Effect of irrigation and variety on oxygen ($\delta^{18}$O) and carbon ($\delta^{13}$C) stable isotope composition of grapes cultivated in a warm climate

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Abstract

**Background and Aims:** $\delta^{13}$C values from *Vitis vinifera* leaves, whole grape, seed, pulp, skin and/or grape must sugars have been investigated as an integrated marker of vine water status or intrinsic water-use efficiency during berry growth and across region of origin, vintage and variety. The use of $^{18}$O/$^{16}$O isotopic ratio as a marker of water addition, vintage and geographical origin has also been studied. This paper examines the effect of irrigation and grapevine variety on $\delta^{18}$O and $\delta^{13}$C of grape must from eight varieties, all cultivated in the same vineyard to reduce the effects from other variables.

**Methods and Results:** Stable isotope compositions of grape must water and sugar were determined by isotope ratio mass spectrometry. The result of the study showed statistically significant effects of irrigation and vine variety on both $\delta^{18}$O and $\delta^{13}$C. The effect of vintage on $\delta^{18}$O was only significant for non-irrigated vines.

**Conclusion:** This research highlights the effect of variety and irrigation on $\delta^{13}$C and $\delta^{18}$O of grape.

**Significance of the Study:** This is the first report to demonstrate that the varietal effect on $\delta^{13}$C and $\delta^{18}$O of grape is not due only to differences in the vegetative cycle of each variety. It further suggests that water exhibits a lower isotopic discrimination in the indigenous Spanish varieties studied than in non-indigenous varieties.

**Abbreviations**

DI drought index; IRMS isotope ratio mass spectrometry.

**Keywords:** carbon, grape, irrigation, oxygen, stable isotope, *Vitis vinifera* L.

### Introduction

Light isotope forms of compounds containing elements like carbon, hydrogen, nitrogen and oxygen are more volatile, more reactive, are distributed faster and are quicker to take part in natural biochemical reactions. As a result, isotopic differentiation occurs during physical and biological processes, making it possible to use certain isotopic ratios as authenticity markers for biological and chemical samples (origin, identity, adulteration, drug taking, etc.).

Because of photosynthetic differences, C3 plants like vines contain less $^{13}$C than C4 plants like corn or sugarcane (Farquhar et al. 1989). Because of this, must sugar or wine ethanol $\delta^{13}$C values can be used to check for C4 sugar/ethanol addition to wine (International Organisation of Vine and Wine 2006). In addition, $\delta^{13}$C from vine leaves, whole grape, seed, pulp, skin and/or grape must sugars has been investigated as an integrated marker of vine water status or intrinsic water-use efficiency during berry growth (Gaudillere et al. 2002, de Souza et al. 2005, Poni et al. 2009), or as an indicator of region of origin, vintage (Day et al. 1995) and of *Vitis vinifera* variety (Yunianta et al. 1995, Van Leeuwen et al. 2001).

The isotopic ratio $^{18}$O/$^{16}$O of water in wine is used to determine water addition (International Organisation of Vine and Wine 2006). The uptake of water by the roots of the vine and the subsequent transport of water to the leaves are not associated with any significant isotope fractionation, and can therefore be neglected (White 1989, Ziegler 1989). $\delta^{18}$O is higher in grape water than in soil water as isotopic enrichment takes place mainly in the leaves and also in the grape skin because of evaporation and or exchange with atmospheric vapour (Martin et al. 1988, Tardagula et al. 1997, Roßmann et al. 1999). The isotopic ratio of grape water is controlled by the isotopic composition of available soil water, relative humidity, interacting time and isotopic ratio of atmospheric vapour. All these variables mean that isotopic ratio changes are
dependent on seasonal climatic conditions, and therefore annual databases are required (Roßmann et al. 1999). The $^{18}$O/$^{16}$O isotopic ratio has also been studied as a marker of vintage and geographical origin (Roßmann et al. 1999, West et al. 2007). More recently, a model has been published for estimating $^{18}$O from meteorological data (Hermann and Voerkelius 2008). In warm, dry regions, vine irrigation is a key factor both for vegetative and fruitful growth, and for physiological and biochemical functioning. However, there have been fewer studies dealing with the oxygen than with the carbon stable isotope ratio of grape. Little is known about how the $^{13}$C isotopic ratio is affected by irrigation and whether this can compromise its use as an indicator of water addition, or if the isotopic ratio is affected by irrigation and whether this can compromise its use as an indicator of water addition, or if the isotopic ratio is affected by irrigation and whether this can compromise its use as an indicator of water addition, or if this parameter could be used alone or along with $^{13}$C as a variety marker.

