Reducing alcohol content in wines by combining canopy management practices and biological techniques

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Abstract. The main objectives of this study was to decrease wine alcohol content by combining several techniques, from the vineyard to the cellar. The combination of these techniques should allow to decrease alcohol in wine by 2% in volume. A two years trial was focused on two main cultivars of the south-west of France vineyard: Tannat N and Gros Manseng B. By now, in the context of climate changing, the grapes oftenly raise a high level of sugars, up to or more than 15 % of potential alcohol. In the need to delay ripening and create more digest wines, three cultural techniques were tested and compared to a control: leaf removal on the upper part of the canopy, reduced canopy by late hedging and anti-transpirant spraying on the full canopy. Those techniques were combined with a biological process to reduce alcohol production using yeast with a low alcoholic yield. Four replicates were vinified with both low yield yeast of *Saccharomyces Cerevisiae* and control yeast. Results showed that late hedging was the most efficient to reduce ripening on both cultivars but had also an impact on others parameters such as acidity and polyphenols. The other viticultural techniques assessed showed also efficiency in reducing ripening. During winemaking, low alcoholic yield yeast leads to lower alcohol content with more acid wines and lower volatile acidity content.

1 Introduction

Climate change is admitted by the majority of the scientific community [1]. The rise in temperature caused by this phenomenon results in an increase in the alcohol content of the wines [2]. This excess of alcohol is not desirable due to harmful effects on the health of consumers and civil restrictions [3].

In this context, several studies have been conducted to counter the effect of the increase in alcohol content in wines. The main cultural practices tested are based on the modification of the leaf area to fruit mass ratio to lower sugar concentration in grapes and on harvest date to reduce maturation period. Vitcultural techniques as leaf removal, hedging height, harvest date, foliar application of growth or antitranspirant regulators were assessed in the last ten years. The apical portion of the foliage is composed of young leaves, the photosynthetic activity is higher. Poni *et al*, 2013 [4] demonstrated that the removing of 6 or 7 leaves from the apical zone, at the pre-veraison and post-veraison stages, could delayed the accumulation of sugar in the berries by one week. In 2017, Zhang *et al.* [5] confirmed these conclusions by practicing the defoliation at veraison. An alternative technique is to trim the vine canopy to reduce the sugar accumulation in the grapes [6, 7]. The latter indicated that the effect was even more marked when the trimming was severe and early. The foliar application of antitranspirant would lead to a reduction of leaves transpiration which would reduce their photosynthetic capacity. A decrease of 1% in the wine alcoholic content can be achieved [8]. In the same way, post-veraison application allowed to reduce the sugar content of the grapes from 1 to 2.2 brix units compared to a control [9].

In parallel, pre-fermentation and winemaking practices as dilution and nanofiltration have been used to remove sugar from grape must at the cellar. Studies on microbiological practices aimed to discover or create oenological yeasts with low alcohol yield. The most recent work consisted in developping 'low-alcohol' yeast strains which redirect their carbon metabolism away from ethanol production to other metabolites, such as glycerol. A number of interspecific hybrids (*Saccharomyces.cerevisiae* / *Saccharomyces.uvarum*) with these properties had significantly reduced the alcohol content of wines compared to a control [10]. By now, a new *S.cerevisiae* strain using adaptive evolution-based strategies [11] is now commercialized IONYSTMWF (International Patent N◦WO2015/11411).

The objective of this study was to assess the efficiency of reducing alcohol content in wines of Tannat N and Gros Manseng B varieties by combining canopy management practices at the vineyard and microbiological techniques at the cellar.

2 Materials and methods

2.1 Plant material and experimental field site

The trial was carried out in a commercial vineyard located in Saint Mont (Designation of origin Saint-Mont), South-West of France and was established on two plots of Tannat N (TA) and Gros Manseng B (GM) located respectively at 43°38'07.7"N 0°07'14.3"W and 43°38'13.6"N 0°07'30.0"W. Tannat clone 398 on rootstock 3309C is planted on a sandy soil with 2.2m x 1m spacing, trained on Vertical shoot positioning (VSP) system and single Guyot pruned. Gros Manseng clone 439 on rootstock 3309C is planted on chalky-clay soil with 2.3m x 1m spacing, trained on VSP system and single Guyot pruned. Both plots are seated at the top of hillsides.

