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# Viticultural techniques of canopy management to mitigate the effects of global warming

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UNIVERSIDAD
DE LA RIOJA

## DEPARTAMENTO DE AGRICULTURA Y ALIMENTACIÓN

# Viticultural Techniques of Canopy Management to Mitigate the Effects of Global Warming

## TESIS DOCTORAL

## WEI ZHENG

## JULIO 2017

Man's dearest possession is life. It is given to him but once, and he must live it so as to feel no torturing regrets for wasted years, never know the burning shame of a mean and petty past.

Nikolái Ostrovsky [Russia]

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# INFORME DEL DIRECTOR DE TESIS

**Fernando Martínez de Toda Fernández**, Catedrático del Área de Producción Vegetal (perfil Viticultura) en el Departamento de Agricultura y Alimentación de la Universidad de La Rioja y Director de la Tesis que, como compendio de publicaciones, presenta **Wei Zheng** para la obtención del grado de Doctor, de título: "Técnicas vitícolas de manejo de la vegetación para mitigar los efectos del calentamiento climático" emite el siguiente

**INFORME:**

La Tesis, que se presenta como compendio de publicaciones, recoge la principal actividad investigadora del Doctorando en los últimos años. Dicha actividad se enmarca dentro de las líneas de investigación, desarrolladas en la Unidad de Viticultura de este Departamento, sobre diferentes experiencias de técnicas vitícolas para paliar los efectos del calentamiento climático.

La actividad investigadora del Doctorando en estos años ha dado lugar a una serie de publicaciones y comunicaciones que paso a describir y a valorar:

**GRUPO 1**: Publicaciones objeto de la Tesis: son publicaciones fundamentales en relación con el tema de la Tesis, en las que el papel del Doctorando es muy relevante, publicadas en revistas indexadas en las bases de datos internacionales.

**Artículos publicados como primer autor:**

**Zheng, W.**, del Galdo, V., García, J., Balda, P., & Martínez de Toda, F. (2017). Use of Minimal Pruning to Delay Fruit Maturity and Improve Berry Composition under Climate Change. *American Journal of Enology and Viticulture*, 68(1): 136-140

SCI (Q1); CIRC (Grupo Excelencia); factor de impacto in 2015: 1.58; factor de impacto en los últimos 5 años: 1.66

**Zheng, W.**, García, J., Balda, P., & Martínez de Toda, F. (2017). Effects of severe trimming after fruit set on the ripening process and the quality of grapes. *VITIS-Journal of Grapevine Research*, *56*(1), 27-33.

SCI (Q2); CIRC (Grupo A); factor de impacto en 2015: 0.99; factor de impacto en los últimos 5 años: 0.83

**Artículos aceptados como primer autor:**

Does Full Exposure of Clusters have any negative effects on Tempranillo (*Vitis vinifera* L.) Grape Quality in La Rioja, Spain? The Use of a Severe Cluster-Zone Leaf Removal after Berry Set.

Aceptado por South African Journal of Enology and Viticulture

SCI (Q2); CIRC (Grupo A); factor de impacto en 2015: 0.92; factor de impacto en los últimos 5 años: 0.89

**Artículos publicados como coautor:**

Martínez de Toda, F., Sancha, J. C., **Zheng, W.**, & Balda, P. (2014). Leaf area reduction by trimming, a growing technique to restore the anthocyanins: sugars ratio decoupled by the warming climate. *VITIS-Journal of Grapevine Research*, *53*(4), 189-192.

SCI (Q2); CIRC (Grupo A); factor de impacto en 2015: 0.99; factor de impacto en los últimos 5 años: 0.83

**Artículos enviados como primer autor:**

Effects of late winter pruning at different phenological stages on vine yield components and berry composition in La Rioja, North-central Spain.

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**GRUPO 2:** Publicaciones y comunicaciones que no se incluyen en esta Tesis como compendio de publicaciones pero que se citan en este informe porque demuestran, también, la formación y actividad investigadora más general del Doctorando.

**Artículos publicados como coautor:**

García, J., **Zheng, W.**, Balda, P., & Martínez de Toda, F. (2017). Varietal differences in the sugar content of red grapes at the onset of anthocyanin synthesis. *VITIS-Journal of Grapevine Research*, *56*(1), 15-18.

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## Artículos técnicos:

Balda, P., Sancha, J. C., García, J., **Zheng, W.**, & Martínez de Toda, F. (2016). Caracterización agronómica y enológica de las variedades tintas minoritarias de la DO Ca Rioja. *Enoviticultura*, (41), 4-15.

Martínez de Toda, F., García, J., & **Zheng, W**. (2015). Efectos de las altas temperaturas y los golpes de calor en la vid. Estrategias frente al cambio climático. *Tierras de Castilla y León: Agricultura*, (228), 94-99.

Martínez de Toda, F., **Zheng, W.**, Del Galdo, V., García, J., Balda, P., & Sancha, J. C. (2015). La poda mínima del viñedo como herramienta de adaptación al cambio climático. *Agricultura: Revista agropecuaria*, (981), 112-115.

Balda, P., Palacios, A., Carrillo, D., Zaldivar, E., Borinaga, I., Martínez de Toda, F., & **Zheng, W**. (2013). Expresión de pirazinas en Maturana Tinta de Navarrete. *La Semana vitivinícola*, (3413), 2132-2139.

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**Zheng, W.**, García, J., Balda, P., & Martínez de Toda, F.2016. Efectos del recorte severo de los pámpanos sobre el retraso y características de la maduración de la uva. Actas de Horticultura, Comunicación Técnicas Sociedad Española de Ciencias Hortícolas, II Jornadas de Grupo de Viticultura. 3-4 noviembre, 2016. Madrid, España.

Martínez de Toda, F., **Zheng, W.**, Del Galdo, V., García, J., & Balda, P. 2015. Minimal pruning and severe trimming as two helpful techniques to increase the anthocyanin:sugars ratio in grapes. 9° edizione Enoforum, 5-7 may, 2015. Vicenza, Italy.

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García, J., **Zheng, W.**, Balda, P., & Martínez de Toda, F. A new approach in the adaptation of varieties to global warming: varietal differences in sugar content at veraison. In Proceedings of the 19th International GiESCO Viticulture Congress. 1-5 June, 2015. Gruissan-Montpellier, France.

Reynolds, A.G., Brown, R., Jollineau, M., Shemrock, A., Kotsaki, E., Lee, H-S., & **Zheng, W.** Application of remote sensing by unmanned aerial vehicles to map variability in Ontario Riesling and Cabernet franc vineyards. 11th International Terroir Congress. 10-14 July, 2016. Willamette Valley, Oregon, United States.

**Zheng, W.**, García, J., Balda, P., & Martínez de Toda, F. 2016. La poda mínima como herramienta para retrasar la maduración y mejorar la composición de la uva en condiciones de temperaturas elevadas. Actas de Horticultura, Comunicación Técnicas Sociedad Española de Ciencias Hortícolas, II Jornadas de Grupo de Viticultura. 3-4 noviembre, 2016. Madrid, España.

García, J., **Zheng, W.**, Balda, P., & Martínez de Toda, F. 2016. Effectos de la temperatura sobre el desacoplamiento entre antocianos y azúcares de la variedad Garnacha en La DOC La Rioja. Actas de Horticultura, Comunicación Técnicas Sociedad Española de Ciencias Hortícolas, II Jornadas de Grupo de Viticultura. 3-4 noviembre, 2016. Madrid, España.

Se cuenta con la conformidad de los coautores de las publicaciones que son objeto de esta Tesis.

Logroño, a uno de julio de dos mil diecisiete

Fdo: Prof. F. Martínez de Toda

# Viticultural techniques of canopy management to mitigate the effects of global warming

## ABSTRACT

The overall aim of this thesis was to assess the effects of different cultural techniques on grape yield components and fruit quality. In particular, within the climate change scenario, their effectiveness of delaying grape technological ripening and improving the anthocyanins-to-sugar ratio was the main priority of the research. All the trials were conducted under field conditions. The three main cultural techniques were: severe shoot trimming (SST) after berry set, minimal pruning (MP) and late winter pruning (LWP). In addition, basal leaf removal (LR), a wildly used cultural technique, was also studied in order to evaluate the impacts on grape and wine characteristics.

The results show that SST could delay grape ripening by two to three weeks, however, under conditions of low vigor, grapes were not able to mature properly with extremely severe trimming (trimming twice). In spite of the relatively cooler ripening period, the anthocyanin accumulation was not enhanced significantly. Nonetheless, SST sometimes reduced the berry size thus increasing the skin-to-pulp ratio, in this way, juice anthocyanin concentration was increased. SST helped to improve organic acid composition by increasing the tartaric acid and reducing the malic acid.

The experiment on MP demonstrated clearer results in the size of the berry: the significantly smaller berries contributed to the darker color of MP juice, but the ability of berry skin to synthesize anthocyanins was not improved by the ripening delay. LWP at stage G (LWPG) and H (LWPH) delayed berry ripening to a larger extent and created much cooler ripening conditions than normal winter pruning. However, this achievement was at the cost of a considerable yield loss and only LWPH succeeded to improve the anthocyanins-to-sugars ratio of grape juice. LWP before or during stage F did not affect yield components nor fruit composition.

The full exposure of clusters due to a severe LR after fruit set did not exert any negative effects on grape quality, on the contrary, it also increased the ratio between tartaric acid and malic acid and the results of the sensory evaluation show that LR enhanced wine color and body. Besides, LR reduced the sugar concentration required in the berry for the onset of anthocyanin synthesis.

# Técnicas vitícolas de manejo de la vegetación para mitigar los efectos del calentamiento climático

## RESUMEN

El objetivo general de esta tesis fue el de evaluar los efectos de diferentes técnicas vitícolas de manejo de la vegetación sobre las características de la uva y, más específicamente y en relación con el calentamiento climático, su eficacia para retrasar la maduración de la uva y mejorar la relación entre antocianos y azucares. Todos los experimentos fueron realizados bajo condiciones de campo. Las tres principales técnicas de manejo de la vegetación estudiadas fueron: el recorte severo de pámpanos después del cuajado, la poda mínima y la poda tardía. Además, también se estudió el deshojado basal de la zona de racimos, despues del cuajado, para conocer los efectos de la exposición máxima o total de los racimos a la radiación solar y sus consecuencias sobre las características de la uva y del vino.

Los resultados mostraron que el recorte severo de los pámpanos puede retrasar la maduración de la uva entre dos y tres semanas; sin embargo, bajo condiciones de poco vigor, las uvas no fueron capaces de madurar correctamente ante un doble recorte severo. Aunque la maduración se produjo en un período relativamente más fresco, la acumulación de antocianos no aumentó de forma significativa. No obstante, en algunas ocasiones, el recorte de los pámpanos redujo el tamaño de baya, aumentando la relación hollejo/pulpa y, por lo tanto, aumentando la concentración de antocianos en el mosto. El recorte severo también incrementó la relación entre ácido tartárico y ácido málico.

La experiencia de poda mínima mostró resultados más claros en el tamaño de la baya: las bayas más pequeñas contribuyeron significativamente a una mayor concentración de antocianos en el mosto, pero la capacidad del hollejo para sintetizar antocianos no aumentó como consecuencia del retraso de la maduración.

La poda tardía, en los estadios fenológicos G y H, retrasó la maduración de la uva en gran medida y permitió unas condiciones de maduración mucho más frescas que la poda estándar. Sin embargo, estos efectos se obtuvieron a costa de una pérdida considerable de la producción y sólo la poda en estadio H incrementó la relación antocianos/azúcares del mosto. La poda tardía, antes o durante el estadio F, no afectó a la producción ni a la composición de la baya.

La exposición total de los racimos a la radiación solar, producida por la operación de deshojado basal después del cuajado, no mostró ningún efecto negativo sobre la calidad de la uva ni del vino; al contrario, incrementó la relación entre el ácido tartárico y el ácido málico y el análisis sensorial del vino mostró más color y más cuerpo para el tratamiento de racimos totalmente expuestos. Además, el deshojado basal redujo la concentración de azúcares necesaria en la baya para el comienzo de la síntesis de antocianos.

# INDEX

# CHAPTER 1

# GENERAL INTRODUCTION

# Chapter 1. General Introduction

## 1.1 Climate change and global viticulture

Climate change is an irrefutable fact. Since 1880, the trend of global warming has been clear (Figure 1). Each of the last three decades has been successively warmer than any preceding decade since 1850 and the period from 1983 to 2012 was probably the warmest 30-year period of the last 14 centuries in the Northern Hemisphere (Pachauri et al. 2014). According to the Intergovernmental Panel on Climate Change, the surface of the Earth warmed about 0.74°C during the 20th century (Stocker et al. 2014). The increase in anthropogenic $CO_2$ emissions is considered as the primary cause of the observed warming of the earth and about half of the anthropogenic $CO_2$ emissions between 1750 and 2011 have occurred in the last 40 years (Pachauri et al. 2014). Moreover, the atmospheric $CO_2$ concentration is expected to keep on increasing and the earth surface temperature is predicted to rise over the 21st century (Schultz 2000, Jones et al. 2005). It is very likely that heat waves will occur more often and last longer, and that extreme precipitation will be more frequent in many regions (Pachauri et al. 2014).

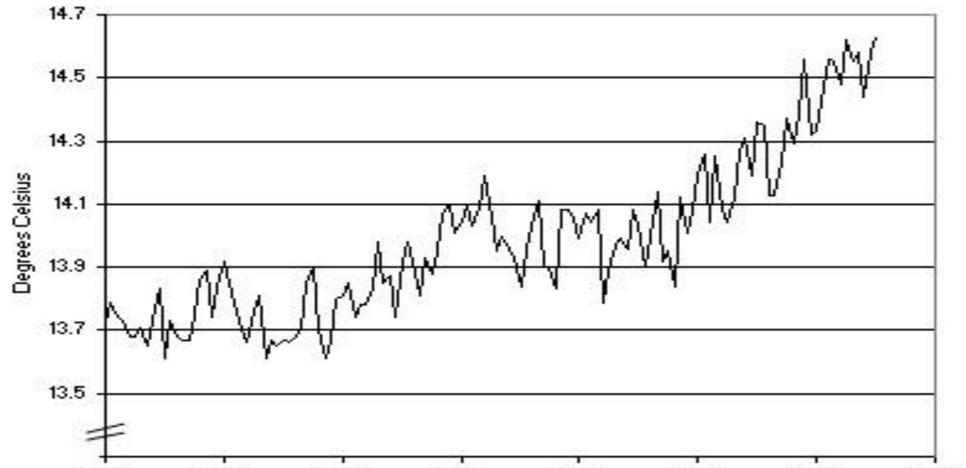

Figure 1. Average global temperature, 1880-2010. Source: NASA GISS (National Aeronautics and Space Administration, Goddard Institute for Space Studies).

The steady trend of climate change has had a profound impact on global viticulture (Schultz 2000) Europe's growing season temperatures have increased by 1.7 ºC from

1950 to 2004 (Jones et al. 2005). Between 2000-2049, temperatures regimes for the important wine regions all over the world are predicted to rise by an average of 0.42 ºC per decade and 2.04 ºC overall (Jones et al. 2005).

It is important to understand the impact of climate change on viticulture because global warming may alter the appropriateness of winegrowing for a number of current and potential wine regions and simultaneously lead to a change of grape variety cultivation (Mozell and Thach 2014). On the one hand, some regions such as the north of Europe (Figure 2) may benefit from the climate change. For example, in recent years, it has been demonstrated that it is possible to grow Merlot and Cabernet Franc in Germany, up to a latitude of 50 degrees north (Hidalgo Fernández-Cano and Hidalgo Togores 2011). Even in the United Kingdom, increasing average temperatures of the growing season provide the country with a good opportunity of winegrowing under cool climates. Actually, the vineyard area in the UK increased 148% from 2004 to 2013 (Nesbitt et al. 2016). On the other hand, many current wine regions have been and/or will be negatively affected by the warming climate. As seen in Figure 2, by 2050, it might no longer be suitable for wine grape growing in the extreme south of Spain and the most part of Greece. Jones et al. (2005) pointed out that many important wine regions in Europe are currently at or near their optimum climate for their respective varieties and wine styles, however, as predicted by the model, by the middle of the 21st century, most of them will lose their competitive advantages due to the increasing temperatures (Table 1).

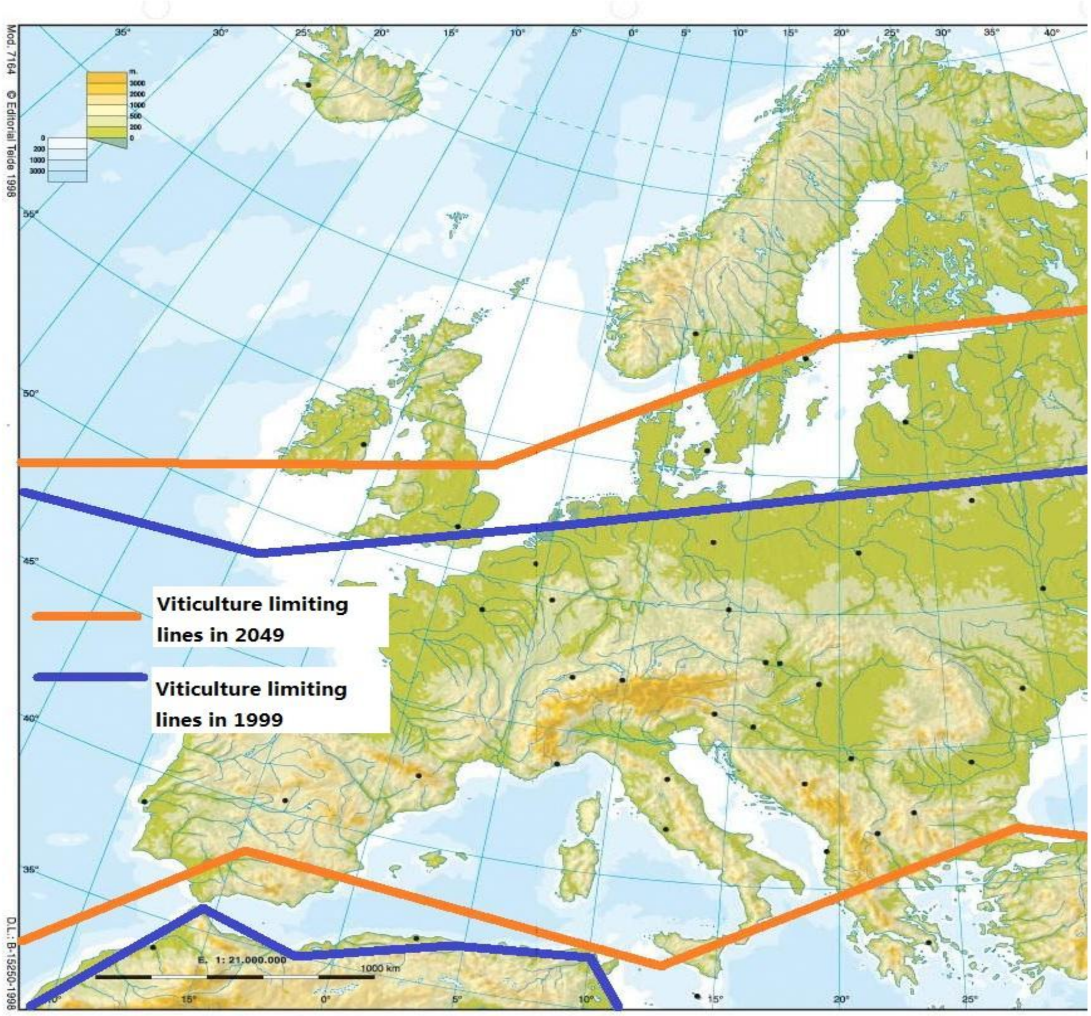

Figure 2. The motion of the limiting lines of viticulture in Europe. Source: Hidalgo Fernández-Cano and Hidalgo Togores (2011)

Table 1 Historical, estimated optimal, and predicted growing season average temperatures (Tavg) for some important wine regions in Europe. This table is elaborated based on Jones et al. (2005)

| | | Tavg (ºC) | | | Estimated optimum tavg (ºC) | Difference between optimum and tavg ºC) | Modeled tavg (ºC) | Difference between optimum and tavg (ºC) |
|---|---|---|---|---|---|---|---|---|
| Region | Category of wine | 1950 - 1999 | 1950 - 1989 | 1990 - 1999 | Modeled | 1990 - 1999 | 2000 - 2049 | 2000 - 2049 |
| Alsace | White | 13.1 | 12.9 | 13.8 | 13.7 | -0.1 | 14.04 | -0.34 |
| Mosel Valley | White | 13.0 | 12.9 | 13.4 | 13.9 | 0.5 | 13.93 | -0.03 |
| Champagne | Sparkling | 14.5 | 14.3 | 15.0 | 15.0 | 0.0 | 15.37 | -0.37 |
| Rhine Valley | White | 14.9 | 14.7 | 15.5 | 15.6 | 0.1 | 15.83 | -0.23 |
| Loire Valley | Sweet white | 15.3 | 15.2 | 15.8 | 16.6 | 0.8 | 16.31 | 0.29 |
| Loire Valley | Red | 15.3 | 15.2 | 15.8 | 16.7 | 0.9 | 16.31 | 0.39 |
| Bordeaux Médoc &Graves | Red | 16.5 | 16.2 | 17.5 | 17.3 | -0.2 | 17.70 | -0.40 |
| Bordeaux St.Émilion | Red | 16.5 | 16.2 | 17.5 | 17.5 | 0.0 | 17.70 | -0.20 |
| Rioja | Red | 16.7 | 16.3 | 18.1 | 17.5 | -0.6 | 18.03 | -0.53 |
| Barolo | Red | 17.8 | 17.5 | 18.8 | 18.6 | 0.2 | 19.21 | -0.61 |
| Southern Rhône Valley | Red | 18.2 | 18.1 | 18.8 | 18.9 | 0.1 | 19.44 | -0.54 |
| Barossa Valley | White | 19.9 | 20.0 | 19.6 | 19.9 | 0.3 | 20.85 | -0.95 |

## 1.2 Impacts of warming climate on vine phenology, yield and berry quality

Vine yield and fruit quality are the most important concerns of viticulture because they directly determine the profits of the viticulturists as well as the wine quality. Despite the fact that both of them are under genetic control, it is entirely possible for environmental variables and cultural practices to alter yield components and fruit composition (Keller 2010a). Therefore, it is essential to gain a deep insight into the impacts of the warming climate on grape yield and quality.

### 1.2.1 Vine phenology

For the existing vineyards, the direct impact of the warming climate is the accelerated phenological stages; earlier dates of phenological events and harvest have been recently observed worldwide. In Rheingau (Germany), budburst and anthesis took place 8-10 days earlier at the beginning of 21st century than the 1950s and the veraison date moved forward 18-23 days during the same period. Besides, according to the data from Johannisberg (Rheingau), the first harvest date was, on average, 2-3 weeks earlier in the 2000s than the 1900s (Stock et al. 2005). In Alsace (northeastern France), from 1972 to 2002, all the phenological events moved forward and the period between budburst and harvest shortened significantly (Duchêne and Schneider 2005). Likewise in France, in Bordeaux, harvest dates have moved forward by two weeks in the past 20 years (Jones and Davis 2000). In the Napa and Sonoma valleys (California, United States), due to higher temperatures in winter and spring, the start of the growing season advanced by 18-24 days between 1951 and 1997 (Nemani et al. 2001). Even in the southern hemisphere, based on the model calculations, the harvest dates for Cabernet Sauvignon and Chardonnay grapes will be shifted forward by 2-3 weeks in most of the Australian wine regions in 2050, compared to 1990 (Webb et al. 2007). The greatest consequence of the forward phenological stages is likely to be that, the grape ripening is taking place under warmer conditions than before.

### 1.2.2 Yield components

Vine yield is a function of the number of buds per vine, bud fertility (potential inflorescence numbers), the number of berries per cluster, and the berry weight (Keller 2015). Except the number of buds, which is determined by the pruning method, all the other variables are environment-dependent. Within a winter bud, the formation of grape inflorescences begins at around flowering time of the current season. During the dormancy phase, morphological development cannot be observed and around budburst of the next season, inflorescences growth recommences together with the flower formation

(Lavee and May 1997, May 2000, Vasconcelos et al. 2009). Warm temperatures, high irradiance and an adequate supply of water and nutrition are necessary for the maximum number of inflorescence primordia and extremely high temperatures (> 35 ºC) during the initiation phase might make the buds unfruitful (Keller 2010a). In the following season, after budburst, the weather conditions could also affect the inflorescence numbers (Keller 2015) and it seems that high temperatures before and during flowering lead to rapid shoot growth thus causing the loss of inflorescences, which is commonly known as the phenomenon of "filage" (Champagnol 1984). The flower differentiation and the percentage of fruit set determine the final berry number per cluster and it is thought that the availability of carbohydrate is the determining factor of flower induction and fruit set (Friend and Trought 2007, Vasconcelos et al. 2009, Keller 2015). Similar to the loss of inflorescences, before flowering, high temperatures could cause shoots to grow rapidly and to compete fiercely with the flower formation for the carbohydrate supply, resulting in flower abortion (Champagnol 1984, Bowen and Kliewer 1990). Conditions that favor fruit set are very similar to those that favor inflorescence formation so temperatures below 15 ºC or above 35 ºC and low light could reduce the percentage of fruit set (Keller 2010a). The final berry size is a function of the number of cell divisions and the multiple of the volume expansion of every cell (Coombe 1976). Since the vast majority of cell divisions take place before flowering and the divisions almost stop two weeks after fruit set, a severe water stress during this period may reduce the final berry size (Martinez de Toda 2011). And once again, both low (< 15 ºC) and extremely high temperatures (> 35 ºC) may repress cell divisions thus limiting berry size (Keller 2010a). Heat stress could also reduce cell expansion but only before the lag phase of berry growth so extremely high temperatures before veraison may also reduce the final berry size (Hale and Buttrose 1974, Keller 2010a).

Due to global warming, it is considered that the continuously rising temperatures and atmospheric $CO_2$ concentration will be likely to increase the canopy photosynthetic potential thus increasing the vine productivity (Schultz 2000, Palliotti et al. 2014). Besides, a higher atmospheric $CO_2$ concentration could improve the water-use efficiency of vines which might benefit the yield in arid regions (Schultz 2000). Moreover, in cold viticulture regions, the current and predicted higher temperatures in winter might be beneficial to vine productivity because the risk of winter frost injury is getting lower and the survival rate of dormant buds is higher. However, the higher temperatures in late autumn and early winter might be harmful in terms of cold hardiness as they can compromise cold acclimation so that vine organs may be less hardy during the winter (Ferguson et al. 2011). An earlier spring warming may also have a negative effect because budburst occurs earlier under this condition and growing shoots are very sensitive to low temperatures hence a spring frost could cause a great loss in yield (Keller 2015). Here is a good example: just this year (2017), the low temperatures (as low as - 6 ºC) during the night of 19 April have wiped out entire crops of young buds in some parts of Champagne; actually, the frost was not later than it traditionally has been but the mild weather in March has caused the vines to develop earlier than in the past. In short, the warming climate is a “double-edged sword” from the cold hardiness point of view. On the whole, the warming climate will generally lead to a higher yield but extreme weather conditions such as a heat wave, continuous drought and spring frost may result in severe yield reduction.

### 1.2.3 Fruit composition

The concept of grape quality integrates the following aspects (Martinez de Toda 2011):

1. The desired wine style.
2. Healthy grapes.
3. Matured grapes with appropriate ratio of sugar to acid.
4. Phenolic maturity.
5. Varietal peculiarity.

The berry composition changes continuously during berry development and ripening, as in the case of yield formation, except for the genetic control, environmental factors and cultural practices, and their interaction with the genotype of the cultivar could also play important roles in the final berry composition (Keller 2010a). Considering all the above-mentioned aspects of grape quality, it is necessary to get a good understanding of how the environmental factors and the warming climate could affect sugar, acid and phenolic compounds.

