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

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

ICVV (Universidad de La Rioja, CSIC, Gobierno de La Rioja), Logroño, Spain

#### 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.

K e y w o r d s : 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 anthocyanin to sugar 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

Correspondence to: F. MARTÍNEZ DE TODA, ICVV (Universidad de La Rioja, CSIC, Gobierno de La Rioja), Madre de Dios, 51, 26006 Logroño La Rioja, Spain. Fax: +34-941-299-721. E-mail: fernando.martinezdetoda@unirioja.es

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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 9-10<sup>th</sup> 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 d 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.

#### **Material 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 northsouth oriented with a planting pattern of 1.2 m (within row)  $\times$  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 4.5 L·vine<sup>-1</sup>·day<sup>-1</sup> 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 2/3of 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 ROBIN-SON 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  $(mg \cdot g^{-1})$ 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

We at her conditions: 2014 had a relatively cool Summer but an extremely warm September and October (Fig. 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.



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

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 Tab. 1 and Tab. 2, for 'Tempranillo' in VL and for 'Grenache' in VB, respectively.

In VL, compared to C, T delayed the veraison date by 3-5 d while TT delayed it by 4-8 d. Grapes of C reached the designate TSS level (22-22.5 °Brix) 14-23 d earlier than T and 21-23 d 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 d by T and 15-18 d by TT. In 2014, grapes from C group were harvested at 24 °Brix on Oct 1<sup>st</sup> and, 20 d 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 Fig. 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

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Effects of trimming once (T) and trimming twice (TT) on yield components for 'Tempranillo' vines (2014 and 2015, Logroño, La Rioja, Spain)

		2	2014		2015				
Treatments	Control	Т	TT	Significance level <sup>a</sup>	Control	Т	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 <sup>-1</sup> )	2.89	2.75	2.50	ns	4.95	5.12	4.71	ns	
Leaf area (LA) (m <sup>2</sup> ·vine <sup>-1</sup> )	3.82 a	1.86 b	1.54 b	***	7.45 a	3.25 b	2.89 b	***	
$LA/P(m^2 \cdot kg^{-1})$	1.37 a	0.70 b	0.58 b	*	1.54 a	0.63 b	0.61 b	***	

<sup>a</sup> Data were analyzed with one way Anova; \*,\*\*, \*\*\*, ns: significant at  $p \le 0.05$ ,  $p \le 0.01$ ,  $p \le 0.001$  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  $p \le 0.05$ .

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#### Table 2

		2	2014		2015			
Treatments	Control	Т	TT	Significance level <sup>a</sup>	Control	Т	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-1)	2.58	2.40	2.82	ns	4.35	4.89	4.76	ns
Leaf area (LA) (m <sup>2</sup> ·vine <sup>-1</sup> )	4.66 a	2.09 b	2.02 b	***	5.57 a	2.98 b	0.61 b	***
$LA/P (m^2 \cdot kg^{-1})$	1.99 a	0.84 b	0.70 b	***	1.28 a	0.61 b	0.29 c	***

Effects of trimming once (T) and trimming twice (TT) on yield components for 'Garnacha' vines (2014 and 2015, Badarán, La Rioja, Spain)

<sup>a</sup> Data were analyzed with one way Anova; \*\*\*, ns: significant at  $p \le 0.001$  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  $p \le 0.05$ .



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  $\pm$  standard deviation).

accumulation of anthocyanins relative to sugar were almost the same in 2014 among treatments (Fig. 3). However, in 2015, for every one unit increment of SS, T and TT seemed to accumulate slightly more anthocyanins.

M u st c o m p o s i t i o n : In 2014, for 'Tempranillo' grapes in VL, trimming treatments managed to maintain a relatively high TA at harvest, in particular T (Tab. 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 (Tab. 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 concent



Fig. 3: The relationship between anthocyanins concentration  $(mg \cdot L^{-1})$  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<sup>2</sup> 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.

tration 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

#### Table 3

			2015					
Treatments	Control	Т	TT	Significance level <sup>a</sup>	Control	Т	TT	Significance
	control							level
°Brix at harvest	22.0	22.1	22.2	ns	22.5	22.3	22.3	ns
Titratable acidity (g·L <sup>-1</sup> ) <sup>b</sup>	3.45 c	4.3 a	4.1 b	***	5.15	4.90	4.90	ns
pН	4.12 a	3.98 b	4.02 b	**	3.52	3.55	3.55	ns
Tartaric acid (g·L <sup>-1</sup> )	4.2 b	4.9 a	5.0 a	***	4.2	4.4	4.4	ns
Malic acid $(g \cdot L^{-1})$	3.5 a	3.1 b	3.0 b	*	4.2 a	3.8 b	3.9 b	*
Anthocyanin concentration (mg·g <sup>-1</sup> )	1.51	1.43	1.45	ns	1.21	1.16	1.21	ns
Anthocyanin content (mg·berry <sup>-1</sup> )	2.33	2.39	2.44	ns	2.18	2.18	2.14	ns

# Effects of trimming once (T) and trimming twice (TT) on must composition for 'Tempranillo' vines (2014 and 2015, Logroño, La Rioja, Spain)

<sup>a</sup> Data were analyzed with one way Anova; \*,\*\*, \*\*\*, ns: significant at  $p \le 0.05$ ,  $p \le 0.01$ ,  $p \le 0.001$  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  $p \le 0.05$ . <sup>b</sup> The titratable acidity is expressed as g·L<sup>-1</sup> 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	Т	TT	Significance level <sup>a</sup>	Control	Т	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 <sup>-1</sup> ) <sup>b</sup>	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 <sup>-1</sup> )	7.3 b	7.6 a	6.7 c	* * *	6.3 a	5.2 b	5.6 b	**
Malic acid (g·L <sup>-1</sup> )	1.45 a	1.00 b	1.35 a	***	2.5 a	2.0 b	2.7 a	*
Anthocyanin concentration (mg·g <sup>-1</sup> )	1.44 a	1.37 a	0.80 b	***	0.67 a	0.56 a	0.30 b	***
Anthocyanin content (mg berry <sup>-1</sup> )	2.60 a	2.12 a	1.22 b	***	1.18 a	1.08 a	0.53 b	***

<sup>a</sup> Data were analyzed with one way Anova; \*,\*\*, \*\*\*, ns: significant at  $p \le 0.05$ ,  $p \le 0.01$ ,  $p \le 0.001$  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  $p \le 0.05$ . <sup>b</sup> The titratable acidity is expressed as g·L<sup>-1</sup> tartaric acid.

time in the growing season. In 2014, 0.70 m<sup>2</sup> kg<sup>-1</sup> 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<sup>2</sup>·kg<sup>-1</sup>) 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. <sup>o</sup> 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.

M ust 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. (2005 and 2007) 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 ( $\Delta T = 10$  °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,  $\Delta T \approx 1.0$  °C; for TT,  $\Delta T \approx 1.4$  °C). However, in VB, though grapes of T were ripening under a cooler daily mean temperature than C ( $\Delta T \approx 2.1$  °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 Fig. 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 properly mature 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.

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