Impact of water stress on the phenolic composition of cv. Merlot grapes, in a typical *terroir* of the La Mancha region (Spain)

Effets de la contrainte hydrique sur la composition phénolique des raisins du cv. Merlot, dans un *terroir* typique de La Mancha (Espagne)

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Abstract

The study was carried out in 2006 with Merlot grapes from vines grown using the trellis system, where four treatments were compared with different levels of water stress. These levels were established using irrigation to maintain pre-dawn leaf water potential (Ψ_{PD}) values between two different phenological intervals: flowering-veraison and veraison-maturity. Leaf area index (LAI), exposed leaf area (SA) and production were also measured. Conventional grape parameters (weight, ºBaumé, pH and malic acid) and seed and skin phenolic compounds (anthocyanins, procyanidins, tannins and total polyphenols) were also analyzed. The results showed that when grape weight diminished as a result of water stress, the percentage weight of grape skins with respect to total grape weight was maintained, but seed weight increased. When the water stress integral increased, total polyphenol, procyanidin and tannin concentrations in the seeds also increased.

Keywords: grape, Merlot, phenolic compounds, water stress.

Introduction

Anthocyanins and their combinations with other phenols are the compounds primarily responsible for the colour of red grapes and wines; levels depend not only on the variety but also on climatic conditions and growing practices (Esteban *et al.*, 2001).

To make high-quality wines which evolve well during aging, the raw material must supply an adequate concentration of phenolic compounds. Of the numerous growing factors that influence the concentration of phenolic compounds in the grape, and hence in the wine, irrigation is perhaps the most important, especially in warm, dry regions.

The water status of the vine throughout the cycle is a key factor both for vegetative and fruitful 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).

There are numerous direct and indirect methods of ascertaining the vine water status of an individual plot, but the reference method at this time is measurement of the pre-dawn leaf water potential (Ψ_{PD}) using a pressure chamber (Scholander et al., 1965). Measurement of Ψ_{PD} is the most commonly used method for characterizing vine water stress levels in the Mediterranean area (Payan, 2004).

Although Merlot grapes have traditionally been cultivated in Atlantic climates, they are now being grown more and more in warm and dry areas such as the region of La Mancha. The objective of this study is to help improve the phenolic quality of this grape variety for use in the production of aged wines by controlling irrigation.

Materials and methods

Environmental conditions and plant material

The trial was conducted in 2006 in a vineyard growing Merlot grapes grafted on to Fercal rootstock, situated in a plot representative of the La Mancha growing region. The soil, only slightly fertile with a Petrocalcic soil horizon situated 35 cm below the surface, is Petric Calcisol which has developed on the alluvial part of the River Guadiana.

The plot, situated at an altitude of 670 m, is located in Region VI according to Winkler, with effective temperatures of around 2000º and reaching mean Huglin index values of 2600. Average annual rainfall is slightly more than 350 mm, with reference evapotranspiration (ET_0) of approximately 1300 mm/year. The year 2006 was extremely dry, with only 61.9 mm of rain in spring, and an ET_0 level of almost 1100 mm between 1 April and 30 September.

The vines, grown on trellises and arranged on rectangular frames measuring 3 m x 1.2 m, are trained to a double Cordon Royat system, with 3-4 spurs of 2 buds on each arm. Before flowering, the vine shoots were removed, leaving all the vines with the same number. The average number of bunches per plant was 26.

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.). Daily Ψ_{PD} data were calculated as the mean of 8 measurements recorded in 8 different leaves from the top third of the vine shoots, for each treatment. All measurements were taken before sunrise.

The water stress integral ($S\Psi_{PD}$) adapted (Myers, 1988) was derived for each treatment by adding together the daily means of the $\Psi_{PD} < -0.2$ Mpa in the period between the phenological states of flowering and maturity. It is considered that the grapes to start to suffer stress when the Ψ_{PD} fell below – 0.2 Mpa (Carbonneau, 1998). For convenience, we considered the positive integral value ($-S\Psi_{PD}$).

$$-S\psi_{PD} = -\left[\sum_{\psi PDi < -0,2} (\overline{\psi}_{PDi} + 0,2)\right] \text{ Mpa}$$

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, flowering-veraison and veraison-maturity. The water stress levels in the different treatments shown in Table 1, are consistent with those proposed by Carbonneau in 1998.

	Period		**Water status of vine**
Trtmnt	**Flowering-Veraison**	**Veraison-Maturity**	**Type of stress**
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

Table 1 Predawn leaf water potentials in the different treatments, and water corresponding to vine-stocks

At the time of veraison (64 days after flowering), - $S\Psi_{PD}$ varied from 6.9 to 17.2 Mpa, and at the time of technological maturity (84 days after flowering) from 9.6 to 23.5 Mpa (Figure 1).