#### 1.2.3.1 Sugars

Grape sugar content is usually considered approximately equal to the concentration of total soluble solids (TSS) and it is measured in ºBrix. However, more accurately, sugars make up more than 90% of TSS at harvest, with much of the remainder being organic acid (Keller 2015). Berry sugar accumulation depends on the import of sucrose from photosynthesizing leaves or woody storage organs and under the action of invertases, hexose (glucose and fructose) starts to accumulate rapidly in berries at veraison (Keller 2015). Temperatures play an important role in sugar accumulation and the optimum temperature range for the photosynthesis of grape leaves are 25-30 ºC. However, the optimum temperature of photosynthesis is also related to the previous temperature condition during the growth of leaves, and those leaves which have experienced high temperatures can even reach the peak of photosynthesis at 35 ºC (Keller 2010b). Thus, high temperatures usually lead to an acceleration of sugar accumulation in berries except in extremely hot regions, where temperatures exceed the photosynthetic optimum during a considerable part of the growing season (Keller 2015). Differently from what may seem obvious, despite the fact that high temperatures accelerate grape ripening, the effects on final sugar content are relatively small (Coombe et al. 1987). That is, for a given cultivar, the maximum sugar content has its limit; Grape berries are not likely to achieve a TSS concentration above 25 ºBrix unless the berry dehydration and shrinkage occur (Keller 2015). Wine regions with a relatively cool climate may benefit from the warming climate since grapes could obtain a better technology maturity. For example, in Bordeaux, the best vintage ratings always have coincided with the warmest years with the grapes having elevated sugar content (Jones and Davis 2000). It seems that, with the increasing temperatures, in most of the wine regions across the world, it is easier to produce wines with a high alcohol content. Traditionally, this was a desirable target (Jackson and Lombard 1993). However, nowadays, there is a new trend that more and more consumers prefer wines with a moderate alcohol content due to health reasons (Palliotti et al. 2014). Moreover, the increased alcoholic level may alter the inherent style of wines in some places. One example is the “Txakoli” which is characterized as a very fresh white wine in Vizcaya and Guipúzcoa, north of Spain. However, with the increasing temperatures, the amount of alcohol increases (> 12 or 13%), and this is totally unmatched to the concept of “Txakoli” (Hidalgo Fernández-Cano and Hidalgo Togores 2011). Another concern about the rapid accumulation of sugars is the so called “decoupling between sugars and anthocyanins”. Regarding this issue, it will be discussed in the part of anthocyanins. We also need to mention that a severe water stress during ripening can decrease the photosynthesis of leaves thus delaying berry ripening or even leading to an improper maturity (Santesteban and Royo 2006, Baeza et al. 2007). In this sense, the possible drought conditions brought by the climate change may lead the fruit ripening to another extreme.

### 1.2.3.2 Organic acids

Acidity plays an important role in fruit and wine quality because it not only affects the sour taste but also masks a sweet taste (Jackson 2009). Besides, acids (especially malic acid) also taste astringent and enhance the perception of astringency (Hufnagel and Hofmann 2008). In grape berries, the most important organic acids are tartaric acid and malic acid (Jackson 2014). Most organic acids are accumulated early in berry development and tartaric acid is mainly synthesized between bloom and veraison in both leaves and berries, and its synthesis in leaves mainly occurs when the leaves are expanding (Ruffner 1982). Water deficit before veraison may limit tartrate accumulation (Esteban et al. 1999). After veraison, tartrate content per berry is usually stable due to its insensitiveness to light and temperature and the decrease in tartrate concentration is mainly attributed to the dilution effect caused by berry expansion (Hale 1977, Mira de Orduña 2010). The accumulation of malic acid mostly occurs before veraison as well, and the optimum temperature range for the accumulation is between 20-25 ºC; when temperatures are more than 38 ºC, the synthesis declines greatly (Keller 2015). Like the case of tartrate, malate accumulation also tends to decline under water deficit (Esteban et al. 1999). After veraison, the carbon source for respiration in the berries is switched from glucose to malate so that malate content declines (Gutiérrez-Granda and Morrison 1992). Regardless of the temperature factor, most of the reduction in malate occurs early in ripening, slows at 16-18 ºBrix and often becomes insignificant above 20-21 ºBrix (Keller 2015). Nevertheless, malate degradation is favored by high temperatures since the malate respiration is enhanced as the temperature increases, up to 50 ºC (Keller 2010b). Moreover, high light intensity may also favor the malate degradation despite the fact that the primary cause of this is also the high berry temperature as a result of the high irradiation (Kliewer and Schultz 1964). The sourness of the wine can be perceived by the tongue of human beings since it is in proportion to the concentration of hydronium ions ($H_3O^+$) (Keller 2015). Thus, the juice pH is more indicative than the titratable acid (TA), though pH is generally inversely related to TA (Boulton 1980b). The tartaric and malic acid levels are not accurate indicators of TA but high ratio of tartrate to malate lead to low pH (Boulton 1980a). Moreover, pH is also influenced by metal cations, especially potassium ($K^+$) and sodium ($Na^+$) (Boulton 1980a). Even when most malic acid has already been respired, $K^+$ may continue to be imported into the berries via phloem influx so the pH may keep rising. During the ripening phase, too much water supply or high temperatures could augment $K^+$ concentration thus increasing pH (Mira de Orduña 2010, Martinez de Toda 2011). It is worth mentioning that values of pH above 3.6 are undesirable since they could lead to decreased color intensity and microbial stability (Keller 2015). From the above, it can be concluded that the warming climate is likely to be positive for cool wine regions from the acid point of view. However, in warm regions, the increasing temperatures may result in extremely low TA and high pH; the winemaking process may become more expensive because low-acid grape juice requires addition of tartaric acid to balance the high sugar level and to enhance microbial stability (Keller 2010a).

#### 1.2.3.3 Phenolic compounds

Phenolic compounds contribute greatly to the fruit and wine quality since they determine the color and mouthfeel of wine (Jackson 2009). Moreover, they are the most important substrates for juice and wine oxidation (Keller 2010b). The two main synthesis pathways of phenols in grapes are the shikimate pathway (more important) and the malonate pathway (less important) (Moreno-Arribas and Polo 2009). Based on the basic structures, phenolic compounds can be grouped into two categories in grape berries: 1) non-flavonoids, which are mainly accumulated in pulp; 2) flavonoids, which mainly exist in the skin, seeds and stem (Keller 2010b). Among the phenolic compounds in grapes, anthocyanidins and tannins are of the most importance and they all belong to the flavonoids group (Moreno-Arribas and Polo 2009).

##### *1.2.3.3.1 Anthocyanins*

Mostly, anthocyanidins are glycosylated (Li et al. 2005). The sugar molecule increase the solubility and decrease the antioxidant activity so anthocyanidins can accumulate in the cell vacuoles in the form of anthocyanins (glycosides of anthocyanidins) and have a better chemical stability (Li et al. 2005). Since the aromatic rings of anthocyanins derive from sugars, those factors that favor carbohydrate accumulation are also in favor of the anthocyanin synthesis, especially in the first 5 weeks after veraison when this correlation is very close (Pirie and Mullins 1977). Nonetheless, environmental factors, especially temperatures and light, affect the accumulation of anthocyanins in significant measure (Keller 2015). It is generally thought that high temperatures could negatively affect the anthocyanin accumulation (Winkler et al. 1974, Mori et al. 2005, Mori et al. 2007, He et al. 2010, Mira de Orduña 2010) probably because, under warm conditions, the activity of some key enzymes is likely to be inhibited (Mori et al. 2005) and anthocyanin degradation tends to occur (Mori et al. 2007). Also, high temperatures could delay the onset of anthocyanin accumulation leading to a low anthocyanin level at harvest (Sadras and Moran 2012). For the maximum production of anthocyanins in grape berries, moderate sunlight exposure is necessary, but the extent varies among different cultivars (He et al. 2010) since light exposure has some positive effects on cluster anthocyanin accumulation (Dokoozlian and Kliewer 1996, He et al. 2010) yet intense sunlight could cause sunburn in exposed berries and the associated high temperature can also inhibit the color development, especially in hot regions (Haselgrove et al. 2000, Bergqvist et al. 2001, Guidoni et al. 2008, Chorti et al. 2010). Because of climate change, the sugar accumulation is more and more rapid, berry ripening takes place during a warmer period of the year than before. Depressed by the high temperatures, grape anthocyanin concentration could not reach the maximum value in theory at the conventional TSS level for harvest. Moreover, since the extractability of anthocyanins increases with the process of ripening (Allegro et al. 2016), a shortened ripening period may cause a reduced extractability of anthocyanins at harvest. The combination of a higher TSS level and a lower anthocyanin concentration has been well known as “decoupling between sugars and anthocyanins” (Sadras and Moran 2012). This decoupling may bring about two consequences: 1) If grapes are harvested at the conventional TSS level, the berry quality may not be satisfactory; 2) If the growers postpone the harvest date in order to allow for higher concentrations of Anthocyanins, the berries may be too high in TSS and the alcohol content of the resulting wine will be too high.

#### *1.2.3.3.2 Tannins*

Different from anthocyanidins, which are mostly monomeric compounds, tannins are oligomers or polymers (Cheynier et al. 2006). Tannins can be divided into two classes: 1) condensed tannins (nonhydrolysable tannins), which are composed of flavan-3-ol monomers and proanthocyanidins; proanthocyanidins can break down to anthocyanidins when heated in acidic condition (Moreno-Arribas and Polo 2009). 2) hydrolysable tannins, which are polymerized from non-flavonoids and easy to hydrolysis under acid condition. They derive from oak wood and are less stable than condensed tannins (Li et al. 2005). The perception of astringency is a tactile sensation elicited by precipitation of salivary proteins and it increases with an increasing molecule size and concentration of tannins (Cheynier et al. 2006). On the other hand, the shorter the chain of flavonoid polymers is, the bitter the tannins taste (Keller 2015). That’s why the skin tannins (with four to more than 100 subunits) provide a better mouthfeel than seed tannins (with 2-20 subunits). Grape tannins are synthesized in the skins and seeds during the early stages of berry development. Soon after veraison, the synthesis of tannins almost terminates (Keller 2015). The polymerization of both seed tannins and skin tannins increases at veraison (Li et al. 2005). Accompanied by oxidation, seed tannins bind strongly to cell walls so their extractability declines gradually (Cadot et al. 2006). Given the discussion so far, we can draw a rough conclusion that the so called “phenolic maturity” consists in the accumulation of anthocyanins, the increased extractability of anthocyanins, the polymerizations of tannins and the reduced extractability of seed tannins. More cluster exposure to the sunlight may enhance tannin accumulation in the skin and increases the length of tannin polymers (Cortell and Kennedy 2006, Downey et al. 2006). The formation of tannins in grape berries is likely to increase with increasing temperatures (Keller 2015). So the possible influence of global warming on grape tannins can be that, in warm regions, grape berries may accumulate more tannins if the temperature before veraison is too high. However, as berry ripening is occurring under increasingly warm conditions, the period between veraison and harvest probably becomes shorter thus there is less time for the tannins to “mature”. On the contrary, in cool regions, the increasing temperature may help to enhance the wine body due to the increased accumulation of tannins.

### 1.2.3.4 Aroma components

The aroma substances in wine can be divided into terpenoids, aliphatic compounds and aromatic compounds (Li et al. 2005). For most varieties, the aroma substances in grape berries only exist in the skin and their concentration increases through berry maturity (Li et al. 2005). Grape volatile terpenoids consist of monoterpenes, sesquiterpenes and norisoprenoid (Keller 2015), and 80%-90% of them are glycosylated being the potential aroma or flavor precursors which can be released during wine making or aging (Bönisch et al. 2014). It appears that the production of terpenoids competes with the accumulation of phenolics for carbon substrates (Dudareva et al. 2013). Apart from volatile terpenoids, the majority of the rest of the volatiles in grapes and wines are produced by oxidation of fatty acids and these components are normally accumulated in glycosylated form as well (Jackson 2008). In grape berries, small amounts of non-glycosylated precursors of aroma components also exist, among others, methoxypyrazines have been well documented because of their potential “veggie” aroma (Li et al. 2005, Keller 2015). Methoxypyrazines accumulate early during berry development to a maximum before veraison and then degrade to a great extent during ripening (Keller 2015). It is commonly considered that sunlight can enhance berry monoterpenoids (Baumes et al. 2002, Mongélard et al. 2011) and reduce methoxypyrazines (Reynolds et al. 1996, Balda et al. 2013). The effects of temperature on aroma and flavor compounds is not well understood and it appears that the accumulation of different compounds has different reactions to temperatures (Keller 2015). The optimum temperature range for the accumulation of terpenoids in grape berries is wide (10 ºC to 20 ºC), but fruit monoterpene concentrations may be negatively correlated with the average daily maximum temperature during ripening possibly because terpenes are more volatile under high temperatures (Marais et al. 2001). In contrast, high temperatures inhibit the accumulation of methoxypyrazines before veraison and accelerate their degradation during ripening. In all, the warming climate may be a “double-edged sword” for the grape aroma compounds. The good thing is that, in cool regions, viticulturists will worry less about the unwanted “veggie” aroma for some certain varieties such as Cabernet Sauvignon as Sauvignon Blanc. However, extremely high temperatures may negatively affect the accumulation of some desirable aroma components.

## 1.3 Strategies of mitigating negative effects of the warming climate

In order to mitigate the negative effects of global warming, it is interesting to delay the grape berry ripening by all means so that the fruits can mature under relatively cool conditions. As discussed above, a cool ripening phase is favorable to the maintaining of acid and aroma components and to the grape phenolic maturity.

According to Martínez de Toda (Martinez de Toda 2011) and Palliotti et al. (2014), three types of viticultural strategies could be used to delay the ripening of grapes:

1. Changes in vineyards site:

New vineyards can be established in areas of high latitude or for the same latitude, in plots with higher altitude and /or with less exposure to the incidence of solar radiation. However, for most of the existed vineyards and wineries, this approach is unrealistic.

2. Changes in plant material:

It is based on the use of new plant material with longer growth cycle. We can replace the existing varieties with late-maturing varieties or maintain the same varieties yet screening late-maturing biotypes or clones. The reselection of rootstocks should also be considered. Those rootstocks which can give an increased production thereby slowing berry ripening may be ideal to counteract the process of too fast sugar accumulation (Palliotti et al. 2014). In any case, this strategy also requires the establishment of new vineyards and cannot be realized in existing vineyards, except by re-grafting.

3. Adopting different cultural techniques:

This strategy is the most interesting because it can be applied directly to an established vineyard. A number of cultural techniques can be applied to delay the grape berry ripening based on three basic principles: 1) the limitation of source to sink ratio; 2) creating carbon and nutritional competition between vegetative growth and reproductive growth; 3) postponing all the phenological stages thus delaying ripening phase.

### 1.3.1 Cultural techniques based on the limitation of source to sink ratio

During the growing season, leaves are considered the main source of carbohydrates since perennial organ cease exporting sugar between bunch closure and veraison (Martínez de Toda 1991, Weyand and Schultz 2006). On the other hand, after the cease of the shoot growth, ripening berries and maturing shoots are the main sinks of sugar within a vine though the starch is also accumulated in roots, dormant buds and perennial woods (Martínez de Toda 1991). So grape quality in a given climatic region is largely determined by their total leaf area (LA) and by the percentage of LA that is exposed to sunlight as well as by the vine yield (Kliewer and Dokoozlian 2005, Martinez de Toda 2011). The ideal leaf area to production ratio (LA/P) for maximum level of total soluble solids, berry weight, and berry coloration at harvest range from 0.8 to 1.2 $m^2$/kg for single canopy (Kliewer and Dokoozlian 2005). Provided LA/P is sufficient, reduced fruit load is unlikely to affect the rate of ripening (Winkler et al. 1974). However, a LA/P value below 0.8 $m^2$/kg may lead to a lower capacity of TSS accumulation in berries thereby slowing the ripening process (Kliewer and Dokoozlian 2005). Before, the reduction in LA/P was always unwanted due to the risk of improper maturity. However, as the climate change has (and will) brought (bring) about a prolonged growing season, even with a low LA/P, berries could also reach a satisfied TSS level though a period of time later (Palliotti et al. 2014). In this way, berries could mature at a relatively cool weather condition which is desirable as discussed above. Since LA/P is determined by two elements (LA and P), we can reduce its value by, on the one hand, reducing LA through shoot trimming (Balda and Martinez de Toda 2011, Filippetti et al. 2011) or post-veraison leaf removal from apical to the bunch zone (Palliotti et al. 2013b) and on the other hand, increasing yield through light pruning or no pruning (Martínez de Toda and Sancha 1998, 1999, Schultz and Weyand 2005). Of course, the limitation of source does not merely consist of the reduction of LA, it can be also realized by limiting the photosynthesis of well-functioning leaves. In this category, it is possible to apply shading nets (Novello and De Palma, Palliotti et al. 2014) as well as antitranspirant sprays (Filippetti et al. 2011).

#### 1.3.1.1 Shoot trimming

Shoot trimming, defined as removing the shoot tip, is a cultural practice variously named shoot tipping, topping, or hedging in the field of viticulture (Keller 2010b). More precisely, tipping was defined by Coombe (1959) as the removal of the apical 8 cm or less of shoot, whereas shoot topping consists of removing the shoot tip and a number of young leaves on the abscised part. Here, we use the term of shoot trimming referring “a severe shoot topping”. It involves both the removal of a major sink for nutrients (shoot tip) and a sharp reduction in the active LA. Trimming stimulates one to several lateral shoots to develop below the cutting point (Wolf et al. 1986, Martínez de Toda 1991). It is true that there is carbon competition between growing laterals and the accumulation of TSS in berries, however, the growth of lateral shoots is highly influenced by the timing of trimming (Molitor et al. 2015) and by the hydrothermal conditions (Palliotti et al. 2014). Besides, provided the weather condition is favorable, laterals may also develop even without shoot trimming since the influence of apical dominance reduces when the main shoot form approximately 18-20 leaves (around the time of bloom) (Keller 2010b). So this competition may not be the main course of the delayed ripening. In contrast, whenever a severe trimming is carried out, a delay in TSS accumulation is likely to be observed as a consequence of the direct reduction in photosynthesis activity (Balda and Martinez de Toda 2011, Filippetti et al. 2011, Rombolà et al. 2011, Herrera et al. 2015, Bondada et al. 2016). More reviews about the effects of shoot trimming are present in Chapter 4.

#### 1.3.1.2 Post-veraison leaf removal apical to the bunch zone

Leaf removal (LR) is a commonly-used canopy management practice. Generally, the intervention is carried out on basal leaves to improve the health conditions of the clusters and the fruit composition (Smart and Robinson 1991). After veraison, basal leaves are no longer the main source of photosynthetic product (Poni et al. 1994) so the removal of them does not affect the ripening process. However, in the same period, if all the leaves above the bunch zone are eliminated, the total photosynthesis activity will decrease considerably because leaves on the apical two-third of the canopy are the most functional ones at the moment (Poni et al. 1994, Palliotti et al. 2014) and as a result, a ripening delay is likely to occur (Palliotti et al. 2014). Palliotti et al. (2013b) demonstrated that a mechanical LR apical to the cluster zone one month after veraison removed 35% of the total LA and reduced LA/P by 36 %; finally, the optimal TSS level of Sangiovese grapes was delayed by 2 weeks. In the same study, authors concluded that leaves should be removed at 16–17 °Brix in order to delay the sugar accumulation effectively. Also, Lanari et al. (2013) reported similar results for Sangiovese and Montepulciano grapes. Poni et al. (2013) found that defoliation above the bunch zone at about 12 °Brix succeeded to delay the technological ripeness of potted Sangiovese grapes by more than one week without affecting color and phenolics. In this PhD thesis, post-veraison LR was not studied. Nevertheless, “early” LR after fruit set was investigated because we wanted to see the effects of full exposure of clusters under our climatic condition. Generally, early LR refers to removing 6-7 basal leaves before flowering with the purpose of reducing fruit set thus increasing cluster looseness (Poni et al. 2006, Tardaguila et al. 2010). However, if the decrease in yield is unwanted, LR should be conducted at least two weeks after fruit set since most berry abscission occur within 2-3 weeks after full bloom (Candolfi-Vasconcelos and Koblet 1990). More reviews about the effects of basal LR after fruit set are present in Chapter 6.

#### 1.3.1.3 Minimal pruning

Winter pruning has been long considered as an effective weapon to meet the following purposes: 1) to maintain the vine in a desirable form which otherwise will expand too much because of acrotony thus facilitating vineyard cultural operations and harvest; 2) to endow the vine shoots adequate vigor that, on the one hand, is enough for floral induction in their buds and for a great degree of lignification to ensure that a certain amount of buds could survive in the winter; and, on the other hand, is not excessive or else vegetative growth dominates being a powerful competitor for sugar against berry ripening. 3) to control the yield by limiting the number of buds and then to give a stable production with high-quality fruit year after year (Winkler et al. 1974, Martínez de Toda 1991). However, pruning has its shortcoming. In general, 85%-98% of the annual growth of the grapevine is removed by traditional hand pruning, which means a great loss of reserves (Winkler et al. 1974). Besides, hand pruning is the most time-consuming as well as the most expensive operation among the cultural practices carried out in the vineyard because of the difficulties in mechanization (Martínez de Toda 1994). In the 1980s, Australian researchers developed an alternative technique called Minimal Pruning (MP) which was remarkable for its low cost and high productivity (Clingeleffer 1984, 1988). In fact, as early as in the 1930s, professor Albert Winkler from UC Davis conducted trials and found that unpruned vines had greater ability to self-regulate. Research over 30 years in Australia showed that traditional severe pruning was unnecessary in a number of viticultural regions and it might lead to low wine quality generally associated with development of shaded, tight bunches with large berries and difficulties in the control of pests and diseases (Clingeleffer 2010). On the contrary, minimally pruned vines generally produce juice with better organic acid composition, greater wine color and higher phenolics (Clingeleffer 2010). In Spain, a long-term study about MP on Grenache vines showed that MP always gave a larger yield under drought conditions of la Rioja (Martinez de Toda and Sancha 1998). Requiring low cost of time and money and producing high yield, MP is a viticultural technique with great application prospect, especially when the climate is warmer and warmer since one of the most conspicuous effects of MP is delaying the berry maturity providing a cooler ripening circumstance for the grapes, in favor of the accumulation of anthocyanins as well as the maintaining of acidity. More reviews about the effects of MP are present in Chapter 5.

#### 1.3.1.4 Application of shading nets

Light is essential for photosynthesis. It is true that leaf shading can reduce photosynthesis thus delaying berry ripening (Smart et al. 1985, Morrison and Noble 1990, Cartechini and Palliotti 1995), which may be applied to fight against the warming climate. However, most of the studies about the effects of shading showed that excessive canopy shading might lead to poor berry quality, which is specifically expressed in high malate level and poor color (Chorti et al. 2010, Palliotti et al. 2014). Morrison and Noble (1990) investigated the effects of both leaf shading and cluster shading on grape composition and it came out that malate, potassium, and pH were higher in fruit from the leaf shading treatments and shading clusters did not affect sugar, acid, or potassium accumulation but reduced the content of anthocyanins and total soluble phenols. Another study carried out by Jeong et al. (2004) showed that cluster shading at veraison significantly reduced the anthocyanin accumulation due to the inhibition of the expression of a gene (VvmybA1) which is involved in anthocyanins synthesis. Thus, it appears that, with the warming climate, leaf shading might be positive since it could slow down the ripening process while cluster shading is undesirable as it might negatively affect the grape color. In all, as concluded by Palliotti et al. (2014), though the application of shading nets is a viable technique, several issues should be clarified: 1) the relationship between timing/duration of shading and the degree of ripening delay; 2) better understanding of the shading effects of different plant portions; 3) the technical feasibility of artificial shading.

#### 1.3.1.5 Application of antitranspirant sprays

In grapevine leaf, the bulk of the total transpiration occur through stomata (Keller 2015). In the meantime, stomata is the main passage way of $CO_2$, which is an essential element of photosynthesis (Keller 2015). Under the natural conditions, the opening and closing of the stomata depends mainly on the illumination and secondly, on the leaf water potential as well as the temperatures (Martínez de Toda 1991). Nonetheless, by spraying antitranspirant, stomata closure can be archived artificially and persistently (Gale and Poljakoff-Mayber 1967). Once the stomata are closed, photosynthesis activity will decrease. This is the physiological background of the application of antitranspirant sprays to reduce yield or to delay the grape ripening. The application of antitranspirant before flowering can effectively reduce yield and bunch compactness thus improving the berry quality (Palliotti et al. 2010). Palliotti et al. (2013a) applied a film-forming antitranspirant after veraison on Sangiovese grape leaves and they found that the treatment slowed significantly the velocity of berry sugar accumulation without affecting negatively the storage of carbohydrates and total nitrogen in canes and roots. The application of antitranspirant sprays is considered as a flexible and easy-to-do technique since the desired effects can be obtained by adjusting dosage, timing and number of sprays and the operation does not require specific equipments or skills (Palliotti et al. 2014).

### 1.3.2 Cultural techniques based on creating carbon and nutritional competition between vegetative growth and reproductive growth

Viticultural practices tend to aim at suppressing shoot growth late in the growing season because unfolding leaves during this period usually grow so slowly that they are unlikely to contribute to berry ripening or carbohydrate storage. On the contrary, such growth may even compete for sugar with the ripening of fruits and shoots (Keller 2015). Generally, the combination of decreasing day length and declining temperatures around veraison seems to halt the shoot apex growth as well as the lateral shoot emergence (Garris et al. 2009, Keller 2015). However, under warm climate, such process can be delayed if water and nutrient availability permits (Keller 2015). In this case, berry ripening is supposed to be delayed by promoting vegetative growth and a possible cultural technique is late irrigation (Freeman et al. 1980, Fernández et al. 2013).

#### 1.3.2.1 Late irrigation

Shortly before or at veraison, shoots begin to form a periderm which means the onset of shoot maturation and along with this process, shoot growth begins to cease (Keller 2015). However, irrigation from this moment could be a useful strategy to resume shoot growth thus reducing available photosynthates for the bunches (Novello and De Palma 2013). The effect of such late irrigation is supposed to be greater if combined with shoot trimming because the latter operation could promote the growth of a number of laterals which could enhance the competition (Palliotti et al. 2014). However, this technique has been applied very little due to the concern about the “dilution effect” and the canopy health (Palliotti et al. 2014). Besides, dense canopies that result from abundant water supply may also be associated with the poor color due to the potential shading problem (Jackson and Lombard 1993). Therefore, compared to other cultural techniques, late irrigation may not be the best choice if the goal is merely to postpone the ripening. Nonetheless, in the case of severe water stress after veraison, photosynthesis can decrease dramatically and leaf abscission can even occur; as a result, grapes may not mature properly (Romero et al. 2010, Keller 2015). In this context, moderate late irrigation is undoubtedly necessary. Many viticulturists habitually think that the application of irrigation during the ripening phase could lead to the dilution of berry composition or even to the rise in yield. However, such fears may be superfluous. In fact, after veraison, xylem flow is blocked while sugar and water increments are linked and phloem sap is their common and unique source (Coombe and McCarthy 2000). Namely, the berry enlargement during ripening depends on the import of photosynthate rather than on the water absorption from roots.

#### 1.3.2.2 Cultural techniques based on postponing all the phenological stages

The timing of budburst exerts a great influence on the subsequent vegetative and reproductive growth (May 2000). Therefore, it is possible to postponing all the phenological stages including technological maturity by simply delaying the budburst date (Martin and Dunn 2000, Friend and Trought 2007). Fortunately, it is not difficult to make it through different pruning methods such as late winter pruning (Frioni et al. 2016, Gatti et al. 2016) and double pruning (Gu et al. 2012).

