es presenta, per exemple, com a una opció per a la selecció de les varietats més adequades a una zona determinada, o per la selecció del moment òptim per adoptar les tècniques de maneig. A més, aquests models ens ajuden a predir el desenvolupament de les diferents fases fenològiques, permetent fer prediccions en condicions d'escalfament global, sempre i quan els models hagin estat desenvolupats en aquestes condicions. Per altra banda, estudis previs de la varietat objectiu d'aquesta tesis, Chardonnay, han demostrat que la reducció en l'aplicació d'aigua en fases prèvies a la verema pot afectar negativament els paràmetres productius i qualitatius del raïm. Per aquest motiu, el període de post-verema es presenta com una fase en què podria ser viable l'adopció d'estratègies de reg deficitari, tenint en compte la importància d'aquest període en l'acumulació de reserves pel nou creixement vegetatiu de la campanya següent.

És per això, que els objectius d'aquesta tesis són el desenvolupament de models fenològics per la predicció de les fases fenològiques clau de la vinya simulant els efectes de l'increment de la temperatura sobre la fenologia; avaluar els efectes d'un estrès hídric en la post-verema sobre l'acumulació de reserves en els principals òrgans de reserva; així com l'avaluació dels efectes acumulats en el comportament vegetatiu i fructífer de la vinya degut a l'adopció d'estratègies de reg deficitari controlat durant la fase de la post-verema. L'obtenció d'aquest

coneixement permetrà una millora en l’execució de les tècniques de maneig coneixent els efectes de l’increment de temperatures en els cultius, així com una millora en la eficiència en l’aplicació de l’aigua de reg.

Resumen

Una de las principales preocupaciones en la agricultura es la incertidumbre de los efectos del cambio climático. Por este motivo, el estudio y la adopción de nuevas técnicas de manejo de los cultivos y de la gestión del agua son elementos esenciales para garantizar la productividad de los cultivos. En la zona del mediterráneo, el viñedo (*Vitis vinifera* L.) representa uno de los cultivos más importantes, el cual se podría ver afectado especialmente en las zonas donde no hay disponibilidad de agua. Además, en los últimos años se ha observado un adelanto en la fenología de la viña, provocando que, entre otros, la fase de la post-vendimia esdevenga una fase crítica debido a su mayor duración y bajo unas condiciones más cálidas y con una menor pluviometría. Debido a que en los próximos años se prevé un incremento en la frecuencia e intensidad de los eventos de sequía, además de un aumento significativo de las temperaturas, los elementos críticos para combatir los efectos del cambio climático son: la disponibilidad de modelos fenológicos para predecir los patrones estacionales de las fases fenológicas; y la selección del momento más idóneo para la adopción de estrategias de riego deficitario, para el ahorro de agua y garantizar unos buenos rendimientos productivos de la vendimia con una composición de las bayas adecuada para la elaboración de vinos y cavas.

En este sentido, el uso de modelos fenológicos se presenta, por ejemplo, como una opción para la selección de las variedades mejor adaptadas a las características de la zona, o por la selección del momento óptimo para la ejecución de las técnicas de manejo. Además, estos modelos nos permiten predecir el desarrollo de las distintas fases fenológicas, permitiendo hacer predicciones en condiciones de calentamiento global, si los modelos han estado desarrollados bajo estas condiciones. Por otro lado, estudios previos de la variedad objetivo de esta tesis, Chardonnay, han demostrado que la reducción en la aplicación de agua en fases previas a la vendimia puede afectar negativamente a los parámetros productivos y cualitativos de la uva. Por este motivo, el periodo de post-vendimia se presenta como una fase en que podría ser viable la adopción de estrategias de riego deficitario, teniendo en cuenta la importancia de éste periodo en la acumulación de reservas para el nuevo crecimiento vegetativo en la siguiente campaña.

Es por ello, que los objetivos de esta tesis son el desarrollo de modelos fenológicos para la predicción de las fases fenológicas clave de los viñedos simulando los efectos del incremento de la temperatura sobre la fenología; evaluar los efectos de un estrés hídrico en la post-vendimia sobre la acumulación de reservas en los principales órganos de reserva; así como la evaluación de los efectos acumulados en el comportamiento vegetativo y fructífero de los

viñedos debido a la adopción de estrategias de riego deficitario durante la fase de la post-vendimia. Éste conocimiento permitirá la ejecución de las técnicas de manejo conociendo los efectos del incremento de temperaturas en los cultivos, así como la mejora en la eficiencia en la aplicación del agua de riego.

Abstract

One of the main concerns in agriculture is the effect of climate change. As a result, studying the adoption of new management techniques and managing water use are essential for guaranteeing crop productivity. In the Mediterranean region, the grapevine (*Vitis vinifera* L.) is one of the most important crops affected by water availability, especially in areas without irrigation. Moreover, over the last years has been observed advancements in grapevine phenology, where the post-harvest period is becoming a critical stage due to their longer duration under warmer and dryer conditions. Due to the expected increases in the frequency and intensity of drought events and rising temperatures, the critical elements for combating the effects of climate change are: the availability of phenological models to predict seasonal patterns in the key phenological stages; and the selection of the most suitable moment at which to apply regulated deficit irrigation strategies. Their study is required to water savings and to guarantee good yields with the desired berry composition for wine and sparkling wine production.

Phenological models are useful tools that help grapevine growers to select the most suitable cultivars for a particular region or the most suitable moment at which to apply crop management techniques. These models also allow us to predict crop development during the different phenological stages and also under conditions of global warming when they have been developed under such conditions. Previous studies carried out with Chardonnay, the cultivar on which this thesis focuses, have reported negative effects on productive and quality parameters relating to yield associated with reducing the application of irrigation water before harvest. As a result, the post-harvest period is presented as a suitable stage at which to apply regulated irrigation strategies. This must be done taking into account the importance of this period on the accumulation of reserves to provide for new vegetative growth during the following growing season.

As a result, the aims of this thesis were to develop phenological models to predict the key phenological stages of vines and to simulate the effects of increases in temperature on plant phenology; the evaluation of the effects of water stress during post-harvest on the accumulation of reserves in the main reserve organs; and the cumulative effects of adopting regulated deficit irrigation strategies during post-harvest on vegetative growth and productive vine performance in subsequent years. This knowledge will allow us to improve the implementation of crop management techniques, make us more aware of the effects of increases in temperature on vines and help us to improve the efficiency with which irrigation water is managed.

Introduction

Introduction

General context

Water is one of the most appreciated natural resources and one that all living beings depend on. It is needed for urban, industrial and environmental uses, including agriculture (Fereres and Soriano 2007). 70% of global freshwater is destined for agriculture, with this sector being the world's major water consumer (FAO 2017). Global population is currently growing at a rate of 83 million people per year, which will imply a significant increase in the demand for freshwater and a 60% increase in the demand for food by 2050 (FAO 2017, United Nations 2017). As a result, providing food will become a major challenge for the agricultural and food industry sectors, as they struggle to cope with a rising population. In addition, there will be mounting pressure and competition for water resources among different sectors.

Climate change has become more and more evident over recent decades and this has led to more frequent and intense extreme weather events (IPCC 2013). Global warming and water shortages have now become unequivocal and challenging environmental impacts that pose important problems for agricultural systems. Droughts and high temperatures are key stress factors which have a major impact on plant physiology, phenology, morphology, plant water relations and production (Raza et al. 2019). As a result of this, research carried out in recent years has been focused on predicting the impact of climate change on crop behaviour and developing strategies to mitigate its possible effects on crop performance (Richardson et al. 2013). The Mediterranean basin is a region which is particularly vulnerable to climate change. Global projections point to pronounced warming in this area, which could lead to a greater occurrence of high temperatures and erratic patterns of rainfall and produce a small decrease in precipitation, particularly during the warm season (IPCC 2013, Gonçalves et al. 2014, Giorgi and Lionello 2008).

The grapevine (*Vitis vinifera* L.) has its origin in the Mediterranean region and its growth cycle has adapted to the semi-arid climatic condition of this area (Terral et al. 2010). The vine is one of the most important crops grown in the regions surrounding the Mediterranean basin, with its cultivation occupying approximately 2,768,000 hectares (OIV 2017). The grapevine has traditionally been a rainfed crop, but due to an increase in the number of new irrigated areas and vine-growers' concerns about the negative impact of water deficits on yield and wine quality (Cancela et al. 2016), the area of irrigated vineyards has significantly increased in recent decades (FAO, 2016). Irrigation systems are also essential for the production of high quality wines because the supply of water, even in small amounts, has a direct effect on grape yield and berry composition (Ferreyra et al. 2004).

In recent decades, several grape-growing areas have reported changes in grapevine phenology, mainly linked to increases in temperature (Petrie and Sadras 2008, Duchêne et al. 2010, Tomasi et al. 2011). One of the expected consequences is earlier phenological development in response to increasing temperatures (Webb et al. 2007, Ramos et al. 2018). These advances may displace the ripening process towards the warmest months of the year, having a negative impact on berry composition and wine quality, and resulting in greater evaporative demands on vines with fewer water resources (Tarara et al. 2008, Bonada et al. 2013). Furthermore, the post-harvest period will be longer and will coincide with warmer temperatures. This therefore points to a scenario in which water demand is likely to increase while water supplies will become more uncertain. Water demand and supply could probably, however, be brought into balance through the sustainable management of water resources, focusing on conserving water and using it more efficiently for irrigation.

In the Mediterranean region, the adoption of regulated deficit irrigation (RDI) on grapevines has been widely recognized as a useful technique with which to save water. It can also be used to control excessive vegetative growth and improve berry composition (Girona et al. 2006, Basile et al. 2011). This strategy is based on reducing irrigation during certain phenological periods, in which a certain degree of water deficit does not affect either crop yield or berry composition, and leaving other periods with their evapotranspiration demands fully covered (Chai et al. 2016). The identification of the correct timing and intensity and of the optimal period during which to apply water stress are key considerations when applying RDI strategies because different responses have been obseved in different grapevine cultivars (Girona et al. 2009, Basile et al. 2012).

Grapevine phenology

Vineyards are climate-sensitive agricultural systems that may be affected by inter-annual weather variability and global warming (Jones and Webb 2010, Mosedale et al. 2016). Their environmental adaptation greatly depends on the timing of the key phenological stages, which can be defined as the periods in which the most important changes take place (Petrie and Sadras 2008). Temperature is widely considered the main climatic driver responsible for crop development when other environmental factors, such as photoperiod and water stress, are satisfied (Pearce and Coombe 2004, Parker et al. 2013, Zapata et al. 2016).

Budbreak, bloom, veraison and berry maturity are the most obvious stages in the growth cycle that are used for timing management practices (Figure 1). However, the time between these phenological stages may vary considerably depending on the grapevine cultivar, climate and geographic location (Jones and Davis 2000).

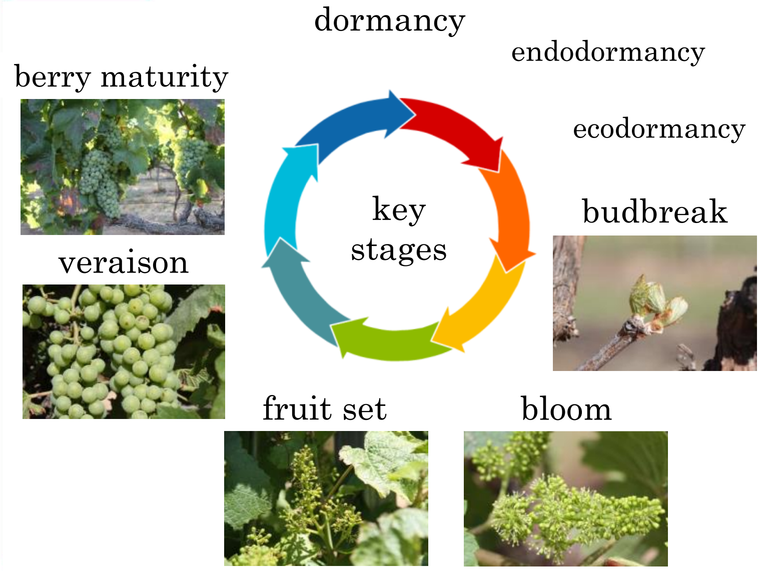

Figure 1. Key phenological stages in the growth cycle of grapevines

In grapevines, budbreak heralds the onset of vegetative growth. It is a key phenological stage because any delay in it could have a significant impact on the seasonal growth cycle (García de Cortázar-Atauri et al. 2009, Duchêne et al. 2010). Before budbreak comes the dormancy stage, which implies a temporary suspension of growth caused by physiological changes in buds (Lang et al. 1987). This stage can be divided into two periods: the endodormancy, when the buds are dormant due to the physiological conditions; followed by the ecodormancy, when the buds remain dormant because of unfavourable environmental conditions. Its timing depends on the exposure of the buds to winter chill which brings to an end the period of endodormancy. This is then followed by a period of spring heat that releases ecodormancy and triggers budbreak (Pope et al. 2014). The mobilization of carbohydrates from plant reserves for the new growth of vegetative organs takes place during the first part of the period from budbreak to bloom, which is also when the development of the floral organs begins. The bloom to fruit set period lasts for only a few days; at this time, the flowers lose their caps and their berries begin to grow. From fruit set to veraison, vines accumulate biomass and their berries actively grow by cell division and enlargement. Finally, from veraison to berry maturity, there is a decrease in the growth rate of the vegetative organs, the ripening process takes place, with the accumulation of soluble sugars in the berries, while there are decreases in acid content, and the berries begin to soften and their skin colour changes. The maturity process also takes place at this time, with an increase in the concentrations of phenolic and aroma berry compounds (Mullins et al. 1992, Lorenz et al. 1995, Caffarra and Eccel 2010).

Models of grapevine phenology

Phenological models have relevant applications in viticulture, from planning viticultural practices to modelling carbon dioxide fluxes (Williams et al. 1985, Richardson et al. 2013). They have been developed to predict the appearance and length of the different phenological stages. These models have mainly taken 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, Zapata et al. 2015).

Budbreak prediction

The models used to predict budbreak are mainly based on the accumulation of heat during spring, starting from a given date and continuing until a specific threshold has been reached. Under spring warming models, the chilling requirements are assumed to be met every year (Pope et al. 2014). These models may, however, be inappropriate for Mediterranean climates, which occasionally have mild winters in which the required minimum amount of chill may not be met (Pope et al. 2014), and for areas in which the climatic conditions tend to produce warmer winters (Luedeling and Brown, 2011), which are conditions that could be expected with global warming. Others, such as sequential models, treat winter chill and spring heat accumulation as independent phases that are fulfilled sequentially (García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010). However, the evaluation of winter chill is not straightforward and yet it is an important element for assessing the impact of climate change (Darbyshire et al. 2017). Moreover, this sequential fulfilment of the chill and heat requirements is often based on a very simplified understanding of the dormancy breaking processes. This is because measuring the specific periods during which buds are influenced by chilling and warming temperatures is a challenging task (Chuine 2000, Luedeling et al. 2009). Complex physiological processes are also likely to be involved in the transition from dormancy to budbreak in grapevines (Fila et al. 2014).

The recently developed Chill Overlap Model attempts to integrate possible interactions between chill and heat accumulation. This model considers the well-documented compensatory relationship between winter chill and spring heat, in which spring heat can compensate for low amounts of winter chill beyond a minimum threshold value, and vice versa, thereby reducing the amount of either chill or heat required to finally trigger budbreak (Chuine 2000, Pope et al. 2017). Furthermore, the accumulation of winter chill is determined using the Dynamic chill accumulation model and the growing degree hour (GDH) ASYMCUR model for the accumulation of spring heat, both of which serve as sub-models of the Chill Overlap Model (Fishman et al. 1987, Anderson et al. 1986). The particularity and interest in using the Dynamic model stem from the fact that it considers fluctuations in temperature and embeds the negation of chill due to high temperatures. In this case, chill is accumulated for a range of temperatures, and periods of warm temperatures are compensated by the cancelation of chill (Fishman et al. 1987). The development of the Chill Overlap Model for grapevines could provide interesting perspectives for budbreak predictions in warm climates due to its capacity to include the effects of high temperatures on the budbreak process.

Predicting grapevine development

The most common phenological models to predict the key stages from budbreak to berry maturity are thermal time models; these use degree-days as their unit of measure. These models strongly rely on the relationship between phenology and heat accumulation (Arnold 1959, Chuine et al. 2013). As temperature plays such an important role in plant behaviour, it is important to analyse vine responses to it. Moreover, responses to climatic change may differ according to the grapevine cultivar, specific phenological stage and magnitude of the temperature changes in question (Petrie and Sadras 2008).

Most of the degree-days models assume that temperature has a linear effect throughout phenological development (Nendel 2010, Parker et al. 2011, Zapata et al. 2015). However, the development of grapevine phenology has been reported to have a non-linear response to temperature, especially during the maturity period. This suggests that other factors, as well as temperature, may be behind the observed shifts (Sadras and Moran 2013, Petrie et al. 2017). The vegetative plant cycle consists of two processes: growth, which is primarily dependent on the ability of plants to capture resources, such as radiation, nutrients and water; and development, which is mainly controlled by non-resource-related environmental factors, such as temperature and photoperiod (Pearce and Coombe 2004, Zapata et al. 2016). Until now, the development of phenological models has focused on temperature effects, while the role of the resources themselves has been widely overlooked (Sheehy et al. 2006). The detected phenological displacements could therefore suggest that there may be a relationship between growth and development processes during certain phenological stages (Sadras and Moran 2013, Petrie et al. 2017).

Physiologically, the effects of temperature on photosynthesis, respiration and the plant development processes are driven by enzymatic activity reactions (Bonhomme 2000). The energy available to a plant increases with photosynthesis, while it decreases with respiration. However, increments and reductions in the amount of available energy are modulated by temperature in a rather complex manner. The amount of net energy available for a plant, and for its development therefore has an optimum temperature (Molitor et al. 2013). Most grapevine phenological models consider the accumulation of degree-days between the different temperature thresholds. The lower threshold is the base temperature, i.e. the temperature at below which plant development ceases (Jones and Davis 2000, Parker et al. 2013, Zapata et al. 2015), while the upper threshold is the temperature above which plant development no longer accelerates. Finally, there is the temperature above which plant development may decrease (Taiz and Zeiger 2010, Molitor et al. 2013) (Figure 2).

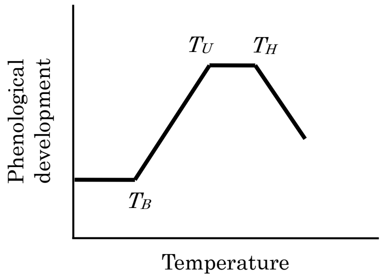

Figure 2. The response of phenological development to a base temperature (T_B), upper temperatures (T_U) and heat temperatures (T_H)

For accurate phenological predictions, it is necessary to make good estimations of the different temperature thresholds. However, thermal time models based on degree-day approaches general fail to consider high temperatures. The incorporation of upper and heat temperature thresholds into phenological models may therefore help to improve their predictions in scenarios such as that of global warming (Molitor et al. 2013).

Water stress during post-harvest

In the Mediterranean region, it is estimated that irrigating vines over a complete growing season at full evaporative demand requires between 350 to 550 mm of water (Ramos and Martínez-Casasnovas 2010, Bellvert et al. 2016). This represents a high level of water consumption and is one of the main concerns for grapevine growers. In some cases, however, it would not be advisable to use full evaporative demand irrigation approaches because of the positive impact on yield of reducing the amount of water applied (Girona et al. 2006). However, for white wine cultivars, water stress has a negative effect on berry composition and wine quality (Basile et al. 2012). This is likely to be critical in a scenario of water shortage or when water restrictions are imposed by local irrigation managers. As a result, in these cases, it is necessary to know the most suitable phenological moments at which to apply water restrictions without adversely affecting the growth, yield or quality parameters of the grapevines.

Under Mediterranean climatic conditions, the post-harvest period coincides with a period of low evaporative demand and late summer rain events. Even so, in the future, it is expected that there will be an increase in the frequency and intensity of drought events throughout the growing cycle and including during post-harvest (Gonçalves et al. 2014, Ramos et al. 2018). Furthermore, in the future, this period may last longer due to the advancement of grape phenology. Grapevine growers do not usually pay much attention to the management of their vines during the period after harvest and particularly not on terrain subject to irrigation management. This can basically be explained by the fact that the harvest has already been completed by this time. Furthermore, the possible consequences of a lack of vine management during this period are not widely known. In a scenario of climate change, the post-harvest period therefore deserves more attention, particularly as this could be a critical stage for determining the performance of the vines in the subsequent growing seasons.

Reserve accumulation

The post-harvest period is particularly important for the accumulation of reserves in storage organs (roots, trunk and shoots), where starch is the primary reserve form for carbohydrates (Zapata et al. 2004). These reserves sustain the mobilization of accumulated carbohydrates for new vegetative growth during the folowing growing season (Vaillant-Gaveau et al. 2014, Köse and Ates 2017). The accumulation of carbohydrates during the previous season is therefore essential for sustaining the mobilization of reserves until photosynthesis becomes the main source of carbon in the following spring (Zapata et al. 2004, Smith and Holzapfel 2009). Carbon assimilation is possible while vines retain functional leaves: until natural leaf fall during the post-harvest period. Any loss of leaf area during this period, either due to water stress or defoliation, could therefore affect vegetative growth and yield in the following growing seasons (Bennet et al. 2005, Vaillant-Gaveau et al. 2014).

The impact of water stress on carbon accumulation after harvest has so far received relatively little attention, especially with regard to root organs, which are the main reserve storage organs for vines (Field et al. 2009, Miranda et al. 2017). Increasing awareness of the contribution of these storage organs to the restoration of carbohydrate reserves could therefore help us to evaluate how important the post-harvest period is for the performance of vines in successive growing seasons.

Yield and berry composition

Regulated deficit irrigation strategies have been studied throughout the vegetative growth period in different grapevines cultivars (Bravdo et al. 1985, Cooley et al. 2017). This technique is normally applied during the crop stages when vegetative growth may be affected and when reproductive growth is relatively slow (Ruiz-Sanchez et al. 2010). In grapevines, most of these studies have been conducted in red-wine producing cultivars and have demonstrated the benefits of reducing this type of vegetative growth without affecting, or enhancing, yield parameters. For instance, applying RDI during post-veraison improves berry composition by enhancing their total soluble sugar content, colour, and concentration of anthocyanin and polyphenols compounds (Girona et al. 2009). All these berry composition parameters are appreciated for the production of premium quality wines (Ojeda et al. 2002).

However, some of the berry composition parameters that are appreciated in red-wine producing cultivars and enhanced by water stress, such as polyphenols, are susceptible to oxidation in white wines (Junquera et al. 1992). Although there is still relatively little information available about the effects of applying RDI strategies to white-wine producing cultivars, overall, it seems that applying water stress during the stages before harvest is not recommendable. Full irrigation during the pre-harvest period seems to be the best irrigation strategy to apply because it ensures a higher malic acid concentration and titratable acidity and better sensory attributes (Basile et al. 2012, Bellvert et al. 2016), which are the quality parameters that growers seek when producing white and sparkling wines. The post-harvest period is therefore a stage in which it is possible to apply RDI as an appropriate water-saving irrigation strategy which should not have any negative effects on relevant paramenters affecting grapevine yields or berry composition in subsequent growing seasons.

As a consequence of this critical review, a number of questions and doubts arose. Several of these were selected to define the objectives of the present PhD thesis.

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Presentation of the work

Presentation of the work

The present PhD thesis is based on an evaluation of the possible effects that phenological shifts resulting from global warming may have on grapevines during the post-harvest period. The thesis is organmised in two parts: i) phenological prediction from the budbreak through to the berry maturity stages (chapters I and II), ii) an evaluation of the effects on vegetative and quality components attributable to the application of water stress during the post-harvest period (chapters III and IV). The target cultivar for this study was Chardonnay.

This thesis consists of four chapters:

Chapter I, which examines the Chill Overlap Model and evaluates its use for the prediction of the onset of the budbreak stage. It also analyses the factors which could have influenced the accuracy of the predictions obtained.

Chapter II, which deals with the most important phenological stages, from bloom until berry maturity. It also analyses the most appropriate degree-day calculation method and evaluates the environmental and physiological factors which influenced the accuracy of the methodology applied, which was based on the forced regrowth technique.

Chapter III, which assesses the contribution of the perennial reserve storage organs to the accumulation of carbohydrates and starch during the post-harvest period, comparing vine growth under well-watered conditions and under water stress.

Chapter IV, which evaluates the impact of applying regulated deficit irrigation during the post-harvest period subject to two different irrigation-threshold values. It was assessed whether the limitation on starch concentration accumulation in the plant roots could influence vine performance in the following season. To elucidate this, the components related to vegetative growth and yield were analysed, as were the berries composition during the maturity period.

This PhD thesis has helped to produce the following published works:

Science Citation Index journals

Maria Teresa Prats-Llinàs, Theodore M. DeJong, Katherine S. Jarvis-Shean, Joan Girona, Jordi Marsal. (2019) Performance of a Chill Overlap Model for predicting budbreak in Chardonnay grapevines over a broad range of growing conditions. American Journal of Enology and Viticulture, 70, 50-59. DOI 10.5344/ajev.2018.18008.

Maria Teresa Prats-Llinàs, Hector Nieto, Theodore M. DeJong, Joan Girona, Jordi Marsal. Using forced regrowth to manipulate Chardonnay grapevine (*Vitis vinifera* L.) development to evaluate phenological stage responses to temperature (currently submitted for publication).

Maria Teresa Prats-Llinàs, Omar García-Tejera, Jordi Marsal, Joan Girona (2019). Water stress during the post-harvest period affects carbohydrate accumulation but not starch concentration and content in Chardonnay grapevine (*Vitis Vinifera* L.) organs. Scientia Horticulturae, 29, 461-470. DOI 10.1016/j.scienta.2019.02.027.

Maria Teresa Prats-Llinàs, Joaquim Bellvert, Mercè Mata, Jordi Marsal, Joan Girona. Post-harvest regulated deficit irrigation in Chardonnay did no reduce yield but at long-term, it could affect berry composition. Agronomy, 9, 328. DOI 10.3390/agronomy906032.

Not included in SCI journals

Maria Teresa Prats-Llinàs, Katherine S. Pope, Theodore M. DeJong, Jordi Marsal. (2018) Modeling budbreak phenology in 'Chardonnay' grapevine using the chill overlap model framework. Acta Horticulturae, 1229, 157-162. DOI 10.17660/ActaHortic.2018.1229.24.

National symposiums

Oral and poster presentation: Maria Teresa Prats-Llinàs, Jordi Marsal, Joan Girona. Variación de la fenología, posibles efectos sobre el cultivo de la vid Chardonnay frente la climatología cambiante y sus efectos sobre la demanda hídrica. XXXV Congreso Nacional de Riegos. Tarragona, June 2017. http//dx.doi.org/10.25028/CNRiegos.2017.A19.

International symposiums

Oral presentation, which obtained the student award for the best oral presentation: Maria Teresa Prats Llinàs, Katherine S. Pope, Theodore M. DeJong, Jordi Marsal. Modelling budbreak phenology in Chardonnay grapevine using the chill overlap model Framework. International Symposium on Flowering, Fruit Set and Alternate Bearing. Palermo (Italy), June 2017.

Poster presentation: Maria Teresa Prats-Llinàs, Joaquim Bellvert, Mercè Mata, Jordi Marsal, Joan Girona. Evaluación de la producción, calidad y ahorro de agua al adoptar estrategias de riego deficitario en viña (cv Chardonnay) durante el periodo de post-vendimia. XIV Simposio Internacional Hispano-Portugués de relaciones hídricas en las plantas. Madrid (Spain), October 2018.

Not included in this PhD document, but also published in SCI journals within the PhD period

Katherine Fyhrie, Maria Teresa Prats-Llinàs, Gerardo López, Theodore M DeJong (2018) How does peach fruit set on sylleptic shoots borne on epicormics compare with fruit set on proleptic shoots? European Journal of Horticultural Science, 83, 3-11. DOI 10.17660/eJHS.2018/83.1.1.

Maria Teresa Prats-Llinàs, Gerardo López, Katherine Fyhrie, Benoît Pallas, Yann Guédon, Evelyne Costes, Theodore M DeJong (2019). Long proleptic and sylleptic shoots in peach (*Prunus persica* L. Batsch) trees have similar, predetermined, maximum numbers of nodes and bud fate patterns. Annals of Botany, 123, 993-1004. DOI 10.1093/aob/mcy232.

Objectives

Objectives

The objectives of this PhD thesis were the following:

1. To evaluate the performance of the Chill Overlap Model for predicting budbreak in the Chardonnay grape cultivar over a broad range of growing conditions.

2.1. To evaluate the environmental and physiological factors that influence the development of the different phenological stages in the Chardonnay cultivar.

2.2. To evaluate variations in response to temperature when predicting the onset of the bloom, fruit set, veraison and berry maturity stages and to establish the most appropriate degree-day calculation method for a range of high temperatures, working with Chardonnay grapevines.

3. To compare the relative contributions of the shoot, trunk and root organs of Chardonnay grapevines to the restoration of carbohydrate reserves during the post-harvest period under well-watered and water stress conditions.

4. To determine the effects of adopting regulated deficit irrigation during the post-harvest period in Chardonnay cultivation on vegetative growth, yield components and berry composition during subsequent growing seasons.

Methodology

Methodology

The following section outlines the methodology used for the PhD thesis. Further information is provided in detail in each of the chapters in this thesis. The cultivar on which all the analyses were performed was the Chardonnay grapevine, which is characterised as an early maturing cultivar that is often grown to produce white wines and sparkling wine.

Data collection

Phenological and weather station data

The phenological datasets used for the parameterization and development of the phenological models, described in **chapter I** and **chapter II**, were obtained from wineries and research institutions at several locations in Spain, including Badajoz and Sant Sadurni d'Anoia, and from several different locations in California (USA). Another part of the phenological dataset was obtained from field experiments conducted at Raïmat, which are described in the next section (*Experimental data*).

Daily maximum and minimum temperature data were acquired from the nearest weather station to each vineyard site. Weather data for Spain were retrieved from the Meteorological Service of the Catalan Government (SMC, www.ruralcat.net/web/guest/agrometeo.estacions), for Raïmat and Sant Sadurni d'Anoia, and from the Irrigation Advice Network of Extremadura (REDAREX, redarexplus.gobex.es/RedarexPlus/), for the Badajoz region. In California, the weather data were obtained from the California Irrigation and Management Information System (CIMIS, www.cimis.water.ca.gov).

Description of phenology

Phenological vine development for the various phenological stages was registered when 50% of the shoots presented signs of having reached a particular stage according to the BBCH scale. This was registered using the following identification codes: 09 - budbreak, 65 – bloom, 71 – fruit set, 81 – veraison (Lorenz et al. 1995) (Figure 3). For the berry maturity stage, we used pre-defined soluble solids concentration thresholds in berries based on criteria for producing white wine or sparkling wine. These measurements were made with a refractometer (Palette PR-32α; ATAGO, Tokyo, Japan).