Rows are oriented east-west and natural grass cover was present between the rows in both plots. Climate is temperate and semi-continental. The experiment was carried out during two years, in 2016 and 2017. The 2016 vintage was characterized by a normal spring for rain and temperature. Summer was very dry associated to thermal amplitudes with hot days but cold nights. The 2017 vintage was characterized by a cold and rainy spring, a hot sunny summer with rains in July and also before harvest.

2.2 Experimental design

The trial was conducted on four rows in a randomized complete block design (latin square) with four replications per treatment. Each experimental unit contained 18 continuous vines.

Three viticultural techniques were evaluated as: a) late hedging (HED), b) leaf removal on the upper part of the canopy (REM) and c) antritranspirant application (VG) (VaporGard®, Miller Chemical, USA) and compared to untreated control (CT).

Canopy management practices, hedging and leaf removal, were applied at mid-veraison on august 24th in 2016 and on august 11th in 2017 to take-off around 60 cm of canopy height or leaves. VaporGard® was applied at veraison in 2016 (24-08-2016) and pre-veraison and veraison in 2017 (02-08-2017 and 17-08-2017). The active ingredient is di-1-p-menthene, a terpene polymere, also known as pinolene. VaporGard was sprayed at 2% concentration in 300L/ha on the entire canopy using a portable sprayer, Stihl SR400, insuring a maximum wet of the leaves.

2.3 Berry growth and yield measurements

From veraison, berry growth and sugar accumulation were tracked by picking every two weeks a sample of 200 berries for each replicate. Sugar content in grape was used to determine harvest date. At harvest, the number of clusters per vine and yield per vine were registered. An average of 15 vines per replicate for Tannat and 9 vines for Gros Manseng were harvested. Total yield per treatment (CT, REM, HED, VG) was also recorded.

A sample of 200 berries per replicate were taken to determine their full composition and weighted. The berries were crushed and centrifuged to enable traditional laboratory measures. A digital hand-held Pocket refractometer PAL (Atago, Japan) was used to determine Sugar concentration (◦Brix). pH was measured with a HI 3221 pH meter (Hana Instruments, France) and titratable acidity was measured according to OIV method [12]. Malic acid was determined by using A Konelab Arena 20 sequential analyzer (Thermo Electron Corporation, USA) associated with enzyme kits (Thermo Fisher Scientific, USA) and tartaric acid was quantified by colorimetric titration[13]. At harvest, carbon isotope discrimination, $\delta^{13}C$, a proxy for vine water status [14] was quantified on each experimental unit according to a published protocol [15]. Anthocyanins and total Total Phenolic Index were determined using an adapted method validated in our laboratory [16] by crushing the berries for two minutes at low speed (600 rpm) using a food blender (Moulinex, Faciclic, France).

2.4 Microvinification and wine tasting

Wines were fermented under microvinification conditions (1L) [15] comparing one control yeast, LA Bayanus (Lamothe-Abiet) for Tannat wines and Zymaflore X5 (Laffort Œnologie) for Gros Manseng wines to Ionys (Lallemand), a low alcohol production yeast. Each replicate was evaluated and separated in two batches, one vinificated with control yeast and the other one vinificated with Ionys (Lallemand). A total of 32 wines for each cultivar were produced in microvinifcation conditions. Same chemical analyses were realized on wine: titratable acidity, pH, malic acid, tartaric acid, anthocyanins, TPI, sulfur content, residual sugar (Konelab Arena 20, Thermo Fisher Scientific), volatile acidity and tanins. Ethanol content was quantified with an alcolyzer M/ME (Anton Paar).

2.5 Data treatment

Statistical analyses were conducted with Xlstat software (Addinsoft, France). All the analytical data measured on the vines at harvest (berries composition) were subjected to a two-way analysis of variance (ANOVA) treatment (treatment x block). Data obtained after alcoholic fermentation were processed by 3 three-way analysis of variance (yeast x treatment x block). The ANOVA were followed by a Newman-Keuls means comparison test at the 5%, with a tolerance of 0.001.

3 Results

3.1 Sugar accumulation in berries

3.1.1 Tannat

Late hedging and leaf removal treatments induce rapidly after their application a delay in sugar accumulation in berries in comparison to control (Figure 1). For the second year, at harvest, both modalities present a significant lower sugar content (respectively -1,3 °Brix and -1,4°Brix). The application of antitranspirant VaporGard ® showed in 2017 (but not in 2016) a significant decrease in sugar content at harvest, with a slower kinetics.