#### 1.3.2.3 Late winter pruning (LWP)

LWP has been well known because it can delay budburst by a few days or a period of time so that the risk of spring frost injury is greatly reduced (Reynier 2002). The mechanism of this phenomenon is the imposition of apical dominance, namely, grapevine shoot growth starts in the distal buds of a cane and the development of the basal buds is often inhibited by the budburst of distal buds (Friend and Trought 2007, Keller 2015). And then, after a late spur-pruning, basal buds/shoots are forced to break/grow (Howell and Wolpert 1978). LWP after budburst removes large amounts of reserves that have been already mobilized by the plant and located in the growing organs. In this sense, the plants are likely to be weakened (Hidalgo Fernández-Cano and Hidalgo Togores 2011). However, from the macro perspective, the grapevine generally has a greater vine capacity under global warming (Keller 2015) so this weakness is not a big concern. In recent years, several studies about LWP have been carried out with a particular purpose of delaying grape ripening in the face of the warming climate. Both Frioni et al. (2016) and Gatti et al. (2016) found that a very delayed winter pruning could slow the sugar accumulation to a large extent but the loss in yield was significant. Thus, the prospects of LWP application will depend mainly on whether a good balance between berry quality and yield can be obtained via this technique. More reviews about the effects of LWP are present in Chapter 7.

#### 1.3.2.4 Double pruning

Double pruning is a very bold method that has been proposed for hot viticultural regions to fight against the warming climate (Gu et al. 2012). It consists of hedging growing shoots to several nodes and removing laterals, leaves, and primary clusters with the aim of forcing the re-growth of vine. According to (Gu et al. 2012), forcing in June shifted fruit ripening from the hot to the cool portion of the growing season, during which the temperatures were more favorable. As expected, forced vines gave smaller berries and their juice showed a lower pH, higher acidity, and higher contents of anthocyanins, tannins, and total phenolics, compared with non-forced vines. Apparently it is a promising technique but there are two preconditions for its application: 1) the newly formed dormant buds should have fertility; 2) the buds should be in the phase of paradormancy. Therefore, the timing of this second pruning is of crucial importance. The formation of dormant buds usually coincides with the rapidly growth period of the shoot (Keller 2015). However, it seems that the first dormant buds in the basal nodes begins to develop as early as 3 or 4 weeks before budburst (Morrison 1991, Keller 2015). Hence, it can be speculated that, on the one hand, it is possible for the double-pruned vines to give production even in the very early phenological stages (i.e. 6-8 leaves separated, stage F based on Baillod & Baggiolini system), nonetheless, the later the operation is carried out, the more production can be obtained. On the other hand, the seconded pruning must be done before veraison, since dormant buds gradually lose the ability to break in 2-3 weeks along with the slowing down of shoot growth (Reynier 2002).

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# CHAPTER 2

# AIMS OF THE STUDY

# Chapter 2. Aims of the Study

The overall goal of the present PhD thesis is to assess the effectiveness of different cultural techniques in improving the quality of grape berry. More specifically, under the context of climate change, an unbalance between technological and phenolic maturity of wine grapes has been observed, accompanied by low acidity and high pH. The most possible cause for these undesirable characters of wine is considered the hotter weather condition during grape ripening phase. Therefore, a number of cultural techniques have been proposed to mitigate the negative effects of the warming climate since these techniques can delay the grape ripening so that berries can mature in a relatively cooler conditions. However, this is only a hypothesis. For each technique, its effects on yield components and berry composition should be studied in detail.

To achieve this, a series of experiments have been conducted. Specific objectives and main hypotheses of each experiment are as follows (a, b, c, d and e correspond with Chapter 2, 3, 4, 5 and 6, respectively):

a) To evaluate the effects of leaf area reduction on the relationship between anthocyanins and sugars.

   A three-year experiment was conducted on *Vitis vinifera* cv. Grenache vines. Shoots were severely trimmed after berry set. Grapes from both control and trimming treatments were picked and analyzed at the same level of total soluble solids (TSS). Our main hypothesis was that a severe trimming could reduce greatly the leaf area-to-fruit ratio and postpone the berry ripening to a cooler period. As a consequence, the anthocyanins-to-sugars ratio would be improved.

b) To evaluate the effects of two levels of severe trimming on grape quality for two varieties under two different environmental conditions.

   A two-year experiment was conducted in Logroño and Badarán, on *Vitis vinifera* cv. Tempranillo (early-maturing variety) and *Vitis vinifera* cv. Grenache (middle-to-late-maturing variety) grapes, with and without irrigation, respectively. Three treatments were carried out: control (C), trimming once (T) and trimming twice (TT). Grapes of all the treatments were intended to be picked and analyzed at the same level of TSS. Our hypotheses were: both T and TT could delay the berry ripening significantly, with TT to a greater extent. Under cooler ripening conditions, grapes from T would have a higher acidity content as well as a higher anthocyanins-to-sugars ratio. With relation to TT, their effects might be better than T but there would be another possibility that grapes from TT could not mature properly, especially for the Grenache vines which had no irrigation facilities.

c) To evaluate the effectiveness of minimal pruning (MP) for delaying grape maturity and its impacts on yield components and berry composition.

   Over a period of two years, field parameters and berry composition (at the same level of TSS) were measured on spur-pruning vines and MP vines of Tempranillo. MP vines had not been pruned since 1996. The main hypothesis was that MP could give rise to a higher yield and delay the berry ripening to a cooler period thus improving the anthocyanins-to-sugars ratio.

d) To evaluate the effects of a sustained bunch exposure through a severe basal leaf removal (LR) on the quality of Tempranillo grapes and wines.

   A three-year experiment was conducted on *Vitis vinifera* cv. Tempranillo vines. LR was conducted two weeks after berry set. Berry TSS was measured when the color began to appear. Grapes from both control and LR were picked and analyzed at the same level of TSS. We did not have a preferable hypothesis for this study. Actually, we really hoped to draw a conclusion if full exposure of clusters had any negative effects on berry quality such as sunburn or poor coloration.

e) To evaluate the effects of late winter pruning (LWP) at different moments on yield components and fruit composition, and to determine a phenological stage from which yield loss would be caused by LWP.

   A two-year experiment was conducted on *Vitis vinifera* cv. Maturana vines. During each year, LWP was performed at three phenological stages. Our main hypotheses were: very late winter pruning would delay the berry ripening to a larger extent and reduce vine yield greatly. Anthocyanins-to-sugar ratio would be improved by LWP.

# CHAPTER 3

## LEAF AREA REDUCTION BY TRIMMING, A GROWING TECHNIQUE TO RESTORE THE ANTHOCYANINS : SUGARS RATIO DECOUPLED BY THE WARMING CLIMATE

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# Chapter 3. Leaf area reduction by trimming, a growing technique to restore the anthocyanins : sugars ratio decoupled by the warming climate

F. MARTÍNEZ DE TODA, J. C. SANCHA, W. ZHENG and P. BALDA

## Summary

The objective of this work consists on the evaluation of the leaf area reduction by trimming, as a growing technique to restore the anthocyanins : sugars ratio decoupled by the warming climate.

Severe shoot trimming were done during a 3-year period (2010-2012). Veraison date was delayed around 20 days. By harvesting the grapes with the same level of soluble solids, the trim treatment increased the total anthocyanin content between 8 % and 21 % compared to control. Therefore, and with significant differences every year, it was observed a higher total anthocyanin content as a result of trim treatment. Delaying berry ripening trough reducing leaf area to fruit ratio could partially restore the anthocyanins : sugars ratio disrupted by elevated temperature. Although it is necessary to study other trimmings intensities as well as other times of intervention, the shoot trimming could be a very simple technique to delay berry ripening and compensate the effects of climate warming.

**Keywords**: decoupled anthocyanins : sugars ratio, delayed ripening, trimming

## Introduction

Many vineyards in the world produce high probable alcohol levels, because viticultural techniques have always been designed in order to produce a higher ripeness. Climatic change has also increased the berry ripeness process naturally (Schultz and Jones, 2010) and, during the last few decades, the berry ripeness has been developed earlier.

Several studies show an earlier stage of development in vine phenology during the last few years in every wine growing region (Jones *et al*., 2005; Duchene and Schneider, 2005). As a result of that, berry ripening is taking place during the warmer part of the ripening period (Webb *et al*., 2007, 2008).

In warm climates, grape varieties reach sufficient soluble solids levels in order to obtain high quality wines, but it is not the same regarding the colour (Iland and Gago, 2002). The temperature levels where the sugar enzymes activity is held (8 to 33º C) are different to the colour enzymes activity (17 to 26º C) (Iland and Gago, 2002; Sadras *et al*., 2007). Temperatures above 30º C after veraison could inhibit anthocyanin synthesis (Mori *et al*., 2007).

Sadras and Moran (2012) speculate that this increase in alcohol could be partially explained by the temperature-driven decoupling of anthocyanins and sugars in berries of red wine varieties; if accumulation of sugars is more responsive to temperature than accumulation of anthocyanins, the harvest date delay in order to obtain higher concentration of anthocyanins would be associated with higher sugar concentration and potential alcohol. Their experiments demonstrate that elevated temperature can decouple anthocyanins and sugars in berries in a temperate environment, and that this decoupling is more likely to be caused by a delayed onset in the accumulation of anthocyanins, rather than relative changes in rates.

According to viticultural strategies, the main objective consists on the production of well balanced grapes, with good quality and a lower soluble solids concentration. The grape growing techniques have not been analyzed strongly enough, in our opinion, to tackle this situation. One of the possibilities consists in the berry ripeness delay taking place during cooler seasons (Stoll *et al*., 2009).

Basically, in viticulture there are three very different strategies used for delaying berry ripening: the vineyard location, the varieties and the management practices. This last strategy is the most interesting one because it could be developed in current vineyards without making any substitution of the vines, as is the case of the first two options.

Considering the plant physiology mechanism, there are different growing techniques which should delay the berry ripening. These techniques could be considered in order to be applied, just to improve the adaptation of our vineyards and their ripeness process to a warmer climate.

The ecophysiology characterization research carried out during the last years all over the world, led to establishing the leaf area to fruit ratio as one of the most important viticultural indexes in order to define a well balanced vineyard which could produce high quality grapes and wines. It is considered that the leaf area to fruit ratio should be between 0.8 and 1.2 $m^2$/kg in order to get a good ripeness (Kliewer and Dokoozlian, 2005). If many experiments show the high influence of the leaf area to fruit ratio on bunch characteristics, it would be very interesting to take a look at research concerning delayed ripeness through the variation of that index.

Stoll *et al.* (2009) argue that leaf area reduction through severity trim or leaf plucking treatments (0.8 y 1.4 $m^2$/kg against 1.9 $m^2$/kg on the control), delay berry ripening on Riesling variety for a period between 15 and 20 days. Intrieri and Filippetti (2009) also consider this reduction in leaf area as a very interesting technique to delay berry ripening.

Martínez de Toda and Balda (2013) show that veraison date is delayed 18-20 days as consequence of a single shoot trimming. This delay of 18-20 days on the veraison date comes to compensate the phenological advance that has occurred in the last thirty years in most of the wine growing regions (Jones *et al.*, 2005; Duchene and Schneider, 2005; Stoll *et al.*, 2009). The ripeness delay due to trim practices involves that berry ripeness takes place in a later period with cooler temperatures. So leaf area decrease, as a consequence of the trim treatment, could be useful to obtain a ripeness delay. When the berry ripeness is developed during cooler periods, phenol developement and aroma synthesis are more adequate (Stoll *et al.*, 2009). This hypothesis is very important in warm wine regions.

The main objective of this work consists in the evaluation of the leaf area reduction by trimming as a growing technique to restore the anthocyanins : sugars ratio decoupled by the warming climate.

## MATERIAL AND METHODS

In 2010, the study was conducted in two commercial vineyards of *Vitis vinifera* cv. ‘Grenache’ and ‘Tempranillo’ located in Badarán (42.36 N, -2.81 W, 615 m) and San Vicente (42.56 N, -2.75 W, 503 m) respectively, inside Rioja appellation (North of Spain). Plantation distance was 1.20 m between vines and 2.70 m between rows. In 2011 and 2012 the study was continued only in the vineyard of ‘Grenache’ variety. Both vineyards were planted in 1998 on bush vines, without trellis system, the vine rows were North–South oriented and the vines were pruned to twelve buds per vine on spurs of two buds each. The vineyards were managed, without irrigation, to standard practices according to the region of Rioja appellation.

A severe manual trim was performed, cutting the shoot on the node located above the last bunch. The treatment was carried out after berry set, when the diameter of the berry was 3-4 mm (near the 1st of July for every year).

Each year, two rows were selected and a completely randomized design consisting in three replicates of ten-vine plots per treatment was made: control (non trimmed vines) and trimmed vines after berry set. The two rows selected were different each year.

Veraison date was established, in the ‘Grenache’ vineyard, following phenological stages of Eichorn-Lorenz (Coombe, 1995) on six vines of each experimental treatment; two vines per replicate.

In order to determine the leaf area of the shoot, the Smart method based on discs technique (Smart and Robinson, 1991) was performed. The leaf area of the shoot at harvest time was measured on 15 shoots per treatment, removing the petioles in order to measure the weight according to the leaf surface. Subsequently, that weight was compared with the weight of one hundred discs of known surface, and the leaf area surface per shoot was obtained. The leaf area surface per vine was obtained multiplying the leaf area surface per shoot and the number of shoots per vine.

The harvest date of the different treatments was determined looking for a comparable level of soluble solids, from 21 to 22 º Brix, and was between October 9 and October 12 for the control and between October 20 and October 28 for the trimming treatment in the tree years. At harvest time, on five vines of each replicate (15 vines per treatment), the yield per vine was determined, as well as the number of bunches.

Berry weight was measured on 200 berries of each replicate. After that, each 200 berries sample was crushed manually to obtain the must for the chemical analysis. The soluble solids were analyzed by OIV standard methods (OIV, 2013). Total anthocyanins were analyzed by Iland method (Iland *et al.*, 2004).

Mean comparisons were performed using t Student test ($p=0.05$). The statistical analysis was performed using the statistical package SPSS 15.0 for Windows.

## RESULTS

**Leaf area to fruit ratio and bunch and berry weight.** As Table 1 shows for 'Grenache', the leaf area to fruit ratio ranged from 0.63-1.83 $m^2/kg$ in the control to 0.50-0.80 $m^2/kg$ in the trim treatment. The bunch weight decrease in the trim treatment was similar to the berry weight decrease; both resulted around 10 % lower.

The results obtained in 'Tempranillo' are shown in Table 3. The leaf area to fruit ratio decreased from 1.88 $m^2/kg$ to 0.64 $m^2/kg$. Berry weight decrease was in the same proportion as the bunch weight decrease; they were reduced by 15% in the trim treatment.

**Veraison date.** As Table 2 shows, significant differences at veraison date were observed every year between the trim treatment and the control. The veraison date was delayed 18-20 days for the trim treatment.

**Anthocyanin content.** Table 2 shows the results obtained from anthocyanin content in 'Grenache' for the three years and for the same level of soluble solids. Every year, the total anthocyanins were increased between 8 % and 18 % in the trim treatment.

In 'Tempranillo' (Table 3) the total anthocyanins were increased around 21 % for the same level of soluble solids.

## Discussion

### Leaf area to fruit ratio and bunch and berry weight

It is interesting to note that in 2012 the leaf area to fruit ratio was smaller than in 2010 and 2011, probably due to lower rainfall in 2012. It is expected that the important leaf area to fruit ratio decrease as consequence of trimming should affect grape ripeness process (Kliewer and Dokoozlian, 2005; Stoll *et al.*, 2009). The reduction of berry and bunch weight (between 10 % in 'Grenache' and 15 % in 'Tempranillo') was similar to that found on the experience carried out by Stoll *et al.* (2009). In the same way that conclude Rombola *et al.* (2011), trim treatment revealed to be an attractive approach for controlling yield and a posible alternative to expensive techniques, such as bunch thinning or early defoliation, the latter often enhancing fruit sugar concentration (Tardaguila *et al*., 2010).

## Delaying ripenning

Ripening in the 'Grenache' control vines started at the beginning of September in the three years studied, when mean temperatures were 19º C, the mean maxim temperatures were 26º C and the mean minimun temperatures were 12,5º C (for the area of the experimental vineyard). Nevertheless, the trim treatment began the ripeness period during the second half of September, when mean temperatures reached 16,7º C, the mean maxim temperatures were 23º C and the mean minimun temperatures were 11,2º C . The ripeness delay due to trim practices involved that ripening process taked place in a later period with cooler temperatures. So leaf area decreasing, as a consequence of the trim treatment, could be useful to obtain a ripeness delay. When the berry ripeness is developed during cooler periods, phenol developement and aroma synthesis are more adequate (Stoll *et al*., 2009). This hypothesis is very important in warm wine regions.

## Anthocyanin content

By harvesting the grapes with the same level of soluble solids, the trim treatment had bigger total anthocyanin content than control. Therefore, and with significant differences every year, it was observed a higher total anthocyanin content as a result of trim treatment.

This behavior is surprising since, although the size of the berry was about 10% lower, the leaf area : yield ratio was around 50% lower for the trim treatment. The only explanation that comes to mind is linked to the lower temperature at which berry ripening took place for the trim treatment, due to the delay of twenty days detected for such treatment in comparison with control.

Sadras and Moran (2012) demonstrated that elevated temperatures can decouple anthocyanins and sugars in berries in a temperate environment. Thus, the trend of increasing anthocyanins : sugars ratio for the trim treatment would be due to the delayed ripening period caused by the trimming. Under this assumption, the trimming would be a possible technique for restoring the anthocyanins : sugars ratio decoupled by warming climate. Delaying berry ripening trough manipulating leaf area to fruit ratio could partially restore the anthocyanin : sugar ratio disrupted by elevated temperature of warming climate.

If the decoupling by elevated temperature is more likely to be caused by a delayed onset in the accumulation of anthocyanins, rather than relative changes in rates (Sadras and Moran, 2012), we could conclude that the trim treatment advances the onset of anthocyanin synthesis. Regarding this aspect, it should be noted that the methodology used in this study did not allow to know the onset of anthocyanin synthesis . To resolve this issue, it would be interesting to study the anthocyanins : sugar ratio for each treatment at different times, beginning at veraison. This is one of the aspects we want to study in subsequent works.

In the same way, for further research, it would be very interesting to study other trimmings intensities as well as other times of intervention.

## Conclusions

Leaf area to fruit ratio decrease, through severe trim treatments after berry set, caused an important grape ripeness delay in ‘Grenache’ and ‘Tempranillo’ varieties. The veraison stage was delayed around 20 days. By harvesting the grapes with the same level of soluble solids, the trim treatment had bigger total anthocyanin content than control. Therefore, and with significant differences every year, it was observed a higher total anthocyanin content as a result of trim treatment. Likely, the explanation is linked to the lower temperature at which berry ripening occurs in the trim treatment, due to the delay of twenty days detected for such treatment.

Delaying berry ripening trough reducing leaf area to fruit ratio could partially restore the anthocyanins : sugars ratio disrupted by the elevated temperatures of the warming climate.

For further research, it would be very interesting to study the anthocyanins : sugar ratio for each treatment with other trimmings intensities as well as other times of intervention.

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**Table 1**

**Leaf area, yield, leaf area/yield, bunch weight and berry weight for control and trimming treatment on'Grenache'in the years 2010, 2011 and 2012**

| | | Control | Trimming |
|---|---|---|---|
| 2010 | Leaf area/Yield ($m^2$/kg) | 1.33 a | 0.80 b |
| | Bunch weight (g) | 309 a | 283 b |
| | Berry weight (g) | 1.62 a | 1.48 b |
| 2011 | Leaf area/Yield ($m^2$/kg) | 1.83 a | 0.82 b |
| | Bunch weight (g) | 271 a | 255 b |
| | Berry weight (g) | 1.46 a | 1.37 b |
| 2012 | Leaf area/Yield ($m^2$/kg) | 0.63 a | 0.50 b |
| | Bunch weight (g) | 388 a | 364 b |
| | Berry weight (g) | 1.57 a | 1.46 b |

Different letters across a row show significant differences between values, according to t Student test (P=0.05)

**Table 2**

**Harvesting and veraison dates, soluble solids and anthocyanin content for control and trimming treatment on'Grenache'in the years 2010, 2011 y 2012**

| | | Control | Trimming |
|---|---|---|---|
| 2010 | Harvesting date | Oct 12th | Oct 28th |
| | Veraison date | Sep 1st a | Sep 19th b |
| | Soluble solids (º Brix) | 21.0 a | 21.2 a |
| | Total anthocyanins (mg/g) | 0.70 b | 0.83 a |
| 2011 | Harvesting date | Oct 10th | Oct 20th |
| | Veraison date | Aug 28th a | Sep 15th b |
| | Soluble solids (º Brix) | 21.5 a | 21.5 a |
| | Total anthocyanins (mg/g) | 0.72 b | 0.78 a |
| 2012 | Harvesting date | Oct 9th | Oct 25th |
| | Veraison date | Sep 5th a | Sep 24th b |
| | Soluble solids (º Brix) | 21.5 a | 21.4 a |
| | Total anthocyanins (mg/g) | 0.83 b | 0.93 a |

Different letters across a row show significant differences between values, according to t Student test (P=0.05)

**Table 3**

**Harvesting date, yield components and soluble solids and anthocyanin content for control and trimming treatment on 'Tempranillo' in the year 2010.**

| | Control | Trimming |
|---|---|---|
| Harvesting date | Oct 9th | Oct 25th |
| Leaf area/Yield ($m^2$/kg) | 1.88 a | 0.64 b |
| Bunch weight (g) | 214 a | 181 b |
| Berry weight (g) | 2.18 a | 1.86 b |
| Soluble solids (º Brix) | 21.5 a | 21.4 a |
| Total anthocyanins (mg/g) | 1.36 b | 1.65 a |

Different letters across a row show significant differences between values, according to t Student test (P=0.05)

# CHAPTER 4

## EFFECTS OF SEVERE TRIMMING AFTER FRUIT SET ON THE RIPENING PROCESS AND THE QUALITY OF GRAPES

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# Chapter 4. Effects of severe trimming after fruit set on the ripening process and the quality of grapes

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## Summary

Some cultural techniques have been proposed in order to delay ripening of wine grapes under global warming. This study on two varieties in a two year period (2014-2015), was aimed at evaluating the effects of severe trimming after berry set on delaying grape ripening as well as on grape quality. The experiment was carried out for Tempranillo in an experimental vineyard in Logroño (VL, with irrigation) and for Grenache in a commercial vineyard in Badarán (VB, without irrigation). Both places are within DOC Rioja and in each of them three treatments were carried out: control (C), trimming once (T) and trimming twice (TT). In both vineyards, trimming treatments reduced leaf area (LA) to production (P) ratio (LA/P) significantly and delayed the veraison dates. In VL, relative to C, T and TT delayed the harvest dates by 14 to 23 days, obtaining a comparable level of total soluble solids (TSS) and a similar total anthocyanin concentration (TAC). In VB, T delayed the harvest dates by 16 to 20 days without significant differences in TSS and TAC from C. However, grapes of TT failed to mature properly due to the serious shortage of LA. From an acid perspective, trimming treatments were likely to improve organic acid composition by increasing the tartaric acid and reducing the malic acid, as long as the LA/P was not too low. The relatively cooler ripening condition caused by trimming seemed insufficient for a better anthocyanin synthesis.

**Key words**: climate change, delayed ripening, anthocyanins : sugar ratio, grape acidity

## Introduction

Global warming is an indisputable fact. The most important climate changed-related effects on wine grapes are the advanced harvest times. With increased temperatures and a warmer maturity period, it would be more natural to produce unbalanced wines characterized by high alcohol levels, low acidities, a modified variety aroma and a lack of color (MIRA DE ORDUÑA 2010, PALLIOTTI *et al.* 2014). This last factor is becoming more well known as the decoupling of anthocyanins and sugars for red varieties which is caused by elevated temperatures (SADRAS and MORAN 2012). That is, under warmer climatic conditions, sugar accumulation in berries is very fast while phenol maturity is much slower, being the possible reasons that high temperatures repress anthocyanin synthesis due to the inhibition of some related key enzymes (MOHAVED *et al.* 2011, MORI *et al.* 2007). As the color is one of the most important indicators of the quality of wine, it is necessary to restore the anthocyanins to sugars ratio decoupled by the increasing temperatures. One of the strategies is to delay the berry ripening in order that it takes place under a cooler condition (PALLIOTTI *et al.* 2014, STOLL *et al.* 2010).

For delaying grape ripening, various management techniques have been proposed such as light pruning (SCHULTZ and WEYAND 2005) , post-veraison apical-to-the clusters leaf removal (PALLIOTTI *et al.* 2013), late winter pruning (FRIEND and TROUGHT 2007), late irrigation (FREEMAN *et al.* 1980), application of antitranspirants (FILIPPETTI *et al.* 2011), double pruning (GU *et al.* 2012) and shoot trimming (FILIPPETTI *et al.* 2011, MARTÍNEZ DE TODA *et al.* 2014).

Among these cultural techniques, shoot trimming has been one of the grower's favorite approaches because of its ease of operation and immediate effect (WOLF *et al.* 1990). It consists of removing shoot tips and a number of young leaves on the abscised part (KELLER 2015). From the physiological point of view, it does not only involve the removal of a substantial source of auxin, but also the removal of a major sink for nutrients and energy and also the reduction of the active leaf area (LA) thus reducing total photosynthesis. Trimming stimulates one to several lateral shoots to develop below the cutting point (MARTÍNEZ DE TODA 1991, WOLF *et al.* 1986) and the growth of lateral shoots is highly influenced by the timing of the first trimming (MOLITOR *et al.* 2014). Conventionally, shoot trimming was mainly used for balancing vine shoot vigor, improving the microclimate of the canopy and providing convenience for mechanized operation (MARTÍNEZ DE TODA 1991). However, trimming could exert more effects depending on its timing and intensity. Before flowering, a mild trimming (15 nodes left) did not diminish the leaf area to fruit ratio significantly, thus it gave similar yield components and must composition to untrimmed vines (PONI *et al.* 2014). Trimming during flowering was reported to improve fruit set (COLLINS and DRY 2009, COOMBE 1970) and besides, since the new laterals, which are stimulated by trimming, would have a bigger functional foliage during the season, berry ripening could be advanced (PONI *et al.* 1994); however, berries could not mature properly if the trimming is too severe with only 6 nodes left and with all laterals cut (PONI and GIACHINO 2000). Early trimming (one week after bloom) at 9-10th node increased yield and total soluble solids (TSS) while reducing acidity for most of the experimental varieties (CARTECHINI *et al.* 2000); this was also confirmed by a study conducted in Turkey for Karasakız grape (DARDENIZ *et al.* 2008), though in the same study it was shown that a severe trimming (one node left above the last cluster) at this time resulted in lower yield and berry quality. Between blooming and veraison, MARTÍNEZ DE TODA *et al.* (2013) reported a significant reduction in TSS and pH for Grenache grapes from vines which were severely trimmed soon after berry set while WOLF *et al.* (1990) found that a light trimming 30 days after bloom lead to higher production and more TSS. Postveraison severe trimming could reduce sugar accumulation without affecting anthocyanin concentration (FILIPPETTI *et al.* 2011, HERRERA *et al.* 2015, ROMBOLÀ *et al.* 2011). Similarly, a very recent study of BONDADA *et al.* (2016) demonstrated that post-veraison severe trimming lowered yield, TSS, pH and cluster compactness without reducing total anthocyanins.