This paper examines the effect of irrigation and grapevine variety on $^{13}$C and $^{18}$O values of grape must from eight different varieties, four foreign and four indigenous to Spain, all cultivated in the same vineyard in an attempt to limit the variables between treatments to irrigation. The assay was conducted over two successive vintages.

**Materials and methods**

**Reagents**

CO$_2$ (99.995) used as reference gas, 0.3% CO$_2$ molar in He used as equilibrium gas and He used as inert carrier gas were from Carburos Metálicos (Barcelona, Spain). Vienna Standard Mean Ocean Water (VSMOW) from the International Atomic Energy Agency (Vienna, Austria) and Ethanol BCR 656 from the Institute for Reference Materials and Measurements (Geel, Belgium) were used for $^{13}$C and $^{18}$O calibration, respectively.

**Plant material and sample preparation**

The trial was conducted on 2007 and 2008 vintages in the same vineyard, where various grapevine varieties were grafted on to Fercal rootstock in a plot representative of the La Mancha growing region. The soil is a petrocalcic soil horizon situated 35 cm below the surface, consisting of petric calcisol that has developed on the alluvial part of the River Guadiana (Elena et al. 1997). The experimental design consisted of 64 vineyard blocks: eight grapevine varieties, two treatments each (irrigated/non-irrigated) and four replicates each one containing 20 vines. Irrigation ground water from a well located in the same vineyard was applied with drip emitters (4 L/h), two per vine. The vines, grown on trellises and arranged on rectangular frames measuring 3 × 1.2 m, were trained to a double Cordon de Royat system, with three to four spurs of two buds on each arm.

The study was carried out on four Spanish indigenous grapevines: Airén, Macabeo (white grapes), Tempranillo and Garnacha (red grapes), and four foreign: Chardonnay, Sauvignon Blanc (white grapes), Cabernet Sauvignon and Merlot (red grapes). In the case of the 2007 vintage, 61 grape samples were taken, 31 from irrigated vines and 30 from non-irrigated vines. In the case of the 2008 vintage, 63 samples were taken, 32 from irrigated vines and 31 from non-irrigated vines.

The sampling was carried out randomly at technological maturity by picking 10–12 berries from the top, middle and bottom of the cluster. We tried to sample berries from both exposed and shaded clusters by picking berries from four to five clusters per vine. The size of the sample was around 2 kg. Grape juice was obtained by pressing grapes in a manual spindle press; SO$_2$ (500 mg/L) was added to the juice and stored at 4°C until analysis.

$^{18}$O/$^{16}$O ratio measurement

An on-line gas equilibration and headspace introduction system model GasBench II (ThermoQuest, Bremen, Germany) was used, equipped with a gas chromatography column (PoraPlot Q, 25 m, 0.25 mm; Varian, Palo Alto, California, USA) operating at 70°C and adapted to an autosampler CombiPAL (CTC-Analytical, Zwingen, Switzerland). Must samples were transferred (500 μL) to 10 mL vials with silicone septa, placed in the GasBench II, flushed with 0.3% CO$_2$ in He for 10 min and then left for 24 h at 27°C before analysis. During this equilibration time, an exchange reaction took place between oxygen in CO$_2$ and H$_2$O (Eqn 1). CO$_2$ was then isolated from the vial headspace and introduced in the isotope ratio mass spectrometry (IRMS) system:

$$^{12}C^{16}O_2 + H_2^{18}O \leftrightarrow ^{12}C^{16}O^{18}O + H_2^{16}O$$ (1)

The GasBench II was coupled to a Delta-Plus IRMS (ThermoQuest) equipped with three Faraday cup detectors to simultaneously and continuously monitor the [CO$_2$] signals for the three major ions at m/z 44 ($^{12}$CO$_2$), m/z 45 ($^{13}$CO$_2$ and $^{12}$CO$^{16}$O) and m/z 46 ($^{12}$C$^{16}$O$^{18}$O). Oxygen isotope composition was expressed as:

$$\delta^{18} O_{\text{sample}} = \left(\frac{R_s}{R_n}\right) - 1 \times 1000$$

where $R_s$ is the $^{18}$O/$^{16}$O ratio in the sample, and $R_n$ is the ratio of the international standard used, VSMOW.