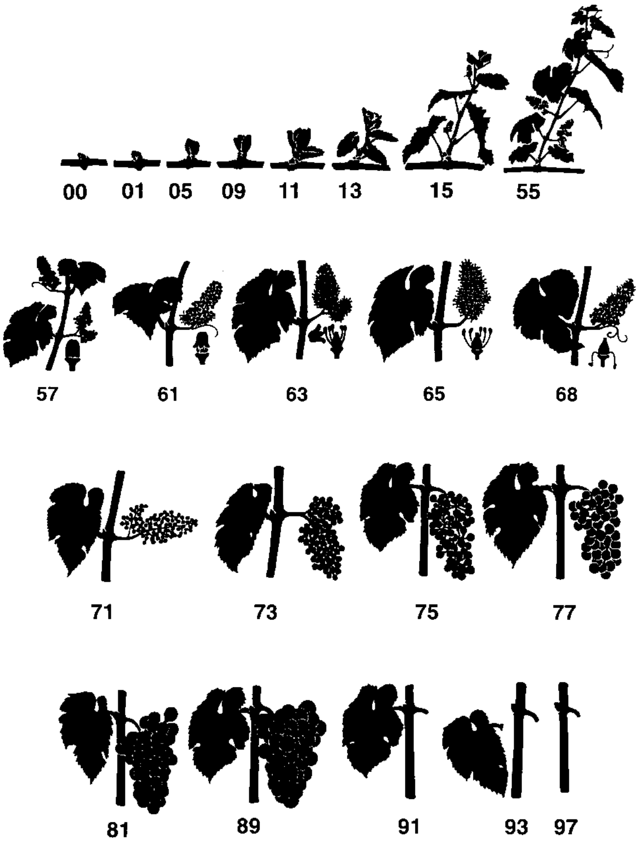

Figure 3. BBCH phenological scale for grapevines (Lorenz et al. 1995)

Experimental data

The field experiments were conducted at Raïmat (41°39'43" N – 0°30'16" E), Lleida (Catalonia, Spain), where disease control and nutrition management were performed according to the wine grape production protocol of the 'Costers del Segre' Denomination of Origin. The experiment described in **chapter III** was conducted with container-grown grapevines grafted onto 1103 Paulsen rootstock in spring 2015 and then planted in 50-L containers with a growing media of loose stones, at the bottom, and a substrate mix consisting of peat, sand and silty-loam soil. The experiment described in **chapter IV** was performed at a 16-ha commercial vineyard of Chardonnay, grafted onto SO4 rootstock, which had been planted in 2006, with a spacing of 2.0 x 3.0 m and a north-south row orientation, and grown in a loam soil.

Phenology models

The model parameterized for the prediction of the budbreak stage was the Chill Overlap Model, which is described in **chapter I**. For the following stages, from bloom to berry maturity, degree-day calculation methods were developed, which are further defined in **chapter II**.

Chill Overlap Model

The Chill Overlap Model fits an exponential declining curve representing the interaction between winter chill and spring heat. It was used to predict the onset of the budbreak stage using phenological observations from Spain and California (USA). These were divided into two independent datasets for the parameterization and validation of the model. The exponential declining curve was defined by equation (1) and the model parameters were tested and set as described in Pope et al. (2014).

$$H_a = \beta_1 + \frac{\beta_2}{e^{(\beta_3\ x\ C_a)}} \qquad (1)$$

H_a, heat accumulation from chill requirement to budbreak
C_a, chill accumulation after chill requirement has been met
β_1, β_2 and β_3, model parameters

The model was fitted using a non-linear regression to represent the possible combinations of chill and heat accumulation required to trigger budbreak, once a certain amount of the chill requirement had been met (Figure 4). The chill requirements and chill accumulation were calculated as chilling portions (CP), using the Dynamic model of Fishman et al. (1987), while the heat requirements and heat accumulation were calculated using the ASYMCUR growing degree hour (GDH) model (Anderson et al. 1986).

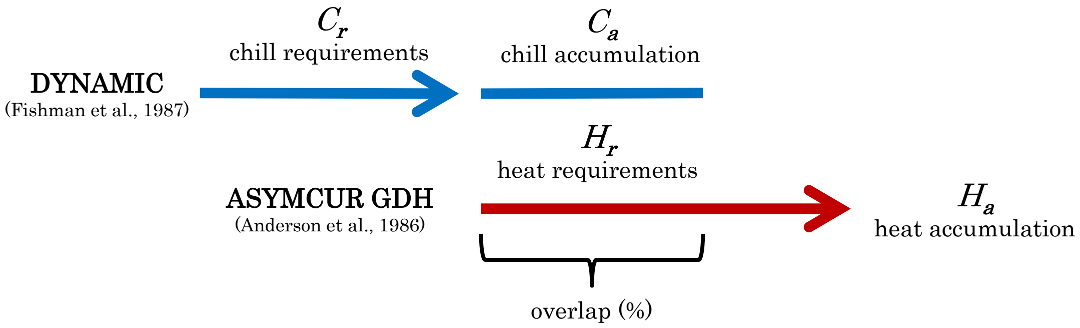

Figure 4. Scheme of the overlap between the chill and heat phases implied in the Chill Overlap Model

Degree-day methods

The two degree-day methods named single triangulation, single sine described in Zalom et al. (1983), the UniFORC (Chuine, 2000) and the single triangle algorithm method (Nendel 2010) were evaluated for each phenological stage: bloom, fruit set, veraison and berry maturity, for the production of white wine and sparkling wine. The phenological data used to develop the methods were obtained from forced regrowth experiments, following Gu et al. (2012). The forced regrowth technique consisted of cutting growing shoots to leave just six nodes and then removing all of the vegetative organs to stimulate new vegetative growth and start a new growth cycle originating from currently growing shoots. To validate the approach, we used phenological data belonging to wineries and research institutions at several different locations in California (USA) and the Spanish province of Badajoz (Spain).

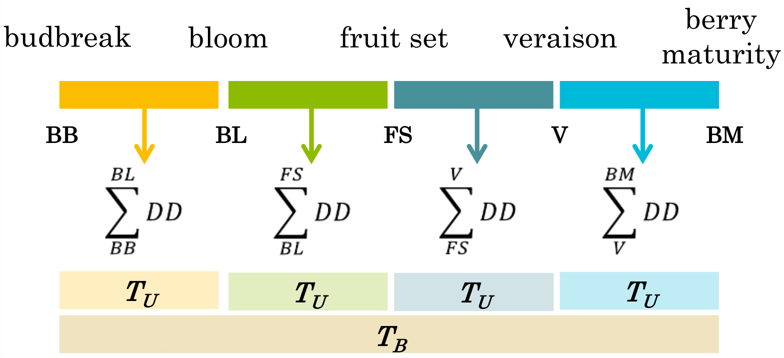

Figure 5. Scheme of the degree-day calculation for each phenological stage with threshold temperatures

Considering a constant base temperature (T_B) threshold, we evaluated a different upper temperature (T_U) threshold for each phenological stage (Figure 5). Then, to predict berry maturity, we tested the influence of resource availability in conjunction with the effects of high temperatures. For such purpouse, it was used a relation between radiation-use efficiency and maximum daily temperature measurenments.

Post-harvest field experiments

In this PhD thesis two experiments were performed that focused on the effects of water stress during the post-harvest period. The experiment described in **chapter III** was carried out with container-grown vines to allow measurements of the whole root system. That described in **chapter IV** was performed with field-grown vines and was used to evaluate the effect of applying regulated deficit irrigation (RDI) to real vines under field conditions.

Shoot, trunk and root growth and starch concentration

The experiment described in **chapter III** was performed at Raïmat, in 2015 and 2016. Two irrigation treatments were applied: a *control* (C), which was irrigated to match ET_C demands; and a *water stress* (WS) treatment, in which irrigation was applied when stem water potential (Ψ_{stem}) reached a threshold of -1.1 MPa. The design was a complete randomized block, with two replications of eight vines (Figure 6). Measurements of the midday stem water potential and leaf net CO_2 assimilation rate were taken from post-harvest until leaf fall. During the same period, vine biomass was also evaluated by splitting vines into above-ground (leaves, shoots and trunk) and below-ground (root system) organs, and starch concentrations were determined in shoots, trunks and thick roots (>2 mm).

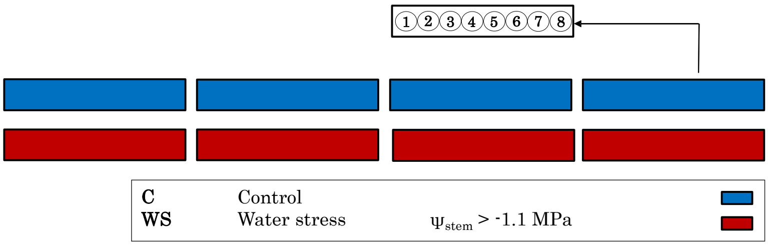

Figure 6. Scheme of the container-grown vine experiment

Post-harvest irrigation treatments and yield determinations

The experiment described in **chapter IV** was carried out on field vines grown at Raïmat. It was conducted from post-harvest 2013 until harvest 2016. Over three consecutive years: from 2013 to 2015, four different irrigation treatments were applied during the post-harvest period: *control* (C), irrigation at full crop evapotranspiration for the whole growing season; *low RDI* (RDI$_L$ SP), full irrigation until harvest to produce sparkling wine, and then different percentatges of ET$_C$ were applied in order to maintain Ψ_{stem} -0.9 MPa; *mild RDI* (RDI$_M$ SP), full irrigation until harvest to produce sparkling wine, and then different percentatges of ET$_C$ were applied to maintain -1.25 MPa; and *mild* RDI (RDI$_M$ W), full irrigation until harvest to produce wine, and also then different percentatges of ET$_C$ were applied to maintain Ψ_{stem} -1.25 MPa.

The experiment consisted of a randomized complete block design with four block replicates (Figure 7). Each block contained four experimental plots with four rows of eight vines per row. The leaf net CO_2 assimilation rate, stomatal conductance and transpiration measurements were determined during the post-harvest period in 2015. In the same year, the roots were sampled to determine their starch concentration. From the 2014 to 2016 growing season, we measured the vegetative growth at the onset of the vegetative cycle, and also evaluated the yield components and berry composition parameters.

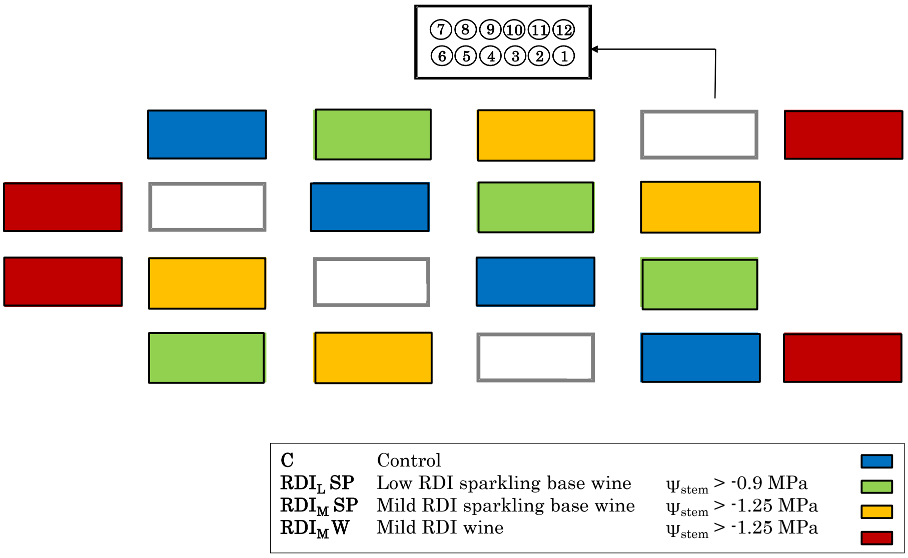

Figure 7. Scheme of the field experiments and their respective irrigation treatments

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Chapters

Chapter I

Performance of a Chill Overlap Model for predicting budbreak in Chardonnay grapevines over a broad range of growing conditions

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Abstract

Predicting phenological stages through modeling has significant implications for planning viticultural practices and for predicting the impact of climate change on phenology. The Chill Overlap Model is based on an exponentially declining curve that integrates the demonstrated compensatory relationship between chill and heat accumulation. It also incorporates recent research-based knowledge of physiological changes during dormancy. The aim of this work was to develop parameters for a Chill Overlap Model to predict budbreak in *Vitis vinifera* cv. Chardonnay grapevines. We also wanted to determine if using a Chill Overlap Model could be better at predicting budbreak than previously developed phenology models. The Chill Overlap Model incorporated the use of the Dynamic chill accumulation model to quantify accumulation of chill exposure in a cultivar with a relatively low chill requirement. Budbreak timing determined in Californian and Spanish winegrape-growing regions, which have a wide range of climates, was used to establish parameters for a Chill Overlap Model for Chardonnay. The newly developed Chardonnay Chill Overlap Model did not predict budbreak better than previous models, but did highlight significant differences between the dynamics of chilling in grapevines and that in other species for which a Chill Overlap Model has been developed. Further research is needed to understand the environmental and vineyard management factors that influence the timing of budbreak to improve the model and better understand factors that influence the completion of dormancy in grapevines.

Key words: chill and heat requirements, chill portions, dynamic chill accumulation model, modeling, phenology, temperature

Introduction

Phenological models have relevant applications in viticulture, from planning viticultural practices (Williams et al. 1985, Caffarra and Eccel 2010) to modelling carbon dioxide fluxes (Richardson et al. 2013, Pope et al. 2014). Recent research has focused on predicting the impact of climate change on plant phenology and developing strategies to mitigate its possible effects on crop behaviour (Chuine 2000, Richardson et al. 2013, Darbyshire et al. 2016).

Budbreak in grape indicates the onset of vegetative growth (Duchêne et al. 2010). Any delay during this stage could have a significant impact on the seasonal growth cycle, making it a key phenological stage, with major site-to-site and cultivar variability (García de Cortázar-Atauri et al. 2009). Ambient temperature is widely considered to be the main climatic driver responsible for phenological development (Williams et al. 1985, Martin and Dunn 2000, Jones 2003, García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010, Duchêne et al. 2010, Nendel 2010). Even so, other factors also modify grapevine phenology, these include soil temperature, soil texture (Jones 2003), photoperiod and water stress (Parker et al. 2013).

Dormancy is a temporary suspension of growth caused by physiological changes in buds (Lang et al. 1987). The timing of its release depends on the exposure of buds to winter chill to end the period of endodormancy, followed by a period of the spring heat that releases ecodormancy and triggers budbreak (Caffarra and Eccel 2010, Pope et al. 2014). Chill and heat are needed to release the corresponding dormancy stages, which translates into specific temperature exposure requirements for different species and cultivars (Chuine 2000).

The grapevine growth models used to predict budbreak are mainly based on the computation of heat accumulation during spring, also known as thermal time (Cannell and Smith, 1983), from a given date until a species-dependent threshold is reached. Under such spring warming models (Hunter and Lechowicz, 1992), the chilling requirements are assumed to be met every year (Pope et al. 2014). However, such models may be inappropriate for Mediterranean climates, which occasionally have mild winters in which the required minimum amount of chill may not be meet (Pope et al. 2014), or for areas in which climatic conditions tend to produce warmer winters (Luedeling and Brown, 2011). Other, sequential models treat winter chill and spring heat accumulation as independent phases that are fulfilled sequentially (Kramer 1994).

Complex sequential models developed for Chardonnay (*Vitis vinifera*) and some other cultivars have provided knowledge about developmental responses to environmental drivers. The BRIN model was developed by adding more biological and physiological explanations about grapevine crops to a phenological modelling framework (García de Cortázar-Atauri et al. 2009). Theirs was the first grapevine model to predict budbreak on the basis of physiological mechanisms as a framework for heat accumulation approach. The FENOVITIS model for the Chardonnay cultivar (Caffarra and Eccel 2010) added complex model parameters to integrate a negative relationship between the chill and heat accumulation stages with previously described set chilling requirements (Cannell and Smith 1983, Chuine 2000, Harrington et al. 2010).

In studies of deciduous trees, it is often assumed that chilling and heat accumulation requirements must be fulfilled one after another, up to a fixed threshold. However, this sequential fulfilment of the chill and heat requirements is often based on a very simplified understanding of the dormancy breaking processes (Luedeling et al. 2009). Measuring the specific periods during which buds are influenced by chilling and warming temperatures is challenging (Chuine 2000). Moreover, complex processes are likely to be involved in the transition from dormancy to budbreak in grapevines (Fila et al. 2014). Although specific proteins appear to contribute to the induction and release of bud dormancy, extensive molecular biological analyses are required to further understand the physiological, biochemical and genetic basis of grapevine bud dormancy (Lavee and May 1997, Nendel 2010).

Dormant buds undergo major changes when chilling requirements are fulfilled. These include changes to membranes and to the fatty acid ratios in their phospholipids (Faust et al. 1997). The relationship between chilling, post-rest and budbreak is complex. Following recent findings on genetic dormancy control (Horvath 2009, Leida et al. 2012), considerable overlap between chill and heat requirements was suggested (Pope et al. 2014). In peach (*Prunus persica*), once the minimum chill requirement has been met, but before bloom occurs, there is a decreased expression of genes responsible for the response to cold with continued chill exposure (Yamane et al. 2011).

Following these principles, recently developed Chill Overlap Models have attempted to integrate possible interactions between chill and heat accumulation. An exponential declining curve was fitted to describe the decreasing requirements for post-chill heat accumulation in response to prolonged exposure to chilling temperatures. This model contemplates a partial compensatory relationship between the chilling and post-chill heat requirements necessary to finally trigger budbreak (Pope et al. 2014). Comparing the conceptual basis for this model with other recently developed models for Chardonnay may increase our understanding of the biological and physiological behavior of grapevines during the dormancy period. Chill Overlap Models with significant improvements over previous prediction models have already been developed for deciduous almond (*Prunus dulcis*) and apple (*Malus domestica*) trees (Pope et al. 2014, Darbyshire et al. 2016).

Furthermore, the Chill Overlap Model calculates chill accumulation using the Dynamic chill accumulation model (Fishman et al. 1987). This chill accumulation performs either better than, or at an equivalent level to, other commonly used chill accumulation methods, when applied to various locations and cultivars (Erez 2000, Ruiz et al. 2007, Luedeling et al. 2009, Pope et al. 2014, Darbyshire et al. 2016). The negation of chill due to high temperatures, which is imbedded in the Dynamic chill accumulation model, has not been tested for grapevines previously. It could, however, provide interesting perspectives on budbreak predictions in warm climates (Dokoozlian 1999, Fila et al. 2014).

The aim of this work was to develop parameters for a Chill Overlap Model for predicting budbreak of the Chardonnay grape cultivar and to determine whether such a model can improve budbreak prediction over a broad range of growing conditions. Budbreak data from different grape growing regions in California and Spain were used to develop and test the predictive capacity of the model. This was done using observations from a range of different locations so as to evaluate the reliability of the model under different climatic conditions.

Materials and methods

Budbreak and weather station data

Budbreak data for the Chardonnay cultivar were used to parameterize and validate the performance of the Chill Overlap Model (Pope et al. 2014). Wineries and research institutions from different parts of California and Spain provided phenological data for different locations (Figure 1). Budbreak was considered to have been achieved when 50% of buds were open. However, it was not possible to apply a specific scale for all of the data sources, so 50% budbreak was estimated for some locations.

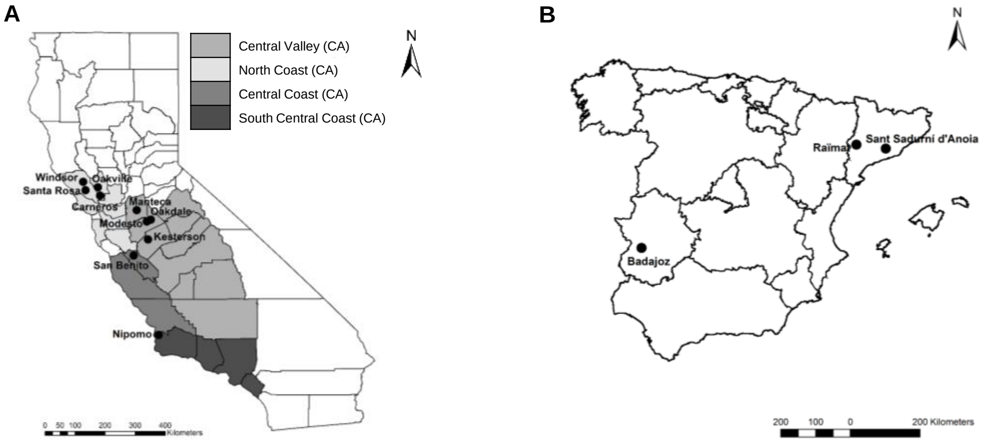

Figure 1 Location of the weather stations (black dots) used in the study in California (A) and Spain (B).

Daily maximum and minimum temperature data were acquired from the nearest weather station to each vineyard site (Table 1). Weather data for California were obtained from the California Irrigation and Management Information System (CIMIS, www.cimis.water.ca.gov). In Spain, weather data were retrieved from the Meteorological Service of the Catalonian Government (SMC, www.ruralcat.net/web/guest/agrometeo.estacions) for the Raïmat and Sant Sadurni d'Anoia locations and the Irrigation Advice Network of Extremadura (REDAREX, redarexplus.gobex.es/RedarexPlus/) for the Badajoz location (Figure 1). Where there was missing temperature data at a specific station, equivalent data were used from the nearest weather station. When several phenology observation sites were located near the same weather station (Windsor, Carneros), mean budbreak data were calculated and used with temperature data taken from the same station.

Phenology data were divided into two independent parameterization (*n*=42) and validation (*n*=39) subsets. The criteria used for these datasets had representative climatic conditions for both subsets. Covering the most representative climatic conditions enabled testing of the model's robustness (Figure 2).

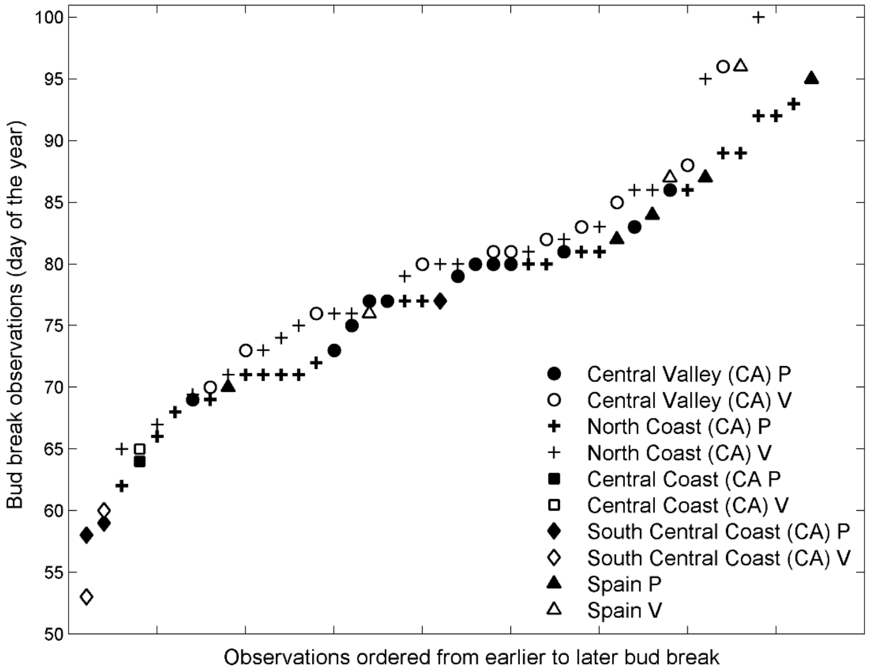

Figure 2 Day of the year budbreak subsets for Chardonnay, ordered from earliest to latest budbreak observations, used for parameterizing: (P; 42 observations, filled symbols) and validating: (V; 39 observations, open symbols) the Chill Overlap Model.

Table 1 Database summary for parameterizing and validating data for Californian) and Spanish locations. Weather station descriptors are latitude, longitude, altitude, number of observation sites associated with each weather station, and the mean distance between them. Data are shown for Californian (www.cimis.water.ca.gov) and Spanish (www.ruralcat.net/web/guest/agrometeo.estacions and redarexplus.gobex.es/RedarexPlus/) weather stations. Average budbreak data are provided for the different observation years. CP is the average value for the chill portions accumulated in the observation years from 1 Oct to 31 March. Daily average maximum (T_{max}) and minimum (T_{min}) temperatures are from 1 Oct to 31 March. DOY, day of year.

Location	Weather stations						Observation years	Budbreak (DOY)	CP	T_{max}	T_{min}
	Station name	Latitude (º)	Longitude (º)	Altitude (m)	Number of observations sites	Mean distance from observations (Km)					
Central Valley (CA)	Manteca	37.83	-121.22	10	1	13	2009-2014	80	55	17.9	4.5
	Modesto	37.65	-121.19	11	1	13.5	2009-2011, 2013, 2014	85	57	21.4	6.2
	Kesterson	37.23	-120.88	23	1	18.5	2009-2014	79	52	23.8	5.5
	Oakdale	37.73	-120.85	50	1	7	2009-2014	77	57	22.8	7.6
North Coast (CA)	Santa Rosa	38.40	-122.80	24	1	8	2012-2015	85	46	21.0	4.5
	Winsdor	38.53	-122.83	92	2	15.5	2007-2013, 2015	82	55	21.3	4.8
	Carneros	38.22	-122.35	2	3	1.5	2004- 2011, 2014, 2015	74	55	21.0	6.1
	Oakville	38.43	-122.41	58	1	1.5	2010, 2012-2016	78	50	22.6	6.7
Central Coast (CA)	San Benito	36.85	-121.36	104	1	2.5	2014	65	35	20.8	4.6
South Central Coast (CA)	Nipomo	35.03	-120.56	78	1	16	2010, 2011, 2014-2016	61	47	19.4	8.8
Spain	Raïmat	41.68	0.45	286	1	5.4	2013	95	49	13.9	3.8
	Sant Sadurni d'Anoia	41.43	1.79	164	1	5	2006, 2012, 2014, 2015	92	42	18.7	6.6
	Badajoz	35.51	-6.39	188	1	0.5	2014-2016	76	59	22.3	8.8

Description of the Chill Overlap Model

The Chill Overlap Model is based on an exponentially declining curve representing the possible combinations of chill accumulation (C_a) and heat accumulation (H_a) that result in budbreak (Harrington et al. 2010). Two sub-models quantified winter chill and spring heat from the onset of dormancy until budbreak (Pope et al. 2014).

Chill accumulation was determined using the Dynamic model (Fishman et al. 1987) and involved a two-step process. In the first step, a chill intermediate was formed or destroyed according to an hourly bell-shaped temperature relationship. The formation of chill intermediates was enhanced in cold temperatures, with an optimal efficacy at 6 to 8ºC, and previously accumulated chill intermediates were negated if temperatures exceed a specific threshold (24ºC). In the second step, the chill intermediate was computed as a single chill portion (CP), which could not be negated by subsequent warmer temperatures. One CP was equivalent to 30 hrs of continuous chill exposure at ≤6ºC (Erez and Fishman 1998).

Heat accumulation was calculated using the growing degree hour (GDH) ASYMCUR model (Anderson et al. 1986). In line with the model described in Anderson et al. (1986), the acquisition of heat was taken as an hourly asymmetric curvilinear model defined by two cosine-type equations based on three threshold temperatures (base temperature = 4ºC, optimum temperature = 25ºC, critical temperature = 36ºC), which determined the accumulation of Heat Units (HU). A base temperature of 10ºC was also tested with this model.

Hourly temperatures were required as inputs for the Dynamic chill accumulation and GDH ASYMCUR models. Daily maximum and minimum temperatures were then interpolated into hourly data following (Linvill 1990), and the specific parameters of each station location were used for both the parameterization and validation data sets (Table 1).

The chilling requirement (C_r) is the minimum amount of accumulated chill required for budbreak to occur, and the heat requirement (H_r) is the minimum amount of accumulated heat required for budbreak to be possible. The C_r should be satisfied before any additional chill (C_a) modifies any specific part of the heat accumulation (H_a) phase and results in an overlap between the two phases. This is defined by Equation 1 and shown in Figure 3:

$$H_a = \beta_1 + \frac{\beta_2}{e^{(\beta_3 \, x \, C_a)}} \qquad (1)$$

where H_a is heat accumulation from C_r to budbreak; C_a is chill accumulation after C_r has been met; and β_1, β_2 and β_3 are model parameters.

The β_1 model parameter, defined the lowest heat accumulation required for budbreak to be possible, is equivalent to H_r. In fitted models, it correlates with heat accumulations experienced in high chill years/climates. β_2 corresponded to the difference in heat accumulation between the highest and the lowest observation values ($\beta_2 = H_o - H_r$), which is estimated by data accumulated during mild winters. The β_3 parameter was related to the shape of the curve, based on values ranging between 0 and 1 that define this shape (Pope et al. 2014).

Development of model parameters and parameterization

The Chill Overlap Model parameters were fitted as described (Pope et al. 2014, Darbyshire et al. 2016). The C_r had to be estimated, as no previous experiments had been conducted to evaluate it. The onset of chilling was considered to occur on 1 Oct (Jarvis-Shean et al. 2015). The minimum value of chill accumulation measured throughout the period for all sites and years (1 Oct to 31 March) was tested as the maximum C_r with 1 CP increments. According to our dataset, the range tested was ran from 1 to 31 CP (Spain: Sant Sadurni d'Anoia in 2012). The overlap interval values tested ranged from 10 to 90%, with increments of 5% (Figure 3).

For each C_r tested, the starting values used to fit the model parameters were estimated from parameterization data sets. The lowest value of H_a was used as an estimation of β_1. The difference between the highest and lowest H_a values was estimated as $\beta_2 = H_o - H_r$, and the starting value for β_3 was 0.0001 (Equation 1).

Non-linear regression algorithms were applied to fit the model. The Levenberg-Marquart algorithm in the curve fitting toolbox of MATLAB software (MATLAB and Statistics Toolbox Release 2014b, The MathWorks, Inc.) was chosen instead of a trust-region algorithm because it required fewer iterations to find the most appropriate fit values for the model. Negative values of β_1 and β_2 where dismissed based on them lacking biological sense: for example, heat accumulation cannot have negative values (Pope et al. 2014).