From above, it is not difficult to infer that whenever a severe trimming is carried out, a delay in berry ripening is likely to occur. However, an early trimming (before fruit set) usually affects the percentage of fruit set thus affects the yield. On the other hand, a late severe trimming (after veraison) causes an irretrievable reduction in leaf area since fewer laterals could be generated at this time and its effect occurs only on the final stage of development of the grape. Therefore, we consider “one week after berry set (when the diameter of berry is 3-4 mm)” to be the optimal moment to experiment the severe trimming, since the development of the berry will be affected during the whole period of berry growth; this means that the berry development would be maximally influenced by the trimming. MARTÍNEZ DE TODA *et al.* (2014) reported that a severe shoot trimming at this moment successfully delayed the harvest date of Grenache by two weeks, maintaining the grapes with the same TSS and a higher anthocyanin concentration relative to those from untrimmed vines. Nonetheless, this increase in anthocyanins was not observed in a similar study for Tempranillo (SANTESTEBAN *et al.* 2016).

The aim of this study was to evaluate the effect of severe shoot trimming after berry set on the grape ripening process, in two different varieties, especially its impact on the anthocyanins to sugars ratio as well as on the acid components.

## Materials and Methods

The study was conducted in two vineyards within Rioja appellation, North of Spain. One was an experimental vineyard, in the University of la Rioja, located in Logroño (42°27′N, 2°25′W, 370 m.a.s.l.) with the variety of *Vitis vinifera* "Tempranillo" (clone CL-306 grafted onto 110-R rootstock) which was planted in 2010 (the vineyard in Logroño is abbreviated to VL). Vine rows were north-south oriented with a planting pattern of 1.2 m (within row) × 2.4 m (between rows). Vines were trained to vertical shoot positioning with two arms and pruned to six spurs (12 buds) per vine. In 2014, the vineyard received a drip irrigation during one and a half months with an average amount of 4.5L/vine/day from mid-July when strong water stress was observed. In 2015, the irrigation was two weeks in advance due to an enduring heat wave starting from the end of June till the end of August. Four rows were selected for the study and each of them had 28 vines. For both years, a severe shoot trimming was performed on 2/3 of the vines in each row when the diameter of the berries was 3-4 mm. 4 weeks later, a second severe trimming was carried out on half of the trimmed vines per row to strictly maintain a low LA. The rest of the vines of each row served as the control treatment, on which only slight shoot topping was carried out to facilitate the field work. Therefore, for each of the rows, three different treatments were randomly applied: control (C), trimming once (T) and trimming twice (TT); The 4 rows served as 4 replicates and each treatment was applied to the same vines in both years. For T, the height of the canopy was cut to about 50 cm high; For TT, the second trimming cut the canopy height back to 50 cm. We kept this canopy height instead of keeping a designable number of nodes because in this way we could simulate the mechanic trimming which would be more practical. Another vineyard was a commercial one of *Vitis vinifera* "Grenache" which is situated in Badaran (42°22′N, 2°49′W, 615 m.a.s.l.; the vineyard was planted in 1998, abbreviated to VB). Vine rows were north-south oriented with the plantation distance being 1.20 m between vines and 2.70 m between rows. The vines were trained by traditional gobelet without trellis system and pruned to 12 buds per vine as well. There were no irrigation facilities in VB. The treatments were totally the same as VL; VB was managed in accordance with standard viticulture practices of Rioja appellation.

Veraison date was recorded when 50% of the berries began to show color. The Smart method (SMART and ROBINSON 1991) was used to estimate LA per shoot; LA per vine was obtained by multiplying LA per shoot and the number of shoots per vine. Yield and final LA were determined at harvest. In both vineyards, grapes from each treatment were attempted to harvest at a similar TSS level. For each replicate, 200 berries were collected for the determination of berry weight. TSS, titratable acidity (TA), pH, tartaric acid and malic acid were all measured based on the OIV standard methods (OIV 2013) and total anthocyanins were determined according to Iland method (ILAND 2004). Total anthocyanins were expressed both as concentration (mg/g berry fresh mass) and as anthocyanin content (mg anthocyanins /berry); the former value would relate closely to wine color while the latter one could reflect the anthocyanin content of a single berry.

In VL, during the maturing period of the vintage 2014, TSS and anthocyanins were measured every ten days or a week in order to evaluate the evolution of both parameters with time and also to establish relationship between themselves. In 2015, this work was conducted as well yet with less frequency. Original climate data were provided by the nearest meteorological stations located in Logroño, for VL and Villar de Torre, for VB.

SPSS 16.0 for windows was used for statistic analysis. In both vineyards, data was analyzed year by year. One-way analysis of variance (Anova) was performed and in the case of the existence of significant differences, the mean separation was carried out with $p<0.05$ using S-N-K method when equal variance assumed and otherwise Dunnett's T3.

## Results

### *Weather conditions*

2014 had a relatively cool Summer but an extremely warm September and October (Figure 1). Besides that, there was an unusually large amount of rainfall throughout September (data is not shown). In 2015, on the contrary, it should be noted there was a hot Spring and Summer as well as a long-lasting heat wave between fruit set and veraison. However, during ripening stage, the temperatures were lower than the previous years' average.

In VL, from veraison to harvest, the mean temperatures for C, T and TT were 20.6°C, 19.8°C and 19.3°C, respectively, in 2014; 20.8 °C, 19.5°C and 19.3°C, respectively, in 2015. In VB, from veraison to harvest, the mean temperatures for C, T and TT were 17.8°C, 16.0°C and 15.5°C, respectively, in 2014; 16.7°C, 14.4°C and 13.5°C, respectively, in 2015.

### *Field parameters and yield components*

Results related to field parameters and yield components are shown in Table 1 and Table 2, for Tempranillo in VL and for Grenache in VB, respectively.

In VL, compared to C, T delayed the veraison date by 3-5 days while TT delayed it by 4-8 days. Grapes of C reached the designate TSS level (22-22.5 Brix) 14-23 days earlier than T and 21-23 days earlier than TT. In 2014, trimming treatments lead to a higher berry weight while in 2015 this trend was not observed. Both cluster weight and production were not significantly affected by trimming treatments. With respect to LA/P, trimming gave rise to a significant reduction in both years, however, there was little difference between T and TT.

In VB, veraison dates were delayed to a large extent by trimming treatments, 13 days by T and 15-18 days by TT. In 2014, grapes from C group were harvested at 24 Brix on Oct 1st and, 20 days later, grapes of T reached a similar TSS level. However, another week later, grapes of TT had still not reached the same level of maturity when botrytis began to occur. Thus, grapes were harvested and must was analyzed at a lower Brix. In 2015, despite the fact that a lower harvest Brix (23 Brix) was set, grapes of TT were unable to ripen properly even at the end of October; Once again, must of TT was analyzed at a lower Brix than C and T. For both years, trimming treatments did not alter any of the yield components in spite of the significant lower LA/P values.

### *Brix and anthocyanins evolution*

As seen in Figure 2, grapes of C always contained a higher sugar concentration than T and TT during maturation stage. However, their patterns of sugar accumulation were quite similar. As the harvest approached, the difference in TSS between T and TT became smaller and smaller. The rates of accumulation of anthocyanins relative to sugar were almost the same in 2014 among treatments. However, in 2015, for every one unit increment of SS, T and TT seemed to accumulate slightly more anthocyanins.

### *Must composition*

In 2014, for Tempranillo grapes in VL, trimming treatments managed to maintain a relatively high TA at harvest, in particular T (Table 3), which was probably due to the high concentration of tartaric acid. However, grapes of C contained more malic acid, which also occurred in 2015, though in this vintage no other differences among treatments were observed from the acid point of view. As to the concentration of anthocyanins, there were no significant differences in either of the two years, as well as the anthocyanin content per berry.

In VB, T gave rise to a higher TA, a higher concentration of tartaric acid and a lower concentration of malic acid relative to C and TT in 2014 (Table 4). In 2015, C lead to more TA, followed by TT, and T had the least. Grapes of C also contained significantly more tartaric acid than T and TT; In regard to the concentration of malic acid, C and TT were significantly higher than T. In both years, grapes of TT accumulated much less anthocyanins than C; however, no difference in this regard was obtained between C and T.

## Discussion

### *Field parameters and yield components*

In all cases of our experiments, trimming treatments delayed both veraison date and harvest date without exception. However, for Grenache in VB, this delay was much larger and as a negative and unexpected result, grapes of TT did not achieve the same maturity as C, the obvious reason being that TT in VB had little LA during most of the time in the growing season. In 2014, 0.70 $m^2$/kg of LA/P was not too low but the given harvested TSS (24 Brix) might be too high for TT; In 2015, the excessively low value of LA/P (0.29 $m^2$/kg) made a proper ripening impossible. Moreover, different from VL, T and TT in VB had a big gap in LA/P in both years, which is probably due to the availability of irrigation system, since in VL water was always applied from before veraison and in VB the only available water was the rainfall. In 2015, the two-week heat wave right after the first trimming left the plants with severe water stress; as a consequence, the recovery of LA after trimming in VB was badly impacted, especially TT. It is also worth mentioning that different varieties might have different capacity of producing lateral shoots (CARTECHINI *et al.* 2000) thus the evolution of LA after trimming might vary with varieties, however, this point is beyond the scope of this research.

Reduction in berry weight due to trimming was not found (in VL, the size of berries of C was even smaller than T and TT in 2014). This is contradictory to the studies of MARTÍNEZ DE TODA *et al.* (2013) and STOLL *et al.* (2010), which stated that trimming could reduce berry size.

### *Brix and anthocyanins evolution*

The high similarity of the sugar accumulation trend during maturation stage in VL indicated that LA of T and TT was not a limiting factor for TSS from at least one month and a half before harvest. The velocity of sugar accumulation might basically depend on the temperatures during this stage. It is worthwhile to note that, the TSS of C, in the majority of the times samples were taken, had less variation (standard deviation) than T and TT, which indicated that grapes of C had a better homogeneity in this regard while ripening.

The relationship between anthocyanins concentration and TSS was quite consistent among treatments and the correlation was very close. Trimming treatments hardly changed the accumulation rate of anthocyanins to TSS. In 2015, the fitted regression lines of T and TT had a slightly steeper slope than C, but it would be too arbitrary to draw a conclusion that trimming helped to improve anthocyanins accumulation, as in the end there was no significant difference in anthocyanins concentration among treatments.

### *Must composition*

In both vineyards, for both vintages, trimming treatments considerably reduced the concentration of malic acid, probably because of a greater loss caused by respiration during a longer ripening period. However, interestingly, in VB, TT gave rise to more malic acid than T in both years, the explanation for this might be that grapes of TT were not as ripe as those of T and C, since malic acid levels are closely dependent on the maturity and the temperatures (MIRA DE ORDUÑA 2010). In VL, trimming treatments tended to increase the concentration of tartaric acid, though in 2015 it was not significant. We attribute this increase to the difference in leaf age between treatments: tartaric acid is mainly synthesized between bloom and veraison in both leaves and berries (KELLER 2015), and its synthesis in leaves mainly occurs when the leaves are expanding (RUFFNER 1982). Therefore, with the occurrence of lateral shoots after trimming, vines subjected to trimming treatments could produce more tartaric acid. In VB, T also helped to improve tartaric acid in 2014; however, grapes of TT had the lowest tartaric acid in both years, as well as those of T in 2015, which is likely to be attributed to the weak growth of the lateral shoots and the subsequent low LA. Furthermore, it could be speculated that trimming treatments might contribute to a better organic acid composition (e.g. a higher tartaric to malic ratio) on condition that the value of LA/P is not too low. Regarding pH, in both sites, our results were not consistent between years so further study is required in this regard.

In VL, despite grapes of T and TT ripened under relatively cooler conditions, the absence of any significant difference in anthocyanins between C and trimming treatments indicated that tiny differences in temperatures during ripening period were unlikely to affect the anthocyanin concentration. MORI *et al.* (2007) and MORI *et al.* (2005) showed that both diurnal and nocturnal higher temperatures reduced anthocyanins content due to the inhibition of relevant synthetases as well as anthocyanins degradation. However, their experiments were conducted under artificial conditions and the differences in temperatures between treatments were enormous (∆T = 10°C or 15°C). MARTÍNEZ DE TODA *et al.* (2014) reported an increase in anthocyanins for grapes from trimmed Grenache vines, the gap of daily mean temperatures between treatments was as much as 2.3°C, bigger than in our case (for T, ∆T ≈ 1.0°C; for TT, ∆T ≈ 1.4°C). However, in VB, though grapes of T were ripening under a cooler daily mean temperature than C (∆T ≈ 2.1°C), still no increase in anthocyanins was observed. TT reduced anthocyanin concentration because of the lower level of TSS relative to C and T, since sugar content is the decisive factor of anthocyanin content (PIRIE and MULLINS 1977).

Comparing the values of anthocyanin concentration between the two years, it is obvious that both Tempranillo and Grenache had more anthocyanins in 2014 than in 2015. This is immediately surprising because in both sites, the daily mean temperatures during the ripening period of 2014 were equal to (in VL) or higher (in VB) than those in 2015.

However, we should not ignore the extremely high temperatures before veraison in 2015: as it is shown in Figure 1, the average temperatures in June and July of 2015 were about 1.0°C and 2.8°C higher than those of 2014. These high temperatures before veraison might greatly delay the onset of anthocyanin accumulation and decouple the anthocyanins to sugar ratio, which was also speculated by SADRAS AND MORAN (2012).

## Conclusion

The severe shoot trimming after fruit set could delay berry ripening and create a relatively cooler maturation condition. Under Rioja viticultural conditions, the trimming treatments delay ripening but they are able to mature properly the grapes. Moreover, trimming treatments could give rise to a better organic acid composition than control treatment by increasing the tartaric acid while reducing the malic acid. During ripening, the differences in temperatures among treatments were so limited that the accumulation of anthocyanins was unlikely to be improved by trimming. Further studies should be focused on different dates and intensities of trimming and combining trimming with other cultural practices such as late winter pruning, in order to delay the berry ripening to a greater extent and to create a considerably cooler ripening conditions which might be in favor of the accumulation of anthocyanins.

## Acknowledgements

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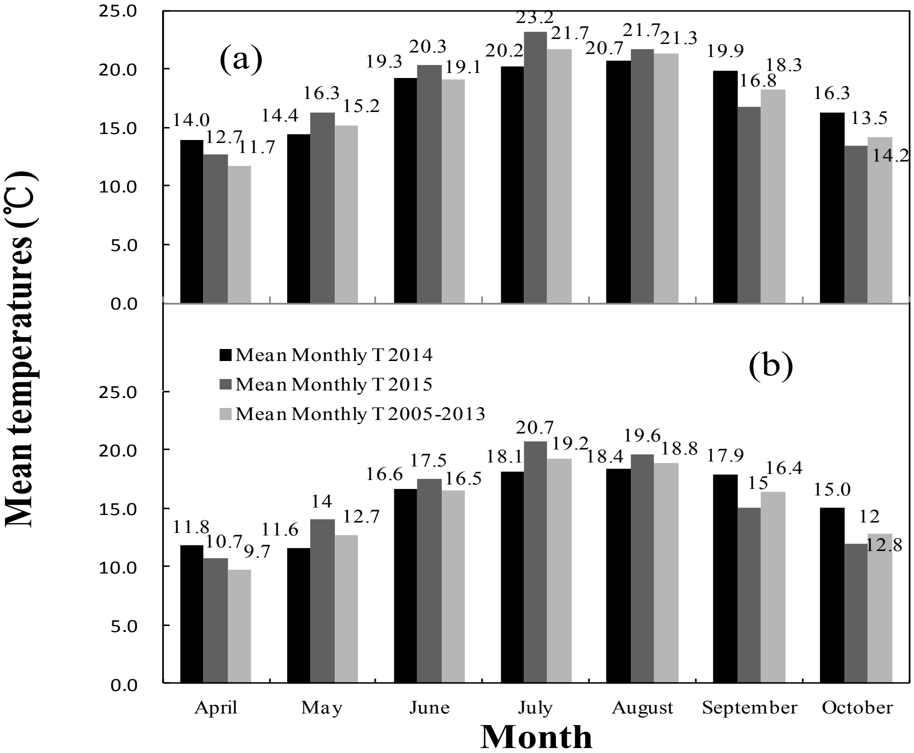

**Fig. 1** Mean monthly temperatures during growing seasons in (a) Logroño and (b) Villar de Torre.

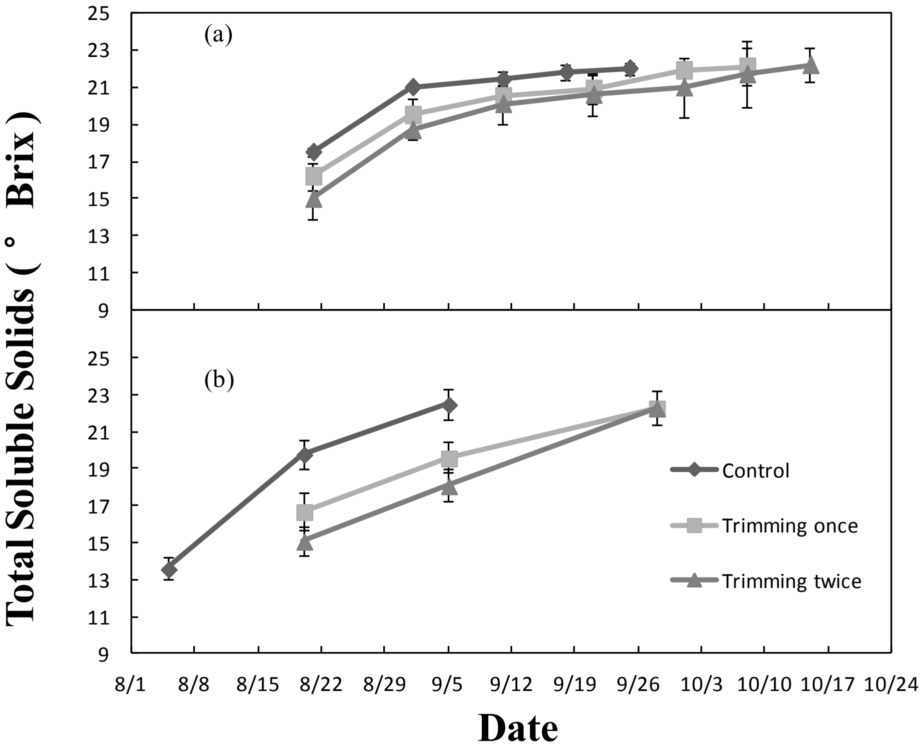

**Fig. 2** Accumulation of total soluble solids (° Brix) over time during ripening period of (a) 2014 and (b) 2015, in Tempranillo berries in the experimental vineyard of Logroño (mean ± standard deviation).

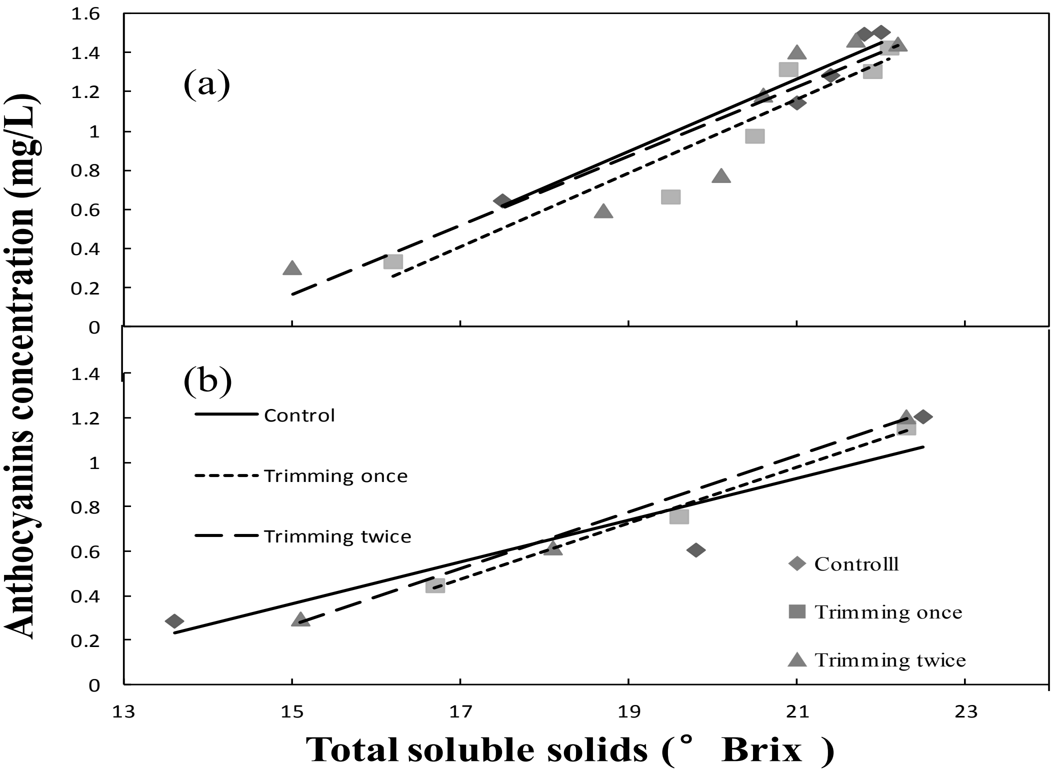

**Fig. 3** The relationship between anthocyanins concentration (mg/L) and total soluble solids (° Brix) in Tempranillo berries in the experimental vineyard of Logroño, during ripening period of (a) 2014 and (b) 2015. In 2014, $R^2$ of the regression lines for Control, trimming once and trimming twice were 0.94, 0.90 and 0.85, respectively; In 2015, they were 0.85, 0.99 and 0.99, respectively.

**Table 1** Effects of trimming once (T) and trimming twice (TT) on yield components for Tempranillo vines (2014 and 2015, Logroño, La Rioja, Spain)

| | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Control | T | TT | Significance level[a] | Control | T | TT | Significance level |
| Veraison date | 8/4 | 8/7 | 8/8 | | 7/28 | 8/2 | 8/5 | |
| Harvest date | 9/25 | 10/8 | 10/15 | | 9/5 | 9/28 | 9/28 | |
| Cluster weight (g) | 175 | 160 | 166 | ns | 266 | 307 | 282 | ns |
| Berry weight (g) | 1.54 b | 1.67 a | 1.68 a | ** | 1.87 | 1.88 | 1.73 | ns |
| Production (P) (kg/vine) | 2.89 | 2.75 | 2.50 | ns | 4.95 | 5.12 | 4.71 | ns |
| Leaf area (LA) ($m^2$/vine) | 3.82 a | 1.86 b | 1.54 b | *** | 7.45 a | 3.25 b | 2.89 b | *** |
| LA/P ($m^2$/kg) | 1.37 a | 0.70 b | 0.58 b | * | 1.54 a | 0.63 b | 0.61 b | *** |

[a] Data were analyzed with one way Anova; *,**, ***, ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at $p \leq 0.05$.

**Table 2** Effects of trimming once (T) and trimming twice (TT) on yield components for Tempranillo vines (2014 and 2015, Badarán, La Rioja, Spain)

| | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Control | T | TT | Significance level[a] | Control | T | TT | Significance level |
| Veraison date | 8/29 | 9/11 | 9/16 | | 8/21 | 9/2 | 9/5 | |
| Harvest date | 10/1 | 10/21 | 10/28 | | 9/29 | 10/14 | 10/31 | |
| Cluster weight (g) | 178 | 160 | 172 | ns | 240 | 272 | 264 | ns |
| Berry weight (g) | 1.81 | 1.55 | 1.53 | ns | 1.75 | 1.92 | 1.75 | ns |
| Production (P) (kg/vine) | 2.58 | 2.40 | 2.82 | ns | 4.35 | 4.89 | 4.76 | ns |
| Leaf area (LA) ($m^2$/vine) | 4.66 a | 2.09 b | 2.02 b | *** | 5.57 a | 2.98 b | 0.61 b | *** |
| LA/P ($m^2$/kg) | 1.99 a | 0.84 b | 0.70 b | *** | 1.28 a | 0.61 b | 0.29 c | *** |

[a] Data were analyzed with one way Anova; ***, ns: significant at $p \leq 0.001$ or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at $p \leq 0.05$.

**Table 3** Effects of trimming once (T) and trimming twice (TT) on must composition for Tempranillo vines
(2014 and 2015, Logroño, La Rioja, Spain)

| | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Control | T | TT | Significance level[a] | Control | T | TT | Significance level |
| Brix at harvest (°) | 22.0 | 22.1 | 22.2 | ns | 22.5 | 22.3 | 22.3 | ns |
| Titratable acidity (g/L)[b] | 3.45 c | 4.3 a | 4.1 b | *** | 5.15 | 4.90 | 4.90 | ns |
| pH | 4.12 a | 3.98 b | 4.02 b | ** | 3.52 | 3.55 | 3.55 | ns |
| Tartaric acid (g/L) | 4.2 b | 4.9 a | 5.0 a | *** | 4.2 | 4.4 | 4.4 | ns |
| Malic acid (g/L) | 3.5 a | 3.1 b | 3.0 b | * | 4.2 a | 3.8 b | 3.9 b | * |
| Anthocyanin concentration (mg/g) | 1.51 | 1.43 | 1.45 | ns | 1.21 | 1.16 | 1.21 | ns |
| Anthocyanin content (mg/berry) | 2.33 | 2.39 | 2.44 | ns | 2.18 | 2.18 | 2.14 | ns |

[a] Data were analyzed with one way Anova; *,**, ***, ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at $p \leq 0.05$.

[b] The titratable acidity is expressed as g/L tartaric acid.

**Table 4** Effects of trimming once (T) and trimming twice (TT) on must composition for Grenache vines (2014 and 2015, Badarán, La Rioja, Spain)

| | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Control | T | TT | Significance level[a] | Control | T | TT | Significance level |
| Brix at harvest (°) | 24.3 a | 23.7 a | 21.8 b | *** | 23.1 a | 22.8 a | 20.9 b | * |
| Titratable acidity (g/L) [b] | 4.85 b | 5.05 a | 4.75 b | * | 7.77 a | 6.04 c | 7.02 b | *** |
| pH | 3.43 | 3.43 | 3.48 | ns | 2.97 b | 3.16 a | 3.14 a | *** |
| Tartaric acid (g/L) | 7.3 b | 7.6 a | 6.7 c | *** | 6.3 a | 5.2 b | 5.6 b | ** |
| Malic acid (g/L) | 1.45 a | 1.00 b | 1.35 a | *** | 2.5 a | 2.0 b | 2.7 a | * |
| Anthocyanin concentration (mg/g) | 1.44 a | 1.37 a | 0.80 b | *** | 0.67 a | 0.56 a | 0.30 b | *** |
| Anthocyanin content (mg/berry) | 2.60 a | 2.12 a | 1.22 b | *** | 1.18 a | 1.08 a | 0.53 b | *** |

[a] Data were analyzed with one way Anova; *,**, ***, ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at $p \leq 0.05$.

[b] The titratable acidity is expressed as g/L tartaric acid.

# CHAPTER 5

## USE OF MINIMAL PRUNING TO DELAY FRUIT MATURITY AND IMPROVE BERRY COMPOSITION UNDER CLIMATE CHANGE

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# Chapter 5. Minimal Pruning as a Tool to Delay Fruit Maturity and to Improve Berry Composition Under Climate Change

Wei Zheng, Vittorio del Galdo, Jesús García, Pedro Balda and Fernando Martínez de Toda

**Abstract.** Minimal pruning (MP) is considered a viable technique to reduce labor costs and produce high quality wine grapes. To evaluate its effects on grapes cultivated in warm areas, a long-term study on Tempranillo (*Vitis vinifera* L.) was conducted in Badarán (La Rioja, Spain). For each vintage between 1999 and 2013, grapes from MP vines and those conventionally hand pruned (CHP) were evaluated for yield and total soluble solids (TSS). On this basis, from 2014, a further study was initiated in which grapes were analyzed at the same TSS to verify the effects of MP on fruit maturation and to determine the effects of MP on fruit quality. The long-term study showed that MP increased yield by 56% and reduced TSS by 9% compared to CHP. Results from 2014 and 2015 demonstrated that MP delayed fruit maturity (22 Brix) by ≈ 17 days. At the same TSS level (22 Brix), MP had lower berry weight by 24% and cluster weight by 57%, and increased yield by 51%. Must from MP fruit had higher total anthocyanin concentration (+17% in 2014 and +21% in 2015). However, this improvement in potential wine color was more likely due to smaller berry size rather than higher anthocyanin synthesis per unit area of berry skin. The study indicates that MP can effectively delay berry ripening and help to improve potential wine color.