$^{13}$C/$^{12}$C ratio measurement

Determination of the carbon isotope ratios of grape must was carried out by on-line analysis using a ThermoQuest Flash 1112 elemental analyser equipped with an autosampler and coupled to a Delta-Plus IRMS (ThermoQuest) through a ConFlo III interface (ThermoQuest). One microlitre of grape must was carefully placed in a tin capsule, and sealed. All of the carbon from the sample was oxidized to CO$_2$ in the reactors of the elemental analyser, passed through a GC column to separate CO$_2$ from the other gases generated and then brought into the mass spectrometer by a helium flow (International Organisation of Vine and Wine 2006). Carbon isotope composition was expressed as:

$$\delta^{13}C_{\text{sample}} = \left(\frac{R_s}{R_n}\right) - 1 \times 1000$$

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where \( R \) is the \(^{13}C/^{12}C \) ratio in the sample, and \( R_a \) is the ratio of the international standard used, Vienna-Pee Dee Belemnite.

All samples were analysed at least in duplicate.

**Statistical analysis**

The data from the must samples were subjected to analysis of variance (ANOVA) and the Student–Newman–Keuls test to identify any statistically significant differences between varieties and irrigation treatment. All statistical tests were performed using SPSS software, version 12.0 (Chicago, Illinois, USA).

**Results**

**\( ^{18}O \) isotope ratio of grape must water**

The La Mancha region, where the study was conducted, has a continental climate, characterized by long, dry summers. Table 1 shows the rainfall recorded in the 2006/2007 and 2008/2009 hydrological years (RY) was 477 and 352 mm, respectively. According to the drought index (DI) established by Tonietto (1999), this vine-growing area was considered as a moderate drought region in the period 1961–1990 (DI = −86). However, because of climate change, this area is currently included in the severe drought group with DI = −135 and −127 (mm) in 2007 and 2008, respectively (meteorological data for DI determination from Centro Regional de Estudios del Agua 2009). Because of this climate trend, irrigation is becoming increasingly necessary in this region, as in other Mediterranean areas (Laget et al. 2008), for adequate vine management. Table 1 also shows rainfall registered in the vineyard during the irrigation period, and the irrigation volume during the summers: 312, 272 and 72 L/grapevine in 2007 summer, and 312, 272 and 72 L/grapevine in the 2008 summer, and 312, 272 and 72 L/grapevine in 2008 in July, August and September, respectively. No statistically significant differences observed in \( ^{18}O \) grape water values of groundwater used for irrigation (−7.9 ± 0.1‰) and rainfall weighted mean \( ^{18}O \) value (−7.7 ± 0.9‰) from the region where the study was conducted (Global Network of Isotopes in Precipitation 2009).

**Table 1.** Mean irrigation volumes, rainfall and \( ^{18}O \) (‰) of grape must water.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Treatment</th>
<th>Irrigation (L/vine)</th>
<th>RDIP (mm)</th>
<th>( ^{18}O ) (‰)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>NI (n = 31)</td>
<td>0</td>
<td>477</td>
<td>37</td>
<td>11.4±</td>
<td>1.0</td>
</tr>
<tr>
<td>2007</td>
<td>I (n = 30)</td>
<td>656</td>
<td>477</td>
<td>37</td>
<td>10.3±</td>
<td>0.9</td>
</tr>
<tr>
<td>2008</td>
<td>NI (n = 31)</td>
<td>0</td>
<td>352</td>
<td>22</td>
<td>13.3±</td>
<td>1.5</td>
</tr>
<tr>
<td>2008</td>
<td>I (n = 52)</td>
<td>640</td>
<td>352</td>
<td>22</td>
<td>10.4±</td>
<td>1.7</td>
</tr>
</tbody>
</table>

NI, non-irrigated vines; I, irrigated vines; RDIP rainfall during irrigation period (from 1st July to 20th September in 2007 and to 15th September in 2008). Different superscripts in the same column and year denote statistically significant differences between irrigation treatments at \( p \leq 0.001 \). * Denote statistically significant differences between data for different year and the same irrigation treatment at \( p \leq 0.001 \).

The last two columns of Table 1 show the mean value and standard deviation for \( ^{18}O \) when samples are grouped by year and irrigation regime. Statistically significant differences (\( P \leq 0.001 \)) between \( ^{18}O \) grape water values of watered and non-watered vines were found for both years: this difference between treatments was higher in the 2008 vintage. When \( ^{18}O \) values from the same watering treatment were compared for the two years, no differences were found for irrigated vines, while \( ^{18}O \) water from non-irrigated vines differed by 2‰ (\( P \leq 0.001 \)).