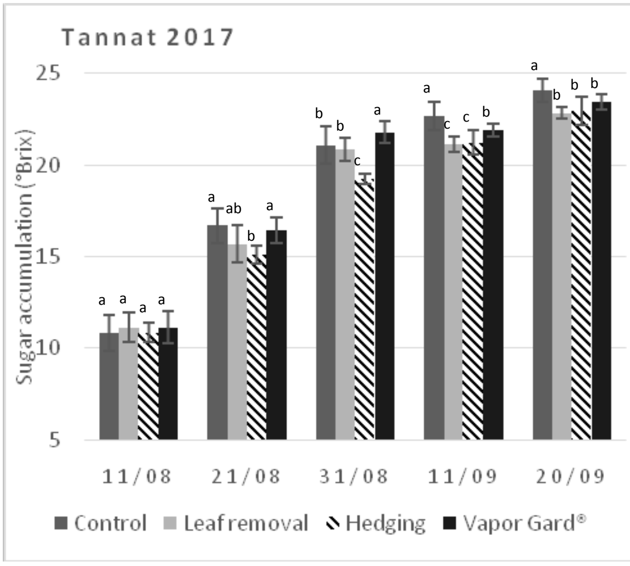

Figure 1: Seasonal accumulation of sugar (°Brix) in 2017 for the three treatments compared with the control. Different letters indicate significant difference with Newman Keuls test at $p<0.05$.

3.1.2 Gros Manseng

The behaviors of sugar accumulation for hedging and leaf removal batches are similar until maturity, with a significant reduction in sugar content as early as 10 days after their application. As for the antitranspirant (VG), the sugar accumulation is slightly lower than control, with a more marked difference in middle of maturation. However, at harvest, the effect seems to have been canceled. On September 25th, both HED and REM showed significant decrease in sugar content in comparison to control (respectively -1 °Brix and -1,1 °Brix).

3.2 Impact of canopy management techniques on yield and chemical parameters at harvest

There is no impact of the different techniques on yield for both cultivars with an average for TA of 7,4 cluster and 3,5 kg per vine in 2016; 5,2 clusters and 2,1 kg per vine in 2017 and for GM of 15.7 clusters and 3,9 kg per vine in 2016; 17,7 clusters and 3,8kg per vine in 2017.

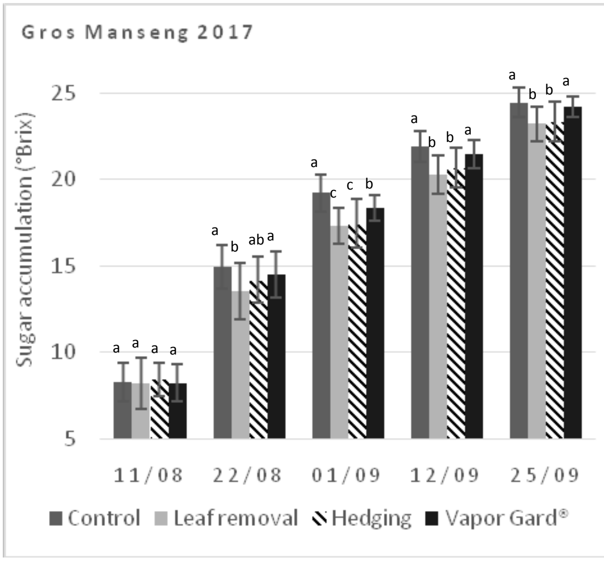

Figure 3: Seasonal accumulation of sugar (°Brix) in 2017 for the three treatments compared with the control. Different letters indicate significant difference with Newman Keuls test at $p<0.05$.

Regarding Total Soluble Solids (TSS in °Brix), leaf removal and late hedging appear to be efficient in reducing sugar content on berries at harvest time for both cultivars. These technics have also a significant effect on anthocyanins on TA and Total acidity and TPI on GM (Table 1). Leaf removal on upper part of the canopy and late hedging appears to have the main effect regarding experiment's objectives. Indeed, they also induce an significant increase in total acidity of 0,2 to 0,5 g/L in Gros Manseng berries and a decrease in anthocyanins contents for Tannat. At harvest, anthocyanin's concentration is significantly 12% lower than control for leaf removal and late hedging (table 1).

3.3 Results on wines: effect of yeasts

In 2017, for the second year, all treatment fermented with Ionys yeast showed a slower fermentation. It was verified that both yeast were well inplanted for each batch. After control, in 2017, Ionys and control yeasts were implanted in all batches in microvinification conditions

Table 1: Yield components and must composition on Tannat and Gros Manseng berries with the four treatments. Data are expressed as the mean of four experimental replicates ± standard deviation.