**Key words:** minimal pruning, ripening delay, anthocyanin, climate change, berry quality

Climate change models predict an average warming in global wine producing regions of 2°C in the next 50 yr (Jones et al. 2005). Under this trend, the greatest problems faced by the wine industry are a decoupling of phenolic and technological maturities of grapes, and excessively high alcohol contents in wine, especially in warm areas such as in Spain (Martínez de Toda et al. 2013).

Anthocyanins are an important component of red wine grape quality. In most cases, factors that favor carbohydrate accumulation also contribute to anthocyanin synthesis, especially in the first 5 weeks after veraison when this correlation is high (Pirie and Mullins 1977). However, high temperatures during berry development can delay the onset of anthocyanin accumulation ultimately leading to low levels at harvest (Sadras and Moran 2012). During berry maturation high temperatures can also cause inhibition of some key biosynthesis enzymes as well as anthocyanin degradation (Mori et al. 2005, 2007). In addition, high temperatures can accelerate grapevine phenological stages (Keller 2010) leading to a decoupling of phenolic and technological maturities (i.e. sugar concentration, titratable acidity and pH of the grape juice). While sugar accumulation becomes earlier and more rapid during a warmer period of the growing season phenolic accumulation is inhibited and berry anthocyanin concentration may not reach a desirable level at harvest. The combination of high TSS and low acidity can produce high-alcohol, unbalanced wine.

For an established vineyard, the negative effects of global warming on fruit maturation could be mitigated by adopting cultural techniques that delay maturation, such as shoot trimming (Martinez de Toda et al. 2013, Palliotti et al. 2014), post-veraison distal leaf removal (Palliotti et al. 2013), late winter pruning (Palliotti et al. 2014), double pruning (Gu et al. 2012), and minimal pruning (MP). Research over 30 years in Australia showed that traditional severe pruning was unnecessary in some viticultural regions and can produce wines of low quality due to the development of shaded, tight clusters with large berries, and difficulties in the control of pests and diseases (Clingeleffer 2010). MP, in most cases, produces higher yields than does hand pruning (Martinez de Toda and Sancha 1998, Morris and Cawthon 1981, Reynolds 1988, Schultz and Weyand 2005), and improves canopy light and vine health conditions by reducing vine vigor (Archer and van Schalkwyk 2007, Clingeleffer 2010). Low bud fruitfulness, small clusters and low berry weight are yield components associated with MP (Bates and Walter-Peterson 2008). While MP can save labor and reduce management costs, it does not perform well for some late ripening cultivars, especially in cool and high-rainfall areas (Schwab 2005). Without crop adjustment, MP tends to over-crop leading to delayed or insufficient ripening (Bates and Morris 2009, Morris and Cawthon 1981), although this effect can be lessened by trimming low-hanging fruiting canes or by applying mechanical crop thinning 20-30 days after bloom (Poni et al. 2000). The propensity for MP to delay maturation could, on the other hand, make it effective for counteracting the effects of climate warming, leading to enhanced accumulation of berry anthocyanins and acidity maintenance. Archer and van Schalkwyk (2007) found that MP resulted in better color in berry skins and in wines with a similar alcoholic level. Holt et al. (2008) found that grapes from mechanically-pruned vines consistently had higher anthocyanin concentration and content than those from cane or spur pruned vines. Based on 30 years of experience in Australia, Clingeleffer (2010) concluded that grapes from minimally pruned vines generally produce greater wine color. In contrast, Morris and Cawthon (1981) found that continuous mechanical pruning led to low TSS and poor color. Similarly Rousseau et al. (2012) found a lower color intensity in wines from MP vines than traditionally-pruned vines.

The main goal of this study was to evaluate the effects of MP on delaying grape maturity under the conditions of the La Rioja Valley in northern Spain. Another goal was to assess the effects of MP on fruit quality including the relationship between berry anthocyanin and TSS.

## Materials and Methods

**Plant material and growth conditions.** The study was conducted in a commercial vineyard of *Vitis vinifera* cv. Tempranillo located in Badarán (42°22′4.4″N, 2°48′33.2″W, 620 m.a.s.l.), La Rioja in northern Spain. The vineyard was planted in 1986, on 41-B rootstock. Spacing was 1.1 x 2.6 m (vine x row) in north-south oriented rows with a density of 3500 vines/ha. The minimal pruning (MP) treatment was applied to vines that originally had a spur pruned free-horizontal cordon (without shoot positioning) at a height of 150 cm, but had not been pruned since 1996. Every 3 or 4 yr, MP vines were subjected to a regular shape maintenance by mechanical trimming to prevent shoots from contacting to the ground and from excessive extension. The most recent trimming was carried out in the summer of 2015. The control (conventional hand pruning, CHP) vines were trained in the traditional gobelet (2-3 arms per vine) and were pruned to 12 buds per vine. The vineyard was subjected to the common viticultural practices in the region. Original climatic data was provided by the nearest meteorological station situated in Villar de Torre. Mean monthly temperatures from 2005 to 2013 were calculated and served as normal monthly average temperatures.

**Experimental design and measurement of variables.** The experiment was conducted in two rows that accommodated a completely randomized design consisting of three replicates of 10-vine plots per pruning treatment which included conventionally hand pruning (CHP) and MP. From 1999 (3 yr after the establishment of MP), for each vintage, CHP and MP grapes were harvested at the same time. Yield and berry juice soluble solids (SS) were measured each year.

In 2014 and 2015, grapes of the two treatments were analyzed at the same TSS level (22 Brix). Veraison date was recorded when 50% the berries began to show color. The maturity was monitored during the entire ripening phase. To estimate leaf area per shoot, the method based on leaf disc sampling was used (Smart and Robinson 1991). 15 shoots per treatment were taken for the measurement. For each of them, the weight of all the leaves (without leaf petioles) and the weight of 100 3.80-cm2 discs were used to estimate leaf area (cm2) per shoot as their quotient X 380. The fruit was harvested when TSS averaged 22 Brix. Yield, clusters per vine and shoots per vine were determined on five vines per plot (15 vines per treatment). Cluster weight was measured on five clusters per treatment replicate. Berry weight was measured on 200 berries per replicate sampled randomly from the harvested fruit. Subsequently, each 200- berry sample was crushed manually to obtain juice for chemical analysis. TSS, pH, titratable acidity (TA), tartaric acid and malic acid were analyzed by standard methods (OIV 2013). Total anthocyanins were determined at 22 Brix according to Iland et al. (2004). Total anthocyanins were expressed by concentration (mg/g berry fresh mass) as well as by anthocyanin "density" (mg anthocyanins /cm$^2$ grape skin surface); the former value indicates the potential wine color while the latter one reflects the anthocyanin synthesis capacity of the grape skins.

Yield and TSS data of the long-term trial were analyzed with a paired-samples t-test ($p=0.05$). Data of 2014 and 2015 was tested for homogeneity of variance using Levene's test and were subjected to two-way (pruning method x year) analysis of variance (ANOVA), using the general linear model and F-test; since interaction between treatments and years was observed for some of the parameters, pruning systems were also analyzed as one way ANOVA for each year. The statistical analysis was performed using statistical package SPSS 16.0 (SPSS Inc., Chicago, US) for Windows.

## Results and Discussion

**Long-term observations.** From 1999 to 2013, yield was higher in response to MP than to CHP (15300 kg/ha vs 9800 kg/ha), which is in agreement with a 10-year MP experiment with Riesling in Geisenheim, Germany (Schultz and Weyand 2005), in which MP led to 25%-75% higher yield. Since the grapes were always harvested at the same time, compared with CHP (20.2 Brix on average), MP grapes had lower TSS (18.4 Brix on average), which would produce a wine with a potential alcohol content of 10.8%. This level was too low for most winemakers 10 yr ago, however, it has become acceptable nowadays with a growing demand of low-alcohol wines. This long-term observation is mostly consistent with a previous study performed in the same region with Grenache (Martinez de Toda and Sancha 1998). It can be concluded that MP is viable as a labor-saving growing technique for certain cultivars under the viticultural conditions of the Rioja wine region.

**Weather conditions.** The 2014 vintage had an unusually warm September and October when the grapes matured (Figure 1). In comparison, the weather in 2015 was unusually hot from May through July however September and October were relatively cool.

**Yield components.** MP effectively delayed veraison by 1-2 weeks (Table 1). In 2014, MP increased yield by 77% as compared to CHP, whereas yield per vine was not affected by the pruning treatments in 2015. MP vines had 10-11 times more shoots but only 20%-40% of them bore fruit compared with 100% of shoots on CHP vines. Berry weight was 12% to 35% lower and the number of berries per cluster was 47% to 53% lower in response to MP compared with CHP. These effects of MP on yield components are consistent with previous studies (Bates and Walter-Peterson 2008, Poni et al. 2000, Schultz and Weyand 2005).

Often the ratio of leaf area to fruit production (LA/P) is used to assess potential berry maturation and quality. Normally, the LA/P required during maturation should range from 0.8 to 1.2 $m^2$/kg (Kliewer and Dokoozlian 2005), according to which, in both years, MP had enough LA to support fruit ripening. However, almost all the expected attributes of MP (delayed veraison, delayed TSS accumulation, lower berry weight, fewer berries per cluster, etc.) were found. Champagnol (1984) found that clusters are mainly supported by leaves on the same shoot, although nutrient transfer from other shoots occurs during maturation. In this study, as is typical, MP vines had many non-fruiting shoots whose leaves could contribute only indirectly to berry composition. In addition, due to more retained buds and earlier budburst, MP vines develop canopies more quickly than do conventionally pruned vines (Lakso, 1993). In this study shoots had fewer leaves on MP vines (10, on average) than on CHP vines (> 15). Final canopy size was attained earlier by MP vines than CHP vines which continued to generate new leaves and lateral shoots.

Poni et al. (1994) reported that leaves normally reached maximum photosynthetic capacity at 30-35 days of age. From about 50 days, it started to decline persistently and 4-month-old leaves retained 45% of the maximum photosynthesis capacity. Hence it is not difficult to infer that during the ripening phase, the “source” of MP vines are all “old leaves” while CHP vines still possess enough high-efficiency leaves. All these above factors contribute to a low “source to fruit” ratio for MP in most of the time.

**Must composition** For both seasons, grapes of both treatments were analyzed at the same TSS level (Table 2). MP delayed maturation by ≈ 17 days. In 2014, grapes from MP vines had higher TA and better organic acid composition (i.e. higher tartaric acid and lower malic acid concentration), which is consistent with Clingeleffer (2010). The pH of berries was surprisingly high considering their high TA. In 2015, berry TA and pH were both lower in response to MP than CHP. These results indicate that further study of MP effects on berry acidity is warranted.

At 22 Brix, compared with CHP, MP produced grapes with higher total anthocyanin concentration (mg/g) for both years. Regarding anthocyanin synthesis capacity (mg anthocyanins / $cm^2$ skin surface), there was no difference between the pruning treatments, which suggests that wines made from MP grapes would be more intensely coloured mainly as a result of smaller berry size rather than enhanced anthocyanin synthesis per unit area of berry skin. The lack of a response to pruning treatments by skin anthocyanin content is surprising given that it has been reported that light pruning to increase fruit exposure can enhance anthocyanin biosynthesis independent of berry size (Holt et al 2008), and that the canopy of MP vines can be more porous thus enhancing fruit exposure (Lakso 1993, Reynolds 1988). Our results may indicate that there was no pruning effect on fruit exposure but we did not evaluate treatment effects on canopy density and fruit exposure. Ambient temperatures may also have influenced our results. The studies of Mori et al. (2007) and Mori et al. (2005) observed that higher temperatures during the day or night led to a decrease in anthocyanin accumulation. However, their experiments were conducted under greenhouse conditions and the difference in temperature between control and high temperature groups was substantial ($\Delta$ T = 10°C or 15°C). In this experiment, from veraison to harvest maturity (22 Brix), daily mean air temperatures for CHP and MP were 17.8°C and 16.9°C in 2014, 17.6°C and 17.1°C in 2015, respectively. These differences were unlikely to affect anthocyanin synthesis. Martínez de Toda et al. (2014)

reported an increase in anthocyanins : sugars ratio for Grenache in response to severe trimming compared with non-trimming treatment; and with a corresponding mean temperature difference during maturation of 2.3°C. Therefore, delaying maturation by creating cooler conditions during ripening might be an effective manner to restore anthocyanin : sugars ratio, but the difference in temperature must be considerable.

## Conclusions

MP produced moderately higher yields and delayed berry development under the study conditions of La Rioja Valley. Berry ripening was achieved under MP, and the higher anthocyanin concentrations in MP compared with CHP fruit resulted from smaller berries rather than anthocyanin synthesis capacity. The slightly cooler ripening conditions caused by MP seem insufficient to enhance anthocyanin accumulation. Further studies should be done to confirm and evaluate the delayed maturation caused by MP compared with CHP and its effect on fruit quality in other varieties and under different climatic conditions.

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**Table 1** Effects of minimal pruning (MP) and conventional hand pruning (CHP) on yield components, vine leaf area and leaf area to production ratio for Tempranillo vines in 2014 and 2015

| Pruning treatment | Veraison date | Shoots/ vine | Production (P) (kg/ha) | Clusters/ vine | Clusters/ shoot | Cluster weight (g) | Berries/ cluster | Berry weight (g) | Leaf area (LA) ($m^2$/vine) | LA/P ($m^2$/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| **2014** | | | | | | | | | | |
| CHP | 15 Aug | 11 | 8900 | 10 | 0.91 | 263 | 156 | 2.19 | 5.91 | 2.34 |
| MP | 31 Aug | 108 | 15700 | 40 | 0.37 | 105 | 74 | 1.42 | 7.09 | 1.58 |
| Significance level[a] | | *** | ** | *** | *** | *** | ** | ** | ns | *** |
| **2015** | | | | | | | | | | |
| CHP | 13 Aug | 8 | 6100 | 9 | 1.13 | 219 | 131 | 1.67 | 4.67 | 2.69 |
| MP | 20 Aug | 92 | 7700 | 21 | 0.23 | 104 | 70 | 1.47 | 6.98 | 3.19 |
| Significance level | | *** | ns | *** | *** | *** | ** | ns | ns | ns |

[a] The difference between treatments was assessed with independent-samples t-test; **, ***, ns: significant at $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively.

**Table 2** The effects of minimal pruning (MP) and conventional hand pruning (CHP) on must composition and berry anthocyanins for Tempranillo vines in 2014 and 2015

| Pruning treatments | Date of fruit maturation (22 Brix) | Titratable acidity (g/L) | pH | Tartaric acid (g/L) | Malic acid (g/L) | Total Anthocyanins (mg/g)[a] | Anthocyanins (mg/cm$^2$ skin surface)[a] |
|---|---|---|---|---|---|---|---|
| **2014** | | | | | | | |
| CHP | 1-Oct | 3.85 | 3.41 | 4.4 | 3.1 | 1.31 | 0.37 |
| MP | 21-Oct | 5.30 | 3.60 | 4.9 | 2.7 | 1.53 | 0.39 |
| Significance level[b] | | ** | *** | *** | ** | * | ns |
| **2015** | | | | | | | |
| CHP | 15-Sep | 7.55 | 3.39 | ---[c] | 3.2 | 0.96 | 0.25 |
| MP | 28-Sep | 6.34 | 3.23 | 5.3 | 3.5 | 1.16 | 0.25 |
| Significance level | | *** | *** | --- | ns | * | ns |

[a] Measured at 22 Brix.

[b] The difference between treatments was assessed with independent-samples t-test; **, ***, ns: significant at p≤0.01, p≤0.001 or not significant, respectively.

[c] Missing data.

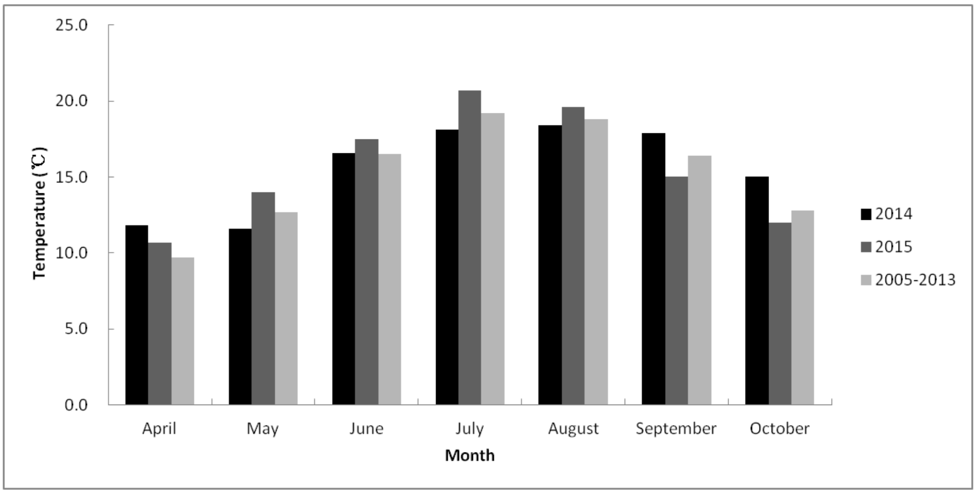

**Figure 1** Mean monthly temperatures during growing season (Villar de Torre, La Rioja, Spain)

# CHAPTER 6

## DOES FULL EXPOSURE OF CLUSTERS HAVE ANY NEGATIVE EFFECTS ON TEMPRANILLO (*Vitis vinifera* L.) GRAPE QUALITY IN LA RIOJA, SPAIN? THE USE OF A SEVERE CLUSTER-ZONE LEAF REMOVAL AFTER BERRY SET

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# Chapter 6. Does Full Exposure of Clusters have any negative effects on Tempranillo (*Vitis vinifera* L.) Grape Quality in La Rioja, Spain? The Use of a Severe Cluster-Zone Leaf Removal after Berry Set

W Zheng, J. García, P. Balda, F. Martínez de Toda

Key words: cluster full exposure, leaf removal, berry quality, warm climate

## Abstract

A three-year experiment about a severe leaf removal (LR) was carried out on Tempranillo grapes in Logroño, North-central Spain. For the LR treatment, six basal leaves along with the basal lateral shoots were removed two weeks after fruit set. Berry total soluble solids (TSS) was examined when the color began to appear. Grapes from both LR and control (C) vines were analyzed at the same TSS level (≈22 Brix). LR advanced the onset of anthocyanin synthesis slightly but significantly. Yield components were not affected by LR and no symptoms of sunburn were observed. Both treatments showed similar juice pH and titratable acidity, nevertheless, tartaric acid increased with LR while malic acid decreased with it. In spite of failing to increase the final anthocyanin concentration of grape juice, LR achieved to enhance the color and body of wine. These results indicate that a relatively early LR could be a viable way to improve the quality of grape and wine under the climatic conditions of Rioja wine region.

## Introduction

Due to climate change, grape sugar ripeness is no longer a big concern for the majority of the viticulturists around the world, especially in warm countries such as Spain. Instead, for the sake of the wine balance, more attention is being paid to acid aspects such as titratable acidity (TA), organic acid composition and pH as well as to the phenol ripeness (Martínez de Toda & Balda, 2014). Under this context, a number of cultural practices have been considered interesting to resynchronize polyphenolic ripening with sugar and the core strategy is to delay the grape berry ripening so that the fruits can mature under relatively cool conditions (Palliotti *et al.*, 2014). On the other hand, those cultural techniques which may directly enhance the accumulation of polyphenols should also be taken account of, among others, leaf removal (LR).

Basal LR has been proved to be an effective practice to reduce the disease incidence of grape bunches as well as to improve the fruit composition thanks to a better illumination and air circulation in the cluster zone (Bledsoe *et al.*, 1988; Smart & Robinson, 1991; Poni *et al.*, 2006; Tardaguila *et al.*, 2010). Generally, it is more befitting under cool and wet conditions where botrytis bunch rot is common and the grapes usually lack total soluble solids (TSS) and color (Reynolds *et al.*, 1986; Jackson & Lombard, 1993; Lee & Skinkis, 2013). On the other hand, under warm conditions, excessive exposure of the fruits may compromise the grape color and acid (Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001) and even result in berry sunburn (Chorti *et al.*, 2010).

The effects of LR depend largely on its timing. Before flowering, LR tends to lead to a lower yield by reducing the fruit set rate (Poni *et al.*, 2006; Tardaguila *et al.*, 2010; Sivilotti *et al.*, 2016). Since most berry abscission occur within 2-3 weeks after full bloom (Candolfi-Vasconcelos & Koblet, 1990), LR should be conducted at least two weeks after the fruit set if the alteration in yield is unwanted. On the contrary, late LR may suddenly expose the clusters to the strong mid-summer sunlight, which could cause berry sunburn (Smart & Robinson, 1991; Downey *et al.*, 2006; He *et al.*, 2010). It is worth mentioning that most previous LR experiments were conducted by only removing the basal leaves, with lateral buds or shoots left. In this way, the remaining leaves and the newly grown lateral shoots may have a higher assimilation rate, which could compensate the reduction of the leaf area (LA) caused by LR (Poni *et al.*, 2006; Tardáguila *et al.*, 2008; Diago *et al.*, 2012). Moreover, cluster shading could reappear due to the lateral shoots, which means that the LR operation has to be repeated when necessary (Smart & Robinson, 1991). Thus, in order to exert the influence of LR to a great extent, it is necessary to apply a full exposure of grapes by removing both basal leaves and lateral shoots surrounding the clusters, as long as the yield is not affected.

A great number of studies exist about the effects of LR on TA, pH and anthocyanins, etc. However, the results varied. In many studies, the mentioned parameters were determined at different levels of TSS for LR treatment and the control group. In many cases, LR led to higher sugar concentration than control when harvested at the same time, meanwhile the fruits which were exposed to the sunlight usually had higher anthocyanin concentration while TA and malic acid were reduced (Kliewer, 1977; Reynolds *et al.*, 1986; Bledsoe *et al.*, 1988; Smith *et al.*, 1988; Poni *et al.*, 2006; Diago *et al.*, 2012). However, with global warming and the market tendency for low-alcohol wine (Palliotti *et al.*, 2014), a high TSS level is increasingly undesirable. Additionally, to evaluate the effects of a wine-growing technique on wine composition, it is more interesting to compare the parameters associated with acid and pigment at the same TSS content, since they are closely correlated with the TSS level. Otherwise, it is difficult to evaluate the direct impact of the technique. At a similar TSS level, Martínez de Toda & Balda (2014) and Mosetti *et al.* (2016) reported that LR after berry set reduced juice pH and malic acid, however, this reduction in pH was not found in the studies of Lee & Skinkis (2013) and Sivilotti *et al.* (2016). Similarly, regarding TA, results also differed among previous studies. Another key parameter of the grape juice quality is the concentration of anthocyanins. Light exposure can exert some positive effects on cluster anthocyanin accumulation, in contrast, since grape color enzymes activity ranges from 17℃ to 26℃ (Iland & Gago, 2002), high temperatures tend to repress the anthocyanin synthesis (He *et al.*, 2010) and even delay the onset of anthocyanin accumulation (Sadras & Moran, 2012). Coincidentally, both increased light exposure and high berry temperatures are the consequences of LR. To our knowledge, the total effects of LR on anthocyanins are still unclear, especially under warm conditions, though it was stated that a high degree of bunch exposure might be harmful for anthocyanin accumulation (Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001; Guidoni *et al.*, 2008; Chorti *et al.*, 2010). Furthermore, few investigators have studied the impact of LR on the TSS content at the onset of anthocyanin synthesis; however, this value could influence the final anthocyanin concentration (Sadras & Moran, 2012).

The aim of this research was to evaluate the effects of a sustained bunch exposure through a severe basal LR on the quality of Tempranillo grapes under the environmental conditions of La Rioja, Spain and, more specifically, on its chemical composition and on the quality of the wine.

## Materials and Methods

### *Plant material and growth conditions*

During a period of three years (2014-2016), the field trial was carried out in an experimental vineyard (42°27′N, 2°25′W, 370 m.a.s.l.) of the University of La Rioja, Logroño, Spain. *Vitis vinifera* L. cv. Tempranillo (clone CL-306 grafted onto 110-R rootstock) planted in 2010 was used for the experiment. Vine rows were roughly north-south oriented at a 2.4 m (between rows) × 1.2 m (between vines) spacing and the vines were trained to vertical shoot positioning with two cordons and pruned to six spurs (12 buds) per vine. The cordons were supported by a single wire 70 cm above the ground and the canopy were constrained and protected by three pairs of foliage wires at the height of 100, 150 and 200 cm, respectively. In 2014 and 2016, the vineyard was drip-irrigated with an average amount of 4.5L/vine/day from mid-July, when a moderate-severe water stress was observed (70 % of the shoots ceased growing), until the end of August. In 2015, the irrigation was started two weeks prior due to an enduring heat wave starting from the last week of June. Before veraison, trimming was performed once to prevent the shoots from extending to the street which would make it difficult for us or tractors to get through.

### *Treatments*

Four adjacent rows were selected for the study and on each of them, two homogenous plots (5 vines per plot) were assigned randomly to control (C) and LR treatments (one plot for C and the other for LR). The 4 rows served as 4 replicates and both C and LR were performed on the same vines in all the experiment years. Every year, the first six basal leaves (bunches are situated at 3-5 nodes) were removed manually from the LR vines two weeks after berry set, along with all the lateral shoots/buds in the basal zone.

### *Measurement of TSS at the onset of anthocyanin synthesis*

When 50% of the berries began to show color, for each of the treatments, 30 randomly selected berries with a slight sign of color change were sampled for the TSS measurement and a digital refractometer (ATAGO CO., LTD, Japan) was used. In addition, based on 3-year data, within each treatment group, the correlativity between TSS at which anthocyanins synthesis was initiated and the effective accumulated temperature (The sum of the daily effective temperature. The daily effective temperature refers to the difference between the daily mean temperature and 10 °C, provided that the daily mean temperature is above 10 °C, otherwise it is 0 °C) from budburst to veraison was studied.

### *Measurement of LA*

LA were estimated when the LR operation was conducted as well as at harvest. The method based on leaf disc sampling (Smart & Robinson, 1991) was used to estimate the leaf area per shoot. Fifteen shoots per treatment were taken at random for the measurement. For each of them, all the leaves (without leave petioles) were removed and weighed. Meanwhile, 100 3.80-cm$^2$ discs from randomly selected leaves were weighed as well. LA per shoot was calculated by multiplying the quotient of the two weights by 380. In the same way, the removed LA of LR shoots was calculated and then the percentage of LR on the whole canopy was estimated.

### *Radiation and berry temperature measurements*

On August 7th of 2016 (a representative summer day in the region, with burning sun and cloudless sky), photosynthetically active radiation (PAR) at cluster zone on both sides of the cordon was measured. The measurements were taken at three moments: four hours before solar noon (10:00 h.), at solar noon (14:00 h) and four hours after solar noon (18:00 h). PAR was measured using a handheld Li-Cor LI-189 quantum 1 m length sensor (Li-Cor, Inc., Lincoln, NE) and ten measurements per replicate were carried out. The sensor was placed on a horizontal position on each side of the cluster zone along the cordon. Cluster sunlight exposure was expressed as the average percentage of both sides of the cordon related to the maxim PAR, which was measured perpendicularly to sun radiation. Independently, in the midday, to evaluate the effects of radiation on berry temperature, 20 berries respectively from exposed (LR treatment), partly exposed (C treatment but with gaps between leaves) and shaded (C treatment totally covered by leaves) clusters were selected for the temperature measurements with an infrared thermometer of “pistol type” (Optris LS, Mesurex SL, Berlin, Germany).