When samples were grouped by vine variety, the differences between water treatments observed previously in the \( ^{18}O/^{16}O \) ratio persisted in both harvests (Table 2). The 2007 crop of Tempranillo was the only case in which the difference was not statistically significant, although the trend was the same as in the other varieties, with a higher \( ^{18}O \) value for non-irrigated vines. The lowest irrigation-dependent variation observed in \( ^{18}O \) grape water was 0.692‰ for Merlot in 2007, and the highest was for Macabeo in the 2008 crop season. The mean differences observed in \( ^{18}O \) between the treatments were 1.065 in 2007, and 2.965 in 2008.

**Table 2.** \( ^{18}O \) (‰) values of grape must water classified according to irrigation regime and variety for 2007 and 2008 harvests.

<table>
<thead>
<tr>
<th>Grape Variety</th>
<th>( ^{18}O ) (‰) Harvest 2007</th>
<th>( ^{18}O ) (‰) Harvest 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-irrigated</td>
<td>Irrigated</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Airén</td>
<td>4</td>
<td>10.7±</td>
</tr>
<tr>
<td>Macabeo</td>
<td>4</td>
<td>10.4±</td>
</tr>
<tr>
<td>Tempranillo</td>
<td>3</td>
<td>10.5±</td>
</tr>
<tr>
<td>Garnacha</td>
<td>4</td>
<td>10.8±</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>4</td>
<td>11.6±</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>4</td>
<td>12.1±</td>
</tr>
<tr>
<td>Merlot</td>
<td>4</td>
<td>12.2±</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>4</td>
<td>12.6±</td>
</tr>
</tbody>
</table>

**Irrigation, variety and grape \( ^{18}O \) and \( ^{13}C \) values**

Different superscripts in the same column and year denote statistically significant differences between varieties (Student-Newman-Keuls, \( \alpha = 0.01 \)), *** and ** denote statistically significant differences in the same line at \( p \leq 0.001 \), 0.01 and 0.05 respectively between irrigation treatments.

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The effects of variety on $\delta^{18}$O were determined by one-way ANOVA, and the Student–Newman–Keuls post hoc test results established three subsets in 2007: subset A, which included the Airén, Macabeo, Tempranillo and Garnacha varieties, all considered indigenous Spanish grapes, with the lowest $^{18}$O/$^{16}$O ratios, while the foreign varieties were classified in subsets B and C. The results for the second year did not group varieties so clearly, but again the indigenous varieties presented lower $\delta^{18}$O values with the only exception of Cabernet Sauvignon, which, in 2008, showed $^{18}$O values lower than those for some Spanish varieties. Table 3 shows that when varieties were grouped into indigenous Spanish (Airén, Macabeo, Garnacha and Tempranillo) and foreign (Sauvignon Blanc, Chardonnay, Merlot and Cabernet Sauvignon), the $^{18}$O/$^{16}$O ratio was statistically significantly lower in the indigenous varieties irrespective of the year or the irrigation regime. In order to determine if differences on $\delta^{18}$O between varieties were caused by the date of sample collection or to a varietal factor, Figure 1 shows the behaviour of the $^{18}$O/$^{16}$O ratio versus the date of sampling. Note that $\delta^{18}$O was higher in foreign varieties regardless of the sampling date in the 2007 harvest in both irrigated and non-irrigated grape-vines. In 2008, $\delta^{18}$O values tended to be lower in the late-ripening varieties, possibly because of the cumulative rainfall during this period, which was higher than in 2007; however, in the foreign varieties, $^{18}$O/$^{16}$O ratios were consistently higher for the same sampling dates.

**Table 3.** $\delta^{18}$O (‰) values of grape must water classified according to irrigation regime and geographical origin of varieties for 2007 and 2008 harvests.

| Year/treatment | Spanish indigenous | | | Foreign | | | Difference |
|---------------|-------------------|---|---|----------------|---|---|
|               | $n$ | Mean | SD | $n$ | Mean | SD | |
| 2007 Non-irrigated | 15 | 10.6 | 0.5 | 16 | 12.1 | 0.7 | 1.6* |
| 2007 Irrigated | 15 | 9.5 | 0.5 | 15 | 11.0 | 0.5 | 1.5* |
| 2008 Non-irrigated | 15 | 12.4 | 1.2 | 16 | 14.1 | 1.3 | 1.7* |
| 2008 Irrigated | 16 | 9.2 | 1.4 | 16 | 11.5 | 1.1 | 2.2* |
| Total | 61 | 10.4 | 1.6 | 63 | 12.2 | 1.5 | 1.8* |

*Denote statistically significant differences in the same line at $P \leq 0.001$.

