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## Delaying berry ripening through manipulating leaf area to fruit ratio

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### Summary

The viticulture practices have always been focused on the production of grapes with higher sugar level, and this tendency has been pronounced by the climate change. The objective consists on delaying berry ripeness by decreasing the ratio between the leaf area and yield, performing intense trimming treatments after berry set and establishing the consequences on grapevine productivity in the following years. Severe shoot trimming with two different intensity treatments were done during a 3-year period (2010–2012). Phenological, vegetative and productive parameters were examined. Veraison date was delayed around twenty days. Regarding the same harvesting date, the trim treatments had lower soluble solids level (15 % reduction), lower pH (0.1–0.3) and less total anthocyanin content (10–27 % reduction). The trim effect was also reflected in berry weight; as a consequence of that, bunch size and yield were also reduced by around 9 and 15 %.

Overall, single trim treatment was superior to double trim treatment because it can achieve the same results in delayed ripening, reduced °Brix, pH and production but with a smaller reduction in anthocyanin content and without having any negative impact on following years.

**Key words:** berry ripeness, summer pruning, leaf area, yield, vine capacity.

### Introduction

The main objective of the viticulture throughout history has focused on the sugar level increase in the produced grapes. Should be noted that grapes have always been paid for according to the probable alcohol level. Nowadays, the alcohol level is still the most important factor on the prize definition, in most of the viticultural areas.

During the last few years, the importance of the sugar content in berries has been changing. Most vineyards can easily produce high probable alcohol levels. Clonal selections have provided new clones for grape growers according to this objective, because it has been one of the main historical selection criteria considered on mass selection programs. Viticultural techniques have always been designed in order to produce a better ripeness. Apart from this historic evolution, in the last few years, this aspect has been increased because of two different synergic facts: on

the one hand, climatic change has increased the berry ripeness process naturally (SCHULTZ and JONES 2010); and on the other, the latest market tendency for more full bodied wines have delayed harvesting date, in some cases for a long period, just to obtain a better phenol ripeness. An important percentage of red wines are between 14 and 16 degrees alcohol, and their pH is around 4.

The disadvantages of an excess of alcoholic degree are difficulties of alcoholic and/or malolactic fermentation, wines with higher volatile acidities, unbalanced wines, especially when the service temperature is high, some countries apply higher taxes when the wines present a high alcoholic degree and the consumers usually refuse wines with a high alcohol content.

To tackle this situation, in the last few years, new technical solutions have been developed in order to avoid the effects of climate change; they are focused mainly on low degree alcohol wines (KONTOUDAKIS *et al.* 2011). According to viticultural strategies, the main objective consists on the production of well balanced grapes, with good quality and a lower soluble solids concentration.

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, DUCHÊNE 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). Although there are many studies regarding several temperature indexes aimed at determining the main changes in the variety profile in viticultural areas (KENNY and HARRISON 1992, SCHULTZ 2000, STOCK *et al.* 2005), the grape growing techniques have not been analyzed strongly enough, in our opinion. One of the possibilities consists in the berry ripeness delay taking place during cooler seasons, when phenols and aroma are more interesting (STOLL *et al.* 2009).

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 33 °C) are different to the colour enzymes activity (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).

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 (KLEWER and DOKOOZLIAN 2005). It is considered that the

leaf area to fruit ratio should be between 0.8 and 1.2 m<sup>2</sup>·kg<sup>-1</sup> in order to get a good ripeness (KLIEWER and DOKOOZLIAN 2005). 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 and 1.4 m<sup>2</sup>·kg<sup>-1</sup> against 1.9 m<sup>2</sup>·kg<sup>-1</sup> in the control), delay berry ripeness on 'Riesling' for a period between 15 and 20 d. INTRIERI and FILIPPETTI (2009) also consider this technique as a very interesting one. But none of these authors studied a more drastic leaf area reduction (below 0.8 m<sup>2</sup>·kg<sup>-1</sup>) and their effects on subsequent years.