### *Yield estimation, berry sampling and must analysis*

Intensive monitoring of TSS content was conducted from late August and fruits were harvested as soon as their average TSS reached 22 Brix, which is a common value for most of the red grapes in the region. For each treatment, cluster number per vine was obtained by counting clusters for 8 vines (two for each plot) and cluster weight was measured on ten randomly cut clusters per repetition. Finally, yield per vine was estimated by multiplying both parameters (cluster number and cluster weight). Average berry weight was determined on 200 randomly sampled berries per repetition and then these berries were crushed manually for the juice analysis. pH, titratable acidity (TA), tartaric acid and malic acid were analyzed by standard methods (OIV, 2014). The concentration of the total anthocyanins was measured based on Iland *et al.* (2004a).

### *Winemaking, wine analysis and sensory evaluation*

Every year, the surplus grapes of both C and LR were harvested at 22 Brix for micro-fermentations. Three 3L-volumn-jars of wine were elaborated per treatment and each of them was filled with about three kilograms of grapes which had been de-stemmed manually. Grapes were crushed by hand inside the jar and 3 ml 6% (6g/100ml) of sulfur dioxide ($SO_2$) solution was added to the juice. Afterwards, 1.2 g of activated commercial yeast strain (*Saccharomyces cerevisae*, OPTI-RED®, Lallemand, Montreal, Canada) was inoculated. A round plastic cover with a hole in the middle was placed inside every jar to keep the berry skin in contact with the juice throughout the fermentation; in this way no manual punching down was performed. The fermentation was carried out at a constant temperature of 25℃ . About two weeks later, after the alcoholic fermentation was finished, wine was pressed and $SO_2$ was adjusted to 30 mg/L. Wine was kept in a cold storage at 2℃ for two weeks before being racked. After bottling, wine bottles were placed horizontally and stored at about 18℃ for two months before the chemical and sensory analysis. TA and pH of wine were measured according to standard methods (OIV 2014). Color intensity (CI) was estimated by adding together the absorption values at 420 nm, 520 nm, and 620 nm (Glories, 1984). Total phenols index (TPI) was estimated by measuring the absorption at 280 nm (Ribéreau-Gayon, 1970). For both CI and TPI, the absorption was measured in a 1 mm optical path cell and then the results were multiplied by 10 since the measure is conventionally referred to the optical path of 10 mm. The sensory evaluation was done for the wine of 2015 and 2016 using a discrimination testing (paired comparison test) (Iland *et al.*, 2004b). In order to identify if the wine of LR had particular attributes, some alternative questions with respect to acidity, astringency and off-flavor were posed. For each year, 10 experienced tasters participated and each of them repeated the paired comparison five times, so the number of paired tests conducted was 50 per year.

### *Statistic analyses*

Statistical package SPSS 16.0 (SPSS Inc., Chicago, US) for Windows was used for the statistic analyses. Independent samples t-test was conducted for the comparison of TSS at which anthocyanins' synthesis was started between treatments. Pearson correlation method with two-tailed test was applied for the correlation analysis. Data of yield components, berry composition and wine composition were tested for homogeneity of variance using Levene's test and were subjected to two-way (treatment x year) analysis of variance, using the general linear model and F-test. When there were significant differences among years, S-N-K method (equal variances assumed) or Dunntt's T3 method (equal variances not assumed) was used to separate the means. Data were also analyzed year by year with independent samples t-test since interaction between treatments and years was observed for some of the parameters. The results of sensory analysis were interpreted based on the two-tailed test statistical table sourced from Amerine & Roessler (1976).

## Results

### *Weather conditions*

The summer of 2014 was cool and the temperatures in September and October were much higher than the average (Fig. 1). Besides, there was a lot of precipitation throughout September (56.0 mm). The weather conditions of 2015 were contrary to those of 2014: the three months of summer were extremely hot but the autumn was chilly. Moreover, it should be emphasized that there was a long-lasting heat wave during a period of two weeks at the end of June and the beginning of July. The season of 2016 suffered from a lack of rain (data is not shown) after a cold April, though the temperature in the summer was close to the annual average.

### *TSS concentration at the onset of anthocyanin synthesis*

In each year, LR grapes had a lower TSS concentration than C when the berries began to show color and this difference was significant in 2014 and 2016 (Fig. 2). It is also observed that the TSS level was strongly proportional to the effective accumulated temperature from budburst until veraison (Fig. 2), that is, the higher the temperature before veraison, the higher the TSS level when the anthocyanins synthesis was started, although this correlation fail to be significant statistically due to the data of only 3 years.

### *Cluster sunlight exposure and berry temperature*

It is obvious that LR grapes received much more illumination during the daytime than C (Fig. 3). As a result, the berry temperature of LR grapes was supposed to be higher than those of C grapes during the majority of the day. For example, our measurements showed that, at midday, the average surface temperatures of exposed (with an average illumination of 2000 μmol/m$^2$s) , partially exposed (120 μmol/m$^2$s) and shaded (4 μmol/m$^2$s) berries were 36.6℃ , 30.3℃ and 27.3℃ , respectively, and the air temperature at that time was 30.7℃ .

### *Field parameters and yield components*

Only in 2014, LR lead to a significantly lower berry weight compared to C and both cluster weight and production (P) per vine were not altered by LR in any vintage (Table 1). Due to the fact that about 57% of the canopy LA was removed by LR, at harvest, LR had significantly less leaf area (LA) per vine compared with C. Nevertheless, the values of LA/P of both treatments were always greater than 1.0 m$^2$/kg. All of the above-mentioned parameters varied with years and among the three years, 2015 could be characterized as a vigorous and productive season with considerably bigger berries and clusters.

### *Must composition*

At the same level of TSS, grape juice of C and LR tended to have a similar concentration of titratable acidity as well as a similar pH (Table 2). However, with respect to tartaric acid and malic acid, significant differences were observed. LR juice usually contained a higher concentration of the former acid and a lower concentration of the latter one. Thus, compared to C, a higher tartaric acid:malic acid ratio was obtained via LR. In 2014, LR resulted in a higher concentration of total anthocyanins, however, this trend was not confirmed in the next two years. Likewise, not any significant difference in anthocyanin content (expressed as mg/berry) was found between treatments. Comparing each year of the experiment, it is noteworthy that the highest anthocyanin concentration was reported in 2016, followed by 2014, and the grapes of 2015 had the poorest color, though the difference between 2014 and 2015 was not significant.

### *Chemical analysis and sensory evaluation of wine*

Wine made from LR grapes significantly had higher CI and TPI (Table 3). Partially in accordance with the results of anthocyanin analysis for grape juice, the wine of 2015 showed the lowest color intensity while there was no significant difference between the wine of 2014 and 2016 in this aspect. Regarding the sensory analysis, in 2015, the vast majority of the tasters considered the LR wine to be more acid (Table 4). Perhaps because of this, the LR wine of 2015 was considerably preferred to the C wine of the same year among tasters. On the other hand, the sensory analysis for the wine of 2016 did not indicate any significant difference in all the aspects.

## Discussion

Throughout the experiment, we did not observe any symptoms of sunburn (brown patches or russet) on the berries, even under the extremely hot conditions which lasted up to two weeks in 2015. It could be explained by the fact that some plant secondary metabolites (i.e. phenolic compounds) could be produced in response to UV-B irradiation and these substances might contribute to the detoxification process and be able to protect the berries from further damage caused by intense solar radiation (Frohnmeyer & Staiger, 2003; Keller, 2010; Webb *et al.*, 2010). Generally, a sudden exposure to the sunlight under hot conditions is the most likely to induce sunburn (Smart & Robinson, 1991; Kuai *et al.*, 2009). In our experiment, due to a basal leaf removal being carried out quite early, the fruits were supposed to have enough time to precondition and react so that sunburn was avoided. Moreover, since drip-irrigation was applied during most of the time from July to August, the vines might regulate its temperature by enhanced transpirational water loss thus reducing the sunburn sensitivity (Winkler, 1974; Van Den Ende, 1999).

The results about the TSS concentration at the onset of anthocyanin synthesis are notable. We attribute the earlier onset of anthocyanin synthesis for LR to the positive effect of cluster exposure on the synthesis of anthocyanins. It seems that the positive effect of increased exposure of berries to light predominates over the possible negative effect of higher temperature. It is well known that, after veraison, the accumulation of anthocyanins mainly depends on the TSS level, as glucose is the precursor of all the anthocyanins in the grapes (Pirie & Mullins, 1977). Thus, a lower level of TSS (due to LR) at which the anthocyanin synthesis is initiated might be an indicator of a higher final concentration of anthocyanins at a given final TSS level, despite the fact that anthocyanins are also related to sunlight exposures and berry temperatures during the period of ripening (He *et al.*, 2010). Sadras & Moran (2012) reported that elevated temperatures could delay the onset of anthocyanin synthesis in grapes of Shiraz and Cabernet Franc. Similarly, the same trend was found in our study when comparing the results of the three years (independent on treatments). Overall, for a given variety, both cultural practices and pre-veraison weather conditions might alter the required TSS for the onset of anthocyanins. For instance, in addition to early LR, water deficit is also likely to accelerate the pigmentation process of grapes (Herrera & Castellarin, 2016).

The reason for the smaller berry size of LR grapes in 2014 might be that, after the basal leaves were removed, the division of mesocarp and skin cells were negatively affected because of the reduction in photosynthesis, as these cells would not cease dividing until 3-5 weeks after anthesis (Keller, 2010). However, in 2015 and 2016, the difference in berry size was not observed between treatments. Mosetti *et al.* (2016) reported that basal LR 25 days after anthesis did not affect the berry size. In the study of Sivilotti *et al.* (2016), even performed 15 days after flowering, basal LR failed to change the berry mass. Nonetheless, in our view, to avoid the risk of yield loss, LR ought not to be conducted within two weeks after fruit set. The values of LA/P at harvest indicate that this parameter was neither a limiting factor for the grape quality of both treatments nor a key factor which caused differences in juice composition between treatments.

Being exposed to direct solar radiation, LR berries might have a considerably higher surface temperature than C in the daytime. Accordingly, the differences in light conditions and berry temperatures between treatments might give rise to the different juice compositions. From the perspective of acid, high light intensity and berry temperature might affect metabolic processes that convert sugar to acids, which could provide an explanation as to why the LR berries contained more tartaric acid (Keller, 2010). On the other hand, high berry temperatures were likely to accelerate the respiratory malate degradation, which resulted in lower malic acid (Keller, 2010). Compared to malic acid, tartaric acid has a higher metabolic stability as well as a higher microbiological stability. Besides, it could provide wine with a crisp and fresh mouthfeel while malic acid tastes tart. Overall, a higher tartaric acid:malic acid ratio is usually what a winemaker wants and, fortunately, it seems possible to achieve this objective by applying a LR shortly after fruit set. With regards to TA, the absence of significant difference between treatments is not surprising as both tartaric and malic acids are not accurate indicators of TA (Boulton, 1980b). These findings agree with what Martínez de Toda & Balda (2014) found for Maturana grapes in the same region. However, in their study, a significant reduced pH was achieved by increasing grape sunlight exposure whereas we did not find this trend in our experiment being the possible reason that pH is usually influenced by the concentration of potassium and sodium ion in the juice (Boulton, 1980a).

In 2014, the significantly higher concentration of anthocyanins of LR grape might be caused by its smaller berry size, since no considerable difference in anthocyanin content between treatments was found in the same year. Throughout the three-year data, LR did not exert positive (nor negative) impacts on the final pigment content of grape, in spite of the reduced level of TSS at the onset of anthocyanin synthesis. Namely, under our experiment condition, the repressed anthocyanin synthesis due to the high berry temperature could be canceled out by the better fruit light conditions (He *et al.*, 2010). On the other hand, the higher CI and TPI indicate that, in relative to C, LR wine had darker color and stronger body, which might be ascribed to that sunlight exposure improved the extractability of anthocyanins during fermentation and enhanced the accumulation of skin tannin (Cortell & Kennedy, 2006).

Since LR grapes were exposed to the sunlight during a long period, one of our concerns about LR wine was some possible unpleasant aromas or off-flavors related to the high temperatures, which is similar to raisin. However, such defects were not detected in the sensory evaluation. Moreover, in spite of the higher TPI, LR wines were not considered more astringent by the tasters. In brief, as a conservative conclusion, the cluster full exposure has no negative effects on wine quality under our experiment conditions.

## Conclusions

Under the environmental conditions of La Rioja, a severe basal LR (with also lateral shoots/buds removed) two weeks after fruit set is not likely to alter yield components of Tempranillo grapes. The full exposure of clusters did not bring about problems of sunburn, on the contrary, several positive impacts such as a reduced TSS level at the onset of anthocyanin synthesis as well as a better acid composition were obtained. Additionally, LR turned out to be an effective way to improve the wine color and body. Viticulturists in the Rioja wine region might apply this wine-growing technique to improve the grape berry and wine quality.

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TABLE 1
Impacts of basal leaf removal (LR) on field parameters and yield components for Tempranillo vines during a period of 3 years (2014-2016).

| | | Budburst date | 50% Veraison date | Harvest date | Berry weight (g) | Cluster weight (g) | Production (P) (kg/vine) | Leaf area (LA) ($m^2$/vine) | LA/P ($m^2$/kg) |
|---|---|---|---|---|---|---|---|---|---|
| 2014 | Control | 6-Apr | 4-Aug | 1-Oct | 1.54 a[2] | 175 | 2.89 | 3.67 a | 1.27 |
| | LR | 6-Apr | 5-Aug | 25-Sep | 1.43 b | 135 | 2.46 | 2.81 b | 1.14 |
| | Sig[1] | | | | *** | ns | ns | *** | |
| 2015 | Control | 7-Apr | 28-Jul | 11-Sep | 1.87 | 266 | 5.27 | 7.45 a | 1.41 |
| | LR | 7-Apr | 30-Jul | 11-Sep | 1.68 | 205 | 3.85 | 5.62 b | 1.46 |
| | Sig | | | | ns | ns | ns | * | |
| 2016 | Control | 4-Apr | 3-Aug | 7-Sep | 1.37 | 220 | 3.84 | 5.34 a | 1.39 |
| | LR | 4-Apr | 3-Aug | 7-Sep | 1.49 | 210 | 3.74 | 4.13 b | 1.10 |
| | Sig | | | | ns | ns | ns | ** | |
| Treatment (T) | Control | 6-Apr | 1-Aug | 16-Sep | 1.59 | 220 | 4.00 a | 5.49 a | 1.36 |
| | LR | 6-Apr | 2-Aug | 14-Sep | 1.56 | 183 | 3.35 b | 4.19 b | 1.23 |
| | Sig | | | | ns | ns | ns | *** | |
| Year (Y) | 2014 | 6-Apr | 5-Aug | 28-Sep | 1.48 b | 155 b | 2.68 c | 3.24 c | 1.21 |
| | 2015 | 7-Apr | 29-Jul | 11-Sep | 1.78 a | 236 a | 4.56 a | 6.53 a | 1.44 |
| | 2016 | 4-Apr | 3-Aug | 7-Sep | 1.43 b | 215 a | 3.79 b | 4.74 b | 1.25 |
| | Sig | | | | *** | * | *** | *** | |
| T×Y | Sig | | | | * | ns | ns | ns | |

[1] Sig: Significance level; data within each year were analyzed with independent samples t-test; data of three years were analyzed with two-way Anova (treatments×years); *, **, ***, ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively.

[2] S-N-K method (equal variances assumed) or Dunntt's T3 method (equal variances not assumed) was used to separate the means when there were significant differences among years; different letters (a, b, c) represent different means at $p \leq 0.05$.

TABLE 2
Impacts of basal leaf removal (LR) on Tempranillo grape juice during a period of 3 years (2014-2016).

| | | TSS at harvest (Brix) | Titratable acidity (g/L)[2] | pH | Tartaric acid (g/L) | Malic acid (g/L) | Anthocyanin concentration (mg/g) | Anthocyanin content (mg/berry) |
|---|---|---|---|---|---|---|---|---|
| 2014 | Control | 22.3 | 3.45 | 4.12 | 4.2 | 3.5 a[3] | 1.29 b | 1.97 |
| | LR | 22.6 | 3.65 | 4.04 | 4.6 | 2.9 b | 1.45 a | 2.31 |
| | Sig[1] | ns | ns | ns | ns | * | *** | ns |
| 2015 | Control | 22.5 | 5.15 | 3.52 | 4.2 b | 4.2 a | 1.21 | 2.03 |
| | LR | 22.4 | 4.85 | 3.55 | 4.4 a | 3.7 b | 1.35 | 2.26 |
| | Sig | ns | ns | ns | * | *** | ns | ns |
| 2016 | Control | 22.2 | 4.64 | 3.36 | 4.5 b | 4.3 | 1.65 | 2.23 |
| | LR | 22.4 | 5.14 | 3.31 | 5.0 a | 4.1 | 1.66 | 2.45 |
| | Sig | ns | ns | ns | ** | ns | ns | ns |
| Treatment (T) | Control | 22.3 | 4.41 | 3.67 | 4.3 b | 4.0 a | 1.38 | 2.08 |
| | LR | 22.5 | 4.55 | 3.63 | 4.7 a | 3.6 b | 1.49 | 2.34 |
| | Sig | ns | ns | ns | *** | *** | ns | ns |
| Year (Y) | 2014 | 22.5 | 3.55 b | 4.08 a | 4.4 b | 3.2 a | 1.37 b | 2.14 |
| | 2015 | 22.5 | 5.00 a | 3.53 b | 4.3 b | 3.9 b | 1.28 b | 2.15 |
| | 2016 | 22.3 | 4.89 a | 3.34 c | 4.8 a | 4.2 a | 1.65 a | 2.34 |
| | Sig | ns | *** | *** | *** | *** | ** | ns |
| T×Y | Sig | ns | ** | ns | ns | ns | ns | ns |

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[1] Sig: Significance level; data within each year were analyzed with independent samples t-test; data of three years were analyzed with two-way Anova (treatments×years); *, **, ***, ns: significant at p≤ 0.05, p≤ 0.01, p≤ 0.001 or not significant, respectively.

[2] The titratable acidity is expressed as g/L tartaric acid.

[3] S-N-K method (equal variances assumed) or Dunntt's T3 method (equal variances not assumed) was used to separate the means when there were significant differences among years; different letters (a, b, c) represent different means at p≤ 0.05.

TABLE 3
Chemical analysis for the wine originated from control (C) and leaf removal (LR) grapes of Tempranillo.

| | | Total acidity (g/L)[2] | pH | Color intensity (CI) | Total phenols index (TPI) |
|---|---|---|---|---|---|
| Treatment (T) | C | 4.3 | 4.25 | 9.60 | 39.2 |
| | LR | 4.7 | 4.19 | 12.86 | 45.5 |
| | Sig[1] | ns | ns | *** | * |
| Year (Y) | 2014 | 4.3 b[3] | 4.28 | 12.89 a | 47.4 |
| | 2015 | 4.3 b | 4.24 | 8.62 b | 41.3 |
| | 2016 | 4.9 a | 4.15 | 12.19 a | 38.4 |
| | Sig | * | ns | ** | ns |
| T×Y | Sig | ns | ns | ns | ns |

[1] Sig: Significance level; data were analyzed with two-way Anova (treatments×years); *,**, ***, ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ or not significant, respectively.

[2] Total acidity is expressed as g/L tartaric acid.

[3] S-N-K method (equal variances assumed) or Dunntt's T3 method (equal variances not assumed) was used to separate the means when there were significant differences among years; different letters (a, b, c) represent different means at $p \leq 0.05$.

TABLE 4
Sensory analysis of the wine originated from control (C) and leaf removal (LR) grapes of Tempranillo with discrimination testing.

| | Question: Which wine is more prominent concerning the following characters? | | | | |
|---|---|---|---|---|---|
| | Unpleasant aroma | Off-flavor | Sensation of acidity | Astringency | Overall preference |
| 2015 | | | | | |
| C | 28[1] | 28 | 14 | 29 | 14 |
| LR | 22 | 22 | 36 | 21 | 36 |
| Sig[2] | ns | ns | ** | ns | ** |
| 2016 | | | | | |
| C | 27 | 30 | 23 | 31 | 18 |
| LR | 23 | 20 | 27 | 19 | 32 |
| Sig | ns | ns | ns | ns | ns |

[1] The value represent the number of times the corresponding answer was recorded.

[2] Sig: Significance level; the two-tailed test statistical table sourced from Amerine & Roessler (1976) was used to determine if the number was sufficiently high to draw a statistically significant conclusion; Since 50 paired tests were conducted, the number of corresponding answers is necessary to be 33 or higher to be significant at the 5% level and 35 or higher to be significant at the 1% level; **, ns: significant at $p \leq 0.01$ or not significant, respectively.

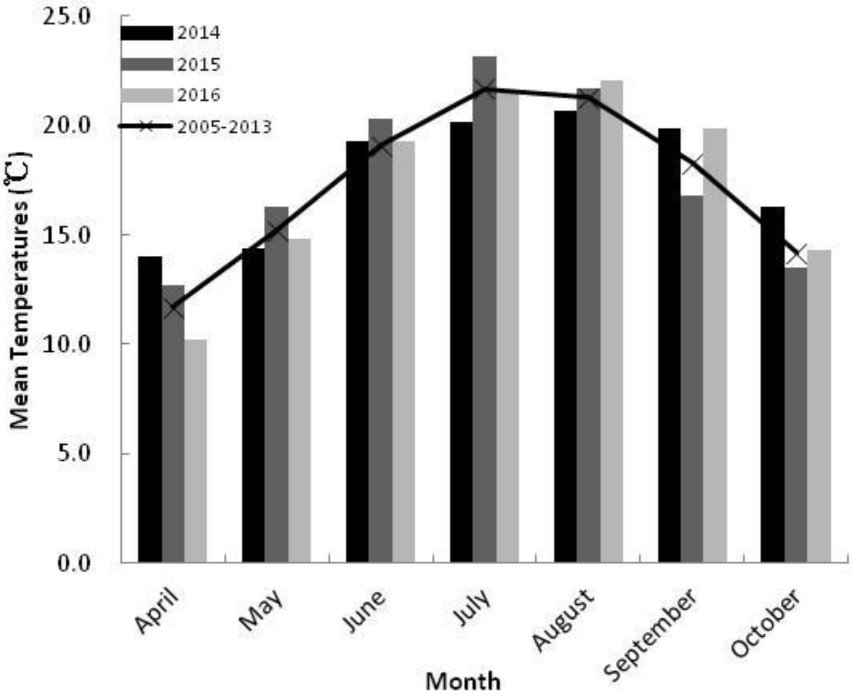

FIGURE 1

Mean monthly temperatures during growing seasons in Logroño.

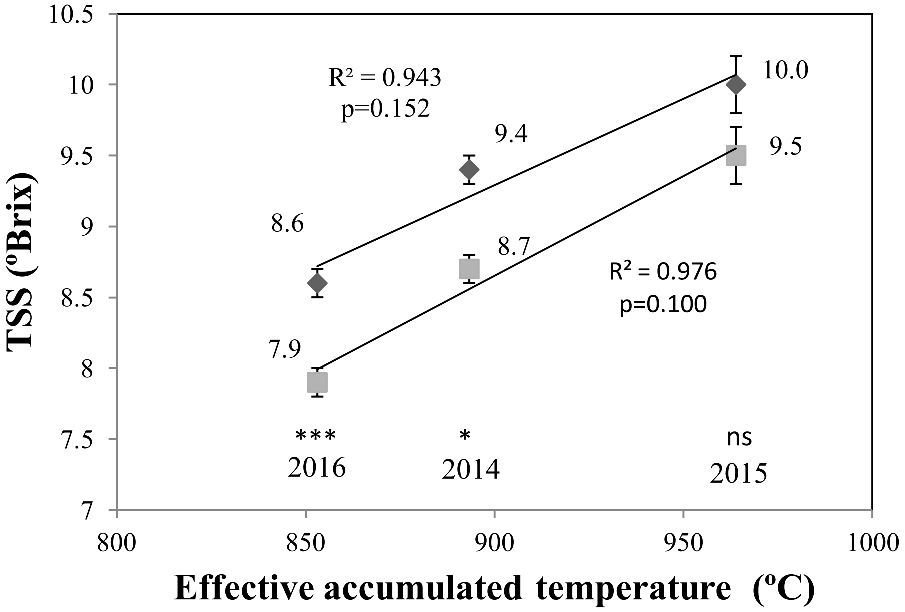

FIGURE 2

Linear correlation between Total soluble solids (TSS) concentration at which anthocyanins synthesis was initiated and the effective accumulated temperature (The sum of the daily effective temperature. The daily effective temperature refers to the difference between the daily mean temperature and 10 °C, provided that the daily mean temperature is above 10 °C, otherwise it is 0 °C) from budburst to veraison, based on data of 3 years (2014, 2015 and 2016). Pearson correlation method with two-tailed test was applied, significant at p≤ 0.05. Values are mean ± SE. (◆): Control; (■): LR. TSS was also compared between treatments with independent samples t-test; *, ***, ns: significant at p≤ 0.05, p≤ 0.001 or not significant, respectively.

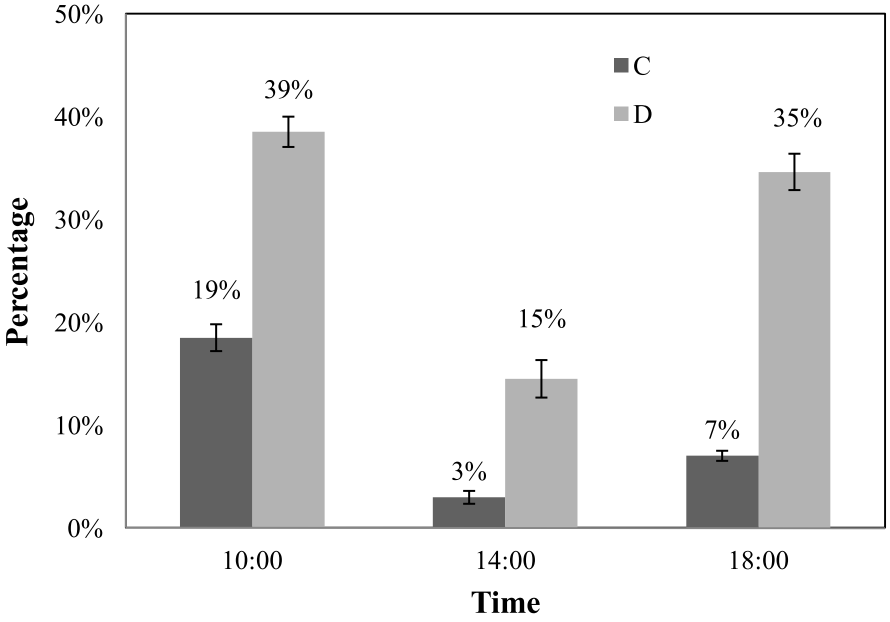

FIGURE 3

The percentage (the average of both sides of the cordon) of the received sunlight radiation of both control (C) and leaf removal (LR) clusters respect to the real-time maximum radiation of a representative summer day (August 7th of 2016) in Rioja wine region. Values are mean ± SE.

