Oxygen isotope composition of must-water in grapevine: effects of water deficit and rootstock

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Abstract

The influence of soil moisture and different rootstock genotypes on the $^{18}O/^{16}O$ ratio of must-water in grapes was investigated. Grapevines (*Vitis vinifera* L.) with cv. Cabernet Sauvignon as scion grafted on rootstocks 101-14, 420A and 41B were subjected to three levels of plant-available soil-water from veraison to harvest. Significant differences were observed in the ^{18}O level of must-water, the differences being related to water regime and rootstock genotype, with ^{18}O increasing as soil-water became less available. The higher the canopy–air vapour pressure gradient (VPG), the larger was the oxygen isotope content of must-water, indicating that VPG is an important factor in determining the degree of must-water enrichment under water deficit conditions. In all water regimes of soil, 41B induced the highest degree of ^{18}O enrichment in must-water. The differences between rootstocks in must-water ^{18}O could not fully be explained either by the rootstock effect on VPG or on root distribution.

Abbreviations

PAW plant-available soil-water; **RH** relative humidity; **VPD** leaf–air vapour pressure difference; **VPG** canopy–air vapour pressure gradient; Δt canopy–air temperature difference; $\delta^{18}O$ ‰ measured $^{18}O/^{16}O$ ratio in relation to an international standard $^{18}O/^{16}O$ ratio.

Introduction

The analysis of either hydrogen or oxygen isotope ratios in the water of different plant organs offers a powerful tool for understanding the dynamic of water utilisation (White 1988, Ehleringer and Dawson 1992, Flanagan 1993). A model of isotope fractionation, originally developed by Craig and Gordon (in Flanagan et al. 1991) for processes occurring during the evaporation of water from the ocean, has been used as model for leaf-water isotope composition. Furthermore, the absence of isotope fractionation in water during its uptake by roots and stem transport was demonstrated (White et al. 1985, Dawson and Ehleringer 1991, Förstel 1982). Leaves are the primary site of evaporative enrichment in plants: water vapour molecules containing lighter oxygen and hydrogen isotopes escape the leaf more readily than molecules containing heavier isotopes, so that during transpiration, the leaf water becomes enriched in heavier isotopes (Zundel et al. 1978, White 1988, Flanagan et al. 1991). The degree of water enrichment depends on the leaf–air vapour pressure difference (VPD), isotope composition of stem water and isotope composition of atmospheric water (Flanagan et al. 1991). Water isotope composition of fruits, where water loss can also occur, has been similarly enriched relative to soil-water (Bricout 1982, Förstel and Hützen 1984).

Plant water status influences growth and physiology (Hsiao 1993). Water deficit affects grape composition and quality (Williams and Matthews 1990, Matthews and Anderson 1988). Water stress effects on the isotope level of leaf-water have been observed (Farris and Strain 1978, Yakir et al. 1990a, b, Flanagan and Ehleringer 1991), even though different conclusions were reached. A relationship between the variability of oxygen isotopes in wines and fruit juices and different environmental conditions has been reported (Bricout 1978, 1982, Dunbar 1982, Förstel 1982, Förstel and Hützen 1984, Versini et al. 1995) but more environmental studies on the influence of specific ambient factors are required. With this in mind, it was of interest to study the effect of water deficit on isotope composition in must-water of grapes or wine.

The use of rootstocks to overcome diseases and soil problems is an old practice, used worldwide and being developed continuously. Recently, more attention has been paid to the effect of rootstocks on wine quality (Cirami et al. 1984, Belvini et al. 1992, Bertamini et al. 1995, pp. 64-70, Tardaguila 1995). As clear differences in the isotope composition of both

hydrogen and oxygen in plant-water have been observed between species (Leaney et al. 1985, Flanagan et al. 1991) and cultivars of a single species (Walker and Lance 1991), a possible effect of rootstock on isotope composition of water in fruit trees including grapevines may be expected and needs to be investigated.

The investigation here reported was done to test how soil-water availability and rootstock influence the stable isotope composition of berry juice of grapevines (*Vitis vinifera* L.) grown in the field.

Materials and methods

Plant cultivation and sampling

The observations were carried out in 1992 on three-year-old vines of cv. Cabernet Sauvignon, clone 341 (*Vitis vinifera* L.) grafted on rootstocks 101–14 Mgt, 420A and 41B. The vines were grown at a density of 4600 plants/ha in a sandy loam soil in the valley of the river Adige, Trentino region, northern Italy (latitude c. 46° north). They were trained according to the Guyot system (cane-pruning, trellis height c. 0.6 m above the ground) with upright shoots that were not trimmed.

