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Carbon isotope discrimination (so-called δ¹³C) measured on grape juice is an accessible tool to monitor vine water status in production conditions

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Assessment of vine water status is needed to understand the effect of environmental factors and management practices on dry-farmed and irrigated vineyards. Among plant-based indicators, carbon isotope discrimination (δ¹³C) is easily accessible, reliable, and inexpensive. As it provides a *post-hoc* assessment of vine water status during the berry ripening period, it can be useful for assessing the results of vineyard management practices during the season, and to map water status in the vineyard for precision management puposes. Possible applications and limitations of this technique for practical vineyard management are discussed in this article.

Assessment of vine water status

Vine water status is a key parameter in grape growing, affecting both yield and berry composition. In turn, vine water status is driven by soil water availability (dependent on soil water holding capacity), climatic parameters (rainfall and evapotranspiration), training system (leaf area per hectare), plant material (rootstock and cultivar) and management practices (vineyard floor management and irrigation).
Many methods for evaluating vine water status have been developed and can be grouped into: (1) soil-based measurements, (2) water balance modelling, and (3) plant-based measurements. Accuracy of soil-based and modelling approaches is limited by assumptions regarding the soil water holding capacity (SWHC) of the vine root zone, which is highly dependent on the rooting depth and root functioning, parameters that cannot be precisely assessed in field conditions. Plant-based indicators, however, naturally integrate the root zone SWHC of the vine and thus provide more reliable results. Among plant-based measurements, water potential by pressure chamber is widely used. Carbon isotope discrimination (δ¹³C) measured on berry juice is another plant-based indicator of vine water deficit with a huge potential for application in vineyard management. Although the first articles on this technique were published more than 20 years ago[1], it is still not widely adopted by vineyard managers.

The principle of carbon isotope discrimination

Isotopes of an element have the same number of protons and electrons, but differing numbers of neutrons, and hence a differing atomic mass. There are three isotopes of carbon, but only ¹²C and ¹³C are stable in the environment, making them useful for the study of so-called *isotope discriminating processes* in nature. ¹²C is by far the most abundant carbon isotope in nature, with a proportion of approximately 99:1 compared to ¹³C.
During photosynthesis, there is discrimination in favor of CO_2 molecules containing ¹²C isotopes, due to its higher reactivity with the enzymes in the photosynthetic reaction and a better diffusion through the stomata and the mesophyll. Hence, ¹³C/¹²C ratios in the sugars produced are lower when compared to atmospheric CO_2.
When plants experience water deficit, these ¹³C/¹²C isotope ratios are further modified as stomata close and block diffusion of CO_2 into the intercellular space of the leaves. This causes the relative concentration of $^{13}CO_2$ to $^{12}CO_2$ to rise in the intercellular space, and hence in the sugars produced. During ripening, these sugars are accumulated in the berry juice, with the measured ¹³C/¹²C ratio in this juice providing an indication of whether the vines were subject to water deficit and, if so, its severity[2].
The ratio ¹³C/¹²C can be measured by isotope mass spectrometry with great precision. The δ¹³C is then obtained comparing the results against a standard with a known ¹³C/¹²C ratio.

TABLE 1. Water potential and δ¹³C values with respect to vine water deficit thresholds. Water potential thresholds refer to the lowest values recorded during the grape ripening period.

	δ¹³C in grape juice (‰)	δ¹³C in wine or spirit (‰)	Midday Stem Water Potential (MPa)	Pre-dawn Leaf Water Potential (MPa)
No water deficit	< -26	< -27.7	> -0.6	> -0.2
Weak water deficit	-25 to -26	-26.7 to -27.7	-0.6 to -0.9	-0.2 to -0.3
Moderate water deficit	-24 to -25	-25.7 to -26.7	-0.9 to -1.1	-0.3 to -0.5
Moderate to severe water deficit	-23 to -24	-24.7 to -25.7	-1.1 to -1.4	-0.5 to -0.8
Severe water deficit	> -23	> -24.7	< -1.4	< -0.8

Classification of water deficit stress levels

δ¹³C measured in grape berry juice sugars range between -28‰ from vines experiencing no water deficit, up to -20‰ from vines suffering severe water deficit stress. The classification of water deficit intensity across this range differs slightly across publications. Differences in response thresholds may result, in part, from the fact that δ¹³C values are also influenced by the grapevine variety[3], and the diurnal variation of the midday plant water potential can be significantly different across climatic conditions during the growing season. Thresholds in Table 1 are computed from van Leeuwen *et al.*, 2009[2] and Santesteban *et al.*, 2015[4] and apply to regions with temperate climatic conditions. More precise site- and variety-specific threshold values can be set locally by combining δ¹³C and water potential measurements. Alternatively, a relative comparison can also be obtained: a variation of 1‰ in δ¹³C corresponds to a difference of ~0.2 MPa in midday stem water potential during the grape ripening period[5].