	200 berries weight [g]	TSS (°Brix)	Total Acidity [g/L H2SO4]	pH	Tartaric Acid [g/L]	Malic Acid [g/L]	Available Nitrogen [mg/L]	Total Polyphenol Index	Anthocyanins (Puissant Léon method) [mg/kg]
Tannat									
CT	339 ± 15 *a* [(1)]	24,5 ± 0,25 a	5,8 ± 0,34 *a*	2,99 ± 0,05 *a*	3,9 ± 0,19 *a*	5,5 ± 0,27 *a*	60,7 ± 2,7 *a*	152 ± 9 *a*	2200 ± 78 *a*
REM	355 ± 13 *a*	23,1 ± 0,26 b	5,8 ± 0,24 *a*	3,02 ± 0,04 *a*	3,5 ± 0,25 *a*	5,7 ± 0,40 *a*	59,7 ± 2,5 *a*	144 ± 5 *a*	1938 ± 40 *b*
HED	343 ± 13 *a*	23,2 ± 0,63 b	5,9 ± 0,29 *a*	3,00 ± 0,05 *a*	3,7 ± 0,21 *a*	5,8 ± 0,20 *a*	64,7 ± 7,2 *a*	139 ± 6 *a*	1915 ± 71 *b*
VG	335 ± 7 *a*	23,6 ± 0,36 b	5,6 ± 0,38 *a*	3,04 ± 0,07 *a*	3,7 ± 0,29 *a*	5,68 ± 0,46 *a*	65,9 ± 5,2 *a*	150 ± 10 *a*	2078 ± 146 *ab*
Sig.	ns [(2)]	**	ns	ns	ns	ns	ns	ns	*
Gros Manseng									
CT	234 ± 15 *a*	24,4 ± 0,70 a	8,2 ± 0,40 *bc*	2,70 ± 0,03 *a*	9,6 ± 0,54 *a*	4,9 ± 0,49 *a*	148 ± 27 *a*	8,74 ± 0,27 *a*	
REM	237 ± 10 *a*	23,3 ± 0,80 b	8,4 ± 0,30 *b*	2,67 ± 0,03 *a*	9,6 ± 0,33 *a*	5,4 ± 0,83 *a*	161 ± 10 *a*	8,30 ± 0,47 *bc*	
HED	233 ± 17 *a*	23,4 ± 0,99 b	8,7 ± 0,37 *a*	2,72 ± 0,07 *a*	9,6 ± 0,61 *a*	5,4 ± 0,72 *a*	158 ± 17 *a*	8,06 ± 0,38 *c*	
VG	235 ± 9 *a*	24,1 ± 0,52 a	8,0 ± 0,32 *c*	2,70 ± 0,01 *a*	9,7 ± 0,51 *a*	4,9 ± 0,44 *a*	167 ± 24 *a*	8,50 ± 0,55 *ab*	
Sig.	ns	**	**	ns	ns	ns	ns	**	

(1) Different letters in the same line indicate treatments are significantly differents from one other ($p \leq 0,05$, Newman Keuls test).

(2) *,**,** and ns represent significance at p <0,05; p <0,01; p< 0,001 and not significant, respectively.

A significant difference is observed for both cultivar for all parameters according to the yeast used. Ionys allowed to decrease ethanol content in wine for an average of 0,6 % vol. for Tannat and 0,5 %vol. for Gros Manseng, independently of the tested treatment. A second predictable difference between the yeasts was the increase of total acidity with Ionys (1,4g/L H_2SO_4 for Tannat and 0,6 g/L H_2SO_4 for Gros Manseng) and decrease of volatile acidity (0,15g/L acetic acid for TA and 0,49g/L acetic acid for GM. These results confirm the ones obtained in 2016.

3.3 Results on wines: effect of combining canopy management practices and low alcohol yield yeast

Combining the use of low alcoholic yield yeast to canopy management practices appears to be efficient in reducing alcohol content in wines. Indeed, the combined use of leaf removal or late hedging to Ionys yeast induce a decrease of alcohol content in wine of 1,1% vol. and 1,3 % vol. respectively for Tannat in 2017. For Gros Manseng, the effect is also significant with a decrease in alcohol content of 1,4% vol. both years.The combination of yeast and canopy management practices showed also in 2017 a significant effect on other parameters as pH, tartaric acid, PTI and anthocyanins for Tannat and pH, tartaric acid, malic acid for Gros Manseng (table 2).