Total leaf area was determined from the leaf area index (LAI), using light extinction measurements by a LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, Nebraska, USA) (Romero et al., 2005). Measurements were taken on a total of 10 vines per treatment. The exposed leaf area (SA) was calculated by analysis of digital images taken from the same plants as were used to determine the LAI.

Yields and analysis of grapes

The production for each treatment was calculated at the time of technological maturity as the mean of 10 randomly-selected vines. Analyses of pH, concentration of sugars expressed in degrees Baumé and malic acid were performed following the OIV international methods. Anthocyanins were determined by decolouring with sulphur dioxide (Ribereau-Gayon & Stronestreet, 1965) and total polyphenols by measurement of absorbance at 280 nm following conventional dilution of the sample (Somers & Evans, 1976). Total flavan-3-ols (Procyianidins) 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).

Skin and seeds extracts

One hundred healthy grapes were weighed and finger-pressed to remove the pulp. The remaining skins and seeds were washed three times in water (Milli-Q) and gently dried twice by patting them between sheets of filter paper. The dried skins (16 g) and seeds (2 g) were extracted twice with 100 ml of a mixture 50:48.5:1.5 (v/v) of $CH_3OH/H_2O/HCOOH$ (Gao et al., 1997), using a homogenizer (Heidolph DIAX 900) for 2 min and then centrifuging at 2500 g for 15 min.

Statistical analysis

Data were analysed by multiple comparison of means and a study of the Pearson correlation coefficients, using version 15.0 of the SPSS package.

Results and Discussion

Figure 1 shows the history of $-S\Psi_{PD}$ for the different treatments, from flowering (22 May) to maturity (11 August). Veraison took place on 25 July that is 64 days after flowering.

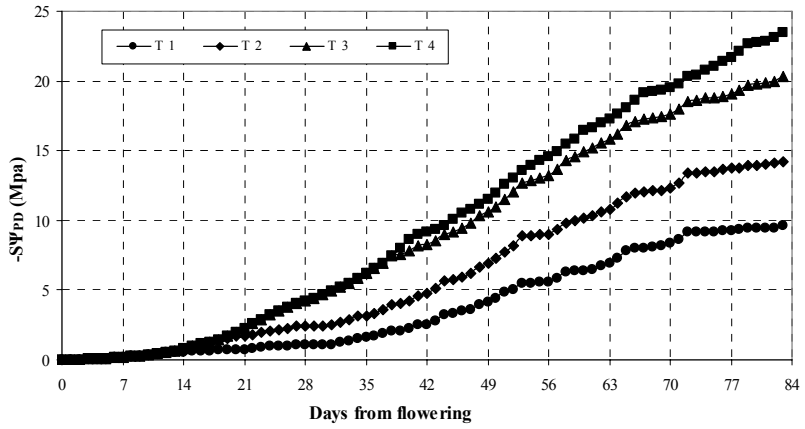

Figure 1. History of $-S\Psi_{PD}$ from flowering to technological maturity under the different treatments.

Table 2 shows some of the Pearson correlation coefficients derived from among agronomic parameters and for grapes. The Pearson correlation coefficient between the parameters $-S\Psi_{PD}$ / Berry weight was -0.941, with a level of significance of $\alpha < 0.01$. Recent studies with the Merlot variety have shown that the average unit weight of grapes at harvesting is determined by the cumulative water stress ($-S\Psi_{PD}$) during the first month of grape growth following flowering (Martínez et al., 2007). Water stress also influences vegetative growth; a high degree of correlation exists between LAI and SA with - $S\Psi_{PD}$.

	- $S\Psi_{PD}$ (Mpa) (n=16)	**LAI** (m^2/ml) (n=16)	**SA** (m^2/ml) (n=16)
- $S\Psi_{PD}$ (Mpa)	1	-0.812**	-0.845**
Berry weight (g)	-0.941**	0.838**	0.843**
Malic acid (g/L)	-0.760**	0.748**	0.711**
Total polyphenos in seeds[1]	0.691**	-0.519*	-0.548*
Tannins in seeds (g/kg)	0.633**	ns	-0.555*

1: mg gallic acid/kg of grape.

Table 2 Correlation of Pearson coefficients among agronomic parameters and grapes (ns: not significant, * significance $\alpha < 0.05$, **significance $\alpha < 0.01$)

Table 3 shows that when -$S\Psi_{PD}$ increased, there was a statistically significant loss of grape weight (24% between treatments 1 and 4) and consequently of yield**.** Like other authors (Sipiora & Gutierrez-Granda, 1998), we found no differences in the unit 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. On the other hand, the weight of the skins as a proportion of the weight of the grape remained unchanged.