![Figure 1](image.png)

**Figure 1.** A plot of the $\delta^{18}$O of grape must water in the 2007 and 2008 harvests versus the date of sampling. Samples: ■, Airén; ●, Garnacha; ▲, Macabeo; ★, Tempranillo; □, Cabernet Sauvignon; ○, Chardonnay; △, Merlot; ☆, Sauvignon Blanc. (a) 2007 non-irrigated; (b) 2007 irrigated; (c) 2008 non-irrigated; (d) 2008 irrigated.
$^{13}$C/$^{12}$C isotope ratio of grape must sugar

Analysis of $^{13}$C of grape sugars during 2008 harvest confirmed the effect of water availability on this isotope ratio. Irrigated grapes showed significantly ($P < 0.001$) lower $^{13}$C/$^{12}$C ratios than non-irrigated grapes (Table 4).

When grapevine variety was considered as a variable, the effect of irrigation on $^{13}$C was observed in all cases, with differences ranging from 1.196 for Sauvignon Blanc to 2.117 for Cabernet Sauvignon (Table 5). This table also shows that $^{13}$C was lower in the foreign than in the indigenous varieties in both irrigated and non-irrigated vines. As it was also observed for $^{18}$O in 2008, the only exception to this general trend was Cabernet Sauvignon, which showed higher $^{13}$C values than some Spanish varieties under both irrigation treatments. Roßmann et al. (1999) asserted that the influence of the variety on $^{13}$C values is an indirect one because those differences are caused by the date of harvest, because of the strong impact of climate during the ripening and harvesting of grapes. Consequently, water in grape varieties that mature early has higher $^{13}$C values than water in later ripening varieties. As Figure 1 shows, the differences between varieties are not due only to the date of harvest, because grapes of different varieties sampled at the same time showed a 1.583‰ variation in the $^{13}$C/$^{12}$C ratio ($P < 0.001$).

Discussion

The $^{18}$O/$^{16}$O isotopic ratios observed in this work were much higher than those found in the relevant literature for Italian, French and German wines of different vintages. $^{18}$O values have been found to range from $-3.3\%$ in some German wines to $7\%$ in Italian wines, while French wines present intermediate values (Roßmann et al. 1999, Hermann and Voerkelius 2008). $^{18}$O/$^{16}$O isotopic ratios for La Mancha grape juices are also higher than those for Korean grape juices, which consistently show negative $^{18}$O (Bong et al. 2008). This can be explained because precipitation and groundwater show higher $^{18}$O in southern Europe than in northern Europe (Global Network of Isotopes in Precipitation 2009), and also because the weather in La Mancha during grape growing is warmer and dryer than in most of the areas of Germany, France or Italy, which increases transpiration through leaves and grape skin, and consequently $^{18}$O/$^{16}$O isotopic ratio of grape water (Roßmann et al. 1999).

The data presented in Table 1 show the effect of irrigation on $^{18}$O of grape juice. The fact that the isotopic enrichment is lower for this isotopic ratio in irrigated vines means that the ratio between the water absorbed by roots and the water evaporated is higher. However, the effect of irrigation on $^{18}$O (around 2‰ in the 2007 and 3‰ in the 2008 vintages) was comparable to or smaller than the effect of varietal differences: up to 2.4 and 4.8‰ in 2007 and 2008 vintages, respectively. Roßmann et al. (1999) asserted that the influence of the variety on $^{18}$O values is an indirect one because those differences are caused by the date of harvest, because of the strong impact of climate during the ripening and harvesting of grapes. Consequently, water in grape varieties that mature early has higher $^{18}$O values than water in later ripening varieties. As Figure 1 shows, the differences between varieties are not due only to the date of harvest, because grapes of different varieties sampled at the same time showed a 1.583‰ variation in the $^{13}$C/$^{12}$C ratio ($P < 0.001$).
time and taken from the same vineyard showed different $^{18}$O/$^{16}$O isotopic ratios. It was observed that $^{18}$O values were usually higher in foreign than in indigenous Spanish varieties. This suggests some physiological differences that lead to a lower water isotopic discrimination in the indigenous Spanish varieties studied than in non-indigenous varieties.