Evaluation of model parameters

Three indices were evaluated to obtain values for the model parameters. The information-theoretic approach akaike criterion (AIC_C) was used to make comparison within each C_r, with the model with the lowest value of AIC_C as described (Burham and Anderson 1998). Models with different C_r could not be compared because of the change in the response variable that resulted in lower C_r values associate with earlier heat accumulation (Pope et al. 2014). Candidate model parameters were also evaluated considering R^2 and root mean square error (RMSE) values. The models with the best parameters were evaluated in the same way, using the validation data set.

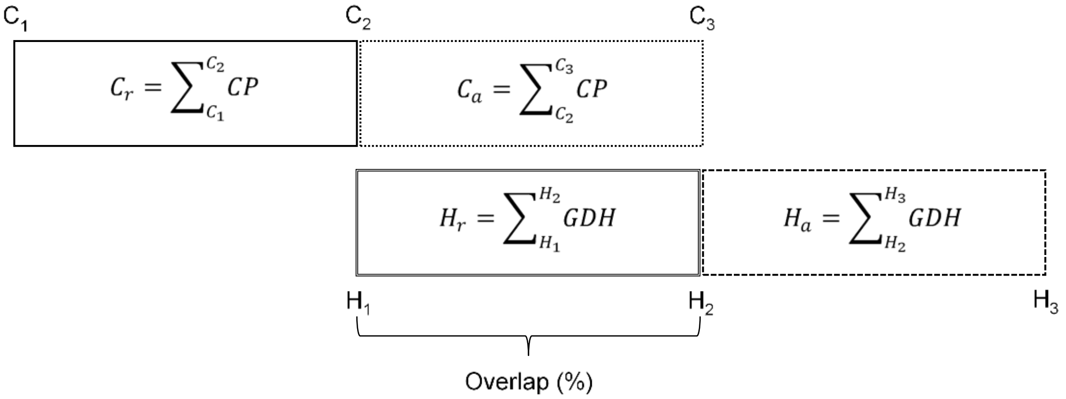

Figure 3 Explanation of the overlap between the chill and heat phases implied in the Chill Overlap Model. C_1, C_2 and C_3 exemplify the different accumulation times for chill, while H_1, H_2 and H_3 show the different accumulation times for heat. The rectangles with solid lines show measures of fixed chill/heat, and the rectangles with dashed lines show variable amounts of accumulated chill/heat. The overlap where the additional accumulated chill (CP, chilling portions calculated with Dynamic chill accumulation model) reduces the heat sum (GDH, growing degree hour determined with GDH ASYMCUR model) occurs when C_a, the chill accumulated from C_2 to C_3, and H_r, the heat accumulated from H_1 to H_2, are determined simultaneously for the same period.

Results

Selected candidate models prioritized according to the lowest AIC_C, highest R^2 and lowest RMSE values using parameterization data are presented (Tables 2 and 3). Testing several overlaps (from 10 to 90% with increments of 5%) for CPs from 1 to 31, an AIC_C with a value of 346.62, an R^2 of 0.54, and a RMSE of 8.86 were obtained with a C_r of 9 CP and a 40% overlap (Table 2, Figure 4A, 4B). The corresponding Chill Overlap Model parameter values were 6110, 9657 and 0.0463 for β_1, β_2 and β_3, respectively (Table 2). An evaluation of the same overlap with different chilling requirements is presented (Table 3). Changing the C_r value from 9 CP did not improve the performance of the model.

These parameters were then validated by applying the same model parameters to an independent data set. The model fit for the validation data set was better than for the parameterization data set, with a C_r of 9 CP and a chill-heat overlap of 40% resulting in an $R^2 = 0.69$ and an RMSE of 7.32 days (Table 2 and 3, Figure 5). This indicates that the previously chosen model parameters were as valid as could be expected.

Table 2 Example of model fit and performance for overlap estimates at one potential (C_r). Aikaike information criterion ($\mathrm{AIC_C}$) was used to evaluate the models used for the parameterization data set. R^2 and root mean square error (RMSE) were used to evaluate the relationship between the observed and predicted budbreak values by applying fitted model parameters for parameterization and validation data sets. Only significant ($p < 0.05$) models are shown.

C_r (CP[a])	Overlap (%)	Model parameters			Parameterization			Validation	
		β_1	β_2	β_3	$\mathrm{AIC_C}$	R^2	RMSE (days)	R^2	RMSE (days)
9	25	6992	8152	0.0729	352.28	0.53	9.45	0.68	7.87
9	30	7800	7751	0.0813	359.77	0.47	10.33	0.64	9.50
9	40	6110	9657	0.0463	346.62	0.54	8.86	0.69	7.32
9	50	2141	14041	0.0252	400.53	0.56	16.78	0.62	16.44
9	75	8856	11358	0.0636	386.61	0.44	14.17	0.42	14.47

[a]CP, chill portion

Table 3 Model fit and performance for estimates of the chilling requirement (C_r) with the same 40% overlap. Evaluation of R^2 and root mean square error (RMSE) for parameterization and calibration data sets evaluating the relation between the observed and predicted budbreak values by applying fitted model parameters. All the model fits were significant, with $p < 0.05$.

C_r (CP[a])	Overlap (%)	Model parameters			Parameterization		Validation	
		β_1	β_2	β_3	R^2	RMSE (days)	R^2	RMSE (days)
7	40	8591	9236	0.0833	0.48	11.67	0.49	11.72
8	40	7096	9242	0.0577	0.48	10.80	0.65	8.84
9	40	6110	9657	0.0463	0.54	8.86	0.69	7.32
10	40	2836	12225	0.0292	0.60	12.63	0.61	11.78
11	40	7275	8615	0.0662	0.55	10.17	0.61	9.99

[a]CP, chill portion

The analysis of model RMSE was most accurate for data from the Central Valley (CA), with values of 7.09 and 6.13 days for parameterization and validation, respectively. Model performance was less accurate at locations with warmer winter, such as on California's Central Coast (9.00 for parameterization and 10.00 days for validation) and South Central Coast (10.60 for parameterization and 8.49 days for validation) (data not shown), possibly because fewer data points from these locations were available for fitting the model initially (Figure 4B and 5).

Discussion

Model approach

In accord with the structure of the Chill Overlap Model, in addition to the C_r in locations where prolonged chill accumulation occurred, there was a decrease in the heat requirements needed to reach budbreak. In contrast, in warmer locations, where less chill was accumulated, more heat was required to trigger budbreak. In our study, cooler conditions resulted in a later budbreak (in Spain), while warmer conditions produced an earlier budbreak (California's Central and South Central Coast, Table 1). A delay in dormancy induction attributable to high temperatures has been reported previously for Chardonnay (Caffarra and Eccel 2010). This suggests that mild fall temperatures may contribute to earlier dormancy, whereas warm temperatures, above 20ºC, may delay it (Caffarra and Eccel 2010). During the same period, low temperatures led to a more rapid chill accumulation in Chardonnay, and therefore, to an earlier ecodormancy transition (Cragin et al. 2017).

The parameterizing data set seemed to provide sufficient data, including extreme values, to estimate C_r, H_o, and H_r, and consequently fit the model parameters. The estimated parameters presented some differences between the starting values and the fitted parameters (data not shown). According to the interpretation of the model parameters (Pope et al. 2014), the lower the fitted β_3 value is, the more linear the relation between C_a and H_a will be. As a consequence, the value of β_1 should be lower and the value of β_2 should be higher. This was not, however, true of all the cases analysed in the current study (Table 2 and 3). These discrepancies were difficult to explain by the curved relationship between chill and heat, particularly considering the numerous studies that have shown this relationship in temperate perennial species (Chuine 2000, Harrington et al. 2010). Given this failure to find an appropriate model, it is recommended to experimentally determine the value of C_r and to fit the values directly, rather than trying various different C_r options and increasing model curvature to compensate for this lack of knowledge (Dennis 2003, Pope et al. 2014). To provide further insight into the accuracy of models used with fruit trees, it is necessary to add endodormancy break dates. This should then yield more robust projections of phenological changes (Chuine et al. 2016).

Model performance

The Chill Overlap Model developed with this research did not improve the accuracy of budbreak prediction substantially over previous phenological models developed for the Chardonnay cultivar, at specific locations and climatic conditions (García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010). However, compared to the Chill Overlap Model developed for other species, these models were able to improve previous models' accuracy (Pope et al. 2014, Darbyshire et al. 2016). Improving model accuracy was not the first objective of this work; our aim was to evaluate whether the Chill Overlap Model could improve understanding of the processes involved in the dormancy to budbreak transition. In this study, the data used for model development covered a wider geographic area for the Chardonnay cultivar than in previous studies; this may explain its only modest accuracy.

The variations in phenological data sources may also have been an important reason for the limited accuracy of the model developed, as the criteria for determining the exact onset of budbreak were not uniform across sites and locations. Although budbreak was generally considered to take place when 50% of buds were open, determining the exact day of budbreak likely varied depending on the number of days between observations and the person who was collecting the data. In addition, the weather data were not recorded directly adjacent to the vines whose phenology was being observed. Therefore, there may have been differences between the temperatures recorded at the weather stations and those experienced in the vineyards, particularly where there differences in altitude (Nendel 2010; Table 1). Furthermore, it should be noted that maximum and minimum daily temperature data were used to estimate hourly chill and heat accumulation. Using actual mean hourly temperatures would have been more accurate for determining temperature accumulation, but such data were not available for all sites.

Clonal behavior may change depending on location, being mainly affected by microclimates and soils (Fidelibus et al. 2006, Jones et al. 2014). Since in our study, Chardonnay clones were not identified in all locations, we should consider clones as a possible source of variability that is difficult to evaluate for this analysis.

The variability in phenology at a given location may be explained by microclimate differences (Verdugo-Vásquez et al. 2016). This could affect phenological development as a consequence of specific changes in environmental conditions at a very local level. In California, for example, phenology performance may have been affected microclimate differences in the Central Valley and especially in the North Coast regions, where there can be strong local differences in air movement (Figure 4B and 5). Another important factor may have been the distance between the weather stations and the observation vineyards (Table 1). Although the Central Valley is largely flat, the distance between the sites and weather

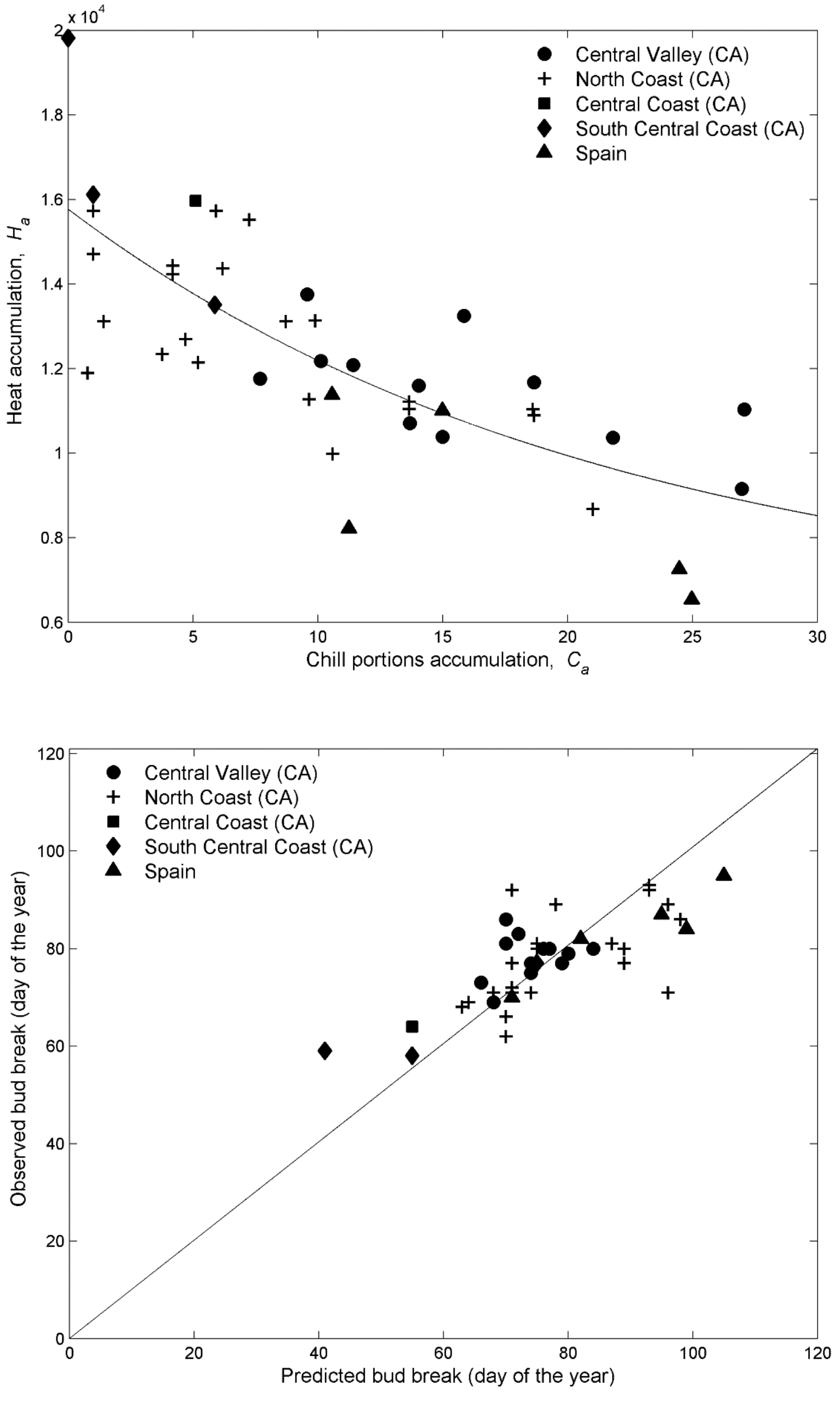

Figure 4 (A) Chill Overlap Model fit parameterization. The chilling requirement (C_r) was 9 chill portions (CP) and there was an overlap of 40% between the chill accumulation (C_a) and the heat accumulation (H_a) phases. Model accuracy was evaluated at an akaike information criterion value of 346.62. (B) Predicted and observed budbreak day of the year based on the Chill Overlap Model using parameterization data (R^2 = 0.54 and root mean square error = 8.86 days). The fitted values were determined after 9 CP corresponding to the C_r were met, with an overlap of 40%.

stations could have been more than 10 km, a potential significant source of error in model performance.

Air temperature has been widely reported to be the main driver of phenology. Depending on net radiation, the differences between air and bud temperature may be 0.5 to 2°C, but on foggy days, this relationship changes to ~0.1 to 0.2°C (Itier et al. 1987). Fog tends to reduce bud temperature and therefore increases chill accumulation. However, the incidence of fog formation is highly variable among years, being the result of many complex and conditional meteorological factors. The general trend in California has been towards fewer winter fog events, which tend to be characterized by sustained periods with air temperatures below 7°C. Possible consequences of less fog include warmer air and more direct sun exposure, which amplifies warming and reduces chill accumulation (Baldocchi and Waller 2014).

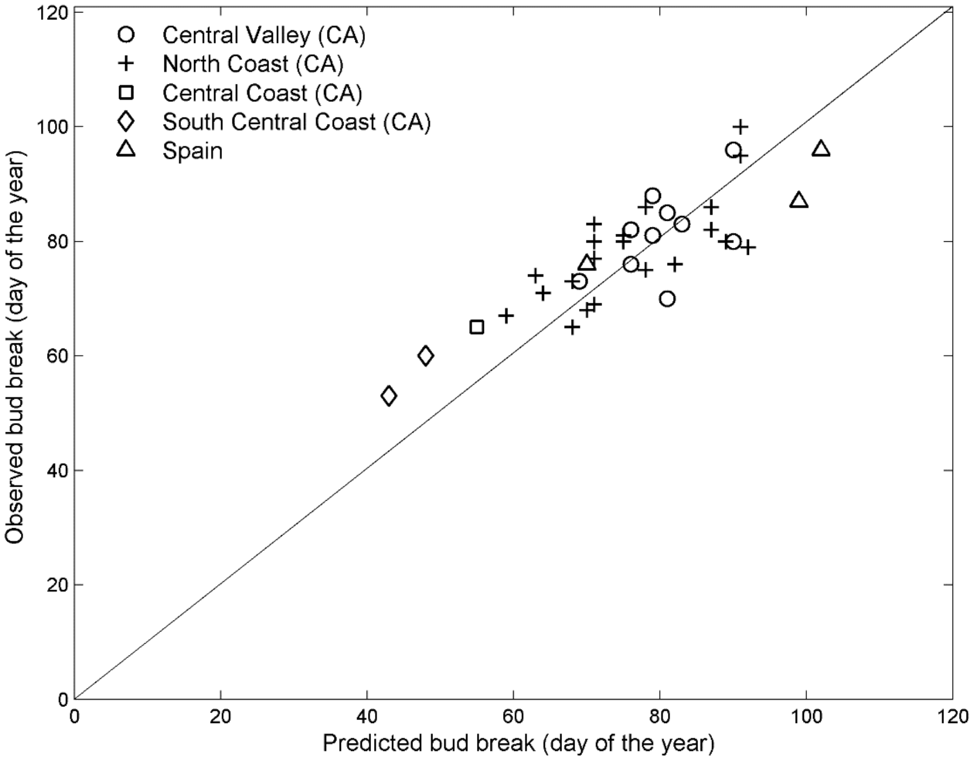

Figure 5 Predicted and observed budbreak day of the year, evaluated with the best performance fit model parameters using the validating dataset ($R^2 = 0.69$ and root mean square error = 7.32 days). The chill requirement was 9 chill portions, with a 40 % chill/heat overlap.

Orchard management may also potentially influence microclimates through effects associated with canopy management, cover cropping, and irrigation regime (Luedeling et al. 2009). Grapevine management practices, for example, have been reported to influence the completion of budbreak. In postharvest irrigation experiments, cutting off irrigation early advanced the budbreak stage of the Perlette cultivar (Williams et al. 1991). Similar responses were observed in an experimental Chardonnay vineyard grown in Raïmat, Lleida, Spain, in spring 2016 (J. Marsal, personal communication). Late pruning slightly delayed budbreak in Cabernet Sauvignon (Martin and Dunn 2000) and Sauvignon blanc grapevines (Trought et al. 2011).

Chill and heat accumulation

Specific changes in dormant buds are not initiated until there has been sufficient chilling to break endodormancy, after which bud growth will respond to warm temperatures (Faust et al. 1997, Chuine 2000). Over a range of temperatures, chill accumulation in grapes was evaluated to be most efficient at 2.8ºC (Caffarra and Eccel 2010). A recent study conducted with Chardonnay canes indicated that three weeks of exposure to temperatures of -3 ºC was also effective for releasing endodormancy (Cragin et al. 2017). The Dynamic chill model, which was used to evaluate chill accumulation in the Chill Overlap Model, considered 6 to 8ºC to be the optimum temperature range for chill accumulation. This model also considers the effects of negation of chill associated with temperatures above 20ºC. To the best of our knowledge, the Dynamic chill model had not been used previously to test grapevine species (Dokoozlian 1999). Even though the chill models described in the literature are often not comparable, because the accumulated chilling units differ from site to site, previously tested chill models and the experiments performed on grapevines provide some basis for comparisons (García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010). The Dynamic chill accumulation model seemed most appropriate for measuring chill in this study because of the range of climates present in the data set.

For most plants, 10ºC is considered the best base temperature for growth. While 10ºC is an appropriate base temperature for calculating heat accumulation to predict grapevine development (Williams et al. 1985), several other reports indicate that a lower base temperature may be more appropriate for predicting budbreak (Duchêne et al. 2010). A base temperature near 5 ºC was found appropriate for budbreak prediction in two studies (Moncur et al. 1989, García de Cortázar-Atauri et al. 2009).

In this study, the GDH ASYMCUR model (Anderson et al. 1986) was used to determine heat accumulation, considering a base temperature of 4ºC. A base temperature of 10ºC during the endodormancy release period was also tested, but no improvements in model prediction capacity were achieved. It therefore seems that a base temperature of below 10ºC may be suitable for predicting budbreak; indeed, this was used in previous models for Chardonnay (García de Cortázar-Atauri et al. 2009).

As the goal of this research did not include determining threshold temperature values for chill and heat accumulation, we used published threshold values. Further research is clearly needed to evaluate temperatures for chill and heat perception by buds during dormancy, but due to other limitations on the data set used in this study, it was not appropriate to pursue that objective. Other factors may also affect the perception of chill and heat by buds, such as the lack of synchrony in bud growth, with apical buds opening before lateral due to their heat requirements being met earlier (Martin and Dunn 2000), and differences in bud vigor and reserves of carbon and nitrogen (Ben Mohamed et al. 2010).

In this study, Chardonnay needed ~9 CP, compared to 13, 21, and 23 CP for the Sonora, Mission and Nonpareil almond cultivars, respectively (Pope et al. 2014), and 34 CP for the Crips Pink apple cultivar (Darbyshire et al. 2016). Compared with other deciduous fruit crops, grapevines grown in Iran do not have very high chilling requirements and need relatively little exposure to chill (Eshghi et al. 2010). Our results were consistent with this report. Since 1 CP is equivalent to 30 hrs of continuous chill at 6°C (Erez and Fishman 1998), the C_r evaluated for Chardonnay may have been similar to a chill exposure of 270 hrs (9 CP x 30 hrs/CP). Previous research using chill hours accumulated between 0 and 10°C found that 200 hrs was the minimum chilling exposure required for normal Perlette grape bud growth (Dokoozlian, 1999), and that 336 hrs at temperatures below 6°C were required for Cabernet Sauvignon (Botelho et al. 2007).

Although there is no exact equivalence between chill hour and CP quantification, as they are not constant in time or space; it is reassuring that the differences between species in terms of C_r are consistent with different chill models and accentuate biological differences between species. Some studies have suggested that the Dynamic chill accumulation model is most accurate for quantifying winter chill and understanding location-specific and year-to-year variability, and that it performs best in warmer areas (Luedeling et al. 2009).

Differences among species can be highlighted by comparing the values for the Chill Overlap Model parameters. In addition to the low C_r in grapevines, the overlap defined by the period with a compensatory relationship between the chill and heat requirements appeared to be smaller in grapevines (40%) than in almonds and apples (75%) (Pope et al. 2014, Darbyshire et al. 2016). The amount of overlap may vary according to the plant species (Pope et al. 2014). Grapevines, for example, appear to have less need for chill than other species, even during the compensatory stage between the two requirements. On the other hand, despite its low chill demand, Chardonnay appeared to require the perception of more heat, as shown in the β_1 fitted model parameter. This suggests that grapevines could be a species in which additional heat may be more effective than additional chill above the minimum C_r.

Based on the Chill Overlap Model, the contribution of chill and heat to budbreak differs between grapevines and apples. Once C_r has been met in both species, cool locations with considerable accumulations of chill and subsequent decreases in heat produce later budbreak in grapevines, but earlier bloom in apples. In warmer locations, more heat was required to meet budbreak conditions, and grapevines reached budbreak earlier, while apples needed additional time to reach flowering (Darbyshire et al. 2016).

Values of β_3 defined similar curves of roughly the same shape, but with different chill and heat requirements from species to species. Additionally, a more precise estimate of chilling requirements obtained by forcing or through growth-room experiments, could increase model curvature (Dennis 2003, Pope et al. 2014). A previous study demonstrated that models calibrated with growth room data provided good accuracy when tested on two different field-based data sets (Fila et al. 2012). The integration of data obtained by growth room experiments with field observation data may yield more accurate model estimates (Fila et al. 2014). Integration of other factors, such as the time of pruning, into the models may also improve their performance (Martin and Dunn 2000).

More research is needed to improve the accuracy and utility of phenology models. For example, the capacity to predict the potential impact of climate change on the suitability of using specific grapevine cultivars for particular growing regions would be useful. The results of the Chill Overlap Model for the Chardonnay grapevine did not improve budbreak predictions significantly compared to simpler phenological models developed previously for the same cultivar (García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010). However, because the model integrates_the overlapping effect of chill accumulation on the subsequent heat accumulation, which has been observed empirically in the field, it would be worthwhile to try to improve the model. This could be done by accounting for several of the sources of potential non-temperature related variability in budbreak highlighted in this work.

Conclusions

This study provided a new set of parameters for modelling budbreak in Chardonnay grapevines using the Chill Overlap Model. Even though model performance did not show substantial predictive improvements over previous budbreak models, the model provides a framework to analyse synergistic interactions between chill and heat accumulation requirements prior to budbreak in grapevines. The results were acceptable, considering the wide range of climates involved and the potential sources of inaccuracy in the data sets used. Knowledge of the possible influences of environmental factors and management practices at specific locations should reduce inaccuracies in the predictions obtained and lead to further model improvement.

The study also confirmed the apparent low C_r of Chardonnay and the fact that temperatures below 10°C seemed to be effective in fulfilling its Cr. On the other hand, the study also supported Chardonnay's need for substantial amounts of warmth to achieve budbreak. These factors must be considered to understand how increases in temperature due to climate change could affect the behaviour and adaptability of this cultivar.

This model includes potential interactions between cold and warm temperatures that could improve understanding of plant physiology and crop behaviour during dormancy and budbreak. Even though all phenological models present simple interpretations to predict complex realities, this model is sufficiently complex and does not require expensive experiments to evaluate its performance.

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Chapter II

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

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Abstract

Time and environmental conditions, such as temperature and photoperiod, are the main drivers governing grapevine development over the growing season. 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 (29.8 °C), while the lowest was observed at veraison (20.9 °C). The RMSE of the model predictions for specific developmental stages ranged from 2 (fruit set) to 9 days (berry maturity). Different temperature responses were found during different phenological stages, with the most temperature-driven stages having the most accurate prediction results (bloom to veraison). Modifying vine growth periods by forcing vine regrowth allowed evaluation of temperature and physiological factors that influence grapevine development.

Key words: Degree-day methods, phenology, physiological factors, radiation use efficiency, upper temperature

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).

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 (Friend and Trought 2007; Gu et al., 2012; Moran et al., 2017, Petrie et al., 2017). 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 Fig. 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. 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.

Materials and methods

Vines and site

Field experiments were conducted in a 16-ha commercial vineyard of Chardonnay grapevines located at Raïmat (41°39’43” N – 0°30’16” 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 *Forced regrowth methodology*), and the second investigated radiation use efficiency based on measurements of vine growth and canopy light interception (section *Berry maturity method*).

In spring 2015, 172 one-year-old Chardonnay grapevines were grafted onto 1103 Paulsen rootstock at Raïmat (41°39'43" N – 0°30'16" 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).

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 (Fig. 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).

Phenological and weather data

Bloom, fruit set and veraison

Phenological data recorded from the vines in Raïmat (Fig. 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 (Fig. 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) (Fig. 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).

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)	Observation years	Phenological stage observations				
			Budbreak	Bloom	Fruit set	Veraison	Sparkling base wine berry maturity
Field vines	Raïmat weather station	2015, 2016	2	2	2	2	2
Forced field vines			3	3	3	3	3
Forced container-grown vines	Automatic weather station	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

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 (Fig. 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) (Fig.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.

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).

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 (°C) 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 (°C)	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

Method development

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) (Eqs. S1-S3, supplementary material). Two of the others methods tested were previously described in Zalom et al. (1983): *Single triangulation* (Eqs. S4-S10, supplementary material) and *single sine* (Eqs. 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 (Eqs. 1-7):

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

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

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

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

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

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

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

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 (°C)
T_U, upper temperature (°C)
T_{max} and T_{min}, daily maximum and minimum temperatures (°C)

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

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 =5°C and T_B = 10°C. 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 20°C to 32°C, while $thresDD_m$ had to be greater than 10 DD. All four methods were tested with respect to each phenological stage.

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):

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

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 °C. 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 daily dry matter production between two successive measuring dates was calculated as follows:

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

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 $T_H = 35°C$ (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.

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.

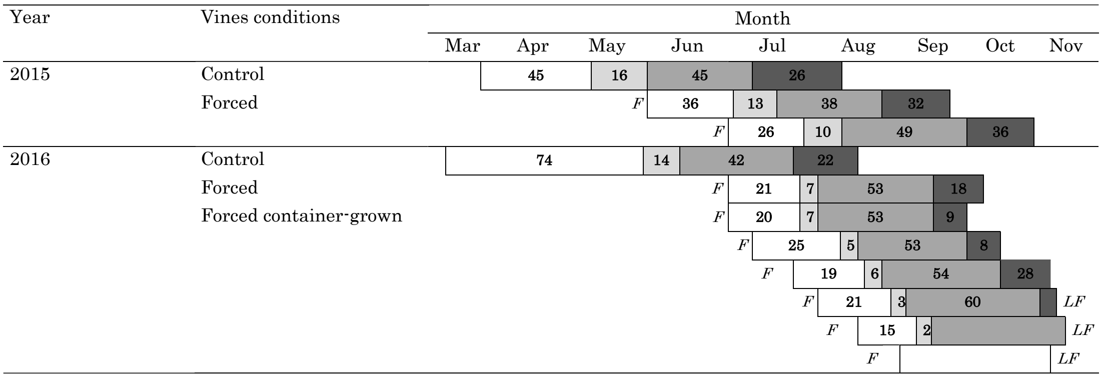

Fig. 1 Phenological data used for the calibration of the degree-day methods for bloom, fruit set and veraison stages, and the cross-validation of the method for berry maturity according with sparkling base wine. The letter *F* indicates when the forced regrowth treatments were performed on field and container-grown vines, 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.

Results

Forced regrowth

All forced regrowth treatments shifted bloom, fruit set, veraison and berry maturity (according to sparkling base wine criteria) phenological stages (Fig 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 (Fig.1).

Degree-day methods

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 (Fig. 1, Table 1a). A base temperature of 5°C 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 25.4°C 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 20.9°C 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 (Fig. 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 (Fig. 2b, Table 3).

