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Impact of the vine water status on the berry and seed phenolic composition of 'Merlot' (*Vitis vinifera L.*) cultivated in a warm climate: Consequence for the style of wine

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Summary

During 2005 and 2006 four irrigation treatments were assayed in a vineyard growing 'Merlot' grapes located in a warm climate region. The treatments were kept for two years and the wine was produced the second year so that the response of the wines to water constraint was consistent. The phenolic parameters of the wines were analysed. The phenolic composition of the seeds was studied for both years and more significant differences were observed in the second. The results showed that when the deficiency increased, so did the concentration of total polyphenols, flavan-3-ols and tannins in the seeds. Pre-dawn leaf water constraint integrals between 20 and 24 Mpa as opposed to levels of 9 to 14 caused statistically significant increases in total polyphenols, flavan-3-ols and the colour index of the wine. These differences were manifested in sensory terms by significant intensification of the colour and body of the wines.

Key words: water constraint, phenolic composition, colour, *Vitis vinifera* L. cv. Merlot, wine.

Introduction

Anthocyanins and their combinations with other phenols are the compounds primarily responsible for the colour of red grapes and wines; levels in grapes depend not only on the variety but also on climatic conditions and growing practices (Esteban *et al.* 2001). The plant water status has an impact on berry phenolic biosynthesis depending on the water level, duration of the water constraint or stress and phenological stage when the vine water status is modified.

The water status of the vine throughout the cycle is a key factor both for vegetative and reproductive growth and for physiological and biochemical functioning. In water-restricted conditions the metabolisms of plant and fruit are affected, and the biochemical development of the berry is what determines the style of the wine (Deloire *et al.* 2005), according to the enological process.

This work aims at contributing to the knowledge of the effects of water constraint, controlled via irrigation, on vineyards cultivated in a warm area: La Mancha, Spain; in particular, the effect on the berry and seed phenolic composition and its consequence for the style of wine.

Material and Methods

Environmental conditions and plant material: The trial was conducted during 2005 and 2006 in a vineyard with 'Merlot' grapes, situated in a plot representative of the La Mancha viticultural region. Average annual rainfall is slightly more than 350 mm. The years 2005 and 2006 were extremely dry years, with only 29.8 mm and 61.9 mm of rain in spring, respectively, and ET_0 levels of almost 1100 mm between 1 April and 30 September.

The vines, grown on trellises, a distance of 3 m x 1.2 m (row by vine spacing), are trained to a double cordon Royat system, with 3-4 spurs of 2 buds on each arm. The canopy has a height of 1.10 m and the topping is performed. Before flowering, some primary shoots and the secondary shoots were removed, leaving all the vines with the same number.

Water regimes and leaf area: The Ψ_{PD} was measured on a total of 34 days, between flowering and maturity, using a pressure chamber (SKPM-1400, Skye Inst. Lim., U.K.). The Ψ_{PD} data were calculated as the mean of 8 measurements recorded on 8 completely developed leaves. The pre-dawn leaf water constraint integral ($S\Psi_{PD}$) that expresses the severity by duration of the constraint above a minimum value was calculated as defined by Myers (1988) using the leaf water potential data. For convenience, we considered the positive integral value ($-S\Psi_{PD}$).

Four irrigation treatments, with 64 vines per treatment and distributed in 2 blocks, were tested in duplicate. The treatments were defined by marking minimum thresholds of Ψ_{PD} during two phenological intervals. The water status in the different treatments shown in Tab. 1, are consistent with those proposed by Carbonneau (1998).

The first week of August, one week before the vintage, total leaf area was determined from the leaf area index (LAI), by a LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, Nebraska, USA). The exposed leaf area (SA) was calculated by analysis of digital images taken from the same plants as were used to determine the LAI.

Seeds extracts and microvinifications: At harvest, 100 healthy berries were weighed and finger-pressed to remove the pulp. The remaining seeds were washed in water (Milli-Q), gently dried and weighed. Two grams of dried seeds were extracted twice with 100 ml of a mixture 50:48.5:1.5 (v/v) of CH_3OH/

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Table 1

Daily values of pre-dawn leaf water potentials in the four treatments, and water status corresponding to vine-stocks

Treatment	Period		Water status of vine
	Flowering-Veraison	Veraison-Maturity	Type of constraint
T1	0 Mpa ≥ Ψ_{PD} ≥ - 0.2 Mpa	Ψ_{PD} ≥ - 0.2 Mpa	None – Slight
T2	-0.2 Mpa > Ψ_{PD} ≥ -0.4 Mpa	Ψ_{PD} ≥ - 0.4 Mpa	Slight – Moderate
T3	-0.4 Mpa > Ψ_{PD} ≥ -0.6 Mpa	Ψ_{PD} ≥ - 0.6 Mpa	Moderate – Intense
T4	-0.6 Mpa > Ψ_{PD}	Ψ_{PD} ≥ - 0.8 Mpa	Intense

H_2O/HCOOH (GAO *et al*. 1997), using a homogenizer for 2 min and then centrifuging at 2500 g for 15 min. When the grapes reached the technological maturity, several vinifications were performed in 2006 (a total of 16 wines, *i.e.* two for each repetition of each irrigation treatment). Ten vines were harvested for each treatment. Fermentation was performed at 20 ºC adding 75 mg of SO_2 per kilogram of macerated must and the selected yeast Uvaferm VN. Maceration was continued throughout the alcoholic fermentation process, which lasted for 6 d, until reducing sugars were exhausted.