At fruit set, the plants were covered with transparent polyethylene film placed 1 m above the canopy in order to prevent wetting by rain. From fruit-set to harvest time, soil moisture in the first 0.5 m depth of the soil was monitored by the Time Domain Reflectometry (TDR) method, using a 1502B Tektronix instrument (Tektronix Instruments, USA). Soil moisture was determined weekly at three different points around the plant (0.2 m from the trunk), using four vines per water regime. There were three such regimes: after veraison, the soil moisture was maintained at three different levels by drip irrigation, namely as near as possible at 15%, 30% and 80% of plant-available soil-water (PAW).

Three plants per scion–rootstock combination and water regime were selected for similar size and pruned to five shoots per vine and one bunch per shoot. Normal cultural practices were applied.

At harvest time, all bunches (in total c. 0.5 kg) of the three grapevines of each PAW × rootstock treatment were picked after midday on a clear day. The grapes were stored in plastic bags at 4ºC until next morning, when they were pressed, and their juice was immediately placed in glass jars (c. 50 mL), frozen, and stored for later oxygen isotope analysis.

Root distribution from 2 replicates per PAW × rootstock treatment was studied using the profile wall method (Swanepoel and Southey 1989). A trench of at least 1.2 m depth was dug parallel to the vine row and 0.3 m from the vine. After the roots were exposed, those falling within a vertically held frame (1.0 m high × 1.2 m wide), containing a 150 mm square grid, were mapped.

Growing conditions and gas exchange

Canopy and air temperature were determined by a hand-held infrared thermometer (510B Infrared AG Multimeter, USA). Ten readings were taken per PAW × rootstock treatment at midday on three sunny days, twice between veraison and harvest. The thermometer, with a 15º field of view, was held at 45º and about 0.75 m away from the sunny side of the canopy surface. Ambient relative humidity (RH) data were collected with a hygro-thermograph (SIAP, Italy) placed at a meteorological station located at about 50 m from the experimental site. Canopy–air vapour pressure gradient (VPG) was calculated as:

$$VPG = e_s - e_a$$

where e_s is the saturation vapour pressure at canopy temperature and e_a is that at the actual ambient pressure:

$$e_a = (RH \cdot e') / 100$$

where e' is the saturation vapour pressure at air temperature.

Leaf transpiration measurements were made on a sunny day between veraison and harvest with an open gas exchange system (ADC-LCD2, UK), using one matured sun-lit leaf on each of two vines per PAW × rootstock treatment.

Oxygen isotope analysis of must-water

Must-water was analysed with an isotope ratio mass spectrometer (IRMS, SIRA II, VG Isotech Ltd., Middlewich, UK), interfaced with a dedicated device (Isoprep 18-VG, VG Isotech Ltd., Middlewich, UK). Oxygen isotope ratio $^{18}O/^{16}O$ measurements in must-water were performed by equilibrating must (2 mL, 25°C, 5 h shaking) with CO_2 in a cone-shaped flask and determining thereafter the oxygen isotope ratio in CO_2 (Epstein and Mayeda 1953). The isotope composition was expressed as $^{18}O‰$ according to White (1988):

$$\delta^{18}O = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R_{sample} is the $^{18}O/^{16}O$ ratio of the sample and $R_{standard}$ that of the international reference standard SMOW (Standard Mean Ocean Water, Baertschi 1976).

The determinations were done in duplicate. The precision of the isotope analysis was about 0.2‰ with a reproducibility of about 0.3 ‰.

Statistical analyses were performed using the SAS/STAT package (SAS Institute 1992).

Results

The grapevines of this experiment were subjected between veraison and harvest to three different levels of plant-available soil-water (PAW, v/v), i.e. high = 80% (SE 8.3%), moderate = 30% (SE 2%) and low = 15% (SE 4.8%).

In Table 1, values of $\delta^{18}O$ of must-water obtained in the various treatments are shown. Note that the more positive the $\delta^{18}O$ value (i.e. the less negative the values in Table 1), the higher the $^{18}O/^{16}O$ ratio, indicating that the sample has greater isotope enrichment. Must-water had always a higher ^{18}O level (about 5.5‰ – 8.5‰) than irrigation water. Significant differences in the oxygen isotope composition of must-water were found between the PAW regimes and between the rootstock varieties. There was a clear

Table 1. $\delta^{18}O$ enrichment of must-water at harvest as function of plant-available soil-water (PAW) and rootstock in Cabernet Sauvignon grape berries.