# CHAPTER 7

## EFFECTS OF LATE WINTER PRUNING AT DIFFERENT PHENOLOGICAL STAGES ON VINE YIELD COMPONENTS AND BERRY COMPOSITION IN LA RIOJA, NORTH-CENTRAL SPAIN

**Submitted to Vine and Wine Open Access Journal (Oeno One)**

# Chapter 7. Effects of late winter pruning at different phenological stages on vine yield components and berry composition in La Rioja, North-central Spain

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## Abstract

**Aims:** Under global warming, the desynchrony between technology maturity and phenolic maturity of wine grapes is a worthy concern. Late winter pruning (LWP) has been proved to be an effective way to delay the grape phenological stages. The aim of this study was to evaluate the effects of LWP at different phenological stages (based on Baillod & Baggiolini system) on the delay of the grape ripening, on vine yield components as well as on berry composition, among others, the anthocyanin to sugar ratio.

**Methods and results:** The two-year (2015 and 2016) trial was conducted in Rioja wine region (North of Spain) on Maturana vines and in each year, four pruning treatments were carried out taking apical buds/shoots as reference: (1) winter pruning at stage A (WPA; dormant bud); (2) LWP at stage C (LWPC; green shoot tip) in 2015 and at stage F (LWPF; inflorescence clearly visible) in 2016; (3) LWP at stage G (LWPG; inflorescences separated); (4) LWP at stage H (LWPH; flowers separated). LWPC failed to delay the late phenological stages and did not exert important influence on vine yield and berry composition. LWPG and LWPH succeeded to delay all the phenological stages of grapes to a great extent and created a considerably cooler and longer ripening period compared to WPA. Vine yield was not affected by LWPF and was reduced significantly (averagely by 41%) by LWPG. LWPH lead to great losses in yield (averagely by 67%), especially in 2015. LWPG did not change the fruit composition while LWPH increased the ratio of anthocyanin to sugar and helped to maintain a relatively high level of acidity in berries.

**Conclusions:** The primary cause of the decline in production seems to be the losses of flowers and/or the reduction in fruit set percentage in the current season, instead of the losses in inflorescences within buds in the previous season. For Maturana grapes, LWP after the stage F would reduce the vine yield and could be applied as an alternative to the time consuming cluster thinning to meet the needs of yield control. Delaying the winter pruning to stage H could improve the fruit quality in spite of the greater risk of botrytis and a serious decline in production.

**Significance and impact of the study:** The outcomes of this research open a door for the winegrowers to realize the yield control in a simple way. Also, for those who only pursue wines of top quality (regardless of production), a very late winter pruning might provide them with high-quality grapes. Moreover, as can be seen obviously from our results, viticulturists could postpone the budburst date to whatever extent they wish thus reducing the risk of spring frost injury to zero, though this point is not our focus in the study.

**Key words:** global warming, viticulture, pruning, grape ripening, vine yield, anthocyanins

## Introduction

The steady trend of climate warming has had a profound impact on European viticulture (Schultz, 2000). In Rioja (North of Spain) wine region, the average growing season temperature for red wine was 16.3 ºC between 1950-1989 and 18.1 ºC between 1990-1999 while the estimated optimum average value is 17.5 ºC (Jones *et al.*, 2005). Moreover, according to model prediction, this value is expected to increase by 1.33 ºC between 2000-2049 (Jones *et al.*, 2005). That is to say, the climate warming is affecting the wine industry of Rioja. One of the greatest problems of high temperatures is the accelerated sugar accumulation which could result in a high alcohol level accompanied with low acidity, high pH and unusual aromas in wine (Keller, 2010; Palliotti *et al.*, 2014). Another worrying problem is the temperature driven decoupling of anthocyanins and sugars in berries of red varieties (Sadras and Moran, 2012), that is, the optimal range of temperature for phenol accumulation in berries is lower than those for sugar accumulation (Iland and Gago, 2002). When temperatures are too high, whether during the day or night, anthocyanin synthesis is repressed (Mori *et al.*, 2005; Mori *et al.*, 2007) and berries would not be likely to attain the maximum anthocyanin concentration at a regular total soluble solids (TSS) level for harvest (Palliotti *et al.*, 2014). To mitigate the mentioned negative effects of global warming above, a number of cultural attempts have been made in different wine regions around the world in order to delay the grape sugar accumulation so that crops may mature under a cooler climatic condition (Gu *et al.*, 2012; Palliotti *et al.*, 2013; Martínez de Toda *et al.*, 2014; Palliotti *et al.*, 2014; Frioni *et al.*, 2016; Zheng *et al.*, 2016).

Among the cultural approaches, late winter pruning (LWP) has been well known and widely applied since it could delay budburst by a few days thus reducing the risk of spring frost injury (Trought *et al.*, 1999; Reynier, 2002). Moreover, the timing of budburst exerts a great influence on the subsequent vegetative and reproductive growth (May, 2000). Accordingly, to retard the budburst is a possible way to postpone the following phenological stages of development including fruit ripening (Martin and Dunn, 2000; Friend and Trought, 2007). Though temperature is the decisive factor that determines the timing of budburst (May, 2000), there is a general agreement that a delayed budburst can be achieved by LWP (Parkin and Turkington, 1980; Frioni *et al.*, 2016; Gatti *et al.*, 2016). The mechanism of this phenomenon is the imposition of apical dominance, namely, grapevine shoot growth starts in the distal buds of a cane and the development of the basal buds is often inhibited by the budburst of distal buds (Friend and Trought, 2007; Keller, 2015). And then, after a late spur-pruning, basal buds/shoots are forced to break/grow (Howell and Wolpert, 1978).

Though LWP is a promising tool to delay ripening, its effects depend largely on the extent to which the winter pruning is delayed (Palliotti *et al.*, 2014). Before the apical buds open, LWP could merely delay budburst and shoot growth by a few days with limited influence on the subsequent phenological stages (Antcliff *et al.*, 1957; Martin and Dunn, 2000). Nonetheless, Friend and Trought (2007) reported that LWP, shortly before budburst, could alter some yield components depending on the year. On the contrary, alteration neither in yield components nor in grape composition was found for vines pruned before budburst in the study of Frioni *et al.* (2016). After the budburst of apical buds, LWP at stage E (leaves unfolded) and F (inflorescence clearly visible) based on Baillod & Baggiolini system (Baillod and Baggiolini, 1993; Coombe, 1995) could delay the budburst date by 17 and 31 days, respectively. However, the losses of yield were significant and LWP at both stages failed to postpone late-season phenological stages under the warm conditions (Gatti *et al.*, 2016). Similarly, Parkin and Turkington (1980) reported that LWP in late October (in the southern hemisphere) delayed the onset of fruit ripening by about 20 days but the fruits matured at about the same time as those from normal-pruned vines; when being carried out even later, LWP in late November could delay the fruit ripening to a large degree and improve the fruit quality at the cost of a great reduction in yield (Parkin and Turkington, 1980). Also, a recent research (Frioni *et al.*, 2016) in central Italy showed that LWP at stage G (inflorescences separated) slowed fruit ripening and reduced yield as well as the number of inflorescences in winter buds, but the LWP berries were lower in TSS while higher in titratable acidity (TA) and in total anthocyanin concentration. In the same study, no yield was obtained by a LWP at stage H-I (40% to 50% of flower caps fallen).

Taken together, LWP is a viable approach to delay grape berry ripening as long as it is carried out late enough. However, extremely late winter pruning may lead to an unacceptable low yield. Therefore, it is vital to find out an appropriate period to realize the LWP with the purpose of delaying fruit sugar accumulation significantly without affecting the yield. To our knowledge, few studies have focused on this point and there is no general agreement. The objectives of this study were to: (1) assess the effects of LWP at different moments on yield components and fruit composition; (2) determine a phenological stage from which LWP would reduce vine yield; (3) verify whether a delayed ripening period due to LWP could improve the anthocyanins to sugars ratio.

## Materials and Methods

The two-year (2015 and 1016) study was conducted in the experimental vineyard of the University of La Rioja (42°27′N, 2°25′W, 370 m.a.s.l.), Logroño, North of Spain. Vines of *Vitis vinifera* “Maturana Tinta de Navarrete” (abbreviated to Maturana in this article) grafted on R110 were planted in 2010. The vineyard was north-south oriented with the spacing of 1.2 m (within row) × 2.4 m (between rows) and each row had 28 vines. Vertical cordon was trained 1.6 m high from the ground and six spurs were left after winter pruning with the lowest one located 0.7 m aboveground. A drip irrigation tube was placed on the ground. Two adjacent vine rows were selected for the trial and each of them were equally divided into four blocks; four treatments of winter pruning were randomly assigned to the four blocks so there were 14 vines for each treatment. Every treatment was applied to the exact same vines in both years. The Baillod & Baggiolini system (Baillod and Baggiolini, 1993) was applied to identify the growth stages of apical buds/shoots and the four treatments were: (1) winter pruning at stage A (WPA; dormant bud); (2) LWP at stage C (LWPC; green shoot tip) in 2015 and at stage F (LWPF) in 2016; (3) LWP at stage G (LWPG); (4) LWP at stage H (LWPH; flowers separated). It is worth mentioning that, in 2015, the second pruning treatment was conducted at stage C, however, LWP exerted very few effects compared to WPA (as will be described in detail below). Since the goal of the study was to delay the fruit ripening, we decided to shift the time of the second pruning to stage F in 2016. Two buds (excluding crown buds) per spur were left through winter pruning. Moderate trimming was performed when the shoots hindered the passing through the inter-rows. In 2015, drip irrigation was applied to all the treatments with an average amount of 4.5L/vine/day from the beginning of July, when strong water stress was observed, until the end of August. In 2016, the same pattern of irrigation lasted from the middle of July till the end of August. Climatic data was obtained from the nearest meteorological station located in Logroño. In both years, for each treatment, dates of budburst, full bloom, veraison and harvest were recorded and the number of days (Nº days), growing degree days (GDD), cumulative precipitation (CP) and Radiation (R) were calculated between every two adjacent phenological dates. Mean temperatures (mean T) during flowering (full bloom date ± 7 days) were also calculated.

Considering the risk of cluster abscission due to the possible environmental stress (Keller, 2015), bud fertility was assessed by calculating the average number of inflorescences per shoot right after the full fruit set of the latest pruning treatment. Leaf area (LA) per vine was calculated at harvest by multiplying LA per shoot by shoot number per vine and the method based on leaf disc sampling (Smart and Robinson, 1991) was used to estimate the leaf area per shoot.

In each year, fruits from all the treatments were harvested and analyzed at the same TSS level (22-23 ºBrix, which is a common range for commercial grapes of Maturana in the region). In the case of LWPH in 2016, the berries did not reach the designated ripening level before a serious botrytis occurred so they were picked at a lower TSS.

For each treatment, cluster number per vine was recorded when assessing bud fertility and cluster weight was measured on 40 randomly cut clusters. Finally, yield per vine was estimated by multiplying cluster number and cluster weight. Average berry weight was determined on 100 randomly sampled berries per repetition (3 repetitions per treatment) and these berries were subsequently crushed manually for the juice analysis. pH and titratable acidity (TA) were analyzed by standard methods (OIV, 2014). The concentration of the total anthocyanins was measured based on Iland *et al.* (2004).

Statistical package SPSS 16.0 (SPSS Inc., Chicago, US) for Windows was used for the statistic analysis. Data of yield components and berry composition were tested for homogeneity of variance using Levene's test and then one-way analysis of variance was run. S-N-K (equal variance assumed) or Dunnett's T3 (equal variance not assumed) method was used for the post-hoc multiple comparisons for means.

## Results

### Weather conditions and phenological stages

The spring and summer of 2015 were hot but the autumn was chilly (Figure 1). Besides, it needs to be pointed out that there was a continuous heat wave during a period of two weeks at the end of June and the beginning of July. In contrast, the season of 2016 had a cold spring and a warm ripening period. Except July and August, the rainfall of both years was low compared to the average of the past decade. Each LWP treatment could effectively delay the budburst date and shorten the time interval between budburst and full bloom (Figure 2, Table 1). The effects of LWPC on delaying the phenological stages were only maintained till veraison, and by contrast, LWPF (as a replacement of LWPC in 2016) successfully delayed the technological maturity of grapes by 20 days. In both years, LWPG and LWPH succeeded to postpone each phenological phase to a great extent and prolong the ripening period by 10-15 days (Figure 2, Table 1).

### Weather conditions between different phenological stages

In both years, the number of days, growing degree days (GDD, ºC), cumulative precipitations (CP, mm) and cumulative radiation (R, $10^6$ MJ/m$^2$) between different phenological stages were calculated and recorded (Table 1), as well as the mean temperature during flowering time. Only LWPH lead to an observably high mean temperature (2-4 ºC higher than WPA) during flowering time. From budburst to full bloom, grapes of LWP treatments received a higher heat energy (expressed as GDD) than WPA, especially LWPG and LWPH. Between full bloom and veraison, in both years, LWPG generated the hottest weather conditions while LWPH brought about the least rainfall. From veraison to harvest, grapes of LWPF, LWPG and LWPH received higher, similar and lower heat energy than WPA, respectively. Besides, vines of LWPF and LWPG received more illumination in relative to WPA while those of LWPH received the highest precipitation amount.

### Field parameters and yield components

LWPC and LWPF did not alter the berry numbers per cluster while LWPG and LWPH reduced the value of this parameter significantly (Table 2). Similarly, the same trend was observed for the cluster weight. In 2015, LWP treatments augmented the berry weight significantly; however, such difference was not found in 2016. In 2015, LWPH reduced the number of clusters per shoot drastically compared to the other treatments. In 2016, a relatively lower number was also observed for LWPH vines, though the difference was not significant. LWPH reduced the yield per vine to a large extent in both years while LWPG only reduced it significantly in 2015, by 52%. Regarding LA to fruit ratio, as a consequence of the lower yield, LWPG had a higher ratio compared to WPA and in the case of LWPH, this value was even higher, especially in 2015.

### Must composition

Must composition was compared at the same TSS level with the exception of the case of LWPH in 2016 (because of the bunch rot). In spite of this, 21.4 °Brix was obtained by the LWPH berries, only 1.6 °Brix lower than WPA (Table 3). In 2015, both LWPC and LWPG increased slightly must pH without affecting TA. In 2016, on the contrary, LWPF and LWPG reduced TA by 0.76 and 1.07 g/L, without increasing must pH significantly. As to LWPH, in both years, the must significantly had a higher TA and a lower pH than all the other treatments. From the anthocyanin point of view, only the latest LWP treatment exerted influences: in 2015, must of LWPH was significantly higher in both concentration (mg/g) and content (mg/berry) of total anthocyanins. In 2016, such difference was not found.

## Discussion

One of the biggest concerns about delayed winter pruning is the fluctuation of vine yield (Friend and Trought, 2007). Vine yield is a function of the number of buds per vine, bud fertility, the number of berries per cluster, and the berry weight (Keller, 2015). Within a winter bud, the formation of grape inflorescences begins at around flowering time of the current season. However, flower initials are not formed before the bud enter in dormancy (May, 2000). During the dormancy phase, morphological development cannot be observed and around budburst of the next season, inflorescences growth recommences together with the flower formation (Lavee and May, 1997; May, 2000; Vasconcelos *et al.*, 2009). Bud fertility is a gene-controlled trait, however, it is also affected by the environmental conditions from before inflorescence differentiation. On the other hand, in the following season, the climatic conditions from budburst to fruit set are as well a deciding factor of the final number of inflorescences and flowers (Keller, 2015). Therefore, a different growing condition caused by delayed winter pruning could have a great influence on the vine yield, not only of the current year but also of the next season.

Friend and Trought (2007) found that a LWP at stage E for Merlot vines significantly increased berry weight thus vine yield thanks to a favorable climatic condition during flowering. However, the finding of Gatti *et al.* (2016) was precisely the opposite: LWP at stage E and F maintained and reduced berry weight, respectively. In our trial, the influence of LWP on berry weight was inconsistent between two years so further study is necessary regarding this aspect. By contrast, the effect of LWP on berry numbers per cluster was clear: extremely late winter pruning (at stage G and H) lead to fewer berries per cluster, which indicated that vines of LWPG and LWPH had fewer flowers and/or a poorer fruit set compared to other treatments. Since the availability of carbohydrate is the determining factor of flower induction and fruit set (Friend and Trought, 2007; Vasconcelos *et al.*, 2009; Keller, 2015), it could be speculated that there was a shortage of carbohydrate supply during the process of bloom and/or fruit set. The lack of carbohydrate supply might be attributed to four possible reasons: (1) normally, vines have the least nutrition reserves in their perennial woods around flowering (Bennett *et al.*, 2005; Weyand and Schultz, 2006); what is more serious is that, in the case of a very late winter pruning, amounts of sources of carbohydrates and nitrogen compounds were removed by pruning otherwise they would contribute to the development of basal shoots (Frioni *et al.*, 2016; Gatti *et al.*, 2016); (2) as seen in Table 1, vines of LWP treatments had a shorter interval between budburst and full bloom in relative to WPA (34~44 days vs 51~57 days). As a consequence, at flowering time, LWP vines' leaves were not as mature as those of WPA and have limited capacity of photosynthesis since one leaf usually attains its maximum photosynthetic capacity at 30-35 days of age (Poni *et al.*, 1994; Gatti *et al.*, 2016), though Gatti *et al.* (2016) also stated that LWP could reduce the required time for foliage to reach its maximum efficiency; (3) before flowering, under warm conditions, a growing shoot is a strong sink creating an intensified competition with flower differentiation for nutrition, resulting in the occurrence of flower abortion, which is well known as the phenomenon of "filage" (Champagnol, 1984). (4) extremely high temperatures (>35 ºC) could reduce fruit set percentage (Keller, 2015). However, this case only happened to LWPH vines in 2015, during the long lasting heat wave.

Another focus is the number of inflorescences per shoot which is the representation of bud fertility (Vasconcelos *et al.*, 2009). Interestingly, for this parameter, we only observed a significantly lower value on LWPH in 2015. Since it was the first year of the experiment, inflorescence differentiation degree was assumed the same among treatments in the previous season. The poorer fertility of LWPH buds should be attributed to the loss of pre-developed inflorescence in the current season. Actually, the reversion of an inflorescence to a tendril is possible, among other circumstances, when the rachis extends rapidly on a rapidly growing shoot under high temperatures (filage) (Champagnol, 1984) and/or when there is a severe source limitation (Gatti *et al.*, 2016). In 2015, for LWPH vines, the mean temperature during flowering was 24.7 ºC and the GDD from budburst to full bloom was 457.9 ºC (Table 1), which might be so high that filage came about. On the contrary, in 2016, under a more normal weather condition (Table 1), no significant difference in bud fertility was found among treatments. To sum up, it seems that LWP does not inhibit the inflorescence differentiation in the season when buds occur and that a very late winter pruning combined with an unfavorable weather condition could give rise to the reversion of inflorescences to tendrils in the following season. Nonetheless, this contention should be supported by long term data, especially after LWP vines are subjected again to a standard winter pruning during bud dormancy. As reported by Frioni *et al.* (2016), after the pruning was switched back to normal, LWPG vines could obtain a normal bud fertility again while vines previously subjected to LWP at stage H-I still had fewer inflorescences per shoot than control.

Based on the two years' experimental results, setting aside every single yield component, the global effects of LWP on vine yield can be summarized as follows: a LWP before or at stage F is unlikely to reduce vine yield. LWPG could significantly reduce vine yield but to an acceptable level (about 30% to 50% less than WPA, Table 2). However, LWPH might lead to an undesirable yield level. If converted into production per hectare, LWPH vines produced 3272 kg/ha and 5304 kg/ha of grapes in 2015 and 2016, respectively. The former value is too low in relative to the average production of red grapes in the region. As a matter of fact, in many commercial vineyards within the Rioja wine region, cluster thinning is annual work as the yield per hectare is strictly limited by the local wine law, though it is quite time consuming (about 40 hours/ hectare). Fortunately, we may dispense with this costly work by simply postponing the pruning date, as long as the extent of the delay is appropriate. The values of LA/P at harvest indicate that vines of all the treatments possessed sufficient LA to mature their berries properly.

We tried to harvest the grapes of all the treatments at the same TSS level because only in this way the rest of the parameters (TA, pH and anthocyanins) of must are comparable, since the perception of acidity in the wine is greatly affected by the alcohol content (Jackson, 2009) and the anthocyanin concentration is closely related to the TSS level (Pirie and Mullins, 1977). However, for LWPH, the sugar accumulation in the ripening period was so slow that the berries did not reach the designated TSS level until the serious botrytis was observed in 2016. The reason for the slow rate of sugar accumulation might be the considerably lower GDD during ripening (Table 1). Though LWPH vines had a much higher LA/P value than other treatments (Table 2), the low temperatures might repress the photosynthetic activity greatly (Keller, 2015); Besides, according to Kliewer and Dokoozlian (2005), excessive LA/P (>1.2-1.5 $m^2$/kg) does not help to achieve a higher maximum level of TSS. On the other hand, low temperatures during the ripening phase of LWPH grapes were exactly what we wanted for the sake of anthocyanins because there is a general consensus that high temperatures could inhibit the anthocyanin accumulation (He *et al.*, 2010). Our results on LWPH also support this standpoint. In 2015, the anthocyanin concentration of LWPH must was 38% higher than WPA. In 2016, though the anthocyanin concentration is similar among treatments, it should not be ignored that the grapes of LWPH had a significantly lower sugar content. In other words, LWPH grapes had a higher anthocyanins to sugar ratio in both years. It is true that a high LA/P is also beneficial to the anthocyanin accumulation, however, like the case of TSS, as long as the LA/P is above 1.2 $m^2$/kg, redundant LA seems unhelpful to the anthocyanin accumulation (Kliewer and Dokoozlian, 2005). Therefore, the best explanation for the improvement of anthocyanins to sugar ratio might be that LWPH created a cooler ripening condition by delaying and prolonging the ripening phase. The mean temperatures from veraison to harvest for WPA, LWPC (2015)/LWPF (2016), LWPG and LWPH were 21.8 ºC, 21.8 °C, 18.9 °C and 16.1 °C, respectively, in 2015; 23.3 ºC, 21.1 °C, 19.3 °C and 18.0 °C, respectively, in 2016. In fact, LWPF and LWPG succeeded to reduce the mean temperature of the ripening phase as well, but the coloration of grapes was not enhanced, indicating that a sharp decline in mean temperature during ripening (>5 °C) might be necessary in favor of the anthocyanin accumulation of Maturana grapes. Actually, Maturana is a minority variety cultivated in the Rioja wine region and it is characterized by very high color content (Balda *et al.*, 2013). In the same region, it was reported that a decrease of 2.3 °C of daily mean temperatures during ripening enhanced the coloration of Garnacha grapes (Martínez de Toda *et al.*, 2014), which usually have much less color than Maturana. So it is conceivable that the sensitivity of anthocyanin accumulation to temperatures might be variety-dependent. From the two years of data, a delayed winter pruning at stage C, F and G did not have much impact on berries from acidic point of view. However, LWPH grapes always kept more acidity and lower pH, being the possible reason that low temperatures repressed the respiratory malate degradation (Keller, 2015).

## Conclusions

LWP at stage G and H could effectively delay all the phenological stages of Maturana grapes to a great extent and create a considerably cooler ripening condition than a standard winter pruning. Vine yield is unlikely to be affected by a LWP before or during the stage F, however, LWPG and LWPH could reduce vine yield by inhibiting flower formation and/or fruit set. With an acceptable yield level, LWPG can be applied as a better alternative to cluster thinning. LWPH can improve the anthocyanin accumulation and help to maintain a relatively high level of acidity in berries. Delaying the winter pruning to the stage H is a promising way to restore the anthocyanin to sugar ratio decoupled by the warming climate, despite the risk of the botrytis and a serious decline in production. Further study should be carried out to evaluate the long-term effects of LWP on grapevines, especially on bud fertility and nutritional reserves of perennial parts. Also, it will be interesting to apply this technique on other varieties to make clear to which degree could a delayed winter pruning help to improve the grape coloration.

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**Table 1 - Climatic parameters during different phenological stages of Maturana grapes for Control (WPA), late pruning at stage C (LWPC) or F (LWPF), at stage G (LPWG) and at stage H (LWPH) in 2015 and 2016**

[a] Flowering period is considered as the period between seven days before full bloom and seven days after full bloom.

[b] T, CP, GDD and R are short for temperature, cumulative precipitation, growing degree days and radiation, respectively.

| Year | Phenological stages | Parameters | WPA | LWPC (2015) LWPF (2016) | LWPG | LWPH |
|---|---|---|---|---|---|---|
| 2015 | Flowering time[a] | Mean T[b] (ºC) | 20.1 | 20.1 | 19.2 | 24.7 |
| | Budburst to full bloom | Nº days | 51 | 44 | 41 | 38 |
| | | GDD (ºC) | 288.3 | 294.0 | 316.0 | 457.9 |
| | | CP (mm) | 20.8 | 4.8 | 69.7 | 74.2 |
| | | R ($10^6$ MJ/m$^2$) | 1.127 | 1.028 | 0.989 | 0.985 |
| | Full bloom To veraison | Nº days | 55 | 53 | 54 | 50 |
| | | GDD (ºC) | 692.6 | 663.0 | 732.4 | 626.2 |
| | | CP (mm) | 114.8 | 114.8 | 56.7 | 55.2 |
| | | R ($10^6$ MJ/m$^2$) | 1.425 | 1.371 | 1.410 | 1.172 |
| | Veraison to harvest | Nº days | 37 | 36 | 50 | 52 |
| | | GDD (ºC) | 434.9 | 421.4 | 438.1 | 310.3 |
| | | CP (mm) | 47.2 | 47.2 | 43.2 | 66.9 |
| | | R ($10^6$ MJ/m$^2$) | 0.789 | 0.760 | 0.930 | 0.764 |
| 2016 | Flowering time | Mean T (ºC) | 20.2 | 19.6 | 22.1 | 22.3 |
| | Budburst to full bloom | Nº days | 57 | 34 | 35 | 34 |
| | | GDD (ºC) | 300.2 | 304.4 | 336.8 | 387.5 |
| | | CP (mm) | 58.9 | 25.6 | 19.7 | 22.3 |
| | | R ($10^6$ MJ/m$^2$) | 1.279 | 0.856 | 0.880 | 0.871 |
| | Full bloom to veraison | Nº days | 62 | 56 | 58 | 56 |
| | | GDD (ºC) | 728.9 | 696.4 | 742.9 | 717.2 |
| | | CP (mm) | 26.5 | 30.5 | 28.7 | 13.7 |
| | | R ($10^6$ MJ/m$^2$) | 1.568 | 1.424 | 1.458 | 1.341 |
| | Veraison to harvest | Nº days | 33 | 46 | 48 | 44 |
| | | GDD (ºC) | 441.9 | 507.9 | 442.6 | 343.0 |
| | | CP (mm) | 9.5 | 1.0 | 8.6 | 11.0 |
| | | R ($10^6$ MJ/m$^2$) | 0.712 | 0.836 | 0.781 | 0.651 |

**Table 2 - Effects of different pruning time on yield components in 2015 and 2016 (Logroño, La Rioja, Spain)**

[a] WPA, LWPC, LWPF, LWPG and LWPH are short for winter pruning at stage A, late winter pruning at stage C, F, G and H, respectively.

