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

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## Summary

**The aim of this work is the evaluation of the leaf area reduction by trimming, as a growing technique to restore the anthocyanins : sugars ratio decoupled by the warming climate. A 3-year period (2010-2012) severe shoot trimming treatment was done after berry-set (berry diameter 3-4 mm) and the veraison date was delayed around 20 days. The grapes were picked at the same level of soluble solids in all the treatments. However, for every year, the trim treatment significatively increased the total anthocyanin content between 8 % and 21 % compared to control. Therefore, delaying the berry ripening process trough the decrease of the leaf area to fruit ratio, could partially restore the anthocyanins : sugars ratio disrupted by elevated temperatures. Although it is necessary to study other trimmings intensities as well as other times of intervention, the shoot trimming treatment could be a very simple technique to delay berry ripening and compensate the effects of climate warming.**

K e y w o r d s : decoupled anthocyanins : sugars ratio, delayed ripening, trimming.

## Introduction

Many vineyards in the world produce high probable alcohol levels, because most of the viticultural techniques have always been designed in order to improve the berry ripeness. Climatic change has also naturally increased the berry ripeness process (Schultz and Jones 2010) and during the last few decades, it occurred 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, berry ripening is taking place during the warmer period of the ripening season (Webb *et al*. 2007, 2008).

Considering warm climates viticulture, 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). Sugar enzymes activity is held between 8 °C and 33 °C, while colour enzymes activity ranges from 17 °C to 26 °C (Iland and Gago 2002, Sadras *et al*. 2007). In addition, temperatures above 30 °C after veraison could inhibit anthocyanin synthesis (Mori *et al*. 2007). Movahed *et al*. (2011) found that anthocyanin accumulation revealed to be very sensitive to high temperatures and that several mechanism such as the inhibition of some key enzymes in anthocyanin biosynthesis and in some anthocyanin degradation pathways, showed to be involved in the inhibitory effect of high temperatures on anthocyanin accumulation.

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

According to viticultural strategies, the main objective consists in the production of well balanced grapes with a suitable quality and lower soluble solids concentration. However, the grape growing techniques should be strongly analyzed to tackle this situation. One of the possibilities is based on 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 in contrast to the others, it does not entail any vineyard replacement and it could be developed in current vineyards.

Considering the plant physiology mechanisms, there are several growing techniques which could contribute to the berry ripening delay. All these techniques should be considered in order to improve the adaptation of the vineyards to warmer climates and subsequently, to achieve a suitable berry ripening process.

The ecophysiology characterization research carried out during the last years, led to establish the leaf area to fruit ratio as one of the most important viticultural indexes in order to define a well balanced vineyard that could produce high quality grapes and wines. It is considered that the leaf area to fruit ratio should range between 0.8 and 1.2 $m^2 \cdot kg^{-1}$ in order to get a good ripeness (Kliewer and Dokoozlian 2005). Although many experiments show the high influence of the leaf area to fruit ratio on bunch characteristics, it also would be very interesting to see the delayed ripeness through the variation of that index.

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Stoll et al. (2009) argue that leaf area reduction through severe trim or leaf removal treatments (0.8 and 1.4 $m^2 \cdot kg^{-1}$ against 1.9 $m^2 \cdot kg^{-1}$ on the control), delay berry ripening on 'Riesling' variety for a period between 15 and 20 d. Intrieri and Filippetti (2009) also consider this reduction in leaf area as a very interesting technique to delay berry ripening. Rombola et al. (2010) and Filippetti et al. (2011) show that a trimming, between 1 and 3 weeks after full veraison, can reduce the sugar accumulation with a better synchronization of sugar and phenolic ripening. Thereafter, it could be interesting to test an earlier trimming (Filippetti et al. 2011).

Martínez de Toda and Balda (2013) show that veraison date is delayed 18-20 d as a consequence of a single shoot trimming after berry set. This delay period on the veraison date could compensate the phenological advance that has occurred over 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. Thereafter, the leaf area decrease as a consequence of trim treatments, 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.

Therefore, the main objective of this work consists in the evaluation of the leaf area reduction by trimming after berry set 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* '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'. Both vineyards were planted in 1998 on bush vines, without trellis system and pruned to twelve buds per vine on spurs of two buds each. The vine rows were north-south oriented. The vineyards were managed according to the standard viticulture practices carried out in Rioja appellation, without any irrigation treatment.

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 all years).

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

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 estimate 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 harvesting 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 100 discs of known surface, and the leaf area surface per shoot was estimated. The leaf area surface per vine was obtained by multiplying the leaf area surface per shoot and the number of shoots per vine.

The harvest date of the different treatments was determined by looking for a comparable level of soluble solids, from 21 to 22 °Brix, and it was fixed between October 9-12 for the control, and between October 20-28 for the trimming treatment during the tree years. The yield per vine was determined at harvest time on five vines per replicate (15 vines per treatment), as well as the number of bunches in order to calculate the bunch weight .

Berry weight was obtained by measuring 200 berries in each replicate. After that, those berries were crushed manually to obtain the must for the chemical analysis. The soluble solids were analyzed according to the OIV standard methods (OIV, 2013) and total anthocyanins were analyzed by Iland method (Iland et al. 2004).