Rootstock	PAW						
	High (80%)		Medium (30%)		Low (15%)		Mean
	Mean	SE	Mean	SE	Mean	SE	
101-14	–4.23	0.57	–3.92	0.36	–3.06	0.43	-3.74^{b}
420A	–4.44	0.21	–3.03	0.46	–2.17	0.21	-3.38^{b}
41B	–3.07	0.32	–2.75	0.25	–1.20	0.27	-2.34^{a}
Mean	-3.91^{b}		$-3.3–3^{b}$		-2.27^{a}		

$\delta^{18}O$ of irrigation water was –9.7‰. Each value is the mean of the juices obtained from all grapes produced by three vines. Means with different superscipts differ significantly ($p \leq 0.05$) according to Duncan's multiple range test.

Table 2. Effects of plant-available soil-water (PAW) and rootstock variety on canopy temperature (Tc) and canopy–air temperature difference (Δt) of Cabernet Sauvignon grapevines.

PAW	Rootstock	Tc (°C)	Δt (°C)†
High (80%)	101-14	33.0^{a}	-3.5^{a}
	420A	32.7^{a}	-2.8^{ab}
	41B	33.0^{a}	-2.5^{a}
	Mean	32.6^{C}	-2.9^{C}
Medium (30%)	101-14	33.3^{b}	-2.3^{b}
	420A	34.9^{a}	-0.6^{a}
	41B	34.0^{ab}	-1.6^{b}
	Mean	34.0^{B}	-1.5^{B}
Low (15%)	101-14	37.4^{a}	1.8^{a}
	420A	37.6^{a}	2.1^{a}
	41B	37.2^{a}	1.6^{a}
	Mean	37.4^{A}	1.8^{A}

†Each value is the mean of 10 measurements per rootstock per plant-available soil-water regime, taken at midday during a day between veraison and harvest. Means with different superscripts differ significantly ($p \leq 0.05$) according to Duncan's multiple range test.

trend for ^{18}O in must-water to increase as PAW decreased. This trend was evident in all three rootstock treatments. On average, the oxygen isotope composition of must-water was not significantly different under conditions of high (80%) and moderate (30%) PAW, but was significantly different and ^{18}O-enriched when grapes were grown at 15% PAW. Similarly, mean $\delta^{18}O$ of the rootstocks 101-14 and 420A, averaged over the PAW treatments, differed significantly from the third, 41B. At moderate and low PAW the lowest ^{18}O level in must-water was found in vines grafted on 101-14.

In attempting to explain the reasons for the treatment differences in $\delta^{18}O$, the effect of the different PAW and rootstocks on leaf transpiration and canopy temperature (Table 2) was examined. The diurnal course of leaf transpiration (Figure 1) for all three rootstock treatments was similar at high and moderate PAW, reaching a maximum of about 6 mmol/m^2·s at around noon. At low PAW however, the rates of transpiration were considerably lower throughout the