4 Discussion

The rate of sugar accumulation in berries is determined by the leaf area to fruit mass (LA/FM) ratio [17]. The application of leaf removal and late hedging reduced by 30% the foliar surface and the spray of pinolene aimed to reduce photosynthesis on active leaves. The three treatments allowed to delay sugar accumulation in berries when applied at veraison without affecting berry growth. Side effects as the increase of total acidity in must for both cultivars and decrease of TPI and anthocyanins concentration on Tannat berries were noticed. These effects were reported by Martinez de Toda, 2013 [7] with a decrease in pH and acidity on Grenache. Nevertheless, this result appear to contradict those of Zhang *et al.* [5], Tessarin *et al.* [18], Poni *et al.* [4] and Stoll et *al.* [17] whom observed a decrease of total acidity on leaf removed Syrah or no significant effect of late hedging and leaf removal on these parameters at harvest on Sangiovese cultivar. In contrary, the decrease of TPI and anthocyanins in berries for Tannat cultivar is in agreement with the bibliography [5,19 and 7] and can be explained by the reduction of foliar surface and modification of the LA/FM ratio [20]. In this study, the use of pinolene (Vapor Gard®) to reduce sugar concentration in berries is variable and not conclusive in opposition to the other treatments (HED, REM) and bibliography [8, 9, 21]. Defoliation above the bunch zone at veraison and post veraison hedging (average 9-11°Brix) was effective in delaying technological ripeness of both cultivar but affecting acidity, colour and phenolics.

This result depend on the compensation capacity for photosynthesis shown by the cultivar [4]. Combination of these technics with late harvest could be investigated to try to minimize these effects.

The selected yeast showed results in accordance with previous experiments [22,23]: a decrease in alcohol content from -0,4% vol to -0,8% vol according to the treatment and an increase in total acidity of around 10% for Gros Manseng and 38% for Tannat.

Table 2: Wine chemistry parameters analyzed on Tannat and Gros Manseng wines in 2017. Data are expressed as the mean of four experimental replicates

(1) Different letters in the same column indicate that treatments are significantly differents from one other ($p \leq 0{,}05$, Newman Keuls test).
(2) *represent significance at $p < 0{,}05$

	Alcohol (% vol)	Total Acidity (g/L H2SO4)	Tartaric Acid (g/L)	Anthocyanins (mg/L)
Tannat				
CT BA	14.0 a	3.7 b	1.0 ab	1640 a
CT IO	13.4 abc	5.2 a	0.4 c	1393 bc
REM BA	13.4 abc	3.7 b	1.1 ab	1468 bc
REM IO	12.3 c	5.0 a	0.5 c	1328 c
HED BA	13.3 abc	3.7 b	1.2 a	1423 bc
HED IO	12.7 c	5.1 a	0.5 c	1294 c
VG BA	13.7 ab	3.7 b	0.9 b	1529 ab
VG IO	13.0 bc	5.2 a	0.3 c	1417 bc
Sig.	*	*	*	*
Gros Manseng				
CT X5	14.8 a	6.1 ab	2.5 ab	
CT IO	14.2 ab	7.0 ab	2.2 b	
REM X5	13.8 ab	6.6 ab	2.9 ab	
REM IO	13.4 b	6.7 ab	2.5ab	
HED X5	13.7 ab	6.6 ab	2.9 a	
HED IO	13.4 b	7.1 a	2.5 ab	
VG X5	14.6 ab	6.1 b	2.6 ab	
VG IO	14.1 ab	6.9 ab	2.3 ab	
Sig.	*	*	*	

Objective of reducing alcohol content in wines was reach during the two years of the study. Combination of late hedging with Ionys appear to be the more efficient technique for two consecutive years. Combination of leaf removal of the upper part of the canopy with Ionys showed an excellent efficiency in 2017. Nevertheless, effect of antitranspirant was lower on wine with no significant difference with control.

5 Conclusion

The reduction of leaf area at veraison by leaf removal in the upper two thirds of the vertical canopy and severe hedging inducing a decrease of 30% of the active canopy showed promising results in delaying sugar accumulation in Gros Manseng and Tannat berries. Combination of these techniques with the use of yeast with a low alcoholic yield allowed to reduce alcohol content in wines from 1%vol. to 2% vol. according the cultivar and the vintage. According to climate change, increase in total acidity is not without of interest even in South west France vineyard.

6 Acknowledgements

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