Mean comparisons were performed using Student's t-tests (p = 0.05). The statistical analysis was carried out with the statistical package SPSS 15.0 for Windows.

## Results

Leaf area to fruit ratio and bunch and berry weight: For the 'Grenache' treatments, Tab. 1 shows that the leaf area to fruit ratio ranged from

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 per vine ($m^2$) | 7.49 a | 4.05 b |
| | Yield per vine (kg) | 5.63 a | 5.06 a |
| | Leaf area/Yield ($m^2 \cdot kg^{-1}$) | 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 per vine ($m^2$) | 7.96 a | 3.35 b |
| | Yield per vine (kg) | 4.35 a | 4.08 a |
| | Leaf area/Yield ($m^2 \cdot kg^{-1}$) | 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 per vine($m^2$) | 3.72 a | 2.76 b |
| | Yield per vine (kg) | 5.90 a | 5.52 a |
| | Leaf area/Yield ($m^2 \cdot kg^{-1}$) | 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).

0.50-0.80 $m^2 \cdot kg^{-1}$ in the trim treatment to 0.63-1.83 $m^2 \cdot kg^{-1}$ in the control. The decrease in bunch weight in the trim treatment was similar to the berry weight decrease; both resulted around 10 % lower.

The results obtained in 'Tempranillo' are shown in Tab. 3. The leaf area to fruit ratio decreased from 1.88 $m^2 \cdot kg^{-1}$ to 0.64 $m^2 \cdot kg^{-1}$, while berry weight decrease was in the same proportion as the bunch weight decrease: both were reduced by 15 % in the trim treatment.

Veraison date: As it is shown in Tab. 2, significant differences at veraison date were observed every year between the trim treatment and the control. The veraison date was always delayed between 18 and 20 d for the trim treatment.

Anthocyanin content: Within the same level of soluble solids, total anthocyanins were increased between 8 % and 18 % in the trim treatment (Tab. 2).

In the 'Tempranillo' variety (Tab. 3), the concentration of total anthocyanins for the trim ttreatment was increased around 21 % within the same level of soluble solids.

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 19 th b |
| | Soluble solids (º Brix) | 21.0 a | 21.2 a |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 0.70 b | 0.83 a |
| 2011 | Harvesting date | Oct 10th | Oct 20th |
| | Veraison date | Aug 28 th a | Sep 15 th b |
| | Soluble solids (º Brix) | 21.5 a | 21.5 a |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 0.72 b | 0.78 a |
| 2012 | Harvesting date | Oct 9th | Oct 25th |
| | Veraison date | Sep 5 th a | Sep 24 th b |
| | Soluble solids (º Brix) | 21.5 a | 21.4 a |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 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, 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 per vine ($m^2$) | 8.49 a | 2.45 b |
| Yield per vine (kg) | 4.52 a | 3.82 a |
| Leaf area/Yield ($m^2 \cdot kg^{-1}$) | 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 ($mg \cdot g^{-1}$) | 1.36 b | 1.65 a |

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

## Discussion

Leaf area to fruit ratio and bunch and berry weight: It is interesting to note that in 2012 the Grenache leaf area to fruit ratio was smaller than in 2010 and 2011, probably due to lower rainfall in 2012 (460 mm from October 2011 to September 2012). It is expected that the important leaf area to fruit ratio decrease as a consequence of a trimming treatment affects grape ripeness process (Kliewer and Dokoozlian 2005, Stoll *et al.* 2009). The reduction of the 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 as conclude Rombola *et al.* (2011), trim treatment revealed to be an attractive approach for controlling yield. It is also an alternative to expensive techniques, such as bunch thinning or early defoliation, that enhances the fruit sugar concentration (Tardaguila *et al*. 2010).

Delaying ripening: Ripening in the 'Grenache' control vines started at the beginning of September in the three studied years, when mean temperatures were 19 °C, the mean maxim temperatures were 26 °C and the mean minimun temperatures were 12,5 °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 16,7 °C, the mean maxim temperatures were 23 °C and the mean minimun temperatures were 11,2 °C. The ripeness delay due to trim practices involved that ripening process took place in a later period with cooler temperatures. So the 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.

Anthocyanin content: Although the grapes were picked with the same level of soluble solids, the trim treatment had higher total anthocyanin content than control for both varieties. Therefore, and with significant differences every year, a higher content of total anthocyanins as a result of trim treatment was observed.

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 most probable explanation would be associated with the lower temperature at which berry ripening took place for the trim treatment, due to the delay of twenty days detected for the treatment in comparison with control. We do not believe that the cluster microclimate is very different as a result of trimming since it is done in a very early time (after the berry set) and there is a rapid lateral growth that re-cover the bunches, decreasing the direct solar radiation.

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 that causes the climate warming.

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 delay in grape ripeness for '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 for the trim treatment. Likely, the explanation is linked with lower temperatures during the berry ripening stage in the trim treatment, due to a delay of twenty days detected for that 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 climate warming.

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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