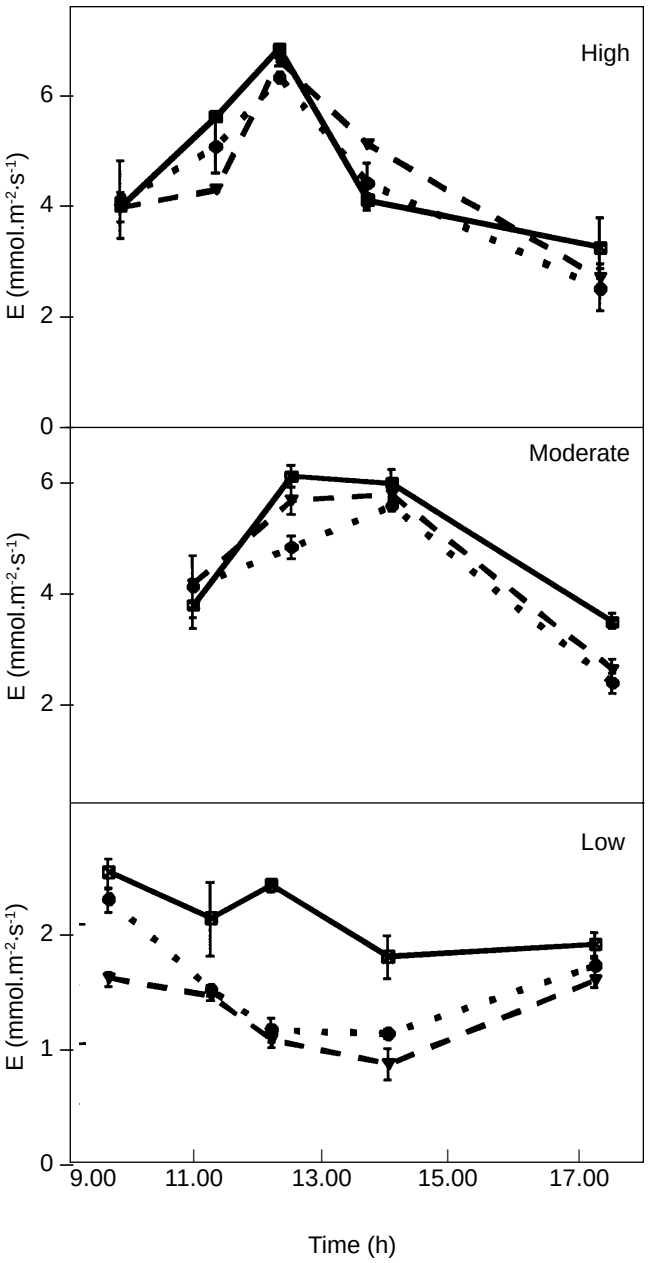

Figure 1. Influence of plant-available soil-water (PAW) and rootstock on leaf transpiration during a day between veraison and harvest. PAW was high = 80%, moderate = 30% or low = 15%. ■ 41B, ▼420A, ● 101-14. Measurements obtained by ADC-LCD2 on one matured sun-lit leaf per vine during a day between veraison and harvest. Each value is the mean ± SE of 2 vines.

day, not raising above 3 mmol/m^2·s and without large diurnal variation; furthermore, large differences in transpiration rate between rootstock treatments became evident at low PAW, with 41B-vines having higher rates than the other two.

Canopy temperatures showed the reverse trend to transpiration rates with changes in PAW — decreases in PAW were associated with increases in temperature. While canopy temperature was lower than ambient temperature at high and moderate PAW, it was higher at low PAW. The temperature response of the three rootstock treatments to changes in PAW was rather inconsistent, perhaps indicating that temperature measurements are less reliable as indicators of plant-water status than measurements of transpiration.

Root distribution of the three rootstocks is shown in Figure 2. 41B had significantly fewer roots in the soil layer where the majority of roots was situated (0 cm–50 cm). There was little difference between the root numbers of the other two rootstocks at all depths. All three rootstocks tended to have similar and low root numbers at depths below 50 cm, although 41B tended to have comparatively slightly more roots there than the other two.

The relationship between VGP (the gradient in vapour pressure from that of the canopy air to that of ambient air), and $\delta^{18}O$ (the oxygen isotope composition of must-water), is shown in Figure 3. The results indicate that the oxygen isotope composition in must-water at harvest was strongly and positively affected by VPG: for each rootstock treatment, the regression of $\delta^{18}O$ on VPG was very highly significant. The vines with the three rootstock genotypes showed significant differences in $\delta^{18}O$ of must-water in response to the three water regimes, even in the absence of significant variations in VPG. This effect was more evident at low PAW.