The statistically significant effect ($P \leq 0.001$) of vintage on $^{18}$O observed for non-irrigated vines (Table 1) can be explained by the rainfall registered in 2007 crop season during the berry development period, mainly July and August, which was much higher than in the 2008 vintage (19 and 1.2 mm, respectively). The effect of other climatic factors apart from rainfall on $^{18}$O/$^{16}$O isotopic ratio seems to have been negligible in the vintages studied because no differences were found between irrigated vines, which received practically the same water input in both years.

The $^{13}$C values found in this work are comparable to those reported by Gaudillere et al. (2002), although the range was smaller in our samples. $^{13}$C is 1.1% of atmospheric CO$_2$, but in C3 plants ribulose-1,5-bisphosphate carboxylase/oxygenase uses preferentially $^{12}$C for sugar synthesis during photosynthesis (Farquhar et al. 1989). When soil water availability decreases, leaf stomata close partially and atmospheric CO$_2$ interchange is reduced, leading to a smaller isotopic discrimination. For that reason, $^{13}$C values reflect water status during photosynthesis and grape ripening (Farquhar and Richards 1984), and make it possible to differentiate between irrigation regimes. $^{13}$C values close to $-20\%$ as found in non-irrigated vines (Table 4) indicate considerable water restriction (Gaudillere et al. 2002), while $^{13}$C values for the irrigated ones are consistent with a lower water restriction but far from those found by Gaudillere et al. (2002) for less stressed vines.

The decrease in the $^{13}$C/$^{12}$C isotope ratio ($-1.583\%$) of grape must from irrigated vines with respect to non-irrigated vines was consistent with that reported in the literature by similar studies. de Souza et al. (2005) observed higher $^{13}$C values of solid parts of grape and leaf in non-irrigated vines. These differences were significant in the case of fully irrigated and non-irrigated vines, while they found intermediate values for partial root zone drying and deficit irrigation. That study reported differences of up to 3% in the $^{13}$C/$^{12}$C isotope ratio of grape pulp, and a good correlation between $^{13}$C and water stress integral. A similar relationship between carbon isotope ratio of sugars of grape must and pre-dawn leaf water potential was found by Gaudillere et al. (2002). However, more recent studies have reported no differences in $^{13}$C determined by irrigation treatments (Poni et al. 2009).

Several researchers who have investigated the genetic variability of carbon isotope composition have concluded that it could serve as an indicator of stomata control in V. vinifera species, and hence as an indicator of resistance to water stress (Gaudillere et al. 2002). Different varieties subjected to the same irrigation treatment showed differences of up to 1.49 and 0.826% in $^{13}$C for non-irrigated and irrigated vines, respectively; these differences are smaller than those reported in the literature: up to 2.6% (Gaudillere et al. 2002). In Table 5, we can see how, as expected, these differences in carbon isotope composition are greater in non-irrigated than irrigated vines, because it is in such stressful conditions that stomata control is most important.

These observations regarding the effect of variety on $^{13}$C corroborate the observations for $^{18}$O and previous studies found in literature. Grape water isotopic enrichment with respect to ground water is mainly caused by isotope fractionation at leaf sites produced by evapotranspiration (Bong et al. 2008, Hermann and Voerkelius 2008) controlled mainly by stomata closure (Loveys et al. 2000). Then, part of the isotopically modified water is transported into the grapes where its isotopic signature is assumed to be more or less preserved and a time average of individual isotopic ratios of different climatology during grape growing (Hermann and Voerkelius 2008). In general, $^{18}$O values were higher in the foreign than in the indigenous Spanish varieties, and hence there was more transpiration; this corresponds to lower $^{13}$C values, indicating less stomata control/closure under water stress conditions. The only exception to this pattern was Cabernet Sauvignon, which in 2008 showed similar isotopic ratios to those for Spanish varieties in both elements. In fact, a statistically significant correlation ($P \leq 0.05$) was observed between $^{13}$C and $^{18}$O values for both non-irrigated ($r^2 = 0.702$) and irrigated ($r^2 = 0.443$) vines.

**Conclusions**

Irrigation had a statistically significant effect in both $^{18}$O of grape water and $^{13}$C of berry sugar. This research, using grape samples from the same vineyard, shows that the variations in $^{18}$O of grape water and $^{13}$C of berry sugar caused by the effect of V. vinifera varieties were not because only of differences in the vegetative cycle of each variety. It further suggests that water suffers a lower isotopic discrimination in the indigenous Spanish varieties studied than in non-indigenous varieties.

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**References**


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