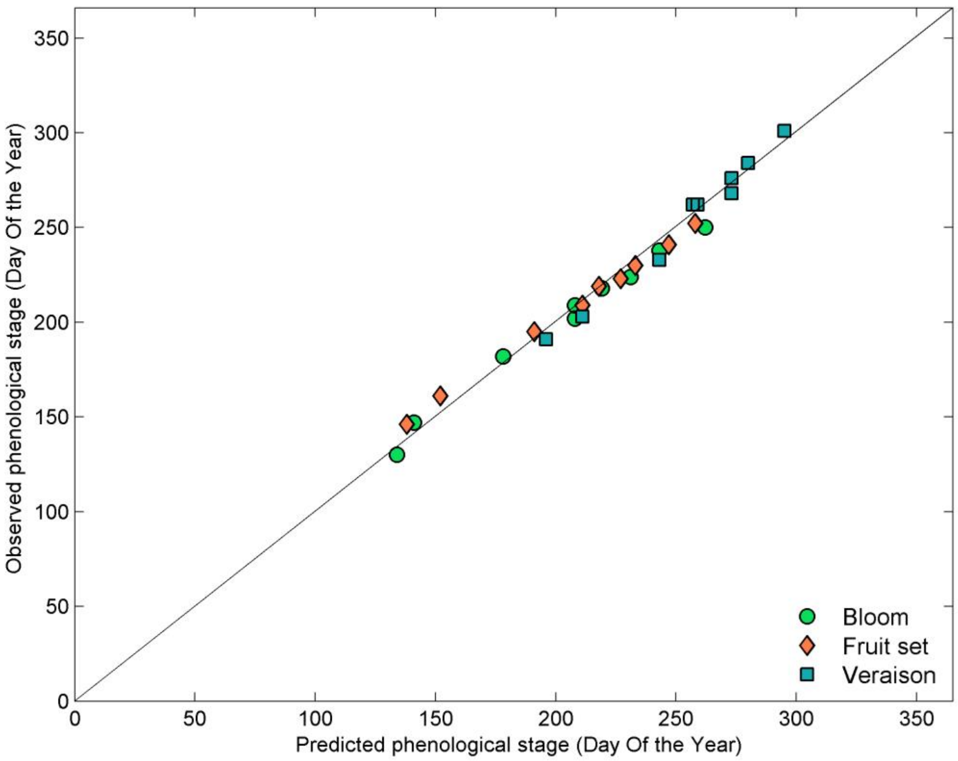

Fig. 2a Comparison between predicted and observed day of the year for bloom, fruit set and veraison for the best fit values on the calibration of the degree-day methods, with the data set shown in Table 1a. All the stages reached their best fit values with T_B =5ºC. Solid line is 1:1 line.

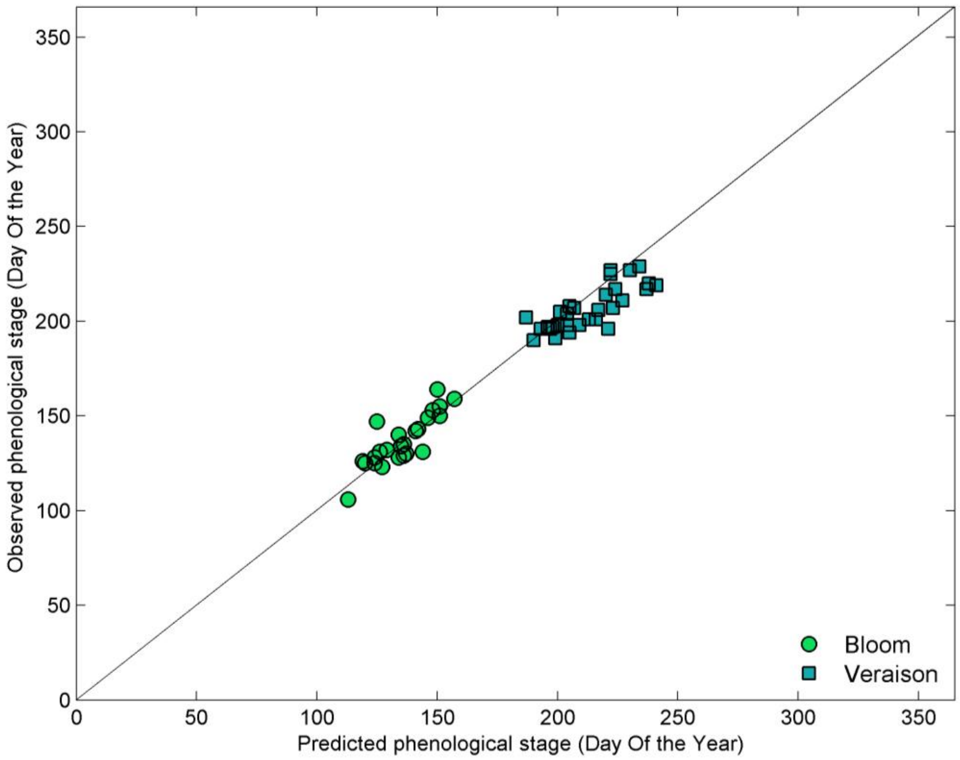

Fig. 2b Comparison between predicted and observed day of the year for bloom and veraison on the validation of the best fit methods with the data set shown in Table 1b. Solid line is 1:1 line.

Table 3. Best fit degree-day methods with a base temperature (TB) of 5°C for the bloom, fruit set and veraison stages. Parameters of the methods of each phenological stage, the statistics descriptors RMSE, R2, MBE and AIC for method calibration and the statistics descriptors RMSE, R2, MBE for method validation. Methods fits were significant (p-value < 0.05).

Phenological stage	Method parameters			Method calibration				Method validation		
	Method	T_U (°C)	*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.

Berry maturity

Three different tendencies were observed in the relationship between T_{max} and RUE measurements (Fig. 3). There was an increase of RUE with temperature from 5°C to 25°C; then, there was a plateau on the curve until 30°C; and above 30°C 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:

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

For berry maturity, a base temperature of 5°C 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 35°C. The method parameters for sparkling base wine were a T_U of 25.7±0.5°C 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 (Fig. 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±1.7°C 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 (Fig. S2b supplementary material, Table 4).

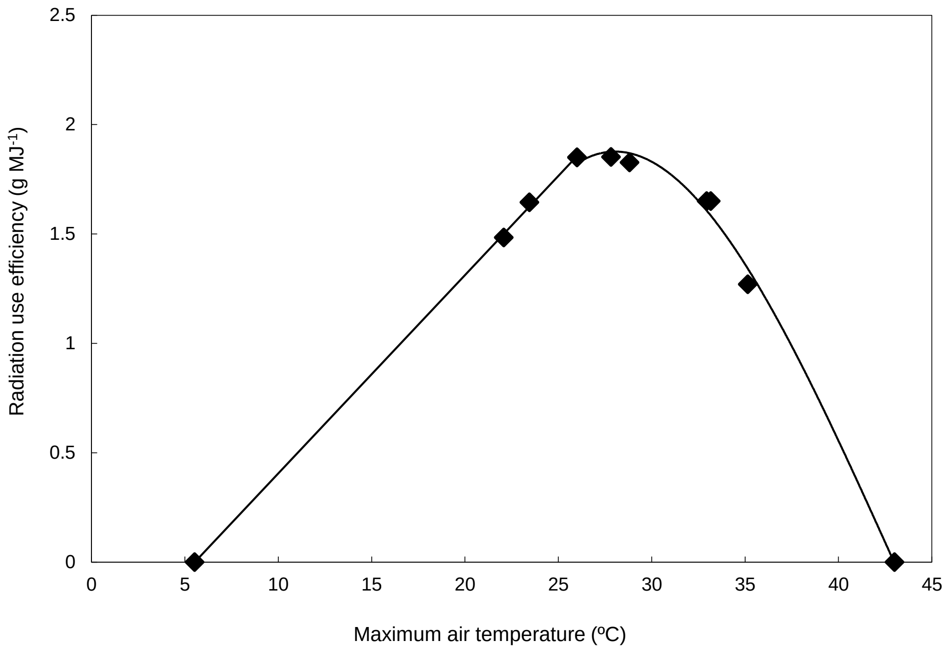

Fig. 3 Influence of resource availability on Chardonnay vine development in conjunction with the effect of high temperatures. Represented by the relationship between the maximum air temperature and the radiation use efficiency for a Chardonnay cultivar from the post bloom to the berry maturity phenological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

Table 4 Best fit degree-day methods with a base temperature (T_B) of 5°C 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. Methods fits were significant (p-value < 0.05).

Berry maturity	Method parameters				Cross-validation		
	Method		T_U (°C)	*thresDD* (*DD*)	RMSE (days)	R^2	MBE (days)
Sparkling base wine	*Single sine* with T_H=35°C	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

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 (Fig. 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 (Fig. 4b).

Discussion

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 (Fig 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

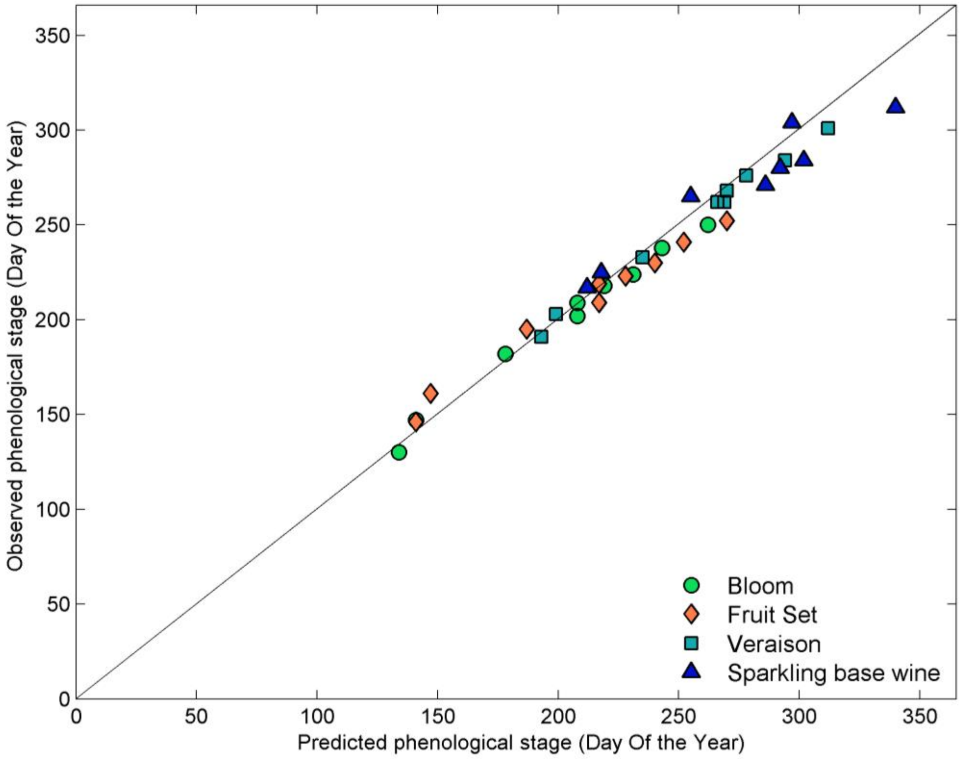

Fig. 4a Phenological prediction from bloom to sparkling base wine berry maturity with the methods selected for each stage. The RMSE statistics for the best methods for each stage were 4.7 (days) for fruit set, 3.4 (days) for veraison and 10 (days) for sparkling base wine berry maturity. Solid line is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

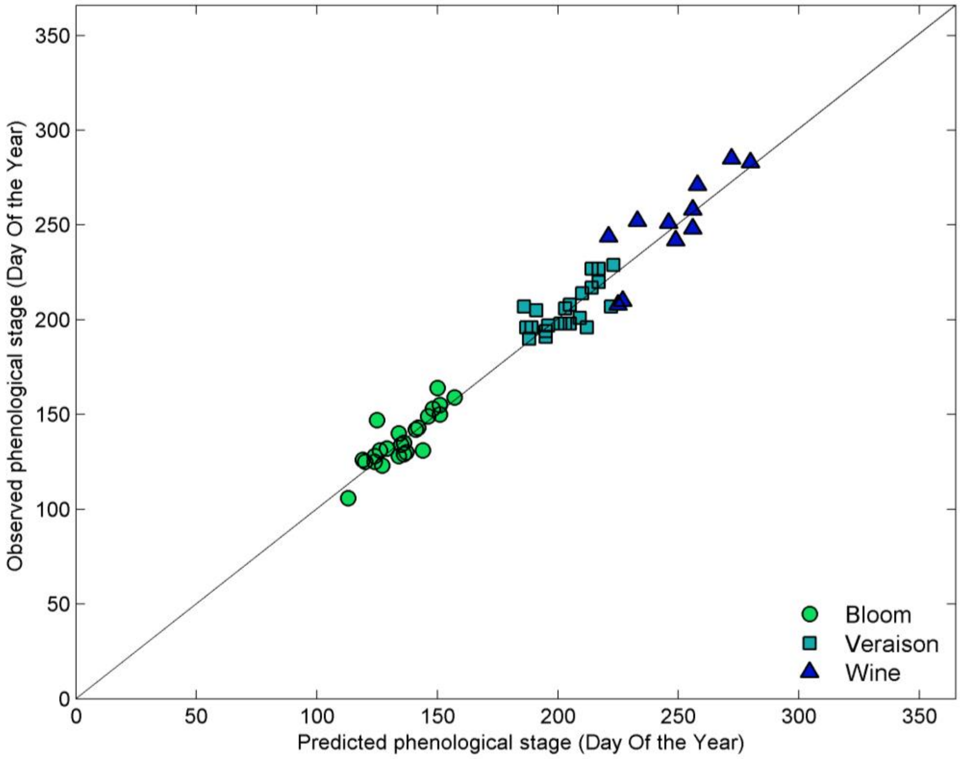

Fig. 4b Phenological prediction from bloom to wine berry maturity with the methods selected for each stage. The RMSE statistics for the best methods for each stage were 8.7 (days) for veraison and 13.3 (days) for wine berry maturity. Solid line is 1:1 line.

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).

Physiological basis

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 (Fig. 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).

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).

Degree-day calculation parameters

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 10°C 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 5°C in all phenological stages (Table 3). In previous Chardonnay studies, a reported T_B for obtaining bloom was 8.2°C, and for reaching veraison was 9.7°C (Zapata et al., 2016); and a range from 7.3 to 7.8 °C was obtained for bloom, and from 1.4 to 3.6°C 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 10°C (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 5°C, which was the T_B threshold providing the best fit. This may indicate that temperatures lower than 10°C 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 10°C 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 (25.4°C), while a lower was observed for veraison (20.9°C) (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).

Berry maturity

The T_U values obtained for the two kinds of berry maturity criteria differed considerably (25.7±0.5°C sparkling base wine, 29.4±1.7°C 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 (20.9°C) (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 35°C (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.

Applicability of the degree-day methods

The predictive capacity of the different methods over a whole growing season (Fig. 4a, Fig. 4b) was evaluated considering that the bloom predictions were the same as those used during method development (Fig. 2a and Fig. 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 terpens: some of the molecules responsible for wine 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.

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 prunings, delaying its onset to different times, allowed us to evaluate the environmental and physiological factors that influence grapevine development. 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.

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Chapter III

Water stress during the post-harvest period affects new root formation but not starch concentration and content in Chardonnay grapevine (*Vitis vinifera* L.) perennial organs

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Abstract

Water stress responses during the post-harvest period were evaluated in a Chardonnay container-grown grapevines grafted onto 1103 Paulsen rootstock. The irrigation treatments were: a control treatment (C) (irrigated to match ET_C demands) and a water stress treatment (WS) (irrigated when midday stem water potential reached a -1.1 MPa threshold). Photosynthesis, biomass and carbohydrate content were determined on five vines in each treatment on specific dates, from harvest until leaf fall. Stressed vines reduced leaf area due to defoliation, while well-watered vines had a higher carbon accumulation allowing the formation of new roots during the post-harvest period. No dry biomass accumulation was observed in the shoot and trunk organs after fruit harvest. Starch concentration and content were not affected by water stress. At the end of the experiment, starch concentrations were lower in the shoots and trunk than in the roots. Water stress induced a variation on biomass accumulation between above and below ground perennial organs, with the roots being the main organs in which biomass and starch concentrations were accumulated and kept, respectively.

Key words: Leaf area, leaf net assimilation rate, reserve organs, stem water potential

Introduction

Water stress can be a limiting factor in perennial crops, affecting important physiological processes such as photosynthesis and respiration (Chaves et al. 2010). Plant growth depends on the carbon balance which, in turn, is linked to photosynthesis and the respiration balance and is often limited by water availability (Flexas et al. 2006). As a result, numerous studies have focused on plant responses to water stress during the vegetative growth period, with the grapevine providing a clear example (*Vitis vinifera* L.) (Girona et al. 2009, Rogiers et al. 2011).

The grapevine has its origin in the Mediterranean basin and its growth cycle has adapted to the climatic condition in this area (Terral et al. 2010). Grape production in the regions surrounding the Mediterranean basin is an important activity, occupying approximately 2,768,000 hectares (OIV 2017). In such regions, the onset of vegetative growth is defined by budbreak (Duchêne et al. 2010). It takes place during spring and is accompanied by a significant mobilization of carbohydrates from plant reserves. This permits new vegetative growth until leaves reach 50% of their final size to become net carbon exporters (Vaillant-Gaveau et al. 2014, Köse and Ates 2017). Depending on the grapevine cultivar, this mobilization of reserves may even be extended almost until anthesis (Zapata et al. 2003). The accumulation of carbohydrates during the previous season is therefore essential for sustaining the mobilization of reserves until photosynthesis becomes the main source of carbon in spring (Zapata et al. 2004, Smith and Holzapfel 2009).

The accumulation of carbohydrates in storage tissues depends on total photosynthesis and the partitioning of carbon among different plant organs (Howell 2001, Smith and Holzapfel 2009). In temperate climate vines, several studies have demonstrated that the majority of the carbohydrate restoration in storage tissues takes place during the post-harvest period, which supports vine reserve recovery (Bennett et al. 2005, Vaillant-Gaveau et al. 2014). Although the rate of photosynthetic activity decreases in line with leaf senescence (Bertamini and Nedunchezhian 2003), functional leaves remain active and help the replenishment of reserves (Scholefield et al. 1978, Loescher et al. 1990). Even in areas with short post-harvest periods, carbohydrate pool replenishment tends to be sufficient to maintain yield levels (Bennett et al. 2005, Vaillant-Gaveau et al. 2014). In most grape-growing regions, vines retain their leaves after harvest (Bennett et al. 2005). However, the length of time that leaves are retained on the vine and the effectiveness of their photosynthetic activity depend on the cultivar, climatic conditions and viticultural practices (Williams 1996, Trought et al. 2011, Hall et al. 2016).

Starch is the primary reserve form for carbohydrates stored in trunk and root organs (Mullins et al. 1992, Pellegrino et al. 2014, Köse and Ates 2017). Although starch concentration seems to be influenced by grapevine cultivar, climate and vine management (Bennett et al. 2005), the majority of starch storage is located in roots (Bates et al. 2002, Zapata et al. 2004). The root system consists of: coarse roots, which provide a structural framework, anchorage, transport and storage for carbohydrates, and nutrients for the woody organs; and fine roots, which are generally responsible for water and nutrient uptake (Comas et al. 2010). Root development in grapevines has been described as cyclical, with two main flushes of growth: in spring, between several days after budbreak and bloom; and in autumn, between harvest and leaf fall (Mullins et al. 1992, Tomasi 2016). Root growth is an energy-dependent process involving endogenous sink-source relations which depend on the availability and partitioning of carbohydrates. The main environmental factors regulating root growth are soil temperature (Kaspar and Bland 1992, Rogiers et al. 2013, Clarke et al. 2015) and water availability. The latter has been described as the most important factor regulating root growth and development (Eapen et al. 2005, Tomasi 2016). However, the impact of water stress on carbon accumulation following harvest has so far received relatively little attention. Furthermore, compared with the above-ground organs (such as leaves, shoots and trunk organs), there are few studies of root processes in grapevines (Field et al. 2009, de Herralde et al. 2010, Holzapfel and Smith 2012, Miranda et al. 2017). This is probably because these types of studies are highly time consuming and, to the best of our knowledge, at certain specific points in the post-harvest period (Bates et al. 2002); this is usually an overlooked period for grapevines (Hall et al. 2016).

In Mediterranean climatic regions, the post-harvest period coincides with low evaporative demand and late summer rain events. As a result, numerous studies have focused on grapevine responses to water stress during the period of vine growth (spring-summer) in which the probability of heat or water stress is high (Eapen et al. 2005, Duchêne et al. 2010). However, according to climate projections, an increase in the frequency and intensity of the drought events is expected, not only throughout the growing cycle of the grapevines, but also during post-harvest (Gonçalves et al. 2014, Ramos et al. 2018). Under such a scenario, the aims of the present study were to compare the relative contributions of the shoot, trunk and root organs of Chardonnay grapevines to the restoration of carbohydrate reserves under well-watered and water stress conditions during the post-harvest period.

Materials and methods

Experimental site and plant material

The experiment was carried out at Raïmat (41°40’37” N – 0°28’38” E), Lleida (Catalonia, Spain), during 2015 and 2016. In spring 2015, 172 one-year-old Chardonnay grapevines that had been grafted onto 1103 Paulsen rootstock were planted in 50-L containers. The growing media consisted of loose stones at the bottom of each container and a substrate mix consisting of peat, sand and silty-loam soil, in equal parts. Disease control and nutrition management were performed according to the wine grape production protocol of the 'Costers del Segre' Denomination of Origin (Catalonia, Spain).

Experimental design, irrigation treatments and water applied

The vines were fully irrigated until the beginning of the experiment, using the crop reference evapotranspiration method (Allen et al. 1998). The post-harvest irrigation study started in late August 2016, after fruit harvest (August 25). For this study, 64 uniform vines were selected and arranged in two rows, of 32 vines each (with a separation between rows of 3 m). The container walls were painted white to prevent excessive root temperatures. The experiment was laid out in a complete randomized block design with two treatments and four replications of eight vines. The experimental unit consisted of eight vines (8 vines × 2 treatments × 4 replications).

Two irrigation treatments were applied: a control (C), scheduled to satisfy full water requirements (100% ET_C), and a water stress treatment (WS). In the latter, irrigation was triggered once the midday stem water potential (SWP) threshold of -1.1 MPa was reached, following Bellvert et al. (2016). The WS vines were scheduled to receive 50%, 15% and 10% of the ET_C, in August, September and October, respectively. The amount of water applied to each experimental unit was monitored using digital water meters (CZ2000-3 M, Contazara, Zaragoza, Spain).

Water status and net assimilation rate measurements

Midday stem water potential (SWP) and leaf net CO_2 assimilation rate (A_n) (μmol CO_2 m^{-2} s^{-1}) measurements were made once per week from post-harvest (August 26) until leaf fall (October 19), measuring one leaf of three of the eight vines per experimental unit in each replication and treatment. Midday stem water potential (SWP) was determined using a pressure chamber (3005-series portable plant water status console, Soil Moisture Equipment Corp., Santa Barbara, California, USA) following the McCutchan and Shackel (1992) procedure. Measurements were made at solar noon on shaded leaves located close to the main trunk. Leaves were covered with plastic sheathes with aluminium foil bags for at least 1 hour before measurements were taken. Leaf net CO_2 assimilation rates were measured with an infrared gas analyser (model LCi; ADC BioScientific Ltd., Hoddesdon, Herts, UK). A portion of each leaf was placed in the chamber window area of 6.25 cm^2 and data were taken after 45 s, when the A_n reading had stabilized. All the measurements were taken in less than an hour. The integrated A_n reading for successive dates and for the whole experiment was calculated according to Basile et al. (2011), as follows:

$$\int A_n = \sum_i^{i+1} \left| \frac{A_{n\,i} + A_{n\,i+1}}{2} \cdot (t_{i+1} - t_i) \right| \qquad (1)$$

Where A_n is the leaf net assimilation rate and t are the measurement days.

The integrated A_n ratio between irrigation treatments was determined as:

$$Ratio\ A_n = \int A_{n\,WS} / \int A_{n\,C} \qquad (2)$$

Subscripts WS and C represent the water stress and control irrigation treatments, respectively.

Biomass determination

Vine biomass was sampled during the post-harvest period on the following dates: August 25 (initial date from which the differential irrigation treatments were applied), September 20, October 4, October 24 and November 28. The first sampling date was scheduled before the start of the irrigation treatment, when five vines were selected. For the following sampling dates, five vines were selected per treatment. The vines were split into above-ground organs (leaves, shoots and trunk) and below-ground organ (root system). Each above-ground organ was dried in a forced-air oven at 65°C to constant weight and then the dry weight of each organ was recorded. Leaf area (*LA*) was measured on a subsample of 20 leaves from each vine, except on the last day, because by then, all the leaves had naturally fallen off the vine. Leaf areas were measured with a leaf area meter (Li-COR 3200; Li-COR, Lincoln, NE, USA). After the sub-samples were measured they were placed in a forced-air oven and dried to a constant weight. The resulting values were then related to the whole vine leaf dry weights to obtain LA measures for each vine.

The root system was washed in a container at the field, and subsequently classified, into 4 categories, in the lab: a) underground stem of the rootstock, b) thick roots (> 2 mm), c) fine roots (suberized), and d) new roots (fine non-suberized). The differentiation between suberized and non-suberized fine roots was made by colour, as the new roots were lighter and finer, and the fine roots were darker (Clarke et al. 2015). The whole root system was dried and the dry weights were recorded as previously described for the above-ground organs.

The proportion of new roots in relation to the total root system was expressed considering the severity and duration of the water stress effect, calculating the water stress integral from the SWP measurements for the period.

Starch accumulation

Vine starch concentration was determined in the shoots, trunk and thick roots (> 2 mm). For each vine, 12 g fresh weight samples were taken for the shoots and trunk and 10 g samples for the thick roots. These were frozen in liquid nitrogen and then dried in a forced-air oven at 65ºC. Once the dry weight was constant, the samples were ground using a hand mill (M20; IKA-WERKE, Staufen, Germany). Starch concentration determination was carried out using a polarimetry technique in line with European regulation CE 152/2009.

Statistical analysis

The effect of the irrigation treatment on leaf net CO_2 assimilation rate, organ dry mass and starch content were evaluated by a one-way ANOVA followed by a Tukey's significant difference test. The same analysis was carried out on the assessment of the effect of the sampling dates on starch concentration. All the statistical analyses were performed using R software (R Core Team 2017) (R version 3.2.4 Revised) and the statistical significance was established at $P<0.05$.

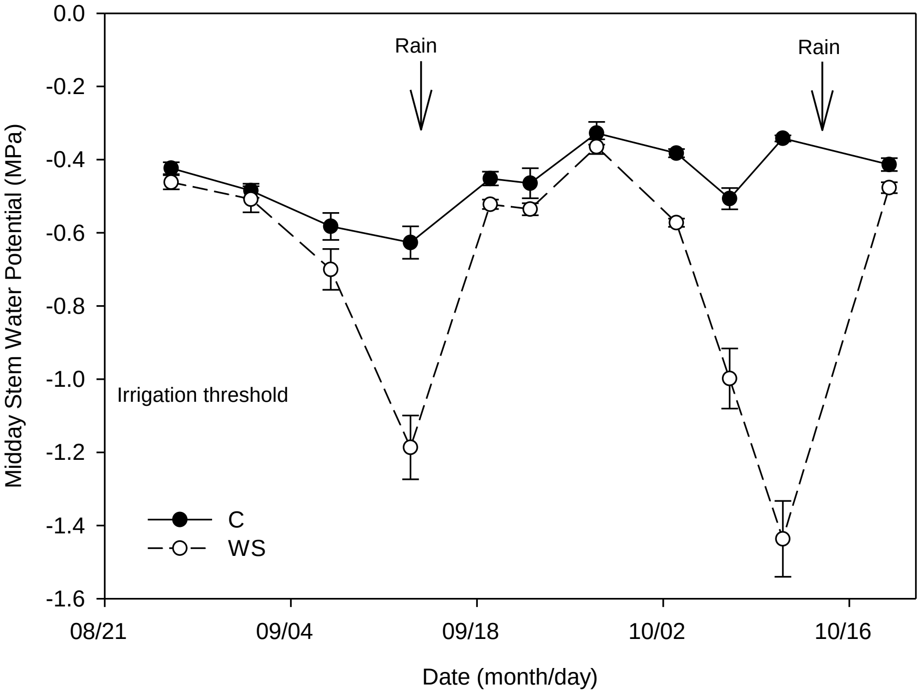

Figure 1. Post-harvest period patterns of midday stem water potential for vines under control (C) and water stress (WS) treatments. Natural leaf fall occurred on October 19. The irrigation threshold in the WS treatment was defined as -1.1 MPa. The bars indicate the standard error of the mean.

Results

Applied water, water status and net assimilation rate

Each vine received 237 L of water from budbreak to harvest prior to the beginning of the experiment. Then, from harvest to leaf fall, the control (C) vines received 116 L per vine, whereas the water stress (WS) vines received 46 L per vine. During this period, the stem water potential (SWP) in the C vines ranged from -0.4 to -0.6 MPa (Figure 1). In the WS treatment, the aim was to subject the vines to moderate stress levels, with a threshold value of -1.1 MPa. The irrigation threshold was exceeded on two occasions: on September 13 (-1.2 MPa) (following a 2.9 mm late summer rain event typical of Mediterranean conditions); and at the end of the experiment (-1.4 MPa), when the vine water status recovered to non-stress values, following a 21.6 mm rainfall event (Figure 1). Since the environmental conditions of the experiment were not favourable for achieving stress after October 19 in the WS vines (Figure 2), that date was taken as the end point of the differential irrigation management.

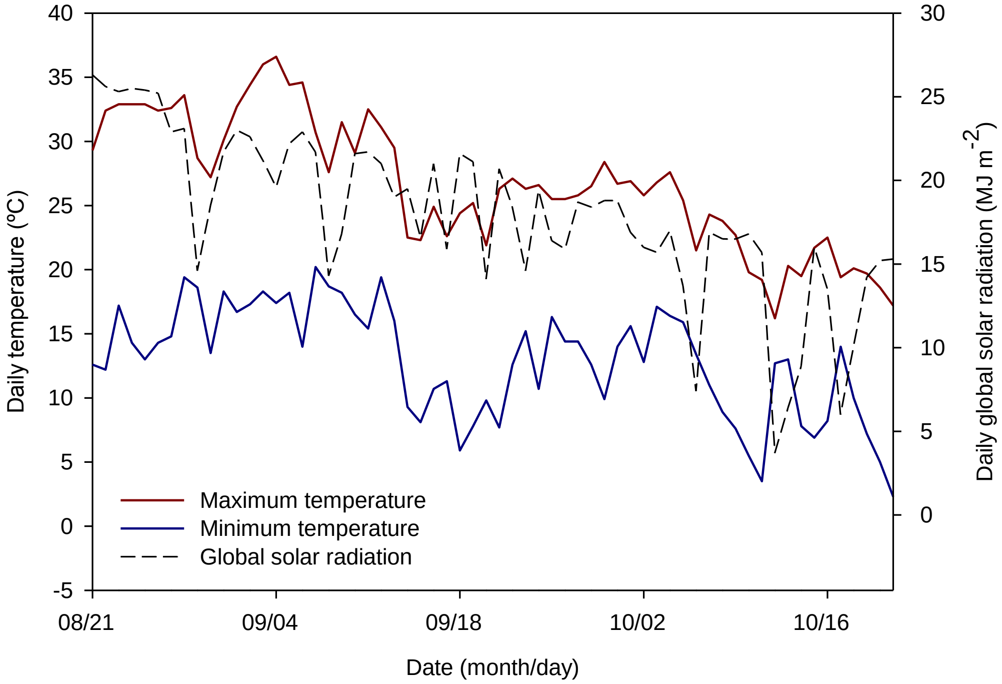

Figure 2. Climate summary during the experimental period with daily maximum and minimum temperature and daily global solar radiation. Environmental data was retrieved from the nearest weather station (1 km) from the study location (Raïmat, www.ruralcat.net/web/guest/agrometeo.estacions).

