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# Quantifying the effect of temperature on decoupling anthocyanins and sugars of the grape (*Vitis vinifera* L. 'Maturana Tinta de Navarrete')

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## Summary

**The objective of this work consists of the quantification of decoupling anthocyanin:sugar ratio produced by higher temperature on *Vitis vinifera* L. 'Maturana Tinta de Navarrete'. Two vineyards were studied in two different climatic areas during a 3-year period (2010-2012). The average Winkler index during the three years in the two studied areas was 1364 °C and 1619 °C, respectively. This implies a difference of 1.3 °C for the mean temperature between both areas from April to October.**

**Both total anthocyanins and the anthocyanins:sugars ratio decreased, significantly, in the warmer area during the three years. The highest anthocyanin sugars decoupling occurred in the warmest year (2012) and the lowest in the coolest year (2010). Given that the grape ripening begins in August, it could be concluded that the above decoupling is higher as higher is the temperature in August.**

**For further research, it would be very interesting to study the anthocyanins : sugar ratio at different times from veraison onwards.**

K e y  w o r d s : climate warming; decoupled anthocyanins; sugars ratio; quantifying decoupling.

## Introduction

Many vineyards around the world produce potentially higher 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, berry ripeness has been developing earlier.

Several studies show earlier 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 part of the ripening period (Webb *et al*. 2007, 2008). There are many studies regarding several temperature indices determining the main changes in the varietal profile in viticultural areas (Schultz 2000, Stock *et al*. 2005),

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 are active (8 to 33 °C) are different to the activity of the colour enzymes (17 to 26 °C) (Iland and Gago 2002, Sadras *et al*. 2007). Temperatures above 30 °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, delaying harvest to search for 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. These authors worked with experiments where temperature was manipulated under realistic vineyard conditions.

Martínez de Toda and Balda (2013) also working in experiments under realistic vineyard conditions argue that the ripeness delay due to trimming practices involves ripening taking place at a later period under cooler temperatures. So decreasing leaf area as a consequence of the trimming treatments, could be useful to obtain a delay in ripening. When the berry ripeness is developed during cooler periods, phenol development and aroma synthesis are more adequate (Stoll *et al*. 2009). This fact is very important in warm wine regions.

The effect of temperature on berry and wine attributes has also been investigated with indirect approaches comparing cool and warm seasons within a region or in thermally contrasting regions (Duchêne and Schneider 2005, Jones *et al*., 2005).

In the indirect approach comparing thermally contrasting regions, it is necessary to consider vineyards as similar as possible in regard to plant material, management practices and soils. Furtheremore these differences between climatic conditions should be comparable to the effect of the studied global warming.

In this regard it is noteworthy that the mean temperature increase projected for 2050 is around 1.5-2.0 °C under some scenarios and modelling conditions of Jones *et al*. (2005).

'Maturana Tinta de Navarrete' is a minority red variety recovered in Rioja appellation in 1991 in a research project, and its planting was authorized by the Regulatory Council in 2007. The genetic profile of 'Maturana Tinta

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de Navarrete' matches with the variety 'Castets' in France, where it has almost disappeared. Nowadays there are more than 50 ha planted recently in Rioja.

'Maturana Tinta de Navarrete' has been cultivated in Rioja as a minority variety, following the traditional viticultural practices of the region for 'Tempranillo', the most common variety in the appellation. 'Maturana Tinta de Navarrete' has always been co-fermented in small percentages with other varieties, so its enological properties have remained unknown until the last few years.

The objective of this work is to quantify the effect of temperature on decoupling anthocyanins and sugars in the variety 'Maturana Tinta de Navarrete', through the behavior of the vineyard into two climatic zones with a mean temperature difference between them, for the period April to October, of approximately 1.3 °C or an equivalent difference of 255 °C in the Winkler index (Winkler *et al.* 1974).

## Material and Methods

Plant material and climate: The study was conducted during the years 2010, 2011 and 2012 in two commercial vineyards of *Vitis vinifera* 'Maturana Tinta de Navarrete' located in Badarán (42.36 N, -2.81 W, 615 m) and Logroño, inside the Rioja appellation, in the North of Spain. In both vineyards, 'Maturana Tinta de Navarrete' was grafted on 110-R rootstock and both were 12 years old. Planting distance was 1.20 m between vines and 2.70 m between rows. The rows were oriented North-South along a 1.5 % sloping terrain. The vines were trellised by simple horizontal cordon and pruned to twelve buds per vine on spurs of two buds each and the vineyard was subjected to the common viticultural practices in the region. It should be noted that planting material was the same in both vineyards as the recovery of this variety was made from only 35 vines found in an old vineyard. Climatic data were obtained from the closest weather stations, located 5 km away from the vineyard of Badaran (the data were corrected for the difference in altitude) and less than 1 km distant in the case of the vineyard in Logroño.