Practical implementation of vine water status assessment with δ¹³C

δ¹³C measurement is carried out on samples of grape juice collected between three weeks after mid-veraison and harvest. Samples are sent to a laboratory equipped with an isotopic mass spectrometer. Some laboratories estimate δ¹³C using Fourier-transform infrared

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spectroscopy (FTIRS), although this method cannot be recommended because it is imprecise. A current limitation for the implementation of δ13C measurement is the small, but steadily increasing number of laboratories that offer the analysis. Only a small amount of juice (+/- 5 µL) is needed for the analyses and can be obtained from samples taken for regular maturity control at the end of the season. These samples, however, need to be representative of the vines or vineyard block being investigated.

The δ13C signature in grape sugar is conserved in wine ethanol, although fermentation induces a shift of -1.7‰ (Table 1[6]). Hence, δ13C analysis can also be performed on wine in order to investigate the corresponding water status of the vines during berry ripening[7, 8].

Applications of water status monitoring with δ13C

δ13C provides insight into the water status experienced by the vines during the grape berry ripening period, which extends approximately from one week prior to mid-veraison to three weeks after mid-veraison. This corresponds, in most cases, to the month of August on the Northern Hemisphere and February on the Southern Hemisphere, a key period during which vine water status can affect yield and wine quality potential.

δ13C can be a useful tool for evaluating irrigation strategies at the end of the season with vine water status being affected by the amount and timing of applied water. In order to optimize wine quality for red wine making, growing vines under mild water deficit is desirable, with values of δ13C between -24‰ and -25‰ indicating vines were not over-irrigated. This can be important information when grapes are purchased from growers, as it provides a tool to check irrigation management *post hoc*. δ13C can also be used to easily evaluate the impact of any vineyard management practice (cover crop, tillage, leaf removal…) on vine water status.

Because δ13C is a highly accessible indicator (easy to measure and not expensive), it can also be used to map vine water status over a vineyard block or a winegrowing estate (Figure 1A[2]). δ13C can be a useful tool in terroir studies of dry-farmed vineyards, where vine water status depends largely on soil water holding capacity (figure 1B) and climate, both important terroir parameters[2, 9].

Such maps of vine water status are very useful for fine-tuning plant material choices and management strategies. In dry areas of the estate, drought resistant rootstocks should be used to avoid severe water stress. In wet areas, wine quality may be impacted by excessive water availability, which can be improved by establishment of a competitive cover crop.

At the intra-parcel level, mapping vine water status with δ13C is a tool for precision viticulture, such as explaining spatial variability in grape phenolics[5]. When measured on wine (see specific interpretation thresholds, table 1), δ13C allows to trace back the water status of the vines which produced the wine. This can be used to explain the effect of vine water status on quality related parameters, like aroma compounds[7].

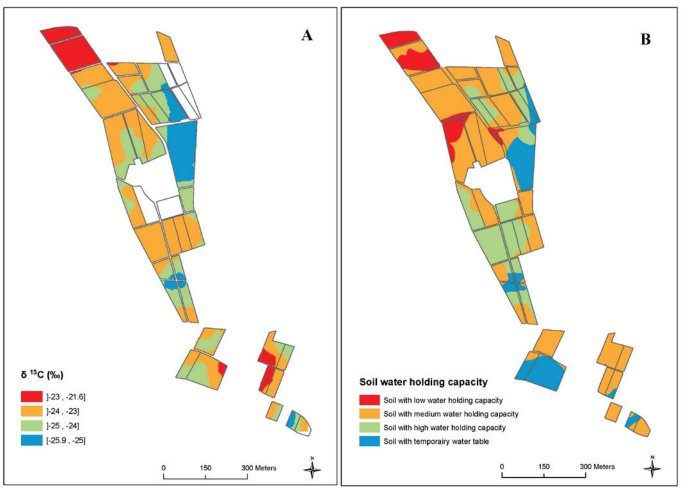

FIGURE 1. A - Vine water status measured on a dry-farmed wine-growing estate in Saint-Emilion (Bordeaux area, 2022). 172 samples were taken over the 23 hectares. B - Soil water holding capacity map drawn from the soil map of the estate.

Limitations of water status monitoring with δ13C

Because δ13C is measured at the end of the season, it is not useful for operational day-to-day irrigation management. For this purpose, the pressure chamber is preferred. Another limitation is that it represents vine water deficit during the period of sugar accumulation in grape berries. Hence, it does not provide a record of early vine water deficits, nor accounts for water deficits experienced after berry sugar loading is achieved. There are also variety specific differences in overall water use efficiency that must be considered when evaluating δ13C responses to water deficits across varieties[3]. The analysis of δ13C is currently offered by only a limited number of commercial laboratories, but will be more widely available as demand increases.

Conclusions

Being able to characterize vine water status is key to understanding how environmental factors and management practices may impact yield and wine quality potential. δ13C measurements on grape must or wine is an easily obtained and inexpensive indicator of vine water status during the berry ripening period and can be useful for managing both dry-farmed and irrigated vineyards. ■

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