Analysis of berries and wines: Anthocyanins were determined by decolouring with sulphur dioxide and total polyphenols by measurement of absorbance at 280 nm. Flavan-3-ols were determined by reaction with dimethylaminocinnamaldehyde (DMACH) and measurement at 640 nm (NAGEL and GLORIES 1991), and the tannins by acid hydrolysis catalysed by ferric sulphate, stabilization with 1-butanol and measurement at 550 nm (PORTER *et al.* 1986). CIELAB parameters were derived using a UNICAM-UV500 spectrophotometer.

Sensory analysis: The sensory analysis was realised three months after the wines were bottled, by a panel of six trained tasters. Wines were scored on a scale of 0 to 10 according to the perceived intensity of descriptors of the wine. 0: undetectable or very weak; 10: very intense.

Results and Discussion

Tab. 2 shows the results obtained. Note that when -$S\Psi_{PD}$ increases there is a significant decrease in production, LAI, SA and berry weight; this last effect was statistically significant for the second year of treatment (24 % decreases between treatments 1 and 4)**.**

Like other authors (SIPIORA and GUTIERREZ-GRANDA 1998), we found no differences in the weight of the seeds over the four treatments (data not shown), but the loss of berry weight meant that the seeds made up a higher percentage of the total weight of the grape. No significant differences were found between treatments as regards the sugar contents (ºBaumé) and the pH of the musts. However, it was found that the concentration of malic acid fell as water constraint increased, with a correlation coefficients of -0.812 and -0.760 ($\alpha < 0.01$) for 2005 and 2006 respectively, which is consistent with the findings published by other authors (SHELLIE 2006, SALÓN *et al.* 2005).

Differences in the constraint on vines produced changes in the phenol concentrations in the seeds, although the concentration increases observed were statistically significant only for 2006: total polyphenols (50 %), flavan-3-ols (59 %) and tannins (62 %). This cannot be attributed solely to the increase in the relative weight of the seeds versus the total grape weight, as the maximum possible value of this is 35 %.

Constraint integrals ranging from 20 to 24 Mpa produced a statistically significant increase in the total phenols and the catechin concentrations in the wines with respect to those in which the values ranged from 9 to 14 Mpa. An increase in the values of the integral caused a reduction in the lightness (L*) of the wines and a corresponding increase of up to 20 % in colour index. There was also a decrease in the parameters a* and b*; in other words, the colour of the wines intensified, losing redness and becoming more blue. Although the differences observed are not significant, wine tannins tend to increase with water constraint. The results showed that chemical differences also translated into statistically significant differences in sensory assessments by the tasting panel.

Conclusions

One of the factors determining the style of wine is the vineyard itself. There are some factors that are difficult or impossible to influence (edaphoclimatic and genetic factors); however, other factors depend exclusively on the wine-grower. Water constraint is perhaps the most important factor since it determines the plant canopy and the yield, and these in turn influence the characteristics of wine.

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T a b l e 2

Means and standard deviations of agronomic, berry and wine parameters, for the four treatments (different superscript letters (^{a,b,c}) indicate statistically significant differences according to the Student-Newman-Keuls multiple comparison of means test, $\alpha = 0.05$)