Experimental design: In each vineyard, the experimental design was a randomized complete block with three replications. The experimental plot consisted of four rows, and each experimental unit consisted of six contiguous vines in each row. Four different treatments were applied, consisting of the combination of vertical shoot positioning (VSP) and free cordon (FC) training systems with or without a leaf plucking treatment (control was without plucking): VSP control (VSP), VSP with leaf plucking (VSP-LP), FC control (FC) and FC with leaf plucking treatment (FC-LP). The leaf plucking treatment consisted of removing the basal leaves of each shoot until the node located above the upper bunch and included the lateral shoots. The treatment was performed at peppercorn size berries (4 mm).

In order to determine the leaf area of the shoot, the Smart method based on discs technique (Smart and Robinson, 1991) was used. The leaf area of the shoot at harvest time was measured on 5 shoots per replicate, 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 surface area per shoot was obtained. The leaf surface area per vine was obtained by multiplying the leaf surface area per shoot and the number of shoots per vine.

At harvest time, between October 25 and October 30 over three years, and on five vines of each replicate, the yield per vine was determined.

A sample of 200 berries for each replicate 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, between the two vineyards and for the four treatments together, using *t* Student test ($p = 0.05$). The statistical analysis was performed using the statistical package SPSS 15.0 for Windows.

## Results

Climate: The values of Winkler Index for each of the three years and its average value in the two studied areas are presented in Tab. 1. The Winkler Index average of the three years for each area was 1364 °C and 1619 °C, respectively.

Table 1

Winkler Index and August mean Tª for the three years in the two areas studied

| | | Badarán | Logroño |
|---|---|---|---|
| | Winkler Index Average (°C) | 1364 | 1619 |
| 2010 | Winkler Index (°C) | 1299 | 1516 |
| | August mean Tª (°C) | 19.9 | 21.3 |
| 2011 | Winkler Index (°C) | 1388 | 1705 |
| | August mean Tª (°C) | 20.6 | 22.5 |
| 2012 | Winkler Index (°C) | 1406 | 1636 |
| | August mean Tª (°C) | 21.6 | 23.1 |

As grape ripening begins in August, the average temperature in August for the three years in each of the areas is also shown. In regard to the average temperature of August, there was a difference of around 1.6 °C between the two areas for the three years.

Leaf area to fruit ratio: As can be seen in Tab. 2, the leaf area to fruit ratio ranges from 1.36 to 3.00 $m^2 \cdot kg^{-1}$ in all cases and is always higher in the vineyard in Badarán. In 2010 and 2011 this increased leaf area to fruit ratio was due to the lower production of the Badarán vineyard compared to the Logrono vineyard. In 2012, however, the lower leaf area to fruit ratio was due to the greater leaf area per vine on the Badarán vineyard.

Soluble solids and anthocyanin content: Tab. 3 shows the results obtained from grape analysis in the two vineyards for the three years. Significant differences were found in the soluble solids between the

Table 2

Leaf area/yield, leaf area/vine and yield/vine for the two areas in the years 2010, 2011 and 2012

| | | Badarán | Logroño |
|---|---|---|---|
| 2010 | Leaf area/yield ($m^2 \cdot kg^{-1}$) | 2.28 a | 1.36 b |
| | Leaf area/vine ($m^2$) | 3.46 a | 4.10 a |
| | Yield/vine (kg) | 1.51 b | 3.00 a |
| 2011 | Leaf area/yield ($m^2 \cdot kg^{-1}$) | 3.00 a | 2.01 b |
| | Leaf area/vine ($m^2$) | 4.92 a | 4.42 a |
| | Yield/vine (kg) | 1.64 b | 2.20 a |
| 2012 | Leaf area/yield ($m^2 \cdot kg^{-1}$) | 2.07 a | 1.49 b |
| | Leaf area/vine ($m^2$) | 4.15 a | 2.46 b |
| | Yield/vine (kg) | 2.00 a | 1.65 a |

Different letters across a row show significant differences between values, according to *t* student test ($p = 0.05$).