On the other hand, some studies have indicated that reducing carbohydrate production during the growing season by defoliation decreased concentrations of overwintering carbohydrate reserves, mostly starch, in both roots and trunks (BENNET *et al.* 2005, ZUFFEREY *et al.* 2012). Yield in the following season, shoot growth and total vine pruning weight were also decreased in vines where carbohydrate reserves were reduced (BENNET *et al.* 2005). These findings suggest that restricted carbohydrate reserve accumulation as a consequence of defoliation may have a negative impact on subsequent grapevine productivity.

The main objective of this work consists in the evaluation of the leaf area reduction, as a growing technique to delay the berry ripeness and establish the consequences on grapevine productivity in the following years.

### Material and Methods

**Plant material:** The study was conducted during 2010, 2011 and 2012, in a commercial vineyard of *Vitis vinifera* 'Grenache' located in Badarán (La Rioja, North of Spain). The vineyard was planted in 1998 on bush vines, the vine rows were north-south oriented and the vines were pruned to twelve buds per vine on spurs of two buds each. The vineyard was 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 July, 1 for every year). In addition, a second trim treatment was carried out around September, 1 (at veraison stage of the control). The shoots were cut at the same level as in the first time. All lateral shoots that had been growing until this time were removed.

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

**Veraison date:** Veraison date was established following phenological stages of Eichorn-Lorenz (COOMBE 1995) on six vines of each experimental treatment; two vines per replicate.

**Leaf area and yield:** In order to determine the leaf area surface of the shoot, the Smart method based on discs technique (SMART and ROBINSON 1991) was per-

formed. The leaf area surface of the shoot at harvest time was measured on 15 shoots per treatment, removing the petioles in order to measure the weight regarding 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.

At harvest time, between October 25 and October 30 for the tree years, on five vines of each replicate (15 vines per treatment), the yield per vine was determined, as well as the number of shoots and the number of bunches.

**Grape composition:** Berry weight was measured on 200 berries of each replicate. After that, each berry sample was crushed manually to obtain the must for the chemical analysis. The soluble solids, pH and total acidity were analyzed by OIV standard methods. Total anthocyanins and phenols were analyzed by Iland method (ILAND *et al.* 2004).

**Impact on the following years:** The effect of trim treatments on vine reserves accumulation was also studied. The vegetative and productive data from the three treatments performed in 2010 were examined also during the years 2011 and 2012; one and two years after the treatments respectively.

The reserves were estimated through the "vine capacity", which is the annual dry matter production of the vine, with exception of roots and trunk growth. It is defined as the addition of dry weight of clusters, pruning weight, and leaves. Clusters dry weight was calculated as the product of yield × 0.23 (cluster dry weight/cluster fresh weight) (MARTÍNEZ DE TODA 1985). Pruning dry weight was determined as the product of pruning weight × 0.47 (dry weight/pruning weight) (MARTÍNEZ DE TODA 1991). Leaf dry weight was calculated as the product of total leaf area per vine (m<sup>2</sup>) × 65 g·m<sup>-2</sup> (specific foliar weight) (MARTÍNEZ DE TODA 1985). During dormancy, vines were pruned to the standard bush vine system as described before, and the pruning weight was determined on five vines per replicate (15 vines per treatment).

**Statistics:** The significance of the differences between treatments was studied by analysis of variance (ANOVA; p = 0.05, p = 0.01, p = 0.001). Mean comparisons were performed using Student-Newman-Keuls (SNK) test (p = 0.05). The statistical analysis was performed using the statistical package SPSS 15.0 for Windows.

### Results

**Leaf area and yield:** As Tab. 1 shows, leaf area per vine is decreased between 26 % (year 2012) and 58 % (year 2011) in the single trim treatment, and between 77 % (year 2012) and 92 % (year 2011) with double trim. The leaf area/yield ratio ranges from 0.63-1.83 m<sup>2</sup>·kg<sup>-1</sup> in the control to 0.50-0.80 m<sup>2</sup>·kg<sup>-1</sup> in the single trim treatment, and to 0.15 m<sup>2</sup>·kg<sup>-1</sup> in the double one, which means a reduction of 48 % and 87 % respectively. Bunch weight decrease level found in both trim treatments is similar to berry weight decrease and it resulted between 8 % and 10 %.