The water stress imposed in the WS treatment induced some early leaf fall in mid-September (Figure 3). Thereafter, the reduction in leaf area (LA) (m^2) was related to leaf senescence. In contrast, in the C treatment, only leaf senescence was responsible for reductions in LA (Figure 3).

The leaf net CO_2 assimilation rate (A_n) for the C treatment ranged from 5.9 to 10.6 μmol CO_2 m^{-2} s^{-1}, while that for WS was between 2.3 and 14.1 μmol CO_2 m^{-2} s^{-1} (Figure 4). From the onset of the experiment until September 22, the WS leaf net assimilation rates were below those of the C treatment; for the remainder of the period, those for WS were greater than for C (Figure 4). The relation between the integrated A_n of WS vines and C vines was 1.00 (calculated by equation 2); this resulted from 412.40 μmol CO_2 $vine^{-1}$ for the WS and

411.02 μmol CO_2 vine^{-1} for the C vine values (calculated by equation 1) (Figure 4).

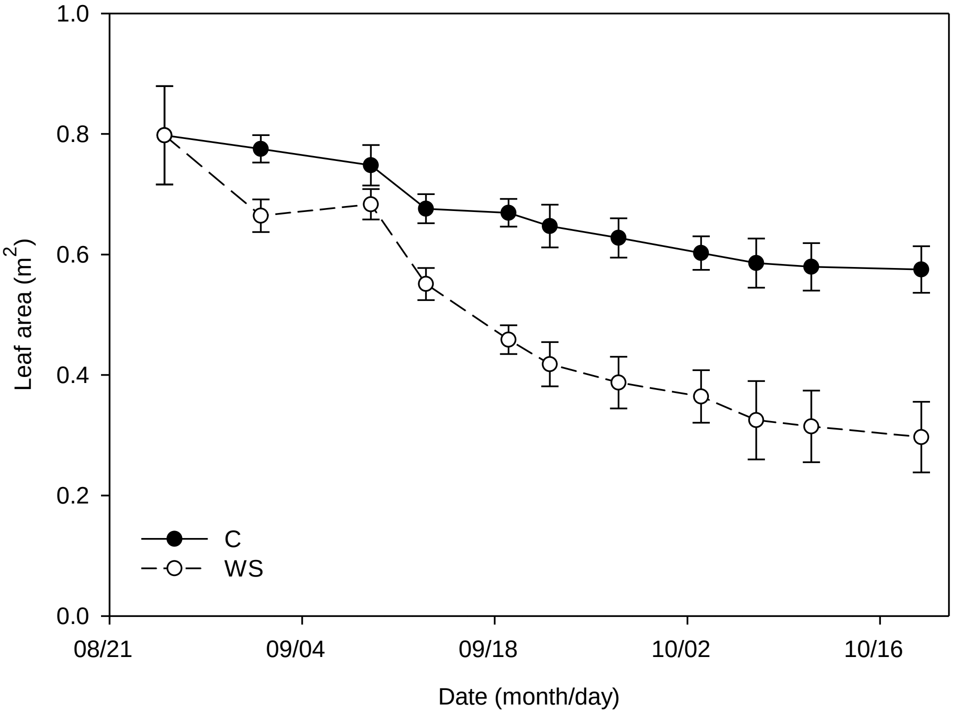

Figure 3. Leaf area pattern until leaf fall on vines under control (C) and water stress (WS) treatments. Natural leaf fall occurred on October 19.The values represent the means for five vines per treatment and the bars indicate the standard error of the mean.

Above-ground and below-ground biomass

Irrigation restrictions did not induce any differences in biomass accumulation of perennial above-ground vine organs. Shoots and trunk did not indicate any significant differences in carbon accumulation for any of the sampling dates during the period analysed (Figure 5a and 5b).

The accumulated biomass measurements for the root systems were not significantly different between irrigation treatments (Figure 5c). Considering only new root biomass, a significant ($P<0.05$) increase was observed for the C treatment with respect to the WS treatment throughout the study; with the October 24 exception (Figure 5c). Differences in accumulated biomass in new roots between the C and WS treatments were 63.4, 44.6 and 50.8% for the September 20, October 4 and November 28 sampling dates, respectively.

The proportion of new root biomass compared total roots biomass was greater in the C treatment compared to the WS treatment and increased as a function of accumulated waster stress (Figure 6). Since leaf fall occurred at the end of October, it was not possible to measure water status on the last measurement date (November 28).

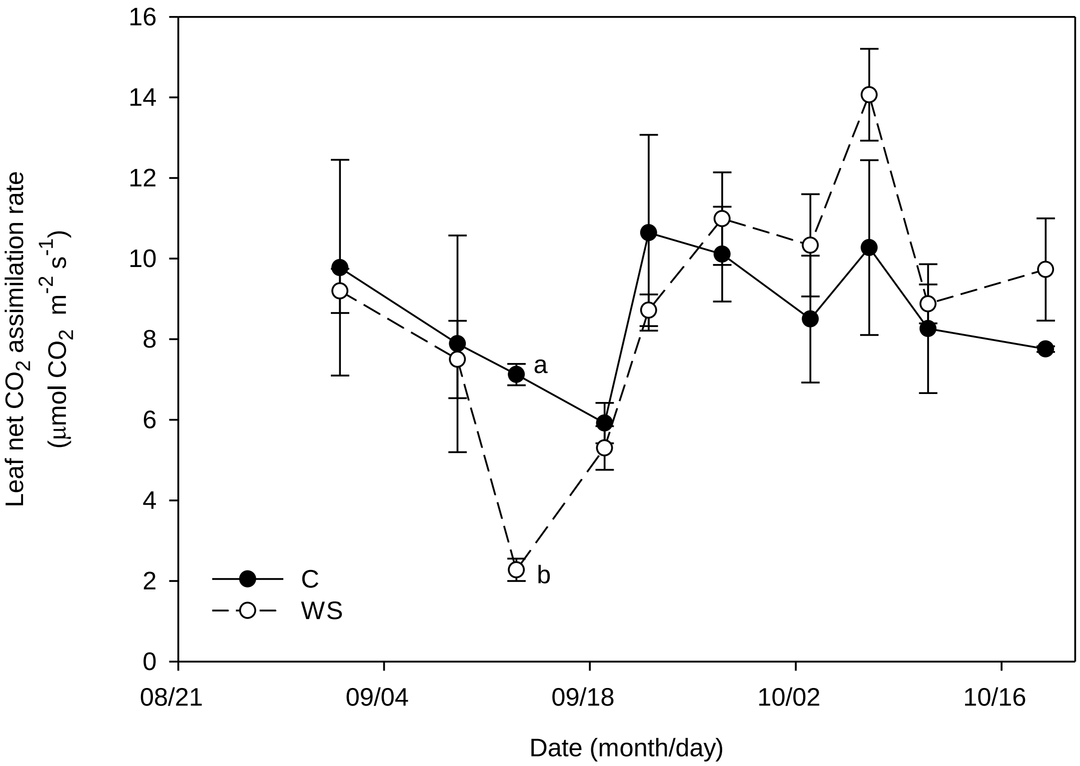

Figure 4. Post-harvest period patterns of leaf net CO_2 assimilation rate for vines under control (C) and water stress (WS) treatments. Natural leaf fall occurred on October 19. Bars indicate the standard error of the mean. Different letters indicate significant differences between irrigation treatments for the same date ($P<0.05$).

Starch accumulation

Starch concentrations during the whole period were significantly different between sampling dates for shoot and trunk organs, in both of the irrigation treatments (Table 1). Significant differences were observed ($P<0.05$) between the treatments on the last measurement date (November 28), when the lowest starch concentrations were registered and ranged from 10.6 and 11.6%. The highest average starch concentration in C treatment shoots was 16.7% for the September 20, October 4 and October 24 sampling dates. The C vines had the highest starch concentrations in the trunk on the September 20 and October 4 sampling dates, with an average of 15.7% (Table 1). The highest starch concentrations in the WS treatment occurred on October 4, in shoots were registered 16.3% and 15.2% in the trunk. No significant differences in starch concentration were found in root organs for any sampling date or irrigation treatment, during the experiment.

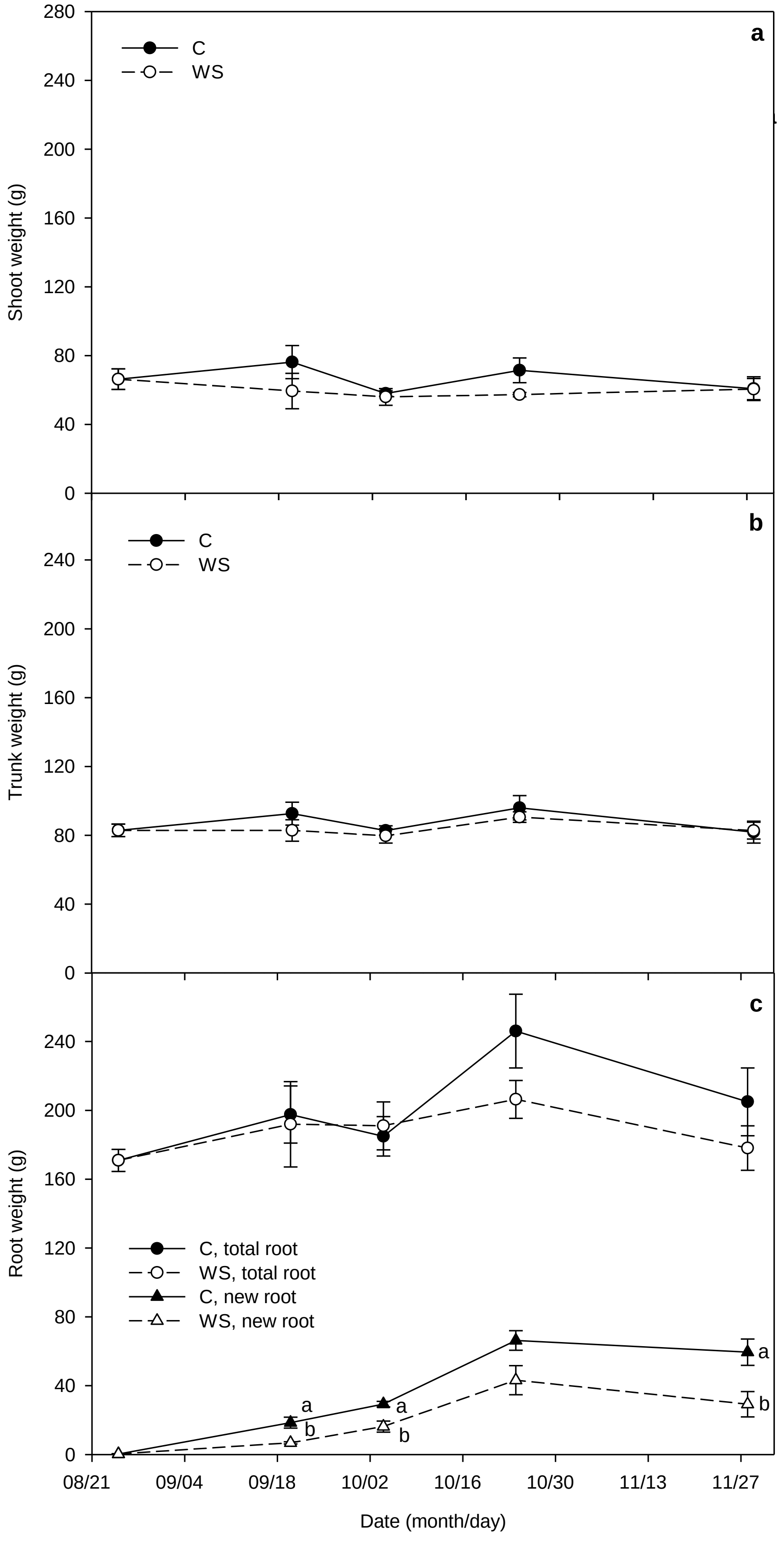

Figure 5. Post-harvest period patterns for dry mass in shoot (a), trunk (b), total root and new root organs (c) under control (C) and water stress (WS) treatments. The values represent the means of five vines per treatment and the bars indicate the standard error of the mean. There were no significant differences between irrigation treatments for the same sampling date ($P<0.05$).

Table 1. Starch concentrations of shoots and trunk (perennial above-ground organs) and roots (below-ground reserve organ) for vines harvested during the course of the post-harvest treatments.

Sampling date (month/day)	Treatment					
	Control (%)			Water stress (%)		
	Shoots	*Trunk*	*Tick roots*	*Shoots*	*Trunk*	*Tick roots*
Starting date 08/25	14.2 ± 0.5^{b}	13.5 ± 0.3^{b}	12.3 ± 1.2^{a}	14.2 ± 0.5^{b}	13.5 ± 0.3^{b}	12.3 ± 1.2^{a}
09/20	16.7 ± 0.2^{a}	15.7 ± 0.2^{a}	12.5 ± 0.8^{a}	15.1 ± 0.5^{ab}	14.9 ± 0.4^{ab}	13.0 ± 1.6^{a}
10/04	16.6 ± 0.2^{a}	15.8 ± 0.2^{a}	14.0 ± 1.0^{a}	16.3 ± 0.2^{a}	15.2 ± 0.2^{a}	12.2 ± 0.4^{a}
10/24	16.8 ± 0.5^{a}	15.0 ± 0.2^{ab}	11.3 ± 0.7^{a}	15.7 ± 0.5^{ab}	14.8 ± 0.4^{ab}	12.5 ± 0.5^{a}
11/28	11.6 ± 0.3^{c}	10.7 ± 0.3^{c}	13.3 ± 1.1^{a}	10.9 ± 0.3^{c}	10.6 ± 0.4^{c}	11.3 ± 0.7^{a}

Different letters mean significant differences on starch concentration between sampling dates in the same organ and in the same irrigation treatment ($P<0.05$).

As observed for dry mass, in all of the perennial organs, the starch content was not significantly different between irrigation treatments for any sampling dates (Figure 7). There were statistical differences between the first (August 25) and the last (November 28) sampling dates within the same irrigation treatments. Shoot starch content did significantly decline from 9.3 g of starch to 7.1 g in the C vines and 6.7 in the WS vines by November 28 (Figure 7a). As with shoots, the minimum starch content in trunk was registered on the last sampling date (November 28), with 8.8 g in both treatments (11.2 g of starch was measured on August 25) (Figure 7b). Over time the starch content in the roots of the C and WS treatments appeared to diverge, but the differences were not statistically significant (Figure 7c).

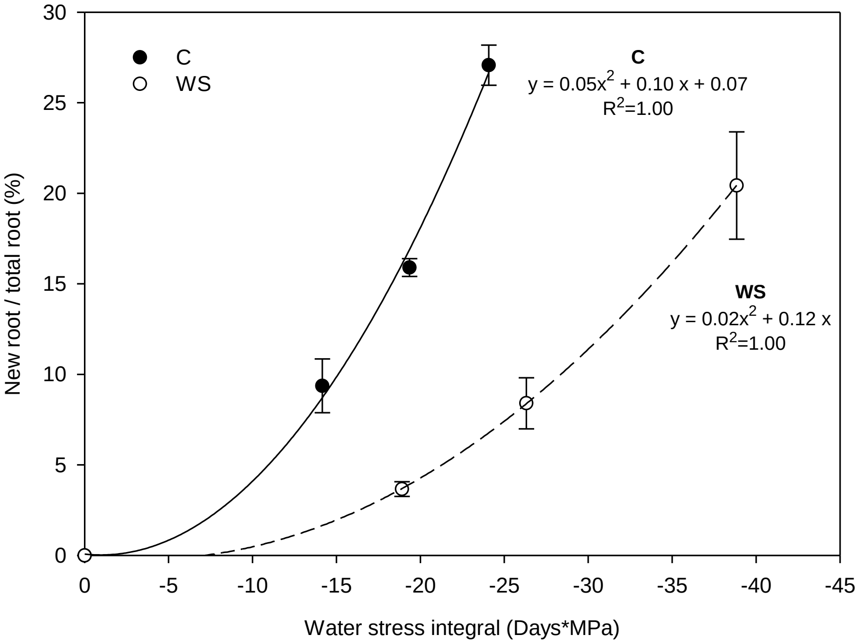

Figure 6. Proportion of new root to total root weight in response to the cumulative water stress integral of vines under the control (C) and water stress (WS) treatments. The values represent means for five vines per treatment on the August 25, September 20, October 4 and October 24 sampling dates. Bars indicate the standard error of the mean. Equations represent the polynomial adjustment of the measures.

Discussion

One of the most noticeable effects of water stress during post-harvest was the rapid reduction of leaf area through defoliation, with plants accelerating the normal process of leaf senescence apparently to compensate for unfavourable water status conditions (Figure 3). During post-harvest, leaf functioning is considered to play an important role in carbohydrate assimilation until leaf senescence (Loescher et al. 1990, Köse and Ates 2017). In spite of the imposed water stress in our study, the total amount of assimilated carbon per leaf surface area was similar for both treatments throughout the experiment ($\int A_{n\ leaf\ WS} / \int A_{n\ leaf\ C} = 1$)

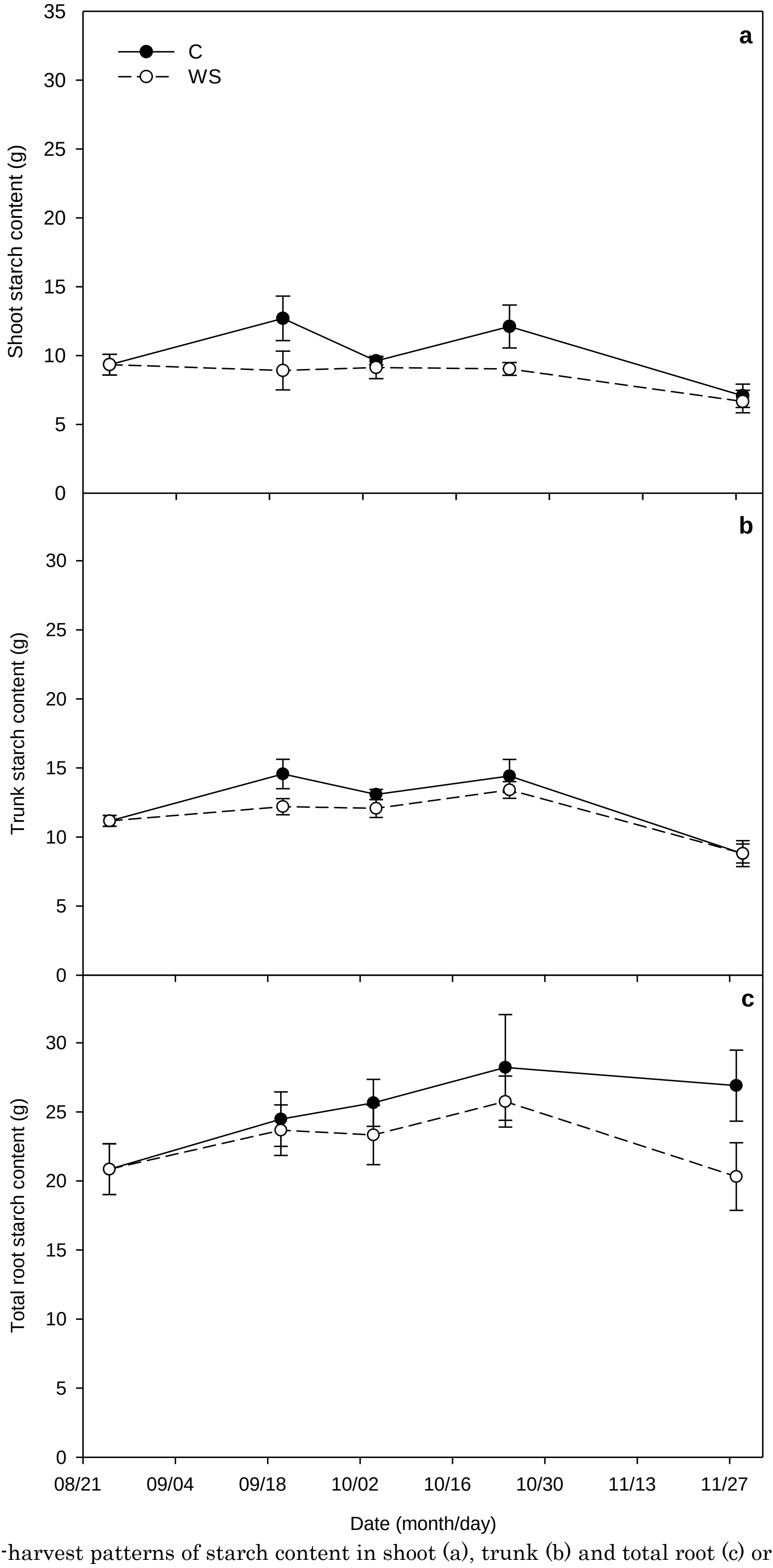

Figure 7. Post-harvest patterns of starch content in shoot (a), trunk (b) and total root (c) organs under control (C) and water stress (WS) treatments. The values represent the means for five vines per treatment and the bars indicate the standard error. There were no significant differences between irrigation treatments for the same sampling date ($P<0.05$).

(Figure 4). However, the leaf biomass in WS vines was considerably reduced in comparison with C vines, due to defoliation (Figure 3), which it could have allowed a lower carbohydrate assimilation. The differences between the treatments with A_n were smaller than expected, because the remaining WS leaves demonstrated greater photosynthetic activity than the C vines (Figure 4). In the C vines, the photosynthetic rate was similar to that reported in other studies (Sauvignon blanc, 5 – 11 µmol CO_2 m^{-2} s^{-1}) (Greven et al. 2016). Abiotic factors such as temperature, light and/or water are known to affect the photosynthetic capacity of vine leaves (Escalona et al. 1999). In the present experiment, however, the main factor responsible for the differences between treatments was the different leaf area (Figure 3). During the stress period, the remaining leaves on the WS vines had a greater A_n, which partially compensated for the impact of the reduction in leaf area attributable to water stress (Figure 4). This compensation may have been the reason why the starch content presented no clear treatment effects (Table 1, Figure 7).

No significant differences were found between the treatments in terms of shoot and trunk dry mass during the course of the experiment (Figure 5a and 5b). The figures 5a and 5b showed a hint of less biomass in the WS vine treatment, but the differences were not statistically significant. At the root level, however, new root formation took place in both treatments, but at different rates (Figure 6). The level of the initial stress imposed on WS on this study may be high enough to restrict the growth rate of new roots and keep this below that of the C treatment, even during periods when the plant water status recovered (Figure 1 and Figure 6). This argument is supported by Figure 6, where is presented a comparison between the water stress integral and the growth of new roots. This was probably related to the reduction in leaf area after the initial stress was applied in WS (Figure 3); this may have limited the photosynthetic capacity of the vine.

Respiration processes necessary for growth and organ maintenance are affected by water stress and, as a consequence, starch content could be also influenced (Flexas et al. 2006, López et al. 2013). Previous studies of vines subjected to water stress conditions have shown differences in starch concentrations and contents (Holzapfel et al. 2010). However, despite the different root growth rates, no significant differences were found in total root biomass (Figure 5c) and the same was true for starch content (Figure 7c). But since the biomass already present in the roots probably was large enough to mask differences, no differences were expected in root biomass and starch content. Although no statistically differences were found on total root dry mass between treatments (Figure 5c), it should not be ignored the apparent differences in root dry mass among vine treatments. Furthermore, no differences between treatments were found in starch concentration (Table 1). The differences that were found were in shoot and trunk starch concentrations corresponding to the last series of measurements taken in the experiment (Table 1). The last measurement date also coincided with the lowest starch content (Figure 7a and 7b) (Greven et al. 2016). In previous studies, starch concentration was evaluated in perennial organs for several grapevine cultivars and locations. Reported trunk starch concentrations ranged from 4 to 14% (Bates et al. 2002, Zapata et al. 2004, Sadras and Moran 2013), and 10% for a Chardonnay cultivar in New Zealand (Bennett et al. 2005). The same studies of vine roots reported starch concentrations of 8 to 30%, and 13% for Chardonnay (in New Zealand). Our starch concentration values were similar to previous Chardonnay study (14% in trunk and 12% in roots, on average) (Table 1). This indicates that the level of water stress applied was not enough to influence the starch concentration in these organs. The differences between the concentrations in the shoots and trunk observed at the end of the experiment may have been related to the conversion of starch into other carbohydrates because no remobilization appeared to take place at the root level (starch values remaining constant, Table 1). The decrease on starch concentration in shoots and trunk after leaf fall (November 28, Table 1) could have been associated with the demand for carbohydrates for new root growth, maintaining organ respiration, or acclimatization of the grapevines to low temperatures. This is because increasing vine hardiness to winter conditions requires the conversion of starch into soluble sugars when temperatures fall below 5°C and the days become shorter in the middle of winter (Hamman et al. 1996, Keller 2010, Zufferey et al. 2012). These environmental conditions were similar to those corresponding to the last sampling measurements taken in the study (Figure 2).

Maintaining starch concentrations and root biomass in the WS treatment responded to a redistribution of carbohydrate assimilates on shoot and trunk reserves organs. Also, it responds to a conservative strategy for preserving carbohydrates in the form of starch to ensure carbon reserves for subsequent spring growth. Water stress is known to affect root growth in vines (Eapen et al. 2005, Comas et al. 2010, Maihemuti et al. 2016). The reduction in root growth induced by the water stress added to the lack of fruits in the post-harvest period reduced the potentially available sinks and favoured the allocation of new photosynthates towards reserves (Iniesta et al. 2009). Furthermore, pre-harvest reserve replenishment has been reported to begin during fruit maturation, when berry sugar accumulation slows; this means that reserve accumulation in roots could have started before and they may be replenished enough by harvest (Candolfi-Vasconcelos et al. 1994, Holzapfel et al. 2006, Rossouw et al. 2017). If this is so, even though the WS vines were stressed, they may have still been able to keep similar concentrations of reserves as the well-watered plants and this would have allowed them to regrow in the next season. This would imply that vines either have a conservation strategy that allows them to maintain carbohydrates in the form of starch, which gives priority to the survival of permanent structures over any increase in vine size (Greven et al. 2016). According to Greven et al. (2016), the carbohydrate dynamics related to storage in reserve organs suggests that the trunk may serve as a relevant, albeit transitional, reserve storage organ between the root system and the rest of the vine, and as the most accessible storage sink. Roots, on the other hand, are widely considered to be the most important storage reserve organs in vines (Scholefield et al. 1978, Loescher et al. 1990, Bates et al. 2002).

Container do not adequately recreate and represent field conditions (Bravdo 2005), because they prevent roots from spreading as widely as they would in soil conditions (Zapata et al. 2001). Furthermore, different stomatal closure processes could be involved with vines grown in soil, such as abscisic acid signalling (Vandeleur et al. 2009). This experiment may point out that water stress during st-harvest “forces” allocation of assimilates towards reserves, thereby allowing plants to maintain their reserves for the following season.

The early defoliation of vines, after several consecutive years under warm conditions and water limitations, has been reported to influence carbohydrate reserves. As a result in the subsequent seasons, it occurred the reductions in yields and poorer vegetative growth, because new growth is dependent on pre-existing reserves (Vaillant-Gaveau et al. 2014,Greven et al. 2016, Köse and Ates 2017). As it has showed on this work, the most significant effect evaluated due to water stress was the reduction of fine root growth, which it may hinder the vegetative growth on the following season. Recent research related to climate change within the same study area has reported reductions in annual precipitation, in both autumn and winter, and an increase in temperatures, especially during summer (Gonçalves et al. 2014). Which it coincided according with the environmental conditions in which the work was tried to be carried out. Moreover, phenological shifts associated with increasing temperatures have been reported in several wine-growing regions (Jones and Davis 2000, Duchêne et al. 2010, Petrie and Sadras 2008). One of the main possible consequences of this shifts may be the enlargement of the post-harvest, its occurrence into warmer conditions and the increase of the irrigation water requirements (Hall et al. 2016). It is substantial to consider the possible interactions in grapevine behaviour, taking into consideration changes in environmental conditions, shifts in phenological events and carbon balances and partitioning (Ollat and Touzard 2014).

Conclusions

The main effect of water stress on grapevines during the post-harvest period was the defoliation of the vines and reduction in their total leaf area. Supplying full water demands until leaf fall permitted the maintenance of photosynthetic leaf area and consequently a higher level of carbohydrate accumulation, along with the formation of new roots which are responsible for high water and nutrient uptake. The lack of water caused vines to respond with variations on biomass accumulation between above and below ground perennial organs, indicating a high response in carbon economy in order to favour the survival of the permanent structures rather than total increases in vine size. Moreover, water stress did not influence the main storage organ, the roots, keeping the biomass and starch concentrations.

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Chapter IV

Post-harvest regulated deficit irrigation in Chardonnay did not reduce yield but at long-term, it could affect berry composition

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Abstract

Future increases in temperatures are expected to advance grapevine phenology and shift ripening to warmer months, leaving a longer post-harvest period with warmer temperatures. Accumulation of carbohydrates occurs during post-harvest and has an influence on vegetative growth and yield in the following growing season. This study addressed the possibility of adopting regulated deficit irrigation (RDI) during post-harvest in Chardonnay. Four irrigation treatments during post-harvest were applied over three consecutive seasons: (i) control (C), with full irrigation; (ii) low regulated deficit irrigation for sparkling base wine production (RDI_L SP), from harvest date of sparkling base wine, irrigation when stem water potential (Ψ_{stem}) was less than -0.9 MPa; (iii) mild regulated deficit irrigation for sparkling base wine production (RDI_M SP), from harvest date of sparkling base wine, irrigation when Ψ_{stem} was less than -1.25 MPa; (iv) mild regulated deficit irrigation for wine production (RDI_M W), from harvest data of wine, irrigation when Ψ_{stem} was less than -1.25 MPa. Root starch concentration in full irrigation was higher than under RDI. Yield parameters did not differ between treatments, but differences in berry composition were detected. Considering that the desirable berry composition attributes of white varieties are high titratable acidity, it would seem inappropriate to adopt RDI strategy during post-harvest. However, in a scenario of water restriction, it may be considered because there was less impact on yield and berry composition than if RDI had been adopted during pre-harvest.