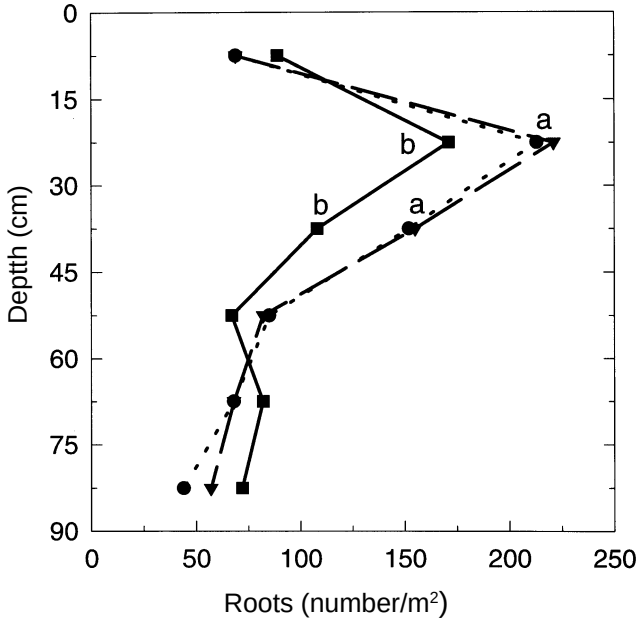

Figure 2. Effect of rootstock on root distribution. Each value is the mean of 6 measurements (2 vines in each of 3 plant-available soil-water regimes (PAW), i.e. 80%, 30% and 15% of PAW. Means plotted for the same soil depth with different letters differ significantly ($p \leq 0.05$). ● 101-14, ▼ 420A, ■ 41B.

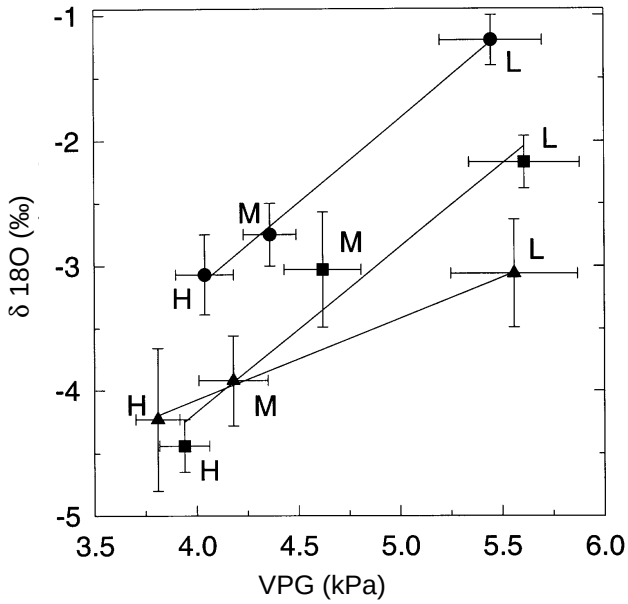

Figure 3. Regressions of ^{18}O content of must-water of grape berries on canopy–air vapour pressure gradient (VPG). Each VPG value is the mean of 10 measurements per rootstock (▲ 101-14; ■ 420A; ● 41B) in each plant-available soil-water regime (H = 80%, M = 30%, L = 15% of PAW) obtained at midday at veraison. Each ^{18}O value is the mean of 3 samples per rootstock in each water regime at harvest. Vertical and horizontal bars represent 2 × SE. Regression equations are: for 41B: $y = -8.61 + 35x$, $r^2 = 0.99$; for 420A: $y = -9.5 + 1.32x$, $r^2 = 0.94$; for 101–14: $y = -6.7 + 0.66x$, $r^2 = 0.99$; $p \leq 0.001$ for all three r^2 values.

Discussion

Our results showed oxygen isotope enrichment of grapevine must-water when the level of plant-available soil-water was low. This agrees with other results obtained for wine: the oxygen isotope ratio was found to be higher in wine from dry, warm countries than from countries with cool climate (Bricout 1982, Förstel and Hützen 1984, Holbach et al. 1994). Versini et al. (1995) confirmed this tendency, observing that the $^{18}O/^{16}O$ ratio in Italian wines was negatively correlated with the geographic latitude of their place of origin. Similarly, Farris and Strain (1978) demonstrated isotope enrichment of leaf water when *Phaseolus vulgaris* L. was kept under water stress, induced under two different environmental conditions, and the effect was greater at low air humidity. Yakir et al. (1990b) also reported that the oxygen isotope composition of water in the leaves of field-grown cotton (*Gossypium hirsutum)* was enriched by c. 5‰ in drought-stressed plants compared to that in well-watered plants. This pattern contrasts with that observed by Flanagan and Ehleringer (1991) who did not note significant differences between control and water-stressed *Cornus stolonifera* L. plants with respect to their ^{18}O content.