Table 1

Leaf area, yield per vine, bunch weight and berry weight for single and double trim treatments and control in the years 2010-2012

		Control	Single trim	Double trim	Sig <sup>1</sup>
2010	Leaf area per vine (m <sup>2</sup> )	7.49 a	4.05 b	0.73 c	***
	Leaf area/Yield (m <sup>2</sup> ·kg <sup>-1</sup> )	1.33 a	0.80 b	0.13 c	***
	Bunch weight (g)	309 a	283 b	282 b	*
	Berry weight (g)	1.62 a	1.48 b	1.48 b	*
2011	Leaf area per vine (m <sup>2</sup> )	7.96 a	3.35 b	0.60 c	***
	Leaf area/Yield (m <sup>2</sup> ·kg <sup>-1</sup> )	1.83 a	0.82 b	0.15 c	***
	Bunch weight (g)	271 a	255 b	251 b	*
	Berry weight (g)	1.46 a	1.37 b	1.36 b	*
2012	Leaf area per vine (m <sup>2</sup> )	3.72 a	2.76 b	0.85 c	***
	Leaf area/Yield (m <sup>2</sup> ·kg <sup>-1</sup> )	0.63 a	0.50 b	0.16 c	***
	Bunch weight (g)	388 a	364 b	363 b	*
	Berry weight (g)	1.57 a	1.46 b	1.46 b	*

<sup>1</sup> ns, \*, \*\*, \*\*\* represent significant differences between treatments at P < 0.05, 0.01 or 0.001 respectively. Different letters within a row show significant differences between values, according to SNK test (P = 0.05).

Table 2

Veraison date for trim treatments and control

	Control	Single trim	Double trim	Sig <sup>1</sup>
2010	Sep 1 <sup>st</sup> a	Sep 19 <sup>th</sup> b	Sep 20 <sup>th</sup> b	***
2011	Aug 28 <sup>th</sup> a	Sep 15 <sup>th</sup> b	Sep 15 <sup>th</sup> b	***
2012	Sep 5 <sup>th</sup> a	Sep 24 <sup>th</sup> b	Sep 26 <sup>th</sup> b	***

<sup>1</sup> ns, \*, \*\*, \*\*\* represent significant differences between treatments at P < 0.05, 0.01 or 0.001, respectively. Different letters within a row show significant differences between values, according to SNK test (P = 0.05).

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

**Grape composition:** Tab. 3 shows the results obtained from grape analysis in the different treatments for the three years. Every year, the soluble solids decreased around 3 °Brix for the single trim treatment and around 3.5 °Brix for the double trim treatment, which means an average reduction of 12 % and 14 %, respectively. The pH was also reduced in both treatments, 0.10 for the single trim treatment and between 0.10 and 0.14 for the double trim treatment in comparison to control. No significant differences in comparison to control were found in the total acidity. The total anthocyanins were reduced around 10 % in the single trim treatment; this reduction was more important for the double trim treatment, reaching 27 %. The total phenols content showed no significant differences neither in any of the trim treatments nor in any of the years.

**Impact on the following years:** Tab. 4 shows the effects of vine trimming in the previous season and two seasons previously on vegetative parameters, yield and vine capacity.

Double trim treatment caused a reduction of 35 % in bunch number per vine in the two following seasons, a re-

duction between 10 % and 20 % in berry weight and between 25 % and 45 % in bunch weight. Smaller bunches on previously trimmed vines, together with fewer bunches per vine, resulted in a reduction in vine yields between 50 % and 70 % in comparison to non-trimmed vines. No differences were found for any yield parameter in the single trim treatment.

Double trim treatment caused a reduction between 20 % and 50 % to the total leaf area per vine in the two following seasons, depending on the vigor of the vine. Vine capacity was halved by the double trim treatment during the two following seasons. No differences were found for leaf area per vine or vine capacity in the single trim treatment.