Key words: Regulated deficit irrigation, root reserves, soluble solids concentration, starch concentration, titratable acidity, viticulture

Introduction

Climate change projections for the Mediterranean region indicate a pronounced warming, which would lead to a greater occurrence of higher temperatures, more frequent drought events and a decrease in precipitation, particularly during the warm season (IPCC 2013, Giorgi and Lionello 2008). Water is probably the most vulnerable resource in the region, but in viticulture it is essential to guarantee stable grape yields and composition (Chaves et al. 2007, Costa et al. 2016). In the Mediterranean region, growing wine grapes is one of the most important crops, occupying approximately 2,768,000 hectares of farmland (OIV 2017). In recent decades, the area of irrigated vineyards has notably increased (FAO, 2016), largely due to vine-growers' concerns about the negative effects of water deficits on yield and berry composition (Cancela et al. 2016), but also because of an increase in the total irrigated area. As a result, we now have a scenario in which water demand is likely to increase while water supplies will probably shrink. It would seem reasonable to think that demand and supply can only be brought into a sustainable balance by implementing the sustainable management of water resources, focusing on conserving water and using it more efficiently for irrigation.

In wine grapes, the adoption of regulated deficit irrigation (RDI) has been widely recognized as a water-saving technique that is effective for both controlling vine vegetative growth and improving berry composition (Williams and Matthews 1990, Chaves et al. 2007). The timing, intensity and also optimal period during the growing season at which to apply water stress have all been widely studied for different grape varieties (Bravdo et al. 1985, Cooley et al. 2017). Most of these studies have demonstrated benefits for berry composition and wine quality, though this has only been achieved in red cultivars, as Cabernet Sauvignon, Shiraz and Tempranillo (Ojeda et al. 2002, Girona et al. 2009). There is still a lack of, and much less definitive, information about the effects of adopting different RDI strategies with white cultivars. However, some recent studies involving cv. Chardonnay (*Vitis vinifera* L.) demonstrated that applying water stress during post-veraison had a negative effect on certain of the sensory attributes of the resulting wines (Reynolds et al. 2007, Basile et al. 2012). Among white varieties, Chardonnay is one of the most commonly used cultivars for producing sparkling wines (Andrés-Lacueva et al. 1996). The most appreciated organoleptic characteristics that enologists look for in base wines destined to produce sparkling wine are the presence of high levels of titratable acidity (TA), a low pH, and a low soluble solids concentration (SSC), all of which are enhanced by adopting full irrigation strategies (Jones et al. 2014, Bellvert et al. 2016). The main concern related to irrigating at full potential is the huge amount of water consumed over a complete growing season; in the Mediterranean region, this has been estimated to range from 350 to 550 mm (Ramos and Martínez-Casasnovas 2010, Bellvert et al. 2016). This is likely to be even more critical in a scenario of water shortage or when water restrictions are imposed by local irrigation managers. Such situations often occur at the end of the growing season and affect the post-veraison and post-harvest stages.

In addition, we must consider that the expected increase in temperatures will also advance grape phenology; this may result in an advance of the ripening period to the warmest months of the year (Webb et al. 2007, Petrie and Sadras 2008, Ramos et al. 2018). As a consequence, post-harvest periods will be longer and will also coincide with warmer temperatures. Irrigation efficiency during the post-harvest stage has often been overlooked, but in a climate change scenario, it may merit more attention. Water applied during this stage accounts for ~26% of total annual crop evapotranspiration (Bellvert et al. 2016). Although it may be appropriate to adopt deficit irrigation strategies, knowledge concerning its possible implications for the following growing season is still currently scant. In addition, the post-harvest stage is particularly important for storage reserve accumulation. It sustains the mobilization of accumulated carbohydrates for new vegetative growth in the subsequent growing season, until photosynthesis once more becomes the main source of carbon (Zapata et al. 2004, Holzapel et al. 2006). After harvest, carbon assimilation is possible while vines still retain functional leaves and through until leaf fall. A loss of leaf area during this period, either due to water stress or defoliation, may therefore affect vegetative growth and yield in the following season (Bennet et al. 2005, Vaillant-Gaveau et al. 2014). To the best of our knowledge, there has so far been no study conducted with white wine grape cultivars that has evaluated the carry over effects of adopting different RDI strategies during the post-harvest period into subsequent seasons.

The current study was based on the assumption that post-harvest RDI can be used as an appropriate water-saving irrigation strategy and that, if properly applied, this may prevent any negative effects on yield and berry composition during the subsequent growing season. The aim of this research was therefore to determine whether adopting different irrigation strategies during the post-harvest stage could influence carbohydrate accumulation in the roots of Chardonnay wine grapes. We also sought to study the influence of RDI on the physiological, yield response and berry composition attributes of wine grapes.

Materials and methods

Study site and plant material

The study was carried out during the 2013 - 2016 growing seasons, at a 13-year-old Chardonnay commercial vineyard, located in Raïmat (41°39'50" N – 0°30'27" E), Lleida (Catalonia, Spain). The vines were grafted onto SO4 rootstock and planted with a 3.0 m x 2.0 m spacing and a north-south row orientation. The soil texture was loam and the effective soil depth was ~60 to 120 cm. The canopy system was trained using vertical shoot positioning (VSP), with a bilateral, spur-pruned cordon located 1.0 m aboveground. Winter pruning left 10 to 15 spurs on each vine. The soil had a loamy texture, with an effective soil depth of between 0.6 and 1.2 m. The local climate was Mediterranean, with an average annual rainfall and reference evapotranspiration (ET_O) of 341 mm and 1,060 mm, respectively. Disease control and nutrition vine management were conducted following the wine grape production protocol of the 'Costers del Segre' Denomination of Origin (Catalonia, Spain).

Experimental conditions

The average air temperature (T_a) and the evaporative water demand (ET_O) during the vegetative growing period were similar from year to year (Table 1). There were, however, significant differences during the post-harvest stage, and particularly in 2014. This was probably due to rainfall of 172.8 mm in the late-summer of 2014 and a resulting higher ET_O.

Table 1 Reference values for average air temperature (T_a), evapotranspiration (ET_O) and rainfall for the different phenological stages during the experiment.

Year / weather variables	*Phenological stage period*					
	Budbreak to harvest			Post-harvest		
	T_a (ºC)	ET_o (mm)	Rainfall (mm)	T_a (ºC)	ET_o (mm)	Rainfall (mm)
2013	-	-	-	18.7	175.7	12.5
2014	17.9	615.9	102.3	20.6	274.5	172.8
2015	20.1	664.0	89.7	19.6	200.1	26.6
2016	17.4	670.8	138.2	-	-	-

Irrigation treatments

Irrigation was applied on a daily basis, using a drip irrigation system with two pressure-compensating emitters, which provided 2.3 L/ha per vine, positioned at regular intervals along the pipe. The vines were irrigated early in the morning, using an individual controller to open and close the solenoid valves in each experimental unit. Meteorological data were gathered from an automated weather station belonging to the Catalonia's official network of meteorological stations (SMC, www.ruralcat.net/web/guest/agrometeo.estacions); this was located 1 km from the study site. Weekly irrigation was scheduled following the water balance method described by Allen et al. (1998). Crop evapotranspiration (ET_C) was calculated using the ET_O Penman-Monteith (Allen et al. 1998) and we used crop coefficients (K_C) obtained from previous experiments conducted in the same vineyard (Bellvert et al. 2016).

The irrigation treatments applied were: (i) *control* (C), irrigation at full crop evapotranspiration ($ET_C = 100\%$) throughout the growing season. The berries were then used to produce sparkling base wine; (ii) *low regulated deficit irrigation for sparkling base wine production* (RDI_L SP), full irrigation until harvest. During the post-harvest stage, weekly irrigation was applied at different percentages of ET_C in order to maintain the midday stem water potential (Ψ_{stem}) at -0.9 MPa. The berries were used to produce sparkling base wine; (iii) *mild regulated deficit irrigation for sparkling base wine production* (RDI_M SP), with full irrigation until harvest. During the post-harvest stage, irrigation was reduced applying different percentages of ET_C until Ψ_{stem} reached -1.25 MPa. The berries were used to produce sparkling base wine; and (iv) *mild regulated deficit irrigation for wine production* (RDI_M W), with full irrigation until harvest. During the post-harvest stage, it was applied weekly irrigation at different percentages of ET_C in order to maintain Ψ_{stem} at -1.25 MPa. The berries were used to produce wine. Percentages of ET_C were decided according to the difference between actual measured Ψ_{stem} and the target Ψ_{stem}, and ranged between 1.3 to 12.4% for RDI_L SP, 1.0 to 43.2% for RDI_M SP, and 0.2 to 42.2% for RDI_M W.

Experimental design

The experimental design consisted of a randomized complete block with four block replicates. Each block contained four experimental plots with four rows of eight vines per row. Measurements were taken from the twelve central vines (six in each row) in the two rows in the middle of each plot, while the others acted as guard vines.

Measurements

Water applied and vine water status

Each day, the volume of water applied was measured and recorded for each plot, using digital water meters (CZ2000-3M; Contazara, Zaragoza, Spain). The midday stem water potential (Ψ_{stem}) was measured on a weekly basis from April to natural leaf fall. Measurements were taken within one hour of solar noon. Two shaded leaves per experimental plot were selected and wrapped in aluminium foil bags one hour before the measurements were taken. The measurements were acquired in less than one hour, using a pressure chamber (plant water status console, model 3500; Soil moisture Equipment Corp., Santa Barbara, CA), and following the protocol established by Shackel et al. (1997). The integrated stem water potential was calculated with Ψ_{stem} readings for successive dates, as described in Basile et al. (2011), during the 2015 post-harvest period.

Leaf net CO_2 assimilation rate, stomatal conductance and transpiration measurements

Leaf net CO_2 assimilation rate (A_n) (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mmol H_2O m^{-2} s^{-1}), and transpiration (T) (mmol H_2O m^{-2} s^{-1}) were determined on a biweekly basis during the post-harvest stage of the 2015 growing season. Measurements were taken, at midday, from five vines per treatment, with one leaf being measured on each vine. The leaf net CO_2 assimilation rate was obtained using an infrared gas analyser (model LCi; ADC BioScientific Ltd., Hoddesdon, Herts, UK). A portion of the leaf was placed in the chamber and data were taken after 45 s of operation, once the A_n reading had stabilized. Stomatal conductance and transpiration were measured with a steady-state porometer, under light-saturated conditions (ModelLi-1600, Li-Cor, Lincoln, Nebraska, USA).

Vine measurements

Once a week, a visual inspection of the vines was performed to determine the phenological stage throughout the whole experiment. The vine phases were recorded when 50% of the shoots on the vines observed presented a certain development stage as recognised according to the BBCH scale (Lorenz et al. 1995). The shoots and inflorescences on each vine and on all of the plots were counted at the onset of the vegetative period.

The canopy intercepted photosynthetically active radiation (f_{IR}) was measured at 11:00 a.m. ± 30 min, using a ceptometer (linear probe length 80 cm; Accupar Linear PAR, Decagon Devices, Inc., Pullman, WA, USA). Measurements were taken throughout the vegetative growing period until leaf fall on five vines per experimental plot, on a biweekly basis. The ceptometer was placed in a horizontal position at ground level and perpendicular to the vines. To cover the vine spacing, five equally spaced measurements were taken on the shaded side of each vine. The incident radiation above the canopy was determined by taking two more measurements in an open space adjacent to each vine. Daily f_{IR} (f_{IRd}) was calculated by using an hourly model of light interception (Oyarzun et al. 2007). The Oyarzun's model was used to estimate the canopy porosity parameter so that the simulated value for the amount of hourly intercepted light at noon equalled the instantaneous value measured in the field. Vine structural parameters such as vine height, and canopy width perpendicular to the row were also measured. The f_{IRd} was then calculated by integrating the diurnal course of the simulated f_{IR}.

The canopy surface area (*SA*) (m^2) of the vines was determined on a biweekly basis throughout the growing season. Measurements of vine canopy height and width were made using a ruler, while the length was considered to be 1 m in all cases. These measurements were conducted in the middle vine in the C, $\mathrm{RDI_M}$ SP and $\mathrm{RDI_M}$ W treatments. Canopy surface area was determined as described by Smart (1985).

Starch concentration

The starch concentration in the vine roots was determined during the winter dormancy period, in December 2015, when the mobilization of reserves was negligible (Sauter and van Cleve 1994). In all of the treatments, two 10 g samples per plot were taken from roots with diameters of > 2 mm; these were extracted from near the trunk, at a soil depth of 0.2 to 0.3 m. The extracted roots were then washed, immersed in liquid nitrogen, and dried at 65°C in a forced-air oven. Once the dry weight was constant, the samples were ground up, using a hand mill (M20; IKA-WERKE, Staufen, Germany). Root starch was then hydrolyzed with acid and subjected to enzyme hydrolysis; this was followed by a spectrophotometric determination of the powdered samples (Boehringer 1984).

Fruit growth

From pea-sized berries (in around June) until harvest, a sample of 12 berries (one berry per vine) was collected from each elemental plot. It were recorded the average berry fresh weight, and berry dry weight, after the berries were dried to a constant weight in a forced-air oven (at 60ºC to prevent the caramelization of their sugar).

Harvest

The harvest was carried out manually and the following yield parameters were measured: total vine yield weight; number of clusters per vine; cluster fresh weight, which was estimated by dividing the total yield by the number of clusters per vine; and the number of berries per cluster, which was estimated by dividing the cluster fresh weight by the mean berry fresh weight.

The soluble solids concentration (SSC) in the berries was used to establish a standard reference for harvesting. The plots were individually harvested once the SSC levels had reached the pre-defined thresholds established by the Raïmat winery for producing either the sparkling base wine and for wine. The pre-defined SSC thresholds established for harvesting were as follows: in 2013, all the treatments were harvested at the same time (day of the year, DOY 239), based on an SSC threshold of 16.8ºBrix; in 2014, the pre-defined thresholds for harvesting were 17.5 and 21.7ºBrix, respectively, for the vines destined for the production of the sparkling base wine (DOY 216) and of wine (DOY 230); in 2015, 17.5 and 21.5ºBrix, respectively, were the values chosen for the vines destined for the production of the sparkling base wines (DOY 215) and of wine (DOY 231). In those years, C was harvested according to the same criteria as the sparkling base wine. Finally, in 2016, the grapes for all the treatments were harvested at the same time according to the criteria for producing sparkling base wines (DOY 224), with the aim to be able to compare all the treatments among them. The moment of harvest for all of the treatments was defined when the SSC of C reached 16.0ºBrix. In 2016, the evolution of the SSC and titratable acidity (TA) in the berries were also tracked from approximately two weeks before harvest and throughout the post-harvest stage (in the case of berries sampled from the guard vines), with six berries being sampled from each plot, every two to three days. At harvest, however, a sample of ten representative entire clusters was analysed from each plot. The SSC was measured using a refractometer (Palette PR-32α; ATAGO, Tokyo, Japan), and the TA was measured from a solution of NaOH until a pH of 8.2.

Analysis of statistical data

The significance of the differences between the irrigation treatments was evaluated using a one-way ANOVA followed by a Tukey's test, as a post hoc test for separation of means. The statistical analysis was performed using the R software (R Core Team, 2017) (R version 3.2.4 revised) and the statistical significance was established at $P \leq 0.05$.

Results

Water applied and vine water status

The average amount of irrigation water applied until harvest over the three growing seasons was 343 mm. During the post-harvest stage, the average amounts of water applied were 107, 67, 16 and 52 mm, respectively, for the C, $\mathrm{RDI_L}$ SP, $\mathrm{RDI_M}$ SP and $\mathrm{RDI_M}$ W treatments (Figure 1A-C). The amount of water applied over the three experimental years differed significantly among treatments. When we only considered the post-harvest stage, the water savings achieved with respect to the C treatment were 37, 85 and 51%, respectively, for the $\mathrm{RDI_L}$ SP, $\mathrm{RDI_M}$ SP and $\mathrm{RDI_M}$ W treatments. On the other hand, when we considered the water savings for the whole growing season, these were 9, 20 and 12%, respectively, for $\mathrm{RDI_L}$ SP, $\mathrm{RDI_M}$ SP and $\mathrm{RDI_M}$ W treatments.

From budbreak to harvest, all the treatments were fully irrigated throughout the three consecutive growing seasons and Ψ_{stem}, which ranged from -0.3 to 0.6 MPa, did not show any significant differences between them (data not shown). Figure 1D-F shows the seasonal pattern of Ψ_{stem} for all the treatments during the post-harvest stage. In 2013, there were significant differences between all the treatments except $\mathrm{RDI_M}$ SP and $\mathrm{RDI_M}$ W (Figure 1D). These two treatments followed the same trend, with their Ψ_{stem} starting to decrease just after harvest until it reached the pre-defined Ψ_{stem} threshold of -1.25 MPa. The Ψ_{stem} of $\mathrm{RDI_L}$ SP also declined just before harvest until reaching the pre-defined threshold of -0.9 MPa. The C treatment had the highest Ψ_{stem} values, which ranged from -0.4 to -0.8 MPa.

In 2014, however, the differences between treatments were not as clear, no doubt due to a series of rainfall events during the months of September and October (Figure 1B, 1E). The maximum differences in Ψ_{stem} occurred just after harvest and in early September and were attributable to differences in the harvest dates of the treatments applied to the vines destined to produce wine and sparkling base wine. The latter ($\mathrm{RDI_L}$ SP and $\mathrm{RDI_M}$ SP) had lower Ψ_{stem} values, particularly during the early post-harvest stage, when they respectively achieved the pre-defined irrigation thresholds of -0.9 and -1.25 MPa. The Ψ_{stem} of both treatments then considerably recovered after the rainfall event of 26 mm in mid-September, with the $\mathrm{RDI_L}$ SP treatment reaching to similar values to the C treatment, while those for the $\mathrm{RDI_M}$ SP

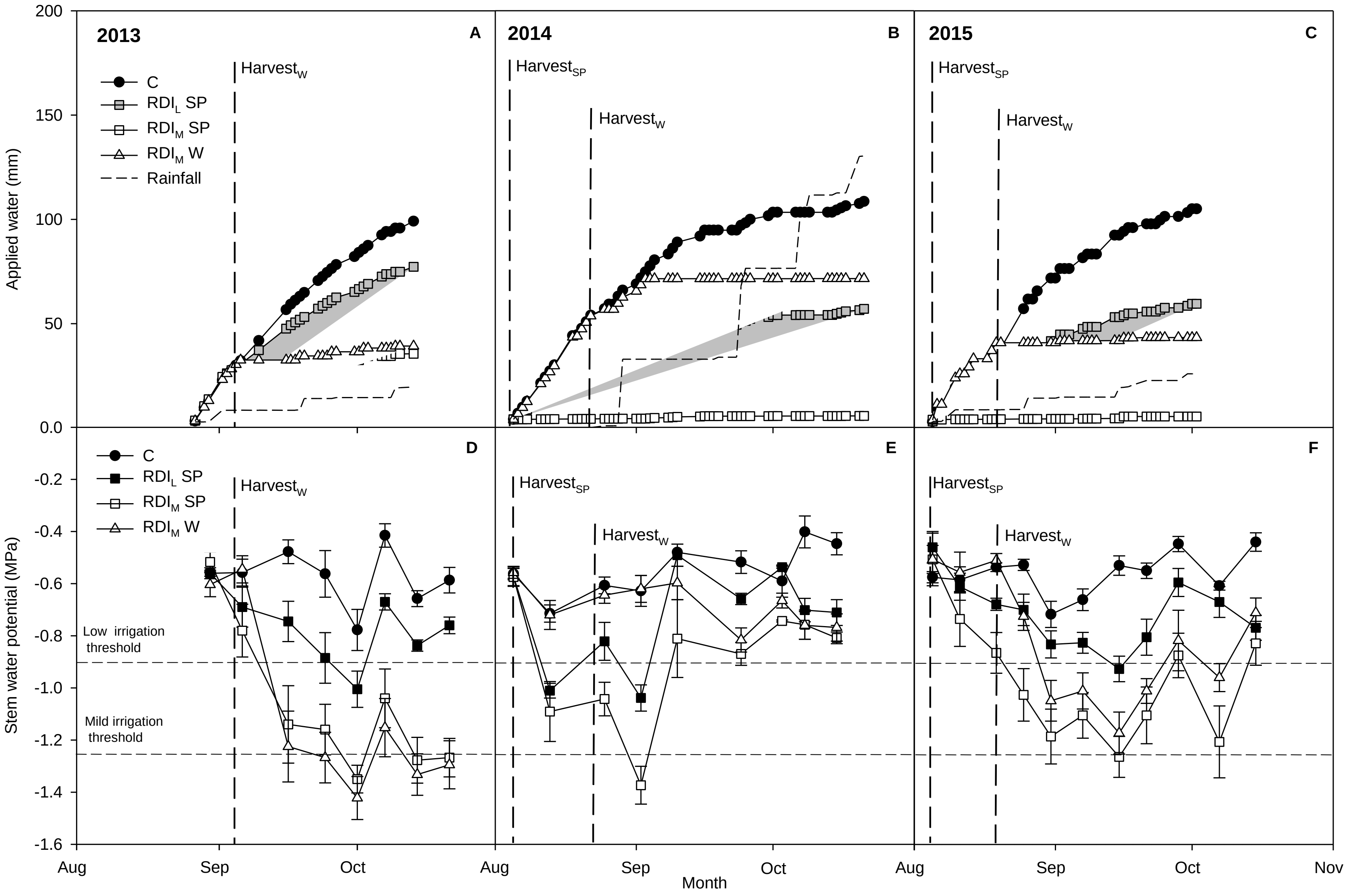
2013
A
2014
B
2015
C
Harvest$_W$
Harvest$_{SP}$
C
RDI$_L$ SP
RDI$_M$ SP
RDI$_M$ W
Rainfall
Applied water (mm)
200
150
100
50
0.0
D
E
F
Low irrigation threshold
Mild irrigation threshold
Stem water potential (MPa)
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
Aug
Sep
Oct
Nov
Month

Figure 1. Amount of applied water and rainfall corresponding to the different irrigation treatments during 2013 (A), 2014 (B) and 2015 (C). Seasonal variation in midday stem water potential (Ψ_{stem}) in response to different irrigation treatments during 2013 (D), 2014 (E) and 2015 (F). Irrigation treatments were C, control; RDI$_{L}$ SP, low regulated deficit irrigation for sparkling base wine production; RDI$_{M}$ SP, mild regulated deficit irrigation for sparkling base wine production; and RDI$_{M}$ W, mild regulated deficit irrigation for wine production. Stem water potential values are the mean values of the treatments, and bars indicate the standard error for eight leaves. Harvest$_{SP}$ and Harvest$_{W}$ indicate harvest time for sparkling base wine and wine criteria, respectively.

Table 2 Analysis of variance for the vegetative growth and yield parameters and their average estimates for the experimental period 2014-2016. The same analysis for measures of the starch concentration for the experimental year 2015.

		P > F							
		Vegetative growth		*Yield parameters*				*Root reserves*	
Source	DF	Shoots	Inflorescence	Kg/vine	Clusters/vine	Berries/cluster	Berry dry weight (g)	DF	Starch concentration (%)
Treatment (T)	3	0.3530[X]	0.6195	0.6536	0.0659	0.2899	0.9266	3	***
Block (B)	3	0.7590	0.2258	0.9575	0.4810	0.2992	0.9760	3	***
Year (Y)	1	***	0.0063**	***	***	***	0.00901**	-	-
	C	44.7	46.0	9.1	35	166	0.33	C	13.21 a[Y]
	RDI$_{L}$ SP	45.0	46.7	8.9	35	168	0.33	RDI$_{L}$ SP	10.13 b
	RDI$_{M}$ SP	43.6	45.7	8.8	33	168	0.34	RDI$_{M}$ SP	9.23 b
	RDI$_{M}$ W	42.8	44.7	8.7	33	161	0.34	RDI$_{M}$ W	10.07 b

[X] Significant codes: '***' P > 0.001, '**' P > 0.01, '*' P > 0.05

[Y] Different letters mean significant differences at P ≤ 0.05 using Tukey's honest significant difference test.

treatment remained at around -0.7 MPa. On the other hand, the minimum Ψ_{stem} values for the RDI$_M$ W treatment were -0.7 MPa, a level that was achieved 37 days after harvest.

In 2015, Ψ_{stem} revealed significant differences between treatments throughout the post-harvest stage (Figure 1F). In general, Ψ_{stem} decreased from harvest onwards, with the maximum differences between the treatments and the minimum values being -1.25, -1.15 and -0.9 MPa, respectively, for RDI$_M$ SP, RDI$_M$ W, and RDI$_L$ SP treatments. The midday stem water potential for C ranged from -0.4 to -0.7 MPa.

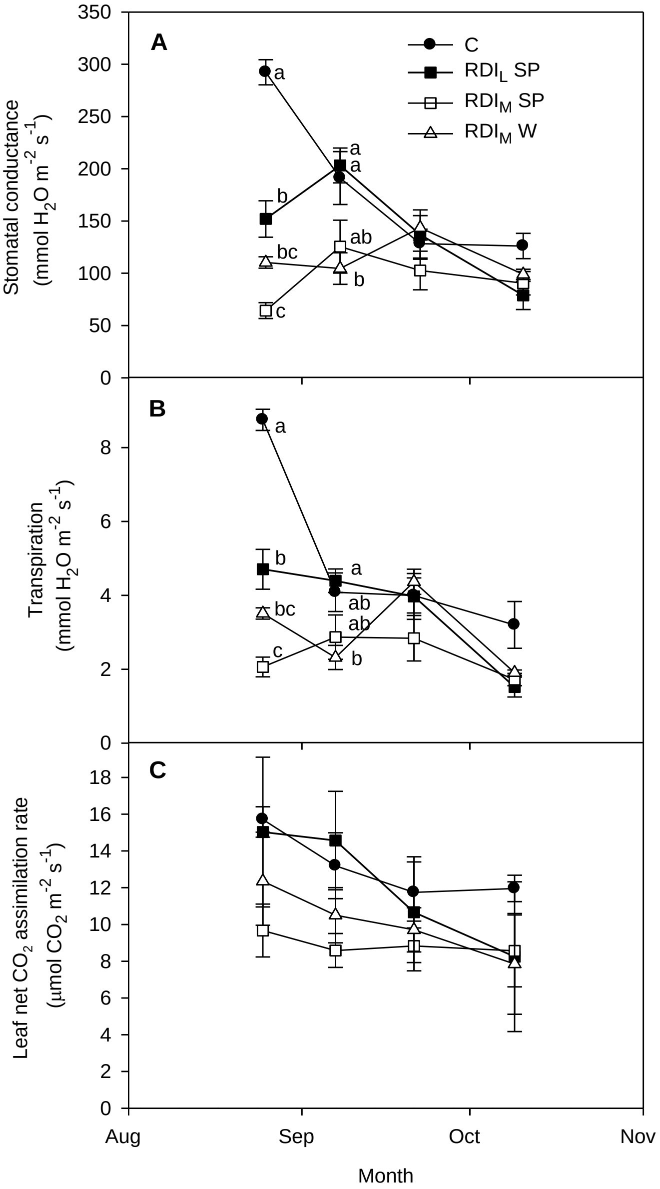

Figure 2 Seasonal variations in stomatal conductance (A), transpiration (B) and leaf net CO2 assimilation rate (C) during the post-harvest period in 2015. Each value represents the mean of eight measurements and bars indicate the standard error of the mean. Different letters indicate significant differences ($P \leq 0.05$).

Physiological measurements

The seasonal trends for g_s and T followed a similar pattern throughout the post-harvest stage (Figure 2A, 2B). The major differences between the treatments were seen during the early post-harvest stage, which pointed to lower g_s and T values in both $\mathrm{RDI_M}$ treatments. Then, as the vine phenology advanced, the measurements for all the treatments tended to become similar. The vines from the C treatment showed a pronounced decline in g_s and T, with these values respectively dropping from 292 to 126 $\mathrm{mmol \cdot H_2O\ m^{-2} \cdot s^{-1}}$, and from 8.8 to 3.2 $\mathrm{mmol \cdot H_2O\ m^{-2} \cdot s^{-1}}$ (Figure 2A, 2B). Similarly, the values for $\mathrm{RDI_L}$ SP also declined during the post-harvest period, although the initial measurements, which were taken just after harvest, were significantly lower than those for C. Although leaf net CO_2 assimilation rate (A_n) did not show any statistically significant differences between the treatments (Figure 2C), the tendencies did slightly vary. For instance, the measurements for $\mathrm{RDI_M}$ SP were slightly lower than those for the other treatments and they remained constant throughout the post-harvest stage, while those for the other treatments tended to decrease as the vine phenology advanced. Thus, the physiological measurements indicated that the water use efficiency (WUE), computed by the ratio A_n / T, was higher for the $\mathrm{RDI_M}$ treatments than for the C treatment (Figure 2B, 2C).

Vegetative growth, yield parameters and starch concentration

In 2016, visual field observations indicated that the date of budbreak differed between irrigation treatments, with the vines from the RDIM treatment being more than seven to ten days more advanced than those in the C treatment.

The ANOVA analysis indicated that adopting different deficit irrigation strategies during the post-harvest stage did not have any significant impact on measurements in the following season, either in relation to the number of shoots and inflorescences, or in vegetative growth and yield (Table 2, Figure 3 and 4). Even so, the source year for the ANOVA was significant for all the vegetative growth and yield parameters (Table 2). This year effect was exemplified by differences in the canopy management, which affected the number of shoots, inflorescences and clusters per vine; the average number of the latter were 29, 33 and 40, respectively for the years 2014, 2015 and 2016. In addition, despite the differences in the amount of water applied between one treatment and another, the ANOVA did not indicate any significant differences in yield parameters (Table 2). In fact, the seasonal evolution of f_{IRd} over the whole study period followed the same pattern for all the treatments through until harvest (Figure 3B, C and D). The f_{IRd} tended to increase throughout the season, reaching maximum values of from 0.48 to 0.52, just before harvest. On the other hand, the f_{IRd} remained lower during the 2016 pre-harvest period, due to more severe pruning (Figure 3D).