[b] Significance level; data were analyzed with one way Anova; *,**, ***, ns: significant at p≤ 0.05, p≤ 0.01, p≤ 0.001 or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at p≤ 0.05.

| | 2015 | | | | | 2016 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WPA[a] | LWPC | LWPG | LWPH | Sig[b] | WPA | LWPF | LWPG | LWPH | Sig |
| Nº berries/cluster | 212 a | 223 a | 132 b | 110 b | ** | 194 a | 173 a | 113 b | 88 b | *** |
| Cluster weight (g) | 270 a | 317 a | 180 b | 158 b | * | 256 a | 228 a | 151 b | 122 b | *** |
| Berry weight (g) | 1.27 b | 1.42 a | 1.37 ab | 1.44 a | * | 1.32 | 1.32 | 1.34 | 1.39 | ns |
| Nº clusters/vine | 14 a | 15 a | 10 ab | 6 b | * | 15 | 16 | 18 | 13 | ns |
| Nº shoots/vine | 13 | 14 | 11 | 14 | ns | 13 | 17 | 16 | 17 | ns |
| Nº clusters/shoot | 1.1 a | 1.1 a | 0.9 a | 0.4 b | ** | 1.2 | 0.9 | 1.1 | 0.8 | ns |
| Production (P) /vine (kg) | 3.78 a | 4.76 a | 1.80 b | 0.95 c | ** | 3.84 a | 3.65 a | 2.72 ab | 1.59 b | * |
| Leaf area (LA)/vine ($m^2$) | 4.70 | 5.29 | 5.02 | 6.50 | ns | 5.10 | 5.31 | 4.47 | 3.86 | ns |
| LA/P ($m^2$/kg) | 1.31 | 1.11 | 2.79 | 6.84 | | 1.29 | 1.49 | 1.64 | 2.31 | |

**Table 3 - Effects of different pruning time on must composition in 2015 and 2016 (Logroño, La Rioja, Spain)**

[a] WPA, LWPC, LWPF, LWPG and LWPH are short for winter pruning at stage A, late winter pruning at stage C, F, G and H, respectively.

[b] Significance level; data were analyzed with one way Anova; *,**, ***, ns: significant at p≤ 0.05, p≤ 0.01, p≤ 0.001 or not significant, respectively. When differences among treatments were significant, S-N-K method was used to separate the means; different letters (a, b) represent different means at p≤ 0.05.

[c] The titratable acidity is expressed as g/L tartaric acid.

| | 2015 | | | | | 2016 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WPA[a] | LWPC | LWPG | LWPH | Sig[b] | WPA | LWPF | LWPG | LWPH | Sig |
| Brix At harvest (°) | 23.0 | 22.6 | 22.5 | 22.4 | ns | 23.0 a | 22.8 a | 22.8 a | 21.4 b | * |
| Titratable acidity (g/L)[c] | 7.15 b | 6.35 b | 6.56 b | 8.89 a | *** | 6.85 b | 6.09 c | 5.78 c | 7.95 a | ** |
| PH | 3.40 b | 3.49 a | 3.53 a | 3.30 c | *** | 3.46 a | 3.52 a | 3.58 a | 3.31 b | ** |
| Anthocyanin concentration (mg/g) | 2.60 b | 2.40 b | 2.72 b | 3.58 a | *** | 3.14 | 3.37 | 3.21 | 3.39 | ns |
| Anthocyanin Content (mg/berry) | 3.44 b | 3.42 b | 3.73 b | 5.17 a | *** | 4.14 | 4.44 | 4.27 | 4.72 | ns |

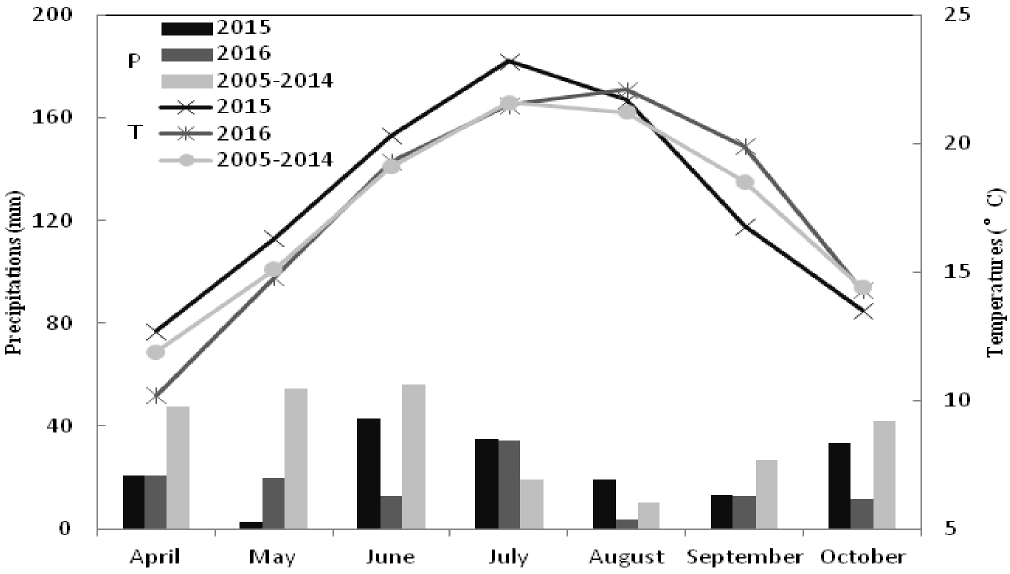

Figure 1 - Mean monthly temperature (T) and monthly cumulative precipitations (P) during growing seasons in Logroño.

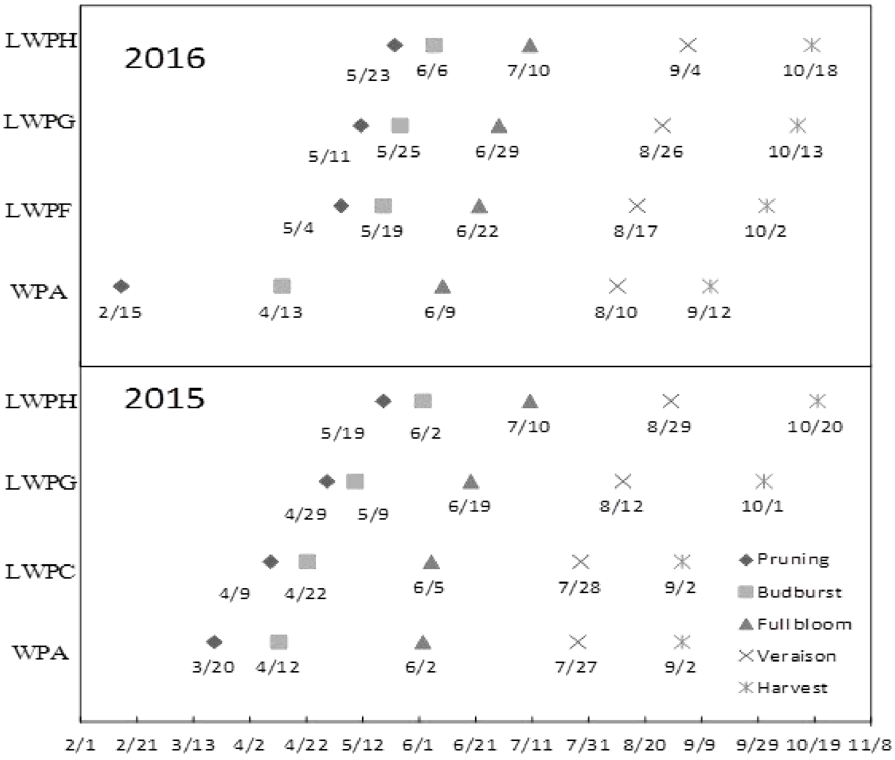

Figure 2 - Dates of pruning, budburst, full bloom, veraison and harvest for Control (WPA), late pruning at stage C (LWPC, in 2015) or F (LWPF, in 2016), at stage G (LPWG) and at stage H (LWPH) in 2015 and 2016.

# CHAPTER 8

# GENERAL DISCUSSION

# Chapter 8. General Discussion

The main objective of this PhD thesis was to evaluate the effectiveness of different cultural practices for delaying the grape maturity and restoring the anthocyanins-to-sugars ratio in the context of climate change. In the meantime, the effects on vine yield components as well as on fruit compositions were also assessed in detail. In this section, different cultural techniques are compared from all the mentioned perspectives. It needs to be emphasized that, though the basal leaf removal (LR) is different from other techniques on the basis of theory, the original intention of the study was the same as the others', that is, to improve grape quality (especially the perspective of anthocyanins) under climate change. Therefore, we put all the techniques together for the general discussion.

## 8.1 Effectiveness of different cultural techniques on delaying grape technological maturity

Except LR, all the techniques succeeded to delay the technological maturity of grape berries. Actually, at the moment of LR, a large portion of the total leaf area (LA) was eliminated. The reason for that no ripening delay was observed might be that, for one thing, the enhanced photosynthetic activity of remaining leaves and newly generated lateral shoots could partially compensate the loss of LA (Poni et al. 2006). In addition to this, the removed leaves (with an average leaf age of 50~60 days) were relatively old and their photosynthetic capacity had been declining. These leaves would not have been an important contributor to the canopy carbon assimilation even if they had been left, especially during the ripening phase (Poni et al. 1994). Interestingly, the case of the severe shoot trimming (SST) is completely different. SST consists of removing the majority of young leaves which otherwise would be the main force of photosynthesis during the ripening period (Vasconcelos and Castagnoli 2000). Despite the fact that lateral shoots can replace the main shoot early or late, the growth of them is unpredictable since it depends largely on the weather conditions and cultural practices (Poni et al. 2014). Moreover, lateral shoots play the role of "sinks" at the beginning thus competing with the growing berries (Wolf et al. 1986). As a consequence, SST could always lead to a delay of sugar accumulation. However, it should be noted that, two-time SST may result in a poor maturity of grapes (low sugar content though harvested very late), in particular under non-irrigation conditions. The water availability seems to determine the effects of minimal pruning (MP) as well. When the water supply is enough, MP can always postpone the berry technological maturity yet without reducing the final sugar content. However, under severe water stress, MP grapes may not ripen properly, as what happened in 2016 (the results in 2016 were not presented in the paper of MP). The growing season of 2016 was so dry that most leaves of MP vines dried-up when the total soluble solids (TSS) level of berries were still unacceptable. Since MP vines always generate a considerably larger LA than spur-pruned vines, their demand for water is supposed to be a lot greater, so it is reasonable that they are more sensitive to water stress (Lakso 1993). Late winter pruning (LWP) has been proved to be very effective on delaying the berry ripening, especially LWP at stage G (LWPG) and H (LWPH), which could delay the technological maturity of grapes to a large degree. However, yield loss seems an ineluctable consequence of LWPG and LWPH.

To sum up, a single severe shoot trimming is the most “safe” technique because it does not affect the vine yield or the final sugar content. MP is also a promising technique on condition that extreme droughts rarely occur or there is an irrigation system. In terms of ripening delay, a very late winter pruning could exert a larger effect than SST and MP, though the yield loss might be unwanted. Of course, it should be interesting to combine the different techniques in order to delay the berry ripening to a large degree without yield loss. For example, with LWP being done at stage F, after fruit set, we could carry out a SST.

## 8.2 Effectiveness of different cultural techniques on restoring the anthocyanins-to-sugars ratio

Anthocyanins play a role of great importance in red grape varieties and their wines. Apart from the aesthetic value, they could give slight astringency to the mouth feel as well as interact with some aroma components (He et al. 2010). So a high level of total anthocyanins is usually desired. The decoupling of anthocyanins and sugars due to climate change has been widely concerned, especially in hot regions (Keller 2010, Sadras and Moran 2012, Palliotti et al. 2014, Herrera et al. 2015, Frioni et al. 2016). To restore the anthocyanins-to-sugars ratio means to maintain or even to increase the anthocyanin concentration of grape berries at a desirable TSS level. Our hypothesis was: by applying different cultural practices, the relatively low temperatures during the postponed ripening phase could help to improve the grape color, due to the fact that high temperatures inhibit the color development (Mori et al. 2005, Mori et al. 2007, He et al. 2010). It seems that berry color could always be enhanced by MP, albeit this improvement in color is more likely due to the reduced berry size than to a better anthocyanin synthesis capacity. We can say that the goal of restoring the anthocyanins-to-sugars ratio is achieved through MP (Even in 2016, though MP berries did not reach a good technological maturity, they still got a higher anthocyanin concentration than those from control vines at the same TSS level). The results of the two studies about SST seems inconsistent: in the paper published in 2014 (Chapter 2), we concluded that delaying the berry ripening by trimming could partially restore the anthocyanins-to-sugars ratio disrupted by elevated temperatures. However, in the recent paper (Chapter 3), we stated that the relatively cooler ripening condition brought about by trimming might be insufficient for a better anthocyanin synthesis. As a matter of fact, the findings are not contradictory because, in the study of 2014, the berry size of SST grapes was always reported to be smaller. Thus, like the case of MP, smaller berries tend to give a higher anthocyanin concentration thanks to their higher skin-to-pulp ratio. Nevertheless, as to anthocyanin content (expressed as mg anthocyanins/berry), both treatments showed similar values indicating that the capacity of anthocyanin synthesis of SST berries might be equal to that of control berries. Moreover, the temperature difference between SST and control during ripening period was different in the two studies. In the study of 2014, the gap of daily mean temperatures between treatments was 2.3°C during the ripening phase of Garnacha grapes. In contrast, in the study of 2017, this gap was 1.0 °C for Tempranillo and 2.1 °C for Garnacha. A bigger temperature difference might contribute to the better anthocyanin accumulation in the study of 2014. A pioneering study of Bobeica et al. (2015) suggested another interesting hypothesis related to severe trimming: carbon limitation might decouple the anthocyanins-to-sugar ratio. In their trials, when only 3 leaves were retained on one shoot after severe trimming, TSS was greatly reduced (-27.1%) while the anthocyanin concentration decreased to an even greater extent (-84.3%). Similarly, looking back at our data of Garnacha in the study of 2017, the results of double trimming showed the same thing. So, we can conclude that the success of the application of SST depends not only on the extent of the ripening delay, but also on the source limitation. The latest winter pruning (LWPH) lead to a higher anthocyanins-to-sugars ratio in both of the experiment years probably due to the fact that grape berries of LWPH vines were ripening under much cooler conditions than those of control vines ($\Delta$ T > 5.0°C). However, though LWPG also created a sharp decline in mean temperature during ripening phase ($\Delta$ T ≈ 3.5°C), the berry coloration was not enhanced. Why? Another important factor may be involved here: the pre-veraison temperatures. Sadras and Moran (2012) presented direct evidence of a temperature-driven decoupling of sugars and anthocyanins in berries of Shiraz and Cabernet Franc and they concluded that such decoupling was more likely to be attributed to the delayed onset of anthocyanin synthesis than the rate of anthocyanin accumulation. Namely, their viewpoint is that, if the temperatures before veraison are too high, the veraison may occur at a higher TSS level than normal so that the final anthocyanin content is poor, provided the accumulation rate is constant during ripening. Throughout our data on anthocyanins, wherever the trials were carried out, for whatever varieties, fruits of the same treatment always contained less anthocyanins in 2015 than in 2014 and/or in 2016. Moreover, we measured the TSS level in the study on LR (Chapter 5) and we found that with the hottest pre-veraison weather (in 2015), the onset of anthocyanin synthesis occurred at the highest TSS content. In contrast, under the coolest pre-veraison weather conditions (in 2016), berry color appeared at the lowest TSS level. This is direct proof of the above viewpoint of Sadras and Moran (2012). Back to the question about LWP, we can readily explain why LWPG failed to improve the berry coloration in spite of the considerably cooler ripening conditions: the pre-veraison weather might be too hot for LWPG grapes. As seen in Chapter 6, the mean temperatures from full bloom to veraison for control, LWPC (2015)/LWPF (2016), LWPG and LWPH were 22.6 ºC, 22.5 °C, 23.6 °C and 22.5 °C, respectively, in 2015; 21.8 ºC, 22.4 °C, 22.8 °C and 22.8 °C, respectively, in 2016. In the case of LWPG, the "advantage" of a cooler ripening condition for anthocyanin accumulation might be cancelled out by the delayed onset of anthocyanin synthesis due to hotter pre-veraison weather conditions.

On the contrary, as discussed in Chapter 5, though an early LR could shift forward the onset of anthocynanin synthesis, this “advantage” might not be maintained if the berries are overheated during the ripening period.

All in all, it can be concluded that delaying maturation thus creating cooler conditions during ripening phase might be an effective way to restore the anthocyanins-to-sugars ratio only when there is a sharp decline in mean temperature. Besides, high temperatures between flowering and veraison could also cause the thermal decoupling of anthocyanins and sugars. Regardless of the temperature factor, a reduced berry size is always helpful to the grape coloration.

## 8.3 Effects of different cultural techniques on vine yield and fruit quality

It was not our original intention to alter the vine yield components by applying the cultural techniques but it always resulted that MP brought about more production while very late winter pruning reduced vine yield. In many viticulture regions around the world, yield per hectare is strictly limited by local wine regulations and severe pruning is widely practiced since it is considered as the cheapest way of fighting against overbearing and vine exhaustion. However, this severe pruning itself decreases vine capacity due to the great loss of reserves (Winkler et al. 1974) and it is not really necessary in some viticulture regions since it might contribute to low wine quality generally associated with development of shaded, tight bunches with large berries and difficulties in the control of pests and diseases (Clingeleffer 2010). Conventional wisdom may suggest that high yield is always linked with poor quality but this taken-for-granted viewpoint has proved to be wrong (Clingeleffer 1993, Archer and Van Schalkwyk 2007, Clingeleffer 2010). Martinez de Toda (2011) proposed four conditions for acquiring grapes of maximum quality: 1) an appropriate variety; 2) an enough leaf area-to-fruit ratio; 3) a moderate vine vigor; 4) healthy grapes. Kliewer and Dokoozlian (2005) stated that the leaf area-to-fruit ratio required for maximum level of total soluble solids, berry weight, and berry coloration at harvest ranged from 0.8 to 1.2 $m^2$/kg. So we can say that vine yield is not a limiting factor to the grape quality as long as there is enough leaf area to aliment the berries.

From this perspective, it is unquestionable that MP is an advantageous and promising cultural technique. In contrast, LWP at stage G and H delayed berry ripening at the cost of yield. As discussed in Chapter 6, LWPG can be used as an alternative to cluster thinning, in order to cater the requirement for production according to the wine legislation. However, the yield loss brought about by LWPH might be too great to be acceptable for the viticulturists. With respect to SST and LR, since the operations were carried out from one to two weeks after fruit set, the cell division process had almost finished so vine yield was not affected significantly.

The effects of different techniques on sugar and pigment have been discussed in the above. Apart from these two parameters, another important quality element is acid since it determines not only the perception of sourness and related organoleptic properties, but also the microbial stability (Boulton 1980b). There are four main parameters related to acid: titratable acidity (TA), pH, tartaric acid and malic acid. The tartaric and malic acid levels are not accurate indicators of TA but high ratio of tartrate to malate lead to low pH (Boulton 1980a). High pH of grape juice is one of the unwanted characters associated with the warming climate. Besides, compared to malic acid, tartaric acid has a higher metabolic and microbiological stability. From this point of view, a higher tartrate-to-malate ratio is desirable. Based on our trials, it can be concluded that SST and LR have the tendency to improve the grape acid composition by increasing the ratio between tartaric and malic acid. As to MP and LWP, no obvious conclusion can be made from the perspective of acid.

## 8.4 Operation costs of different cultural practices

The ultimate goal of the use of these cultural practices is to improve the grape quality, thereby increasing the income of viticulturists or winemakers. Of course, it is unworthy of application if the operation costs are too high. Therefore, it is worth a simple discussion on this issue. Winter pruning is the most time-consuming job and it accounts for 30% of the total labor costs in the vineyard (Martinez de Toda and Sancha 1998). For a skilled worker, manual pruning usually takes about 40h/ha (Martínez de Toda 1994). MP can completely save such labor-hours. It is true that, every few years, trimming operation should be conducted so as to maintain the canopy shape (Clingeleffer 1988), but the trimming can be mechanized and conducted along with other field operations such as soil tillage and chemical spraying. Another advantage about MP is that, since MP vines usually have a balance growth (Clingeleffer 1993), operations such as shoot thinning, repeated trimming and lateral removal can be dispensed (Martínez de Toda et al. 2015). Therefore, the saving of labor and time is even more considerable than what it seems to be. For early LWP operation (before stage F), the workload is similar to normal winter pruning. If LWP is carried out too late (after stage G), the heavy current-growth shoots will increase the amount of pruning, making the operation a little bit time-consuming and laborious. Nonetheless, LWPG can be used as an alternative of cluster thinning, which is also a labor-cost operation. Both shoot trimming and leaf removal can be mechanized and according to Martínez de Toda (1994), each operation takes about 1.5-2.0 h/ha by machine. It seems difficult to mechanize a severe shoot trimming for VSP (vertical shoot positioning) as the trimming is usually exerted above the foliage wires on such a training system (Palliotti et al. 2014). Nonetheless, with the innovative system of pre-pruning Pellenc, it is possible to realize the cutting at any height. On the other hand, mechanical LR can exert similar effects as hand LR, but to a lesser extent. Besides, different from the case of distal LR which can be mechanized perfectly (Palliotti et al. 2013), with mechanical LR in the cluster zone, it is hard to succeed in removing enough LA without inflicting damage to the clusters, whose tips are easy to be cut off by the blades (Intrieri et al. 2008). So, it is better to do LR in the cluster zone manually though the cost will be increased.

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# CHAPTER 9

# CONCLUSIONS

## Chapter 9. Conclusions

1. A severe shoot trimming on grapevines after fruit set delays the berry ripening significantly so that berries from trimmed vines can ripen under relatively cooler conditions.

2. Severe trimming seems beneficial to the anthocyanin accumulation only when the mean temperature during the ripening phase of trimmed vines is much lower than that of untrimmed vines or when the berry size of trimming treatments is significantly smaller than normal.

3. Excessively severe trimming (namely two severe trimmings) after fruit set might lead to improper maturity of grapes, especially under drought conditions. Great shortage of photosynthesis source could even decouple the anthocyanins-to-sugars ratio.

4. Severe trimming treatments have the tendency to improve the fruit acid composition by increasing tartaric acid meanwhile reducing malic acid.

5. In the two-year study, minimal pruning (MP) vines had enough leaf area-to-fruit ratio but compared to spur pruning, MP always lead to smaller berry size and fewer berries per cluster as well as a delayed ripening, indicating that MP vines had more source limitation than spur pruning.

6. Compared to spur pruning, MP always leads to a moderately higher yield and delays the technological maturity of grapes by 2-3 weeks thus creating a slightly cooler condition for berry ripening.

7. Higher concentration of total anthocyanins was observed on MP juice, however, potential improvements in wine color were more likely due to the smaller berry size than to a greater ability of anthocyanin synthesis.

8. MP is a viable and labor-saving cultural practice for Tempranillo grapes within Rioja wine region, though it is better to be accompanied with irrigation facilities in case of extreme drought.

9. A severe basal leaf removal (with also lateral shoots removed) two weeks after fruit set is not likely to alter yield components of Tempranillo grapes under the viticultural conditions studied.

10. After a severe basal leaf removal, the full exposure of clusters did not have any negative effects on the quality of Tempranillo grapes under the experimental conditions. Not only so, like the case of severe trimming, berry acid composition was also improved by this cultural technique.

11. Basal leaf removal two weeks after berry set reduces the required sugar level for the onset of anthocyanin synthesis.

12. Late winter pruning (LWP) at stage G (LWPG) and H (LWPH) (based on Baillod & Baggiolini system) effectively delays all the phenological stages of Maturana grapes to a large degree and creates a much cooler ripening condition than a standard winter pruning.

13. LWPH improved the grape color while LWPG failed to do so, which indicates that delaying grape phenological stages in search of cooler ripening conditions might be an effective way to improve anthocyanins-to-sugars ratio only when there is a sharp decline in mean temperature. Besides, pre-veraison weather conditions should not be too hot.

14. LWP before or during stage F is not likely to alter vine yield while LWPG and LWPH could cause a great yield loss. The primary reason for such reduction in production seems to be the losses of flowers and/or the reduction in fruit set percentage in the current season, instead of the losses in inflorescences within buds in the previous season.

15. From the results obtained on the delay of ripening we can conclude that it would be also viable to combine the different techniques which have been studied. For example, we can conduct a LWP at stage F or G; then, after berry set, if necessary, a trimming could help with the further delaying. By using more than one technique but with less intensity, we could delay the grape ripening to a great extent so that the defect of every single technique might be avoided.

# CONCLUSIONES

1. El recorte severo de los pámpanos, después del cuajado, retrasa significativamente la maduración de la uva, de manera que esta maduración puede desarrollarse en condiciones relativamente más frescas.

2. El recorte severo puede ser beneficioso para la síntesis de antocianos con la condición de que la temperatura durante la fase de maduración de las vides recortadas sea claramente más baja que la de las vides no recortadas o cuando el tamaño de las bayas resultantes del tratamiento de recorte sea más pequeño que en el control.

3. El doble recorte severo puede producir una maduración insuficiente de la uva en condiciones de poco vigor y una fuerte limitación de la fotosíntesis podría, incluso, desacoplar la relación entre antocianos y azúcares.

4. El recorte severo presenta una tendencia a aumentar la relación entre ácido tartárico y ácido málico.

5. Los tratamientos de poda mínima siempre presentaron suficiente relación superficie foliar/producción pero, comparados con la poda tradicional, produjeron bayas de menor tamaño y menos bayas por racimo, así como un retraso importante en la maduración. Esto parece indicar que la superficie foliar de las cepas sometidas a poda mínima presenta una fotosíntesis menor que la de las cepas con poda tradicional.

6. Comparada con la poda tradicional, la poda mínima siempre produce una mayor cantidad de uva y un retraso en la maduración de entre dos y tres semanas, lo que genera unas condiciones ligeramente más frescas para la maduración de la uva.

7. La poda mínima produjo una mayor concentración de antocianos totales en el mosto que la poda tradicional, pero fue más debido al menor tamaño de la baya que a una mayor de síntesis de antocianos en el hollejo.

8. La poda mínima es una técnica de cultivo viable para retrasar la maduración de la uva y aumentar la concentración de antocianos aunque, debido a la mayor

superficie foliar desarrollada, necesita mayor cantidad de agua disponible.

9. El deshojado basal completo (con eliminación de los nietos), dos semanas después del cuajado, no afectó a las características de la producción en las condiciones vitícolas estudiadas.

10. La exposición total de los racimos a la radiación solar, como consecuencia de un deshojado basal completo después del cuajado, no tuvo ningún efecto negativo sobre la calidad de la uva ni del vino; al contrario, incrementó la relación entre el ácido tartárico y el ácido málico y el análisis sensorial del vino mostró más color y más cuerpo para el tratamiento de racimos totalmente expuestos.

11. El deshojado basal completo, dos semanas después del cuajado, redujo la concentración de azúcares necesaria en la baya para el comienzo de la síntesis de antocianos.

12. La poda tardía, en los estadios fenológicos G y H, retrasó la maduración de la uva en gran medida y permitió unas condiciones de maduración mucho más frescas que la poda estándar. Sin embargo, estos efectos se obtuvieron a costa de una pérdida considerable de la producción.

13. La poda tardía realizada en el estadio fenológico H incrementó la relación antocianos/azúcares del mosto mientras que la realizada en el estadio G no lo hizo, lo que indica que retrasar la fenología en búsqueda de condiciones más frescas de maduración podría ser un camino efectivo para incrementar la relación antocianos/azúcares.

14. La poda tardía, antes o durante el estadio F, no afectó a la producción ni a la composición de la baya mientras que las podas realizadas en los estadios G y H causaron una disminución considerable de la producción. La principal razón en el descenso de la producción parece ser la pérdida de flores y/o la reducción en el porcentaje de cuajado en la añada actual, y no la menor iniciación floral de las yemas en el año anterior.

15. De los resultados obtenidos en las experiencias desarrolladas se desprende que también se pueden utilizar combinaciones de las diferentes técnicas estudiadas.

Por ejemplo, podríamos hacer una poda tardía hasta el estadio F o G y, si después del cuajado, queremos retrasar todavía más la maduración, podríamos hacer un posterior recorte de los pámpanos; utilizando más de una técnica, pero con menor intensidad, podríamos retrasar en mayor medida la maduración de la uva sin los efectos negativos de realizar una sola técnica, pero más severa.