## Discussion

**Leaf area and yield:** It is interesting to note that in 2012 there was a smaller leaf area compared to 2010 and 2011, probably due to lower rainfall in 2012. The yield is not affected as much as the leaf area as consequence of the trim treatments (Tab. 1); because of that, the leaf area to fruit ratio is modified mainly by the leaf area loss. It is expected that this important ratio decrease should affect grape ripening process (KLEWER and DOKOOZLIAN 2005, STOLL *et al.* 2009). The reduction of berry and bunch weight (9 %) is similar for both single trim and double trim treatment, and similar to that found in the experiment 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 possible alternative to expensive techniques, such as bunch thinning or early defoliation, the latter often enhancing fruit sugar concentration (TARDAGUILA *et al.* 2008 and 2010).

**Veraison date:** Ripeness stage (Tab. 2) in the control treatment starts during the beginning of September, when mean temperatures are 20 °C and the max. tempera-

Table 3

Grape composition (soluble solids, etc.) for single and double trim treatments and control in the years 2010-2012

		Control	Single trim	Double trim	Sig <sup>1</sup>
2010	Soluble solids (°Brix)	24.4 a	21.2 b	20.7 b	***
	pH	3.10 a	3.01 b	2.98 b	***
	Total Acidity (g·Tar/L <sup>-1</sup> )	7.27	7.11	7.10	ns
	Total anthocyanins (mg·g <sup>-1</sup> )	0.92 a	0.83 b	0.67 c	***
	Total phenols (AU·g <sup>-1</sup> )	2.34	2.46	2.32	ns
2011	Soluble solids (°Brix)	25.7 a	23.1 b	22.5 b	***
	pH	3.24 a	3.14 b	3.10 b	***
	Total Acidity (g·Tar/L <sup>-1</sup> )	6.00	5.88	6.60	ns
	Total anthocyanins (mg·g <sup>-1</sup> )	0.98 a	0.88 b	0.66 c	***
	Total phenols (AU·g <sup>-1</sup> )	1.80	1.78	1.78	ns
2012	Soluble solids (°Brix)	24.5 a	21.4 b	21.0 b	***
	pH	3.17 a	3.07 b	3.06 b	***
	Total Acidity (g·Tar/L <sup>-1</sup> )	6.70	6.70	7.00	ns
	Total anthocyanins (mg·g <sup>-1</sup> )	1.05 a	0.93 b	0.73 c	***
	Total phenols (AU·g <sup>-1</sup> )	1.30	1.15	1.14	ns

<sup>1</sup> ns, \*, \*\*, \*\*\* represent significant differences between treatments at P < 0.05, 0.01 or 0.001 respectively. Different letters within a row show significant differences between values, according to SNK test (P = 0.05).

Table 4

Effect of vine trimming in the previous season and two seasons previously on vegetative and productive parameters and vine capacity

		Control	Single trim	Double trim	Sig <sup>1</sup>
Trimmed in the previous season	Leaf area per vine (m <sup>2</sup> )	7.96 a	7.72 a	4.16 b	***
	Yield per vine (kg)	4.06 a	4.20 a	2.00 b	***
	Bunch number per vine	15.0 a	15.0 a	10.0 b	***
	Bunch weight (g)	271 a	280 a	200 b	***
	Berry weight (g)	1.46 a	1.52 a	1.25 b	***
	Vine capacity (kg)	2.11 a	1.97 a	1.06 b	***
Trimmed two seasons previously	Leaf area per vine (m <sup>2</sup> )	3.72 a	3.60 a	3.02 b	***
	Yield per vine (kg)	5.82 a	5.55 a	1.90 b	***
	Bunch number per vine	15.5 a	15.0 a	9.5 b	***
	Bunch weight (g)	388 a	370 a	200 b	***
	Berry weight (g)	1.57 a	1.62 a	1.21 b	***
	Vine capacity (kg)	1.88 a	1.69 a	0.90 b	***

<sup>1</sup> ns, \*, \*\*, \*\*\* represent significant differences between treatments at P < 0.05, 0.01 or 0.001 respectively. Different letters within a row show significant differences between values, according to SNK test (P = 0.05).

tures are 33 °C (for the area of Rioja Alta). Nevertheless, in the trim treatments the ripening period begins during the second half of September, when mean temperatures reach 14 °C and the maximum temperatures are 25 °C. The ripening delay due to trim practices involves that ripening takes place in a later period with cooler temperatures. So leaf area decreasing, as a consequence of the trim treatments, could be useful to obtain a ripeness delay. When berry ripening takes place during cooler periods, phenol development and aroma synthesis are more adequate (STOLL *et al.* 2009). This fact is very important in warm wine regions.