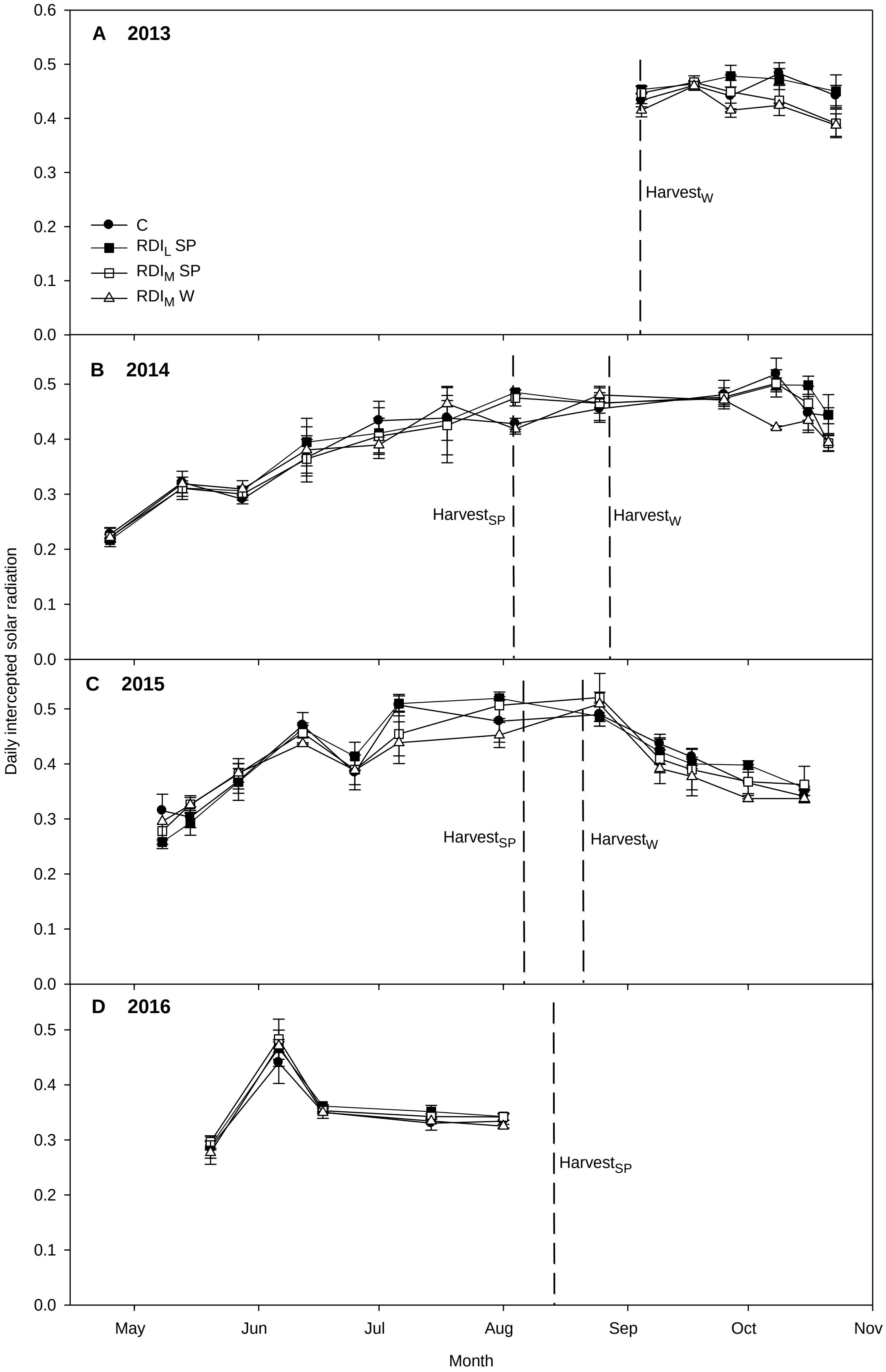

Figure 3 Seasonal variations in daily intercepted solar radiation (f_{IRd}) in response to different irrigation treatments in 2013 (A), 2014 (B), 2015 (C), 2016 (D). Each value is the mean of four measurements and bars indicate the standard error of the mean. No significant differences were found among the observations. HarvestSP and HarvestW indicate the harvest time according to sparkling base wine and wine criteria, respectively.

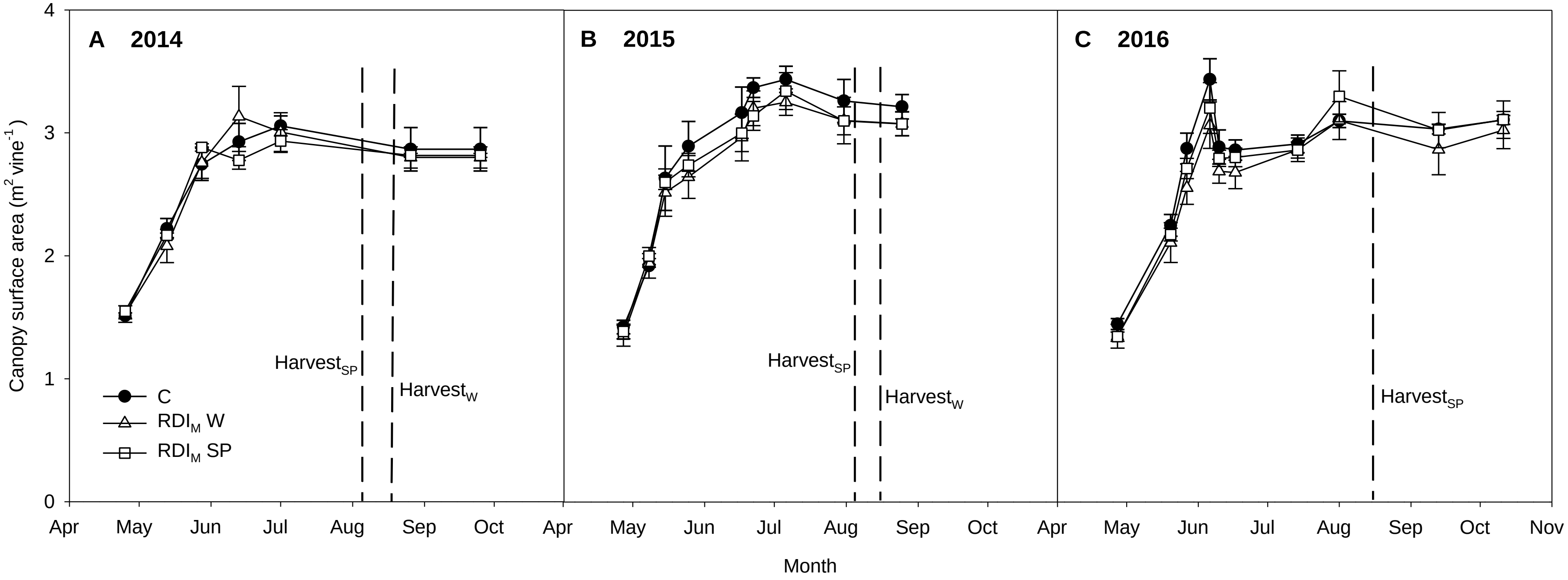

Figure 4 Seasonal variations in canopy surface area for the C, RDIM SP and RDIM W treatments for 2014 (A), 2015 (B) and 2016 (C). Each value is the mean of four measurements and the bars indicate the standard error of the mean. HarvestSP and HarvestW indicate the harvest time according to sparkling base wine and wine criteria, respectively.

Table 3 Mean values of the berry composition parameters for each irrigation treatment in the 2014, 2015 and 2016 growing seasons.

	Berry composition					
Year	2014		2015		2016	
Irrigation treatments	SSC (ºBrix)	TA (g/L tartaric acid)	SSC (ºBrix)	TA (g/L tartaric acid)	SSC (ºBrix)	TA (g/L tartaric acid)
C	17.6	9.7	17.7	11.8^{a}	15.9^{b}	10.9^{a}
RDI$_{L}$ SP	18.4	9.5	17.6	11.3^{ab}	15.9^{b}	10.8^{ab}
RDI$_{M}$ SP	17.2	9.3	17.9	11.1^{b}	16.4^{ab}	10.4^{ab}
RDI$_{M}$ W	21.4	6.7	21.1	8.5	17.0^{a}	9.9^{b}

In 2014 and 2015, vines were harvested on different dates depending on their planned uses (sparkling base wine or wine production). In 2016, the harvest was carried out at the same time and all destined to the production of sparkling wine. Quality parameters are SSC, meaning soluble solids concentration and TA, titratable acidity. Irrigation treatments were C, control; RDI$_{L}$ SP, low regulated deficit irrigation for sparkling base wine production; RDI$_{M}$ SP, mild regulated deficit irrigation for sparkling base wine production; and RDI$_{M}$ W, mild regulated deficit irrigation for wine production. Different letters mean significant differences at $P \leq 0.05$ using Tukey's honest significant difference test.

Although the statistical analysis did not show any significant differences between treatments during post-harvest, there was a noticeable trend which differentiated the treatments that had involved mild stress (RDI$_{M}$ SP and RDI$_{M}$ W) from the others (RDI$_{L}$ SP and C). It seems that the mild stress treatments caused an advance in leaf senescence, resulting in a reduction in the f_{Rd} (Figure 3).

These trend in vegetative growth difference seemed more evident when we analysed the canopy surface area (*SA*) (Figure 4). The seasonal pattern showed that the C vines had a slightly higher *SA* than those in the RDI$_{M}$ W treatment. These differences were more noticeable in 2015 and 2016.

The starch concentration in the root reserves at the end of the 2015 growing season was significantly higher in C than in the RDI treatments (Table 2). Although these differences were not sufficient to affect the yield parameters, the results did show that these treatments had accumulated smaller reserves due to the application of the RDI strategy over the three-year period.

Must and base wine composition

In 2014 and 2015, the harvest was carried out on DOY 216 and 215 for sparkling base wine, and DOY 230 and 231 for wine, respectively, based on the pre-defined soluble solids concentration (SSC) thresholds. The SSC levels for the treatments destined to produce sparkling base wine were not significantly different, while the treatment destined to produce wine had a higher SSC (Table 3). In 2014, the C and RDI treatments for sparkling base wine production had a similar titratable acidity (TA). In 2015, however, the C treatment had a slightly higher TA than the treatments that involved deficit irrigation. In 2016, all the treatments were harvested at the same time on DOY 224, when the total SSC in the C treatment had reached 16°Brix. It seems that those treatments that had been exposed to mild stress (RDI_M SP and RDI_M W) in the previous growing season had been able to synthesize more SSC than the others, while the TA was higher in treatment C than in the RDI treatments.

Discussion

Similar to our phenological observations of budbreak, previous works reported similar responses with studies carried out with wine grapes in which water stress during the post-harvest had affected the date of budbreak in the following growing season (Williams et al. 1991, Ndung'u et al. 1996). Ndung'u et al. (1997) reported that stressed vines had a readily available source of energy for ready for budbreak at the onset of the growing season. This was because the amount of sugar stored at the expense of starch was higher in the shoots, trunk and roots, which is the main reserve organs in vines (Ndung'u et al. 1997).

Despite these differences at the budbreak date, no variations in vegetative growth among treatments were detected. This was probably due to the management of the vine canopy throughout the season, which consisted of adopting a vertical shoot positioning (VSP) trellis system and topping the shoots in summer. This management probably helped to homogenize any slight differences that could have existed between treatments. Although the seasonal evolution of f_{IRd} showed a similar trend for all the treatments (Figure 3), we assume that the leaf area index (LAI) may differ in vines with a VSP system. In this system, vine shoots are trained upward in hedgerow, in order to maximise canopy light interception. The number of leaves and, as a result, the total leaf area per unit of ground surface, may vary despite them intercepting the same amount of light. Although we did not directly measure the LAI, Figure 4 trend to show differences in the canopy surface area (*SA*) between treatments. These differences could mostly be explained by canopy width; suggesting that the treatments that were submitted to RDI would have had slightly smaller *SA*s during the subsequent growing season.

The vine roots are the main storage organ for starch, which is, in turn, the primary reserve form for carbohydrates (Zapata et al. 2004). The two main flushes of root growth for wine grapes occur around bloom and after harvest (Mullins et al. 1992, Tomasi 2016). As a result, post-harvest is a critical period for the restoration of carbohydrates in storage tissues in order to sustain the vegetative growth of vines in the following season, and also to maintain yield levels (Bennett et al. 2005, Vaillant-Gaveau et al. 2014). Several studies have shown the links between the accumulation of carbohydrate reserves and yield parameters and berry weight (Candolfi-Vasconcelos et al. 1990, Sommer et al. 2000, Greven et al. 2016); this demonstrates that post-harvest defoliation and early leaf fall influence carbohydrate accumulation in vine roots. In our study, the fully-irrigated treatment (C) had the highest root starch concentration, which reached up to 13.21%. Other studies have reported similar values in the same cultivar (Bennett et al. 2005, Prats-Llinàs et al. 2019). In contrast, the starch concentrations for all the RDI treatments were significantly lower, averaging 9.81% (Table 2). This decline may be explained by lower carbohydrate assimilation, probably as a result of early defoliation caused by water stress (Figure 3). In addition, the minor carbohydrate accumulation in the reserve organs could, at least in part, be explained by a lower leaf net CO_2 assimilation rate (A_n) (Figure 2C). In fact, this study demonstrates that the starch concentration was significantly affected by water stress and that it exponentially decreased in line with the integrated midday stem water potential increment (Figure 5).

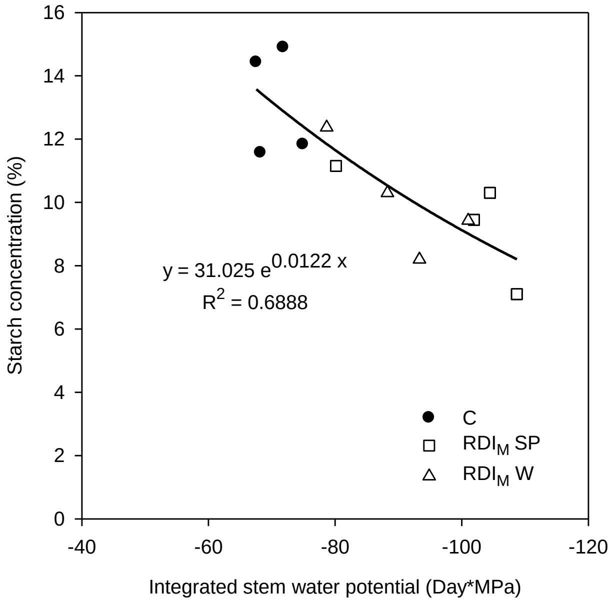

Figure 5 Relationship between the integrated stem water potential in 2015 and the root starch concentration determined at the end of the 2015 growing season.

Interestingly, and as we have previously reported, it seems that the greater ability to accumulate carbohydrates during the post-harvest period in the C treatment, these vines trend to have greater vegetative growth in the subsequent growing season (Table 2, Figure 4). Previous works on wine grapes, which involved defoliating vines during the post-harvest period, indicated a reduction in shoot growth in the next growing season due to a reduced capacity to accumulate carbohydrates during post-harvest (Holzapfel et al. 2006, Greven et al. 2016). Nevertheless, the same long-term studies also reported a decrease in yield due to a lower number of berries per cluster, as a result of the effects of cumulative water stress

(Holzapfel et al. 2006, Greven et al. 2016). In our study, however, apart from the slight differences in vegetative growth between the C and RDI$_M$ treatments, no carry-over effects were detected in yield parameters over the three growing seasons (Table 2). Other studies conducted in early maturing cultivars of Japanese plum (Samperio et al. 2015) and peach (Johnson et al. 1992, Dichio et al. 2007) also reported how, after the adoption of deficit irrigation during post-harvest for a period of three to five consecutive years, the yield parameters did not significantly differ among irrigation treatments. In plum, the adoption of deficit irrigation coincided with floral differentiation and it did not reduce fruit bearing. In peach, there was an increase in the appearance of double fruits, which was explained by the important influence of temperature during the carpel differentiation phase (Johnson et al. 1992). In wine grapes, the process of early flower differentiation mainly occurs during post-harvest (Mullins et al. 1992, Vasconcelos et al. 2009). It may therefore be suggest that, based on the results obtained in this study, it would seem that mild water stress during this period should not have negatively affected the development of flowers in the subsequent growing season. This suggests that in years of water scarcity, or for water saving purposes, the adoption of a moderate RDI strategy during post-harvest could be appropriate as it should not have any negative effects on yield parameters.

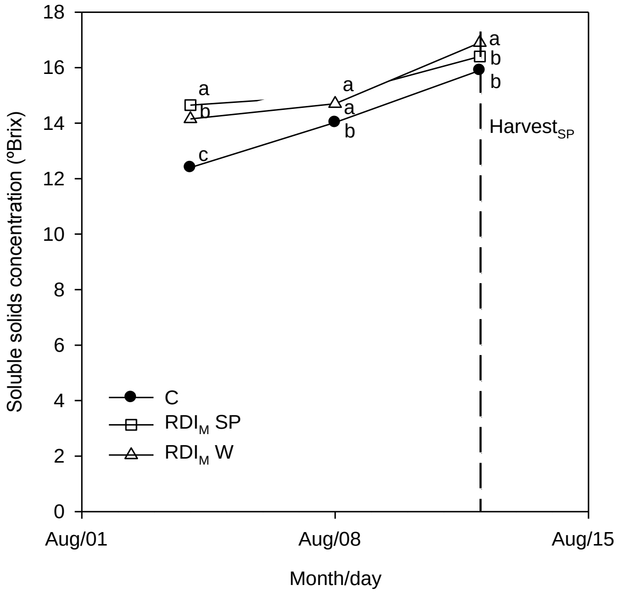

Figure 6 Evolution of soluble solids concentration (ºBrix) during the pre-harvest period in 2016. Different letters indicate significant differences ($P \leq 0.05$).

Among other factors, the synthesis of soluble solids in wine grapes during berry ripening mainly depends on leaf photosynthesis; it is attributable to the translocation of sucrose from the proximal leaves and, to a lesser extent, to its translocation from the storage organs (Mullins et al. 1992). Leaf photosynthesis is a function of the amount of light intercepted by the canopy and of the leaf water potential (Escalona et al. 2003). It has been widely studied that crop load affects berry composition (Bravdo et al. 1985, Reynolds et al. 1994, Uriarte et al. 2016). In our study, the synthesis of SCC in the berries of the RDI_M treatments was higher than in C (Table 3). However, none of the previously stated reasons could explain this as there were not statistically significant differences between the treatments. These differences may therefore be explained, in part, by the higher starch concentration registered in the C vines (Figure 6, Table 2). This effect was also observed by Greven et al. (2016), who reported that vines with lower root starch concentrations tended to have higher SSCs. Similarly, Ndung'u et al. (1996) observed that vines that were stressed during post-harvest had higher SSC levels. They, however, attributed these differences to vine canopy management and to the environmental conditions in which the vines had matured.

The influence of the starch concentration on the SSC/TA ratio is illustrated in detail in Figure 7. Although the irrigation schedules of all the plots belonging to the same treatment were conducted according to the same criteria, differences in soil spatial variability meant that there were some plots with slight differences in vegetative growth or water status. These differences affected the starch concentration and showed that for any determined irrigation treatment, vines with higher starch concentrations also tended to have higher SSC/TA ratios. In contrast, differences between irrigation treatments showed that for a given starch concentration, RDI_M vines had higher SSC/TA ratios than C vines. As previously reported, differences in the canopy surface area, and even slight phenological advances in RDI_M, may have been the reason for these significant differences (Figure 4, Table 2).

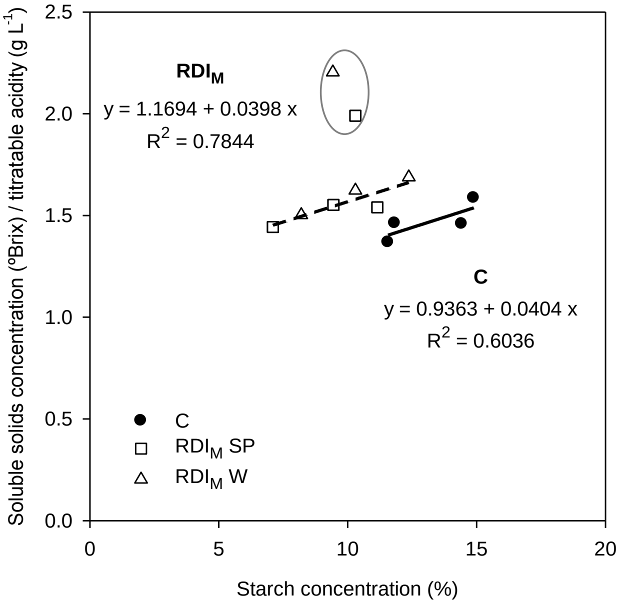

Figure 7 Relationship between the starch concentration in post-harvest 2015 and the soluble solids concentration by the titratable acidity in 2016. The observations inside the grey oval were outliers removed from the RDI_M linear regression.

Water stress during pre-harvest also influenced the SSC/TA ratio. On account of soil spatial variability, two of the RDI_M treatment plots tended to have lower Ψ_{stem} and as also lower f_{IRd} values throughout the growing season, which caused an increase in the SSC/TA ratio. Water stressed vines have a lower transpiration rate and, due to their lower evaporative cooling effect, this may contribute to increased losses in organic acids due to metabolism, resulting in grapes with a lower TA (de Souza et al. 2005, Bellvert et al. 2016).

There is general agreement among viticulturists and enologists that the most desirable organoleptic parameters for white varieties of grape destined for the production of sparkling wines is to have a low SSC/TA ratio (Andrés-Lacueva et al. 1996, Jones et al. 2014). In previous studies, it was demonstrated that deficit irrigation during pre-harvest contributed negatively to TA enhancement (Basile et al. 2012, Bellvert et al. 2016). The results obtained from the present study also suggest that adopting an RDI strategy during post-harvest may not be the most appropriate way to achieve that goal. The irrigation strategy recommended to enhance the berry composition attributes of white grape varieties is full irrigation throughout the growing season. However, in a scenario of water restrictions, the post-harvest period would probably also be the only time at which to reduce irrigation, since its impact on yield parameters and berry composition would be negligible and certainly much lower than during pre-harvest. Other canopy management strategies based on 'crop forcing' have been presented as techniques with which to fight climate change, aiming to shift periods of vine growth by delaying their initiation (Gu et al. 2012). At the same time, they can contribute to obtaining berries with higher TA values However, more research is needed in this direction in order to evaluate the long-term impact of these techniques on both yield and berry composition attributes.

Conclusions

In Chardonnay, it is recommended to conduct a full-irrigation strategy throughout the growing season. Significant differences in the accumulation of starch concentration in roots were detected between irrigation treatments, being C the treatment that tend to accumulate more reserves. Although the adoption of RDI during post-harvest did not negatively affect the yield parameters, it did reduce the vegetative canopy surface area of the vines and increased the SSC/TA ratio of the berries. For any given starch concentration, the SSC/TA ratio tends to increase as the water stress increases. The most desirable berry composition parameters for white varieties focus on enhancing the TA and reducing the SSC/TA ratio; this can be achieved through applying full-irrigation strategies. However, further research is needed to evaluate the long-term impact on yield and berry composition of applying conditions of sustained deficit irrigation during post-harvest.

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General discussion

General discussion

In irrigated vineyards, growers usually water vines until harvest, since yield and vine quality can be affected during this period. In view of advancing harvest dates, the longer duration of the period after harvest, and the tendency for warmer and drier autumns, the post-harvest period currently merits more attention. The efficiency of applying irrigation during this period is another theme that has so far been largely overlooked. For white grape cultivars, there are two matters that require particular attention: the need for models to predict seasonal variations in the different phenological stages; and how to manage vineyards during the post-harvest period, particularly if it is becoming longer and has fewer available resources. It is with this in mind that, in the present PhD thesis, we have presented phenological models to help predict the timing of the different phenological stages under high temperatures, to evaluate water stress during the post-harvest period and its impact on the vegetative and productive vine components in subsequent growing seasons.

Chapter I parameterised the Chill Overlap Model with the intention of studying how the Chardonnay cultivar performs under such a model and improving our understanding of the processes involved in the transition from dormancy to budbreak.

The Chill Overlap Model developed in this work did not improve budbreak predictions compared to previous simpler models (García de Cortázar-Atauri et al. 2009, Caffarra and Eccel 2010). The data used to develop the model referred to a wider geographic area of Chardonnay production than previous studies and this may partially explain its rather modest accuracy. The limited accuracy of the model developed could also be explained by variations in: phenological data sources; orchard management practices, which differ from one observation locations to another;, the microclimatic conditions at specific data collection sites; clonal variability; and distances between weather stations and the vineyards subject to observation (Williams et al. 1991, Martin and Dunn 2000, Luedeling et al. 2009, Trought et al. 2011).

Despite the wide range of climates involved and numerous potential sources of inaccuracy in the data sets used, this study was able to provide a new set of parameters for the Chill Overlap Model which have allowed us to analyse synergic interactions between accumulations of chill and the amount of heat required to trigger budbreak in Chardonnay grapevines. According to the model fit, a base temperature of less than 10°C would seem suitable for predicting budbreak; this is the base temperature commonly adopted as the threshold temperature for grapevines. Grapevines appear to have less need for chill than many other species, even during the compensatory stage between the chill and heat accumulation requirements. In contrast, despite its low chill demand, Chardonnay appeared to require more heat. This suggests that the grapevine could be a species for which additional heat is more effective than additional chill, once the minimum chilling requirements have been satisfied.

Further insight into the physiological behaviour of vines could be provided by adding endodormancy break dates to the model. This would provide a more precise estimate of the chilling requirements and permit an evaluation of the temperatures required for chill and heat perception by buds during dormancy. Having this information would help to improve the accuracy of the model and yield more robust projections of the phenological changes at work (Chuine et al. 2016).

More research is needed to improve the accuracy of current phenology models. It would, for example, be useful to be able to predict the potential impact of climate change and its implications relating to the suitability of using certain grapevine cultivars in particular growing regions. Although the Chill Overlap Model includes the overlapping effect of chill accumulation on subsequent heat accumulation, and this has been empirically observed in the field, it is necessary trying to improve the current model. This could be done by accounting for several of the, previously mentioned, potential sources of non-temperature-related factors that may be responsible for variability in the onset of budbreak. Further research is also needed to understand the environmental and vineyard management factors that influence the timing of budbreak in order to help improve the model and better understand the factors that influence the completion of dormancy in grapevines.

Chapter II developed a degree-day calculation approach for predicting the timing of the bloom, fruit set, veraison and berry maturity stages. This was done to evaluate the influence of environmental and physiological factors and responses to high temperatures on the development of these phenological stages.

The data set used to calibrate the degree-day approach for predicting the bloom, fruit set and veraison stages was obtained from a forced vine regrowth experiment. In this experiment, the annual timing and climatic time window within which these stages normally occur were altered by applying forcing treatments. As a result, such environmental factors as the photoperiod and temperature, which are signals for vine growth cessation and induce dormancy, were modified (Wake et al. 2000, Fennell et al. 2005). This made it possible to assess different phenological stages under the same, somewhat warmer, conditions. When modelling phenology, temperature is normally the main environmental factor taken into account during the calibration of phenological models. In this study, temperature was considered the main factor determining bloom, fruit set and veraison stages, while for berry maturity, we also considered interactions between high temperatures and solar radiation.

Bloom and veraison were the stages that presented the highest correlations with temperature. This is because vine development during this period involves active cell division, which is reflected by an exponential response of vine growth to temperature (Considine and Knox 1981, Rogiers et al. 2014). On the other hand, during berry maturity, major changes take place and, as well as taking temperature into account, several other factors must also be considered. During maturation, the strongest driver responsible for sugar accumulation in berries is the availability of resources, with photoassimilation becoming a limiting factor for berry growth as maturation advances (Williams et al. 1985, Sadras and Moran 2013). Other factors, including crop load, water availability and the source:sink ratio, also influence the rate of maturation (Williams et al. 1985, Petrie and Sadras 2008, Duchêne et al. 2010).

According to the degree-day approach that we developed, the base temperature with the best performance was 5°C, for all stages. This result was in line with the findings of previous studies, in which the development of phenological models under different climatic conditions suggested that the base temperature for grapevine cultivars is less than 10°C (Moncur et al. 1989, Nendel 2010, Molitor et al. 2013, Parker et al. 2011, Zapata et al. 2015). We also evaluated variations in response to temperature between different phenological stages and for different ranges of the upper temperature threshold. As a result, stage-dependent variations in upper temperature were developed based on observed decreases in thresholds. The highest upper temperature threshold value was associated with fruit set, and was 25.4°C, while the lowest was observed for veraison, and was 20.9°C. In a previous study with Chardonnay, it was reported that base temperature thresholds tended to increase over the growing cycle (Zapata et al. 2016). In this work, it was observed an increase in the base temperature threshold while the upper temperature remained the same (Zapata et al. 2016). The stage-dependent variations in each phenological phase were therefore evaluated in a similar way in both studies and as the phenological stages advanced, the possible range of degree-day accumulation declined.

According to the final use of the production, the prediction for berry maturity for producing a sparkling base wine could be obtained with an upper temperature threshold of 25.7°C±0.5°C, whereas one of 29.4±1.7°C would be more suitable for white wine production. This difference was due to the use of the relation with temperature and solar radiation (obtained by maximum temperature and radiation use efficiency measurements) in sparkling base wine production, which improved the accuracy of prediction. In contrast, the predictions for wine production did not work so well with this relation; this was probably because of the high level of variability in the source data.

Even so, the approach developed and presented in this thesis could prove useful for improving grapevine phenology models in scenarios involving warmer climatic conditions. These degree-day method predictions may be further improved by adding more variables. These could include: water availability and soil temperature, as they have been reported to be strong drivers for phenological development (Ramos and Martínez-Casasnovas 2010, Rogiers et al. 2014); maximum daily temperatures (Duchêne et al. 2010); or source:sink relations. The incorporation of high temperature thresholds, like the one obtained from the relation between maximum temperature and radiation use efficiency, into the calibration of phenology models, could also be crucial under warmer climatic conditions (Molitor et al. 2013).

There is increasing concern amongst wine growers about the influence of drought events on grape yields and berry composition. This is because these considerations will ultimately influence wine production attributes, as will irrigation management under such conditions. Until now, regulated deficit irrigation (RDI) strategies had been evaluated based on the phenological stages before harvest. Given that the post-harvest period may become an increasingly important stage for vine performance in subsequent growing seasons, **chapter III** and **chapter IV** of this thesis focus on evaluating the impact of water stress and irrigation efficiency on vines during this period.

In **chapter III**, we evaluated the influence of water stress on root growth and the accumulation of starch. At different dates during the post-harvest period, we assessed the dry mass of all the perennial structures of the vines. This allowed us to compare the relative contribution of the different vine organs to the restoration of carbohydrate reserves under both well-watered and water stress conditions.

Water stress reduced the total leaf area, as a result of defoliation, with the normal process of leaf senescence being accelerated, probably as a result of unfavourable water status conditions. Despite differences in leaf area, the remaining leaves on the water stress vines exhibited a greater rate of leaf net CO_2 assimilation, which partially offset the impact of the reduction in leaf area. This compensation may explain why the starch content did not vary between the stressed and control vines.

There was, however, a clear difference in the case of new root formation, with well-watered vines presenting significantly greater root growth formation. In contrast, no significant differences in growth were observed in shoot and trunk organs. Starch concentration values were similar to those reported in previous Chardonnay studies (Bates et al. 2002, Zapata et al. 2004, Sadras and Moran 2013). This indicates that the level of water stress applied was not sufficient to influence starch concentration in the reserve organs. Moreover, the starch concentrations in shoots and trunks were lower at the end of the experiment; this may have been related to the conversion of starch into other types of carbohydrates. Another reason for this decrease may have been associated with the demand for carbohydrates to produce new root growth, maintain organ respiration, and/or acclimatize the grapevines to low temperatures (Hamman et al. 1996, Keller 2010, Zufferey et al. 2012). On the other hand, there was no remobilization at the root level because there, starch concentrations remained unchanged. Maintaining the same starch concentrations in roots, irrespective of the stress level, may point to a conservative strategy for preserving carbohydrates in the form of starch in order to ensure carbon reserves for subsequent spring growth (Greven et al. 2016).