Table 3

Soluble solids and anthocyanin content for the two regions in the years 2010, 2011 and 2012

| | | Badarán | Logroño |
|---|---|---|---|
| 2010 | Soluble solids (°Brix) | 23.26 a | 22.68 b |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 3.75 a | 3.39 b |
| | Anthocyanins/sugar ($mg \cdot g^{-1}$/°Brix) | 0.161 a | 0.149 b |
| 2011 | Soluble solids (°Brix) | 23.51 b | 24.68 a |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 4.23 a | 3.75 b |
| | Anthocyanins/sugar ($mg \cdot g^{-1}$/°Brix) | 0.180 a | 0.152 b |
| 2012 | Soluble solids (°Brix) | 23.80 b | 25.11 a |
| | Total anthocyanins ($mg \cdot g^{-1}$) | 5.24 a | 3.73 b |
| | Anthocyanins/sugar ($mg \cdot g^{-1}$/°Brix) | 0.220 a | 0.148 b |

Different letters across a row show significant differences between values, according to *t* student test ($p = 0.05$).

two regions for all years. In 2010, the soluble solids content was higher in the cooler area while in 2011 and 2012 that content was higher in the warmer region. Both the total anthocyanins and the anthocyanins:sugars ratio decreased, significantly, in the warmer area during the three years. The reduction in the anthocyanins:sugars ratio for the warmer vineyard was 7.4 % in 2010, 15.5 % in 2011 and 32.7 % in 2012.

## Discussion

C l i m a t e : The difference in average Winkler Index for the three years between the two areas was 255 °C (Tab. 1), which is equivalent to a mean temperature difference between them, for the period April to October, about 1.3 °C. In this regard it is noteworthy that this difference of 1.3 °C is close to the projected increase in the average temperature for 2050 in some scenarios and modeling conditions (Jones *et al*. 2005).

With regard to the average temperature of August, there was also a difference of around 1.6 °C between the two areas for three years. Overall, 2010 was the coolest year and 2012 was the warmest year for both areas. In the month of August, when the grape ripening begins, 2012 was also the warmest year and 2010 the coolest.

L e a f a r e a t o f r u i t r a t i o : To analyze the behavior of these two vineyards, it is important that the leaf area to fruit ratio is sufficiently high to allow proper ripening. Generally, ripening gradually increases with leaf area to fruit ratio and then reaches a plateau (Kliewer and Dokoozlian 2005). This plateau can be considered as the potential ripening level that a vine can achieve under non-source limitation condition. In order to get enough ripeness in the grapes, a leaf area to fruit ratio between 0.8 and 1.2 $m^2 \cdot kg^{-1}$ (Kliewer and Dokoozlian 2005) is needed. As seen in Tab. 2, the leaf area to fruit ratio is, in all cases, much higher than these minimum standards listed so we can say that leaf area has not limited the ripening of the grapes in any of the experimental units.

S o l u b l e s o l i d s a n d a n t h o c y a n i n c o n t e n t : Both the total anthocyanins and the anthocyanins : sugars ratio decreased significantly, in the warmer area over the three years (Tab. 3). The reduction in the anthocyanins : sugars ratio for the warmer vineyard was 7.4 % in 2010, 15.5 % in 2011 and 32.7 % in 2012.

If we consider that the leaf area to fruit ratio was higher than 1.36 $m^2 \cdot kg$ in all cases (Tab. 2) and, therefore, the grape ripening was not limited by insufficient leaf area, we have to consider that this decoupling between anthocyanins and sugars was due to the higher temperature of the warmest area. The only explanation that comes to mind is linked to the higher temperature at which berry ripening occurs in the warmer area.

Sadras and Moran (2012) demonstrate that elevated temperature can decouple anthocyanins and sugars in berries in a temperate environment. Thus, the decreasing anthocyanins:sugars ratio for the warmer vineyard could be due to the higher temperature of this area.

We have not studied 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). Regarding this aspect, it should be noted that the methodology used in this study did not allow the investigation into the onset of anthocyanin synthesis nor its subsequent rate . To resolve this issue, it would be interesting to study the anthocyanins : sugar ratio at different times beginning at veraison. This is one of the aspects we want to study in subsequent works.

With respect to differences between years, the highest anthocyanin sugars decoupling occurred in 2012 with a reduction of 32 % and the lowest in 2010 with a reduction of 7 %, and if we look at the climate data of Tab. 1 and especially temperatures of August, we can see that 2012 was the warmest year and 2010 the coolest. Given that the grape ripening begins in August it could be concluded that the above decoupling is positively correlated to higher temperatures in August.

## Conclusions

Both total anthocyanins and the anthocyanins : sugars ratio decreased, significantly, in the warmer area over the three years. If we consider that the leaf area to fruit ratio was higher than 1.36 $m^2 \cdot kg^{-1}$ in all cases and, therefore, the grape ripening was not limited by insufficient leaf area we have to consider that this decoupling between anthocyanins and sugars was due to the higher temperature of the warmest area. The only explanation that comes to mind is linked to the higher temperature at which berry ripening occurs in the warmer area.

With respect to differences between years, the highest anthocyanin sugars decoupling occurred in 2012 and the lowest in 2010 and if we look at the climate data and especially temperatures in August, we can see that 2012 was the warmest year and 2010 the coolest. Given that the grape ripening begins in August it could be concluded that the above decoupling is higher when higher August temperatures are experienced.

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