	T1 (-$S\Psi_{PD}$ = 9.61)	**T2 (-$S\Psi_{PD}$ = 14.23)**	**T3 (-$S\Psi_{PD}$ = 20.33)**	**T4 (-$S\Psi_{PD}$ = 23.50)**
Parameter	**mean ± sd**	**mean ± sd**	**mean ± sd**	**mean ± sd**
Yield (g/m lineal)	2730^{b} ± 51	2624^{b} ± 340	1917^{a} ± 68	1707^{a} ± 10
LAI (m^2/m lineal)	4.59^{c} ± 0.10	4.11^{c} ± 0.50	3.93^{ab} ± 0.26	3.53^{a} ± 0.03
SA (m^2/m lineal)	2.58^{b} ± 0.10	2.57^{b} ± 0.06	2.31^{a} ± 0.11	2.18^{a} ± 0.12
Berry weight (g)	0.90^{c} ± 0.03	0.83^{b} ± 0.04	0.71^{a} ± 0.03	0.68^{a} ± 0.03
ºBaumé	14.16 ± 0.23	14.25 ± 0.10	14.32 ± 0.19	14.19 ± 0.10
pH	3.49 ± 0.02	3.52 ± 0.08	3.54 ± 0.03	3.47 ± 0.03
Malic acid (g/L)	0.85^{b} ± 0.14	0.76^{ab} ± 0.11	0.61^{a} ± 0.02	0.61^{a} ± 0.07
% Skins	14.1 ± 2.6	14.0 ± 2.3	13.5 ± 1.2	14.6 ± 1.1
% Seeds	4.53^{a} ± 0.07	5.31^{b} ± 0.12	5.76^{c} ± 0.28	6.11^{d} ± 0.28
Anthocyanins in skins[1]	816 ± 65	930 ± 156	882 ± 42	900 ± 83
Catechins in skins[2]	98.5^{a} ± 7.4	122.0^{b} ± 19.0	96.2^{a} ± 6.5	104.2^{a} ± 6.4
Tannins in skins[3]	1.82 ± 0.22	1.76 ± 0.29	1.77 ± 0.24	1.93 ± 0.14
Total polyphenols in skins[4]	628 ± 63	651 ± 100	615 ± 26	631 ± 73
Procyanidins in seeds[2]	855^{a} ± 279	1373^{b} ± 98	1397^{b} ± 101	1348^{b} ± 107
Tannins in seeds[3]	4.03^{a} ± 0.97	6.25^{ab} ± 0.73	6.82^{b} ± 1.67	6.51^{b} ± 0.79
Total polyphenols in seeds[4]	1289^{a} ± 426	1796^{b} ± 29	1959^{b} ± 178	1923^{b}± 172

1: mg malvidin/kg of grape; 2: mg catechin/kg of grape; 3: g/Kg; 4: mg gallic acid/kg of grape

Table 3 Means and standard deviations of agronomic parameters and of grapes 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, α=0.05)

No significant differences were found between treatments as regards the sugar contents (º Baumé) and the pH of the musts, which indicates only very slight differences of maturity between them. These results are consistent with the findings of some authors (Sivilotti et al., 2005) but disagree with some others (Matthews and Anderson, 1998; Sipiora & Gutierrez-Granda, 1998). This may be because the stress levels to which the plants were subjected were different in each study or because of the diversity of climatic conditions between regions.

However, it was found that the concentration of malic acid fell as water stress increased, with a correlation coefficient of -0.760 ($\alpha < 0.01$), which is consistent with the findings published by other authors (Shellie, 2005). The concentration of malic acid in the grape at the time of technological maturity depends basically on the temperatures to which the bunches are subjected during ripening. The fact that there is more vegetation in more intensely irrigated systems reduces the exposure to sunlight and hence the temperature of the bunches, so that the metabolic rate of this acid in the fruit is lower.

As regards the phenolic fraction of the grape, the differences in levels of water stress in the different treatments did not seem to affect the concentration of phenols in the skin. Alteration of the vine's water status only moderately affects flavonoid synthesis in the skin and is dependent on the intensity and the timing of water restriction (Kennedy et al., 2002; Ginestar et al., 1998; McCarthy, 1999). In the present experiment, however, differences in the stress on plants produced changes in the phenol concentrations in the seeds expressed as a percentage of berry weight, and there were significant increases in total concentrations of phenols (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%.

Conclusions

In warm areas, irrigation is a key factor for the type of production to be obtained. The concentrations of phenolic compounds in grapes can be influenced by controlling water supply to grapes. In order to obtain quality grapes with higher phenol contents that can be used to make aged wines, the grapes must be subjected to water stress and to do this water supply must be restricted after flowering.

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