The early defoliation of vines, after several consecutive years subject to warm conditions and water limitations, has been reported to influence carbohydrate reserves. As a result, in subsequent seasons reduced yields and poorer vegetative growth have been observed, no doubt because new growth is dependent on pre-existing reserves (Vaillant-Gaveau et al. 2014, Greven et al. 2016, Köse and Ates 2017). In this study, and our conditions, we did not evaluate variations in carbohydrate reserves in response to water limitations. It could, however, be hypothesised that they could be depleted as the result of a cumulative effect on vine reserves during the post-harvest period.

Chapter IV evaluated the carry-over effects that regulated deficit irrigation (RDI) applied during the post-harvest period had on vegetative components, grape yield and berry composition attributes during the following growing seasons. Our work focused on the hypothesis that applying RDI in the post-harvest period could be used as a water saving strategy. This was based on previous studies that had assessed the negative impact on yield and berry composition when RDI was applied to Chardonny during the pre-harvest period (Basile et al. 2012, Bellvert et al. 2016).

As was hypothesized from the results obtained from the work described in **chapter III**, the most evident carry-over effect was the depletion of root reserves revealed by determining starch concentrations. Moreover, and as reported in previous studies, applying water stress advanced the date of budbreak in subsequent growing seasons (Williams et al. 1991, Ndung'u et al. 1996). Despite the differences between the RDI and control vines in terms of their starch concentrations, no significant differences in vegetative growth were observed in the following growing seasons. One of the reasons for this was porbably the uniform management of the vine canopy throughout the season; this may have helped to homogenize any slight differences between vine treatments. Nevertheless, there was a clear relation between the observed starch concentration and the intensity and duration with which water stress was applied during the post-harvest period. It would therefore seem that the greater ability of well-watered vines to accumulate carbohydrates during the post-harvest period enabled them to have greater vegetative growth in the subsequent growing seasons and that this could be explained by the differences observed in the widths of their respective canopies.

Although no carry-over effects were detected in yield parameters over the three years of the experiments, well-watered vines presented lower soluble solids concentrations in their berries; this could, at least in part, be explained by their higher starch concentrations (Greven et al. 2016). According to most viticulturists and enologists, the most desirable organoleptic parameters for white grape varieties destined for the production of sparkling wines include having low soluble solids concentrations and titratable acidity ratios (Andrés-Lacueva et al. 1996, Jones et al. 2014). The influence of starch concentration on soluble solids concentrations and titratable acidity ratio suggests that vines with higher starch concentrations also tend to have higher ratio. The RDI strategy would therefore not be recommendable for growers pursuing these berry composition parameters.

In previous studies, it was demonstrated that deficit irrigation during pre-harvest had a negative impact on titratable acidity (Basile et al. 2012, Bellvert et al. 2016). The results obtained from this study also suggest that it may not be appropriate to adopt an RDI strategy during post-harvest in pursuit of this goal. The best irrigation strategy to enhance the berry composition attributes of white grape varieties would therefore be the application of full irrigation throughout the growing season. However, in a scenario of water restrictions, the post-harvest period would probably be the only time at which it would be possible to reduce irrigation, as its impact on yield parameters and berry composition would be certainly much lower than during pre-harvest.

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Conclusions

Conclusions

The main conclusions that can be drawn from the chapters presented in this PhD thesis are the following:

- The Chill Overlap Model provides a framework in which to analyse synergistic interactions between chill and heat accumulation requirements prior to budbreak in grapevines.
- The apparently low chill requirements have been confirmed, as has the fact that temperatures below 10°C seem effective in fulfilling this need. Also the fact of providing substantial amounts of warmth to achieve budbreak.
- The earlier stages of development are mainly driven by temperature, while from veraison to berry maturity other factors, such as solar radiation, source:sink ratio, crop load and water availability, must also be considered.
- Phenological grapevine development presents different upper temperature thresholds responses at each of the key stages: bloom, fruit set, veraison and berry maturity.
- Incorporating the relationship between temperature and radiation use efficiency slightly improved the approach for predicting berry maturity in vines destined for making sparkling wines.
- Applying water stress during post-harvest has a direct effect on the defoliation of vines and reduces their total leaf area.
- Vines present a high response in terms of carbon economy which favours the survival of permanent structures to the detriment of total increases in vine size; this also affects the growth of new roots.
- Applying a regulated deficit irrigation strategy during post-harvest has an influence on the accumulation of starch in vine roots, as a result of carry-over effects.
- The soluble solids contents and titratable acidity ratio of berries tend to increase as starch concentrations increase. However, the most desirable wine quality parameters for white varieties are based on enhancing the titratable acidity and reducing these ratios.

- In the Chardonnay cultivar, it is generally recommendable to apply a full-irrigation strategy throughout the growing season in order to ensure the accumulation of reserves and avoid any negative impact on berry composition attributes. However, in a scenario of water restriction, it is possible to consider adopting regulated deficit irrigation during the post-harvest period because this would have less impact on yield and berry composition than if it was adopted during the pre-harvest period.

The final general considerations and observations are:

- The newly developed methods for predicting the stages from budbreak to berry maturity could prove useful for improving grapevine phenology models in scenarios involving warmer climatic conditions. It has been shown that lower values than the widely used base temperature of 10ºC can be used to obtain more accurate predictions at all the key stages. It is also crucially important to incorporate high temperature thresholds into the calibration of phenology models under climate change conditions.
- Water scarcity definitely influences grape yield and quality attributes throughout the growing period. Identifying the phenological stages at which grapevines could be least affected would therefore be of great relevance in the coming years. It is also important to identify the wine attributes that are sought after by wineries and to determine how they could best be achieved by managing vines in the field.

Future research

Future research

After the conclusions and final remarks referring to the whole of the work carried out within this PhD thesis, it would be interesting to outline the next steps to be taken in order to move forward with this research. Although some of these issues have already been mentioned in this document, the aim of this section is to point out the next steps to follow in future research.

Phenological models under warmer conditions

Predicting the onset of the budbreak stage is the most challenging task involved in achieving accurate predictions because of (from a physiological point of view) the complex changes that occur from dormancy to budbreak. Approximations of the type presented in the new model of *Sperling et al. (2017), which link temperature and carbohydrate allocation with environmental cues and phenology, present interesting perspectives that should help us to further improve current phenological models.

It should also be possible to improve the predictive capacity of models for subsequent phenological stages. This should be achieved by considering the influences of: the source:sink relationship, which implies the transport and accumulation of sugars; solar radiation, which is the main source for carbohydrate synthesis; and soil temperature, which is a factor that has an important influence on vine development.

Carry-over effects of water stress during post-harvest

Further research will be needed to evaluate the long-term impact of applying conditions of sustained deficit irrigation during post-harvest on yield and berry composition. A further evaluation of other berry composition parameters, such as aroma sensory attributes and polyphenols, will also be required.

The canopy management strategy based on the forced regrowth of vines has been presented as a technique with the potential to fight climate change. However, more research is required in this direction in order to evaluate the long-term impact that such strategies could have on both yield and berry composition attributes.

*Sperling O., Silva L.C.R., Tixier A., Théroux-Rancourt G. and Zwieniecki M. A. (2017) Temperature gradients assist carbohydrate allocation within trees. Scientific Reports, 7, 1–10.

Annexes

Annex I

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 5°C in all cases.

References

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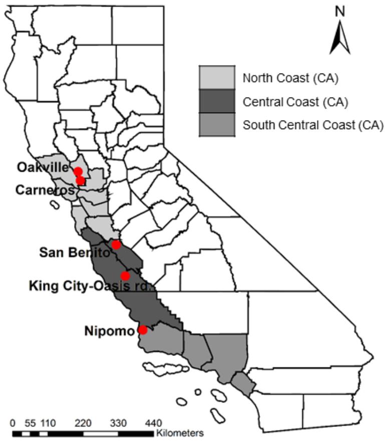
Supplementary figures

(a)



(b)

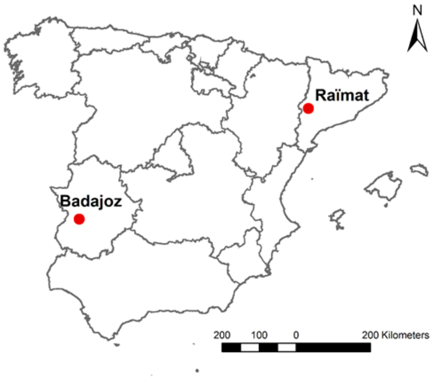


Fig. S1 Location of the weather stations (red dots) used in the study across (a) the California (USA) region and (b) Spain.

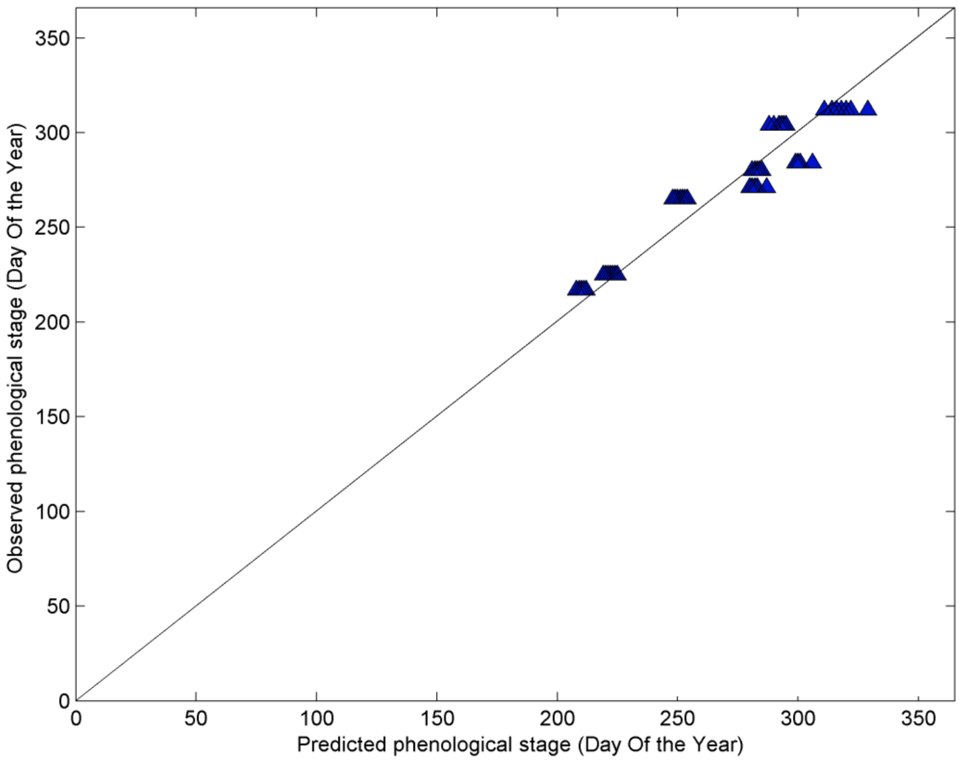


Fig. S2a Predicted and observed day of the year references for sparkling base wine berry maturity (Table 1, dataset) with the best methods based on the cross-validation technique. The statistics for the methods are shown in Table 4.

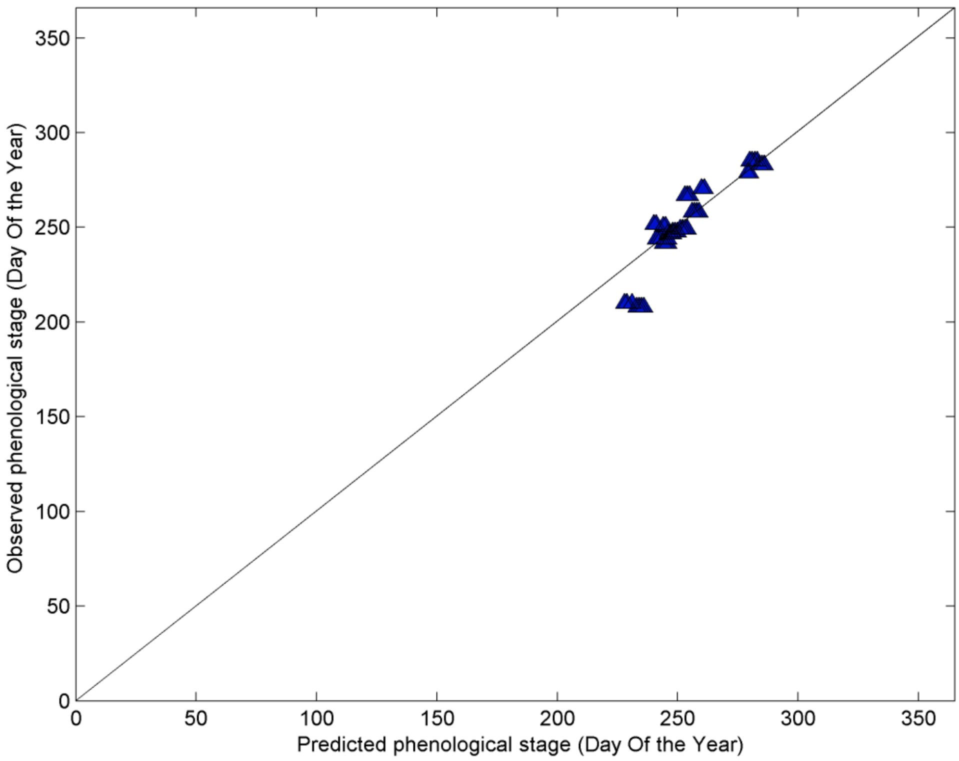


Fig. S2b Predicted and observed day of the year references for the best wine berry maturity (Table 2, dataset) performance using the cross-validation technique. The statistics for the methods are shown in Table 4.

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 (°C)

T_U, upper temperature (°C)

T_{mean}, daily mean temperature (°C)

T_{min}, daily minimum temperature (°C)

T_{max}, daily maximum temperature (°C)

- *UniFORC model* (Chuine, 2000)

$$treshDD = \sum_{i=1}^{m} DD_{UF} \tag{S1}$$

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

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

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

$$treshDD_{ST} = \sum_{i=1}^{m} DD_{ST} \tag{S4}$$

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$DD_{SS} = \frac{1}{\pi} * \left[\left(\frac{T_{max} + T_{min}}{2} - T_b\right) * (\theta_2 - \theta_1) + \alpha\,[\cos(\theta_1) - \cos(\theta_2)] + (T_U - T_B) * \left(\frac{\pi}{2} - \theta_2\right)\right] \quad \text{(S17)}$$

Supplementary tables

Table S1 Degree-day methods with a base temperature (TB) of 5ºC for the bloom, fruit set and veraison stages. Parameters of the methods of each phenological stage, the statistics descriptors RMSE, R2, MBE and AIC for method development using the calibration data set, and the statistics descriptors RMSE, R2, MBE for the evaluation of the methods using the validation data set. Methods fits were significant (p-value < 0.05).

Phenological stage	Method parameters			Method development				Method evaluation		
	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 (TB) of 5ºC for berry maturity Parameters of the methods for each berry maturity criteria, and the statistics descriptors RMSE, R2 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

Annex II

Modeling budbreak phenology in ‘Chardonnay’ grapevine using the chill overlap model framework

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Abstract

Budbreak time in grapevines (*Vitis vinifera* L.) defines growth cycle onset, and is strongly sensitive to temperature. Delays during this stage can have impacts on the whole growth cycle, making it a key phenological stage. Changing temperatures, due to climate change, make the need for accurate models to predict phenological patterns increasingly relevant, potentially affecting vineyard management, establishment and adaptability. ‘Chardonnay’ budbreak data from Californian and Spanish wine regions were used to estimate chilling requirements, and the compensatory relationship of overlapping chill and heat phases during specific temperature accumulation periods. Considerable variation in day of the year observation data, and diversification of climates across locations, enhanced the performance reliability of the model, leading to more accurate predictions over different climates. Preliminary evaluation of the model yielded acceptable model performance. However, variation due to the use of different criteria to define phenological stages, differences in microclimatic conditions, clonal variability among vineyards and vineyard management practices may be important factors to be considered for further increasing model accuracy. The chill-overlap model provided a framework for predicting bud-break in grapes but there is a necessity for deeper analyses in order to develop a more robust global model.

Keywords: chill requirement, heat requirement, chill portions, heat units, endodormancy, ecodormancy

Introduction

Recent research has been focused on predicting climate change impacts on phenology, to develop strategies to mitigate its possible effects on crop management, establishment and adaptability (Richardson et al., 2013). Changing temperatures have created a necessity for development of accurate and reliable phenological models to predict crop behavior.

Grapevine vegetative growth cycle onset is defined by budbreak stage. Shifts in bud-break timing can affect the whole growth cycle making it a key phenological stage (García de Cortázar-Atauri et al., 2009). Temperature is widely reported to be the main driver for phenological development in grapes (Williams et al., 1991; Nendel, 2010).

Dormancy is considered to be divided in two phases: Endodormancy, when buds become dormant due to physiological conditions and broken by the exposure of winter chill; followed by ecodormancy, when buds are still inactive due to unfavorable environmental conditions, and exposure to spring heat is necessary to trigger budbreak (García de Cortázar-Atauri et al., 2009; Caffarra and Eccel, 2010). The minimum requirements of winter chill and spring heat needed to release correspondent dormancy stages are specific requirements for individual species and cultivars that need to be determined for each genotype (Chuine 2000).

The evaluation of chilling requirements (minimum winter chill) is not straight forward, and is an important element for the assessment of climate change impacts (Darbyshire et al., 2017). The lack of physiological understanding of the physiological process involved during dormancy (Nendel, 2010) and the identification of the specific periods is challenging because buds are influenced by both cold and warm temperatures (Chuine, 2000).

Sequential models, which consider the fulfillment of a specific amount of winter chill followed by a subsequent amount of spring heat prior to budbreak, has been a commonly used approach for predicting budbreak (Ashcroft et al., 1977). But in Mediterranean climates there are occasionally mild winters when the minimum amount of chill is not met, but budbreak eventually occurs (Pope et al., 2014). Thus the sequential model appears to represent a simplification of the dormancy release process (Luedeling et al., 2009).

The recently developed chill overlap model (Pope et al., 2014), fits an exponential declining curve representing the interaction between winter chill and spring heat using non-linear regression techniques. It considers the well-documented compensatory relationship between winter chill and spring heat, where spring heat can compensate for low amounts of winter chill beyond a minimum threshold and *vice versa*, decreasing the amount of either required to finally trigger budbreak (Chuine, 2000; Pope et al., 2017). The model integrates possible combinations of chill and heat accumulation overlap that can result in budbreak. The model framework provides enough complexity to be able to do initial model evaluation without the need of the use of forcing experiments, simplifying the evaluation of chill and heat requirements.

The present work was a preliminary evaluation of a Chill Overlap Model for predicting budbreak of the 'Chardonnay' grape cultivar. Chilling requirements were estimated with observation data from both Californian and Spanish wine production regions.

Materials and methods

Observation data, from 2005 to 2016 range years, of budbreak were recorded from Californian and Spanish wine regions by winery companies (Figure 1). Budbreak was considered when 50% of buds were open, but a specific rating scale was not used.

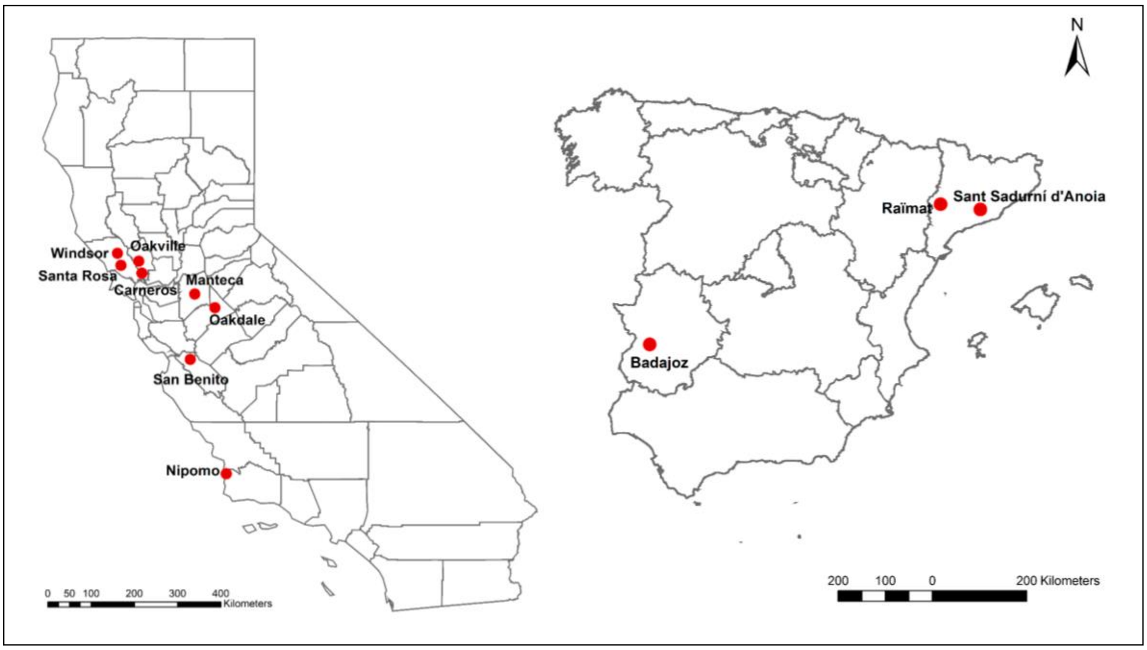

Figure 1. California (USA) (*n*=37) and Spain (*n*=5) budbreak temperature data locations.

Daily maximum and minimum temperature data were acquired from California Irrigation and Management Information System (CIMIS, www.cimis.water.ca.gov) for California (USA), and from Meteorological Service of Catalonian Government (SMC, www.ruralcat.net) and the Irrigation Advice Network of Extremadura (REDAREX, redarexplus.gobex.es/RedarexPlus/) for Spain. Hourly temperatures used for modelling were calculated following the methods of Linvill (1990).

Model parameters (β_1, β_2 and β_3,) for the chill overlap model were tested and set according with Pope et al. (2014). The exponential declining curve defined as $H_a = \beta_1 + \frac{\beta_2}{e^{(\beta_3 \, x \, C_a)}}$ was fit using a non-linear regression to represent the possible combinations of chill accumulation (C_a) and heat accumulation (H_a) required to trigger budbreak, after a certain amount of the chill requirement (C_r) was met. Chill requirements and chill accumulation were calculated as Chilling Portions (CP) using the Dynamic model of Fishman et al., (1987). Heat accumulation was calculated using the Growing Degree Hour (GDH) ASYMCUR model (Anderson et al., 1986). Models were fit with the Levenberg-Marquart algorithm in the Curve Fitting Toolbox of MATLAB software (MATLAB and Statistics Toolbox Release 2014b, The MathWorks, Inc., Natick, Massachusetts, United States). The tested chill requirements ranged from 1 CP to 31 CP, corresponding to the minimum chill accumulated from October 1 to March 31 within the dataset, and the model overlaps examined where 25%, 50% and 75%.

Model evaluations were made using coefficients of determination (R^2), comparing predicted and observed budbreak dates, and Root Mean Squared Error (RMSE) of the difference between predicted and observed dates.

Results

The best three models corresponding to the tested overlaps were prioritized according to evaluation of their model performance.

The chill overlap model appeared to have the best performance with a 25% chill-heat overlap, with a chilling requirements ranging from 9 to 11 CP. Minimum RMSEs ranged from 9.3 to 9.6 days, with maximum R^2 values of 0.53 to 0.59 (Table 1).

Table 1. Model performance with 25% overlap

Model parameters					
Chilling requirements (CP)	β_1	β_2	β_3	R^2	RMSE (days)
9	6992	8152	0.0729	0.53	9.5
10	7416	7428	0.0887	0.56	9.6
11	7725	7051	0.0971	0.59	9.3

All models were statistically significant (p-value<0.05)

CP, chill portions

β_1, β_2 and β_3, Chill Overlap Model parameters

RMSE, Differences between predicted and observed budbreak dates (days)

Chill-heat overlaps of 50% and 75% resulted in poorer performance (Table 2, Table 3). The chill requirements required for model fitting were lower for both amounts of overlap (from 4 to 9 CP); RMSEs were greater than 10.3 days, and R^2s were lower than 0.53.

Table 2. Model performance with 50% overlap

Chilling requirements (CP)	Model parameters			R^2	RMSE (days)
	β_1	β_2	β_3		
5	4514	14216	0.0371	0.50	13.5
7	6888	10933	0.0506	0.51	10.3
9	2141	14041	0.0252	0.53	16.9

All models were statistically significant (p-value<0.05)

CP, chill portions

β_1, β_2 and β_3, Chill Overlap Model parameters

RMSE, Differences between predicted and observed budbreak dates (days)

Table 3. Model performance with 75% overlap

Chilling requirements (CP)	Model parameters			R^2	RMSE (days)
	β_1	β_2	β_3		
4	7342	14529	0.0366	0.46	10.3
5	8146	13061	0.0475	0.40	13.4
7	7884	13023	0.0489	0.42	13.0

All models were statistically significant (p-value<0.05)

CP, chill portions

β_1, β_2 and β_3, Chill Overlap Model parameters

RMSE, Differences between predicted and observed budbreak dates (days)

Model parameter values for determining the shapes of the curve with the best fits for each overlap amount are shown in Figure 2, where the chilling requirements were 11, 7 and 4 CP (for 25%, 50% and 75% overlap, respectively), with the lowest RMSE in each case (9.3 days for 25% and 10.3 days for 50% and 75%) (Table 1, Table 2, Table 3).

Discussion

The estimated chill requirement values derived from this study indicate that the 'Chardonnay' grape cultivar has a relatively low chill requirement (4 to 11 CP, depending on the overlap) compared with other deciduous fruit crops tested with the same model (Pope et al., 2014; Darbyshire et al., 2016). This was expected because grape is generally considered to need relatively little exposure to chill (Eshgi et al., 2010). This study provided the first approximation of C_r using the Dynamic chill model for a specific grape cultivar.

The shape of the chill overlap model curve are defined by β_3 model parameter (Pope et al. 2014). β_3 model parameter values ranged from 0.0366 to 0.0971 (considering an interval of 0 – 1) and similar curve shapes were found for all overlaps tested (Figure 2). This appears to indicate that the R^2 differences found in the regression analyses could be because of the spring heat experienced by the crop and determined by β_1 and β_2 model parameters.

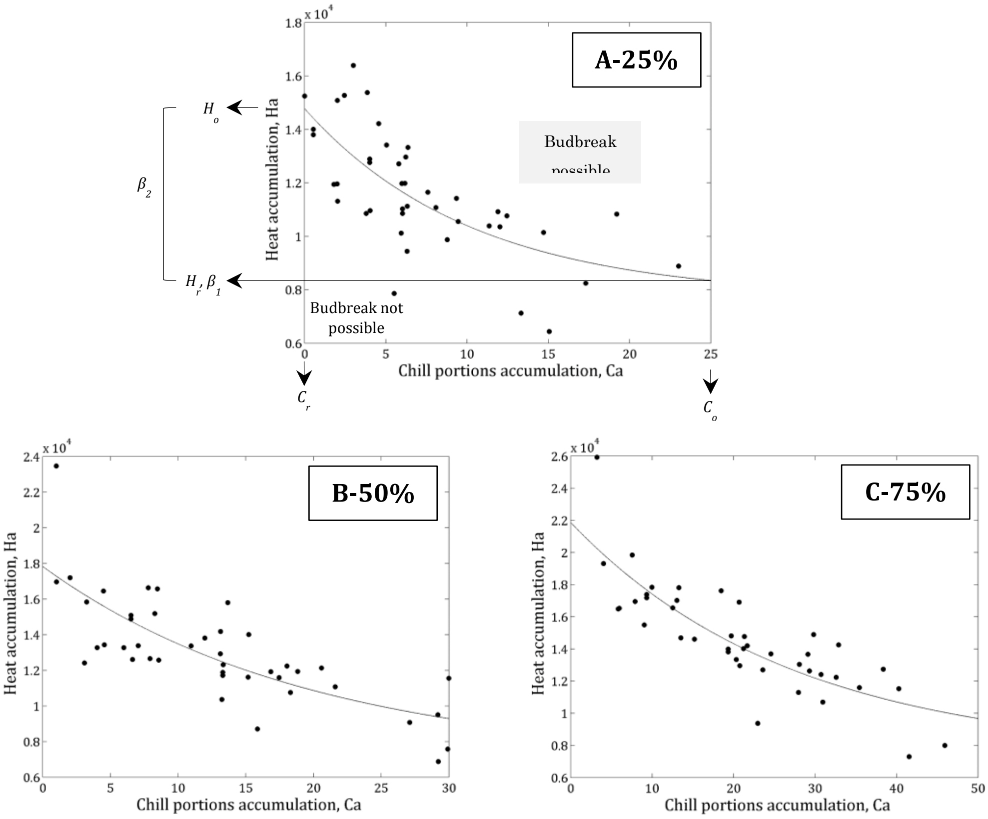

Figure 2. Best fit of the chill overlap model with the model parameters with the lowest RMSE. A, B and C plots belongs to 25%, 50% and 75% overlap, respectively. In A, parameters defining chill overlap model framework (Pope et al., 2014), where: C_r are the chill requirements meet before running the model, C_o is the highest accumulated chill, H_r is the minimum heat need to budbreak, and H_o is the highest accumulated heat.

According with the statistical analysis, the chill overlap model performed best with an overlap of 25%. Meaning that the 'Chardonnay' might be simultaneously influenced by both chill and heat for a short period, and needs higher exposure to spring heat to compensate for low chill accumulation to trigger budbreak.

The wide range of data from variable climates was important for achieving reliability and accuracy of model parameterization. The data used for this study appeared sufficient, considering the accumulation of chill portions ranged from 31 to 59 during the period October 1 to March 31 across the various data collection sites.

The performance of the model appeared to be acceptable as a first evaluation, with a RMSE of 9.3 days for predicting budbreak. It is likely that model accuracy could be improved by taking into consideration limitations of the original data set such as: using clearer criteria to define phenological stages of grape developmental stages, differences in microclimatic conditions of specific data collection sites, clonal variability among cultivars in different vineyards and differences in vineyard management practices.

Conclusions

Considering the results obtained in this preliminary evaluation, the chill overlap modelling framework appears to have promise for modelling budbreak of grapes across broad climatic regions. Although, there is a necessity for deeper analyses in order to develop a more robust global model.

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