This delay of 18-20 d of the veraison date is similar for the single and double trim treatment and can compen-

sate the phenological advancement that has occurred in the last thirty years in most of the wine growing regions (JONES *et al.* 2005, DUCHÊNE and SCHNEIDER 2005, STOLL *et al.* 2009).

**Grape composition:** The results of grape composition (Tab. 3) obtained in the experiment confirm the initial hypothesis: heavy leaf area decrease, lower leaf area to fruit ratio, ripeness process delay and soluble solids, pH and total anthocyanin level delay (KLEWER and DOKOOZLIAN 2005, STOLL *et al.* 2009, INTRIERI and FILIPPETTI 2009). The reduction in soluble solids level and pH was similar for both trim treatments (around 12-14 %) but the reduction in total anthocyanin level was higher for the dou-

ble trim than for the single trim treatment, showing a decreasing gradient that involves an important loss of a wine quality parameter. The observed differences in pH, with no differences in total acidity, are probably due to the smaller amount of malic acid synthesized in trimming treatments which, in turn, causes a lower pH, but we can not say this for sure because we have not analyzed malic and tartaric.

It should be noted that the methodology used in this study did not allow to know if there is just a delayed ripening or incomplete maturation. To resolve this issue, it would be interesting to harvest each treatment in different time and even transform grapes into wine to assess the final quality of the wines.

**Impact on the following years:** As it has been reported by BENNET *et al.* (2005), this study shows a reduction in bunch number per vine and in bunch weight, in response to double trimming treatments carried out one and two seasons before. Such results suggest that a whole-vine effect, in response to previous season's trimming, was mediating the reduction in bunch number and in bunch weight. BENNET *et al.* (2005) suggest that restricted carbohydrate reserve accumulation as a consequence of defoliation may have a negative impact on subsequent grapevine productivity over two seasons.

ZUFFEREY *et al.* (2012) found that higher leaf to fruit ratios resulted in increased carbohydrate reserves which attained the maximum values when the leaf to fruit ratio neared 2.0 m<sup>2</sup>·kg<sup>-1</sup>.

Our results suggest that the double trim treatment has a negative impact on subsequent grapevine productivity, over two seasons, because its leaf area to fruit ratio is too low (0.15 m<sup>2</sup>·kg<sup>-1</sup>, Tab. 1) to ensure adequate reserves. By contrast, the single trim treatment maintains a leaf area to fruit ratio (0.50-0.80 m<sup>2</sup>·kg<sup>-1</sup>, Tab. 1) high enough that doesn't negatively affect the reserves accumulation.

These results seem to indicate that the trim treatments must not reduce the leaf area to fruit ratio below 0.5 m<sup>2</sup>·kg<sup>-1</sup>, in order to ensure sufficient reserve accumulation without having a negative impact on the vine capacity during the following years.

Overall, single trim treatment was superior to double trim treatment because it can achieve the same results in delayed ripening, reduced °Brix, pH and production but with a smaller reduction in anthocyanin content and without having any negative impact on following years. 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, produced an important grape ripening delay. The veraison stage was delayed around 20 d. Regarding the same harvesting date, the trim treatments had lower soluble solid levels (15 % reduction), lower pH (0.1-0.3) and less total anthocyanin content (10-27 % reduction). The trim effect was also reflected in berry weight; as a consequence of that, bunch size and yield were also

reduced by around 9 and 15 %. The trim treatments should not reduce the leaf area to fruit ratio below 0.5 m<sup>2</sup>·kg<sup>-1</sup>, in order to ensure sufficient reserve accumulation without having a negative impact on the vine capacity in the following years.

Overall, single trim treatment was superior to double trim treatment because it can achieve the same results in delayed ripening, reduced °Brix, pH and production but with a smaller reduction in anthocyanin content and without having any negative impact on following years.

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