Parameter		T1 (n = 4)	T2 (n = 4)	T3 (n = 4)	T4 (n = 4)
	Year	mean ± sd	mean ± sd	mean ± sd	mean ± sd
$-S\Psi_{PD}$	2005	10.74	12.95	14.60	21.99
(MPa)	2006	9.61	14.23	20.33	23.50
Production	2005	1.26^{c} ± 0.03	1.22^{c} ± 0.01	1.09^{b} ± 0.07	0.89^{a} ± 0.03
(Kg/m^2)	2006	0.90^{b} ± 0.02	0.87^{b} ± 0.11	0.63^{a} ± 0.02	0.56^{a} ± 0
LAI	2005	1.49^{ab} ± 0.08	1.64^{b} ± 0.18	1.52^{ab} ± 0.18	1.37 ± 0.08
(m^2 leaf area/m^2 soil surface area)	2006	1.51^{c} ± 0.03	1.36^{c} ± 0.17	1.30^{ab} ± 0.08	1.16^{a} ± 0
SA	2005	0.83 ± 0.02	0.83 ± 0	0.81 ± 0.06	0.75 ± 0.03
(m^2 leaf area/m^2 soil surface area)	2006	0.85^{b} ± 0.03	0.85^{b} ± 0.02	0.76^{a} ± 0.04	0.72^{a} ± 0.04
Berry weight	2005	1.09 ± 0.02	1.04 ± 0.07	0.99 ± 0.08	0.96 ± 0.07
(g)	2006	0.90^{c} ± 0.03	0.83^{b} ± 0.04	0.71^{a} ± 0.03	0.68^{a} ± 0.03
% Seeds/berry weight	2005	5.76^{a} ± 0.25	6.14^{ab} ± 0.01	6.15^{ab} ± 0.27	6.73^{b} ±0.01
(w/w)	2006	4.53^{a} ± 0.07	5.31^{b} ± 0.12	5.76^{c} ± 0.28	6.11^{d} ± 0.28
ºBaumé	2005	13.38 ± 0.11	13.31 ± 0.23	13.29 ± 0.43	13.11 ± 0.04
	2006	14.16 ± 0.23	14.25 ± 0.10	14.32 ± 0.19	14.19 ± 0.10
pH	2005	3.34 ± 0.02	3.31 ± 0.01	3.32 ± 0.06	3.35 ± 0.02
	2006	3.49 ± 0.02	3.52 ± 0.08	3.54 ± 0.03	3.47 ± 0.03
Malic acid (g/L)	2005	1.06^{b} ± 0.01	1.02^{b} ± 0.16	0.97^{b} ± 0.21	0.87^{a} ± 0.05
	2006	0.85^{b} ± 0.14	0.76^{ab} ± 0.11	0.61^{a} ± 0.02	0.61^{a} ± 0.07
Flavan-3-ols in seeds	2005	1279 ± 38	1333 ± 39	1372 ± 44	1442 ± 86
(mg catechin/Kg grape)	2006	855^{a} ± 279	1373^{b} ± 98	1397^{b} ± 101	1348^{b} ± 107
Tannins in seeds	2005	4.91 ± 0.10	5.30 ± 0.56	5.6 ± 0.19	5.45 ± 1.20
(g/Kg grape)	2006	4.03^{a} ± 0.97	6.25^{ab} ± 0.73	6.82^{b} ± 1.67	6.51^{b} ± 0.79
Total polyphenols in seeds	2005	2083 ± 91	2290 ± 123	2325 ± 13	2564 ± 250
(mg gallic acid/Kg grape)	2006	1289^{a} ± 426	1796^{b} ± 29	1959^{b} ± 178	1923^{b}± 172
% Alcohol	2006	13.94 ± 0.22	14.15 ± 0.20	14.09 ± 0.32	13.84 ± 0.15
Total acidity (g tartaric acid/L)	2006	3.64 ± 0.09	3.79 ± 0.27	3.80 ± 0.30	3.76 ± 0.20
pH	2006	3.68 ± 0.02	3.64 ± 0.04	3.65 ± 0.10	3.62 ± 0.07
Anthocyanins (mg malvidin/L)	2006	327 ± 22	338 ± 22	378 ± 42	328 ± 14
Flavan-3-ols (mg catechin/L)	2006	215.5^{a} ± 9.9	219.5^{a} ± 25.7	268.3^{b} ± 29.3	275.4^{b} ± 23.7
Tannins(g/L)	2006	1.38 ± 0.28	1.25 ± 0.21	1.54 ± 0.24	1.67 ± 0.21
Total polyphenols (mg gallic acid/L)	2006	815^{a} ± 33	887^{a} ± 83	1064^{b} ± 67	1069^{b} ± 39
L*	2006	18.19^{b} ± 1.25	17.85^{b} ± 0.61	13.00^{a} ± 0.61	12.94^{a} ± 1.30
a*	2006	49.52^{b}± 1.14	49.11^{b} ± 0.79	43.79^{a}± 0.49	43.60^{a}± 1.61
b*	2006	37.06^{b} ± 1.25	36.82^{b} ± 0.69	30.31^{a}± 0.99	30.15^{a}± 2.04
Colour Index (absorbance units)	2006	7.87^{a} ± 0.49	7.98^{a} ± 0.25	9.98^{b} ± 0.33	9.84^{b} ± 0.58
Intensity of colour	2006	6.46^{a} ± 0.28	6.79^{a} ± 0.39	7.33^{b} ± 0.36	7.67^{b} ± 0.27
Intensity of aroma	2006	6.58 ± 0.32	6.75 ± 0.29	6.96 ± 0.92	7.33 ± 0.49
Body	2006	5.79^{a} ± 0.21	6.29^{b} ± 0.32	7.00^{c} ± 0.27	7.17^{c} ± 0.14

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