We have shown that an increase in PAW caused higher rates of leaf transpiration and lower leaf

temperature. A negative relationship between leaf transpiration rates and PAW found in this experiment was to be expected as at low soil moisture levels stomatal conductance is reduced. This in turn leads to diminished transpirational cooling and elevated canopy temperature, as shown by Boyer (1985). Results similar to ours were also obtained with well-watered cotton plants where higher rates of transpiration caused an increase in RH and lower leaf temperature (Yakir et al. 1990b). Grape berry transpiration was shown to be significant both before and after veraison (Greenspan et al. 1994), and the temperature of grape berries was found to be negatively correlated with PAW (Matthews and Anderson 1988, Tardaguila and Bertamini unpublished data). Thus the comparatively low water loss of berries would appear to be still contributing to an increase in VPG when soil-water availability is low.

Based on the Craig-Gordon model (Flanagan et al. 1991), variations of the isotope composition of plant water have been explained as function of the isotope compositions of stem water and of atmospheric water vapour, and of VPD (White 1988, Flanagan et al. 1991, Flanagan and Ehleringer 1991). In our experiment, well-watered vines had higher rates of leaf transpiration that led to lower canopy temperatures and, probably, higher RH in the air surrounding the leaves; thus the VPG was lower than in water-stressed plants. We then found a close relationship between $\delta^{18}O$ and the canopy–air vapour pressure gradient (VPG) in must-water of grapes. Increases in VPG were associated with larger contents of heavy oxygen isotope in must-water. Flanagan et al. (1991) observed that higher VPD levels increased the heavy isotope content of leaf-water. For grapevines, Förstel and Hützen (1984) obtained the same results for leaves but, in contrast to our results, not for grape berries.

The ^{18}O enrichment of must-water could be caused by enriched water flow from the leaves to the bunches and by berry transpiration. There is a limited ability for movement of solutes into the berry through the pedicel xylem after veraison, but such movement from leaf to berry may be possible during the day through the pedicel phloem (Greenspan et al. 1994).

At all three PAW levels, the grapevines grafted on 41B showed higher ^{18}O values in must-water than the vines grafted onto the other two rootstocks. In order to explain the effect of PAW on isotope composition of must-water, we assumed that the increase in VPG at reduced levels of PAW reflected lower stomatal conductance. This argument cannot explain the effect of rootstock on isotope composition in must-water. Although the 41B vines consistently accumulated more ^{18}O in the must-water, their stomatal conductance tended to be similar to that of vines on the other two rootstocks under high and moderate PAW and higher under low PAW. Similarly, vines on 41B showed consistently the greatest rates of leaf transpiration and stomatal conductance at low levels of PAW in gas exchange measurements of leaves described by Tardaguila (1995).

If VPG does not explain the effect of rootstock, other factors must be considered. We can assume that the rootstock was unlikely to alter the isotope composition of atmospheric water vapour. Even though isotope composition of stem water was not determined, we have no indication that it was not enriched in vines on 41B. With the exception of salt-excluding species (Lin and Stenberg 1993), no fractionation has been shown to occur during water uptake by plant roots (White et al. 1985, Dawson and Ehleringer 1991). As for differences in the distribution of heavier water in the soil, Förstel and Hützen (1984) and Thornburn and Walker (1993) noted that shallow layers had a greater $\delta^{18}O$, indicating enrichment with ^{18}O. This may possibly be due to the temperature gradient within the soil profile. It is unlikely that the smaller $\delta^{18}O$ of 41B vines was due to their using more of this ^{18}O-enriched water of the shallow soil layer than the vines on the other two rootstocks, for two reasons. Firstly, root density of all rootstocks was greatest in the uppermost, ^{18}O-rich 40 cm of soil, where 41B had less roots than the other two rootstocks while it had somewhat more roots than the other two in the deeper strata. Secondly, 41B influenced $\delta^{18}O$ in the must-water in all three PAW regimes, not only in the driest one, and also showed similar rooting pattern in all three water regimes (Tardaguila 1995).

Before we commenced our study, there was little information available in the literature to determine whether and how the isotope composition of must-water in grape berries is affected both by environmental and genetic factors. We conclude from our results that plant-available soil-water and rootstock influence the oxygen isotope composition of must-water in grapevines. Although there were genetic differences among the rootstocks causing variations in the amount of ^{18}O in must-water, water availability in the soil was the major factor controlling ^{18}O. The greater the VPG, the larger was the observed heavy isotope content of must-water. However, VPG did not explain the rootstock effect on ^{18}O. More mechanistic studies of the influence of rootstock on isotope composition of must-water are required. Soil water status and rootstock variety should be taken into account as contributors in determining the final isotope status of must-water and wine.

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