Using $\delta^{13}$C and hydroscapes for discriminating cultivar specific drought responses

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ABSTRACT

Measurement of carbon isotope discrimination in berry juice at maturity ($\delta^{13}$C) provides an integrated assessment of vine water status and water use efficiency (WUE) during the period of berry ripening, and when collected over multiple seasons, can provide an indication of drought stress responses. Berry juice $\delta^{13}$C measurements were carried out on 48 different varieties planted in a common garden experiment in Bordeaux, France from 2014 through 2020 and found important differences across this large panel of varieties. Cluster analysis showed that $\delta^{13}$C values are likely affected by the differing phenology of each variety, resulting in berry ripening of different varieties taking place under different conditions of soil water availability within the same year. Accounting for these phenological differences, the cluster analysis created a classification of varieties that corresponds well to our current empirical understanding of their relative drought tolerance. In addition, using measurements of predawn and midday leaf water potential measurements collected over four seasons on a subset of six varieties, a hydroscape approach was used to develop a list of metrics indicative of the sensitivity of stomatal regulation to water stress (i.e. an/isohydric behaviour). Key hydroscapes metrics were also found to be well correlated with some $\delta^{13}$C metrics. A variety’s water potential regulation as characterized by a minimum critical leaf water potential as determined from hydroscapes was strongly correlated to $\delta^{13}$C values under well-watered conditions, suggesting that the latter may be a useful indicator of drought stress response.

KEYWORDS: water use efficiency, carbon isotopic discrimination, water potential, drought tolerance, VitAdapt, hydroscapes, grapevine, Vitis vinifera
INTRODUCTION

Vines are cultivated in a wide range of climates, from very dry (such as Cyprus or Aragon, Spain) to extremely wet (such as the Hunter Valley, Australia). The reason for the commercial success of vineyards across such a large range of environments can be explained by the winegrowers’ ability to adapt through viticultural practices or by choosing plant material adapted to the local climatic conditions. These adaptations are increasingly important as local environmental conditions are changing under the effect of climate change (IPCC, 2021). Rainfall patterns are changing, with precipitation increasing, or decreasing depending on the region. An increase in temperature and thus evaporative demand will induce drier conditions in most winegrowing regions, even in those with unchanged precipitation regimes. As a result, adaptations are needed to maintain commercially viable viticulture in current winegrowing areas (Fraga et al., 2012; Ollat et al., 2016; van Leeuwen and Darriet, 2016).

Vines react to water deficits by closing their stomata (Winkel and Rambal, 1990; Williams and Araujo, 2002), which reduces carbon assimilation (Flexas et al., 1998). Despite this reduction in photosynthesis, mild water deficit has positive effects in fine wine production: micro-climatic conditions are improved (better light penetration in the canopy due to lower vigour); more carbohydrates are available for berry ripening due to reduced competition with shoot growth; and berries become smaller with increased levels of anthocyanins in the produced wines (Roby et al., 2004; Keller, 2010; Carbonneau et al., 2015; Triolo et al., 2019). The positive impact of mild water deficit has also been shown on most aroma compounds in grapes and wines (van Leeuwen et al., 2020) as well as on the sensory properties of aged red Bordeaux wines (Le Menn et al., 2019).

However, water deficits can also lead to yield reductions that may negatively impact commercial viability (van Leeuwen et al., 2019a). Under severe water deficit, yield is highly impacted and grape ripening (e.g., sugar accumulation) can slow down or even stop in extreme conditions (van Leeuwen and Darriet, 2016). Although vines can survive under severe droughts and are unlikely to die (Charrier et al., 2018), commercially viable fine wine production may become more difficult under increased levels of water deficit.

Many options for adapting viticulture to a warmer and drier future are available, such as changes in canopy management practices, vineyard planting densities, or plant material selection (e.g., clones, rootstocks, and/or varieties) (Fraga et al., 2012; van Leeuwen et al., 2019a; van Leeuwen et al., 2019b). To inform future plant material selection, the drought responses of different varieties need to be better characterized. Assessing the regulation of vine water status is fundamental in making this characterization. Measurements of vine water potentials and the use of carbon isotope discrimination are two methods that have proven particularly useful in assessing water status, each with its own caveats (Choné et al., 2001; Gaudillère et al., 2002; Cifre et al., 2005; van Leeuwen et al., 2009; Santesteban et al., 2012).

$\delta^{13}C$ provides an integrative measure of the water status of the plant over a given period of time and can be assessed in leaves and/or berry juice at the end of the season (Gaudillère et al., 2002; Santesteban et al., 2012). During the process of photosynthesis there is a discrimination against assimilation of CO$_2$ containing $^{13}$C isotopes due to the higher reactivity of $^{13}$C isotopes with the Rubisco enzyme in the photosynthesis reaction. In addition, the rate of diffusion of $^{13}$CO$_2$ through the stomata and leaf boundary layer is less than for $^{12}$CO$_2$. This results in lower $^{13}$C/$^{12}$C isotope ratios in carbohydrates photosynthesized at that time when compared to atmospheric CO$_2$ (Farquhar 1989; Santesteban et al. 2012). As stomata close in response to drought stress, the $^{13}$C/$^{12}$C ratio rises in the intercellular space and sugars formed and incorporated into various plant tissues at that time will contain a modified ratio of carbon isotopes (Bchir et al., 2016).

When assessed on berry juice at maturity, $\delta^{13}C$ is a useful measure of the level of water deficit that existed during the time sugars in the berry juice accumulate (van Leeuwen et al., 2009). $\delta^{13}C$ effectively provides a measure of the ratio between the rate of photosynthesis and the stomatal opening over a period of time (Farquhar, 1989). This is similar to the ratio representing water use efficiency (WUE), which at the leaf level is the amount of carbon assimilated by photosynthesis ($A_{\text{leaf}}$) per amount of water transpired ($g_{\text{tr}}$), with a less negative $\delta^{13}C$ corresponding to less stomatal opening and higher WUE (Medrano et al., 2010; Souza et al., 2005; Bchir et al., 2016). A study of eight own-rooted Vitis vinifera varieties found a strong positive correlation between $\delta^{13}C$ and leaf-level WUE (Tomás et al., 2014).

Vine water status can also be assessed by different measures of leaf water potential. Midday leaf water potential ($\Psi_{m}$) is a measure of the immediate negative water potential (i.e., suction) in the leaf in response to many factors, such as vapour pressure deficit, stomatal closure, soil water status, and plant hydraulic conductivity (Choné et al., 2001). Predawn leaf water potential ($\Psi_{\text{pd}}$) is experienced at night when the vines have equilibrated with the soil water potential (Williams and Araujo, 2002) and can be considered as a proxy for soil water availability (Gaudin et al., 2017), or more precisely of the water availability in the most humid soil layer (Améglio et al., 1999).

The amount of stomatal regulation in response to changing $\Psi_{\text{pd}}$ varies among genotypes, a concept which has been referred to as (an)isohydricity (Tardieu and Simonneau, 1998; Hochberg et al., 2018). An anisohydic species allows transpiration and photosynthesis at more negative $\Psi_{\text{pd}}$, while an isohydic species will regulate stomata at less negative $\Psi_{\text{pd}}$, decreasing transpiration and eventually photosynthesis (Tardieu and Simonneau, 1998). Plant species do not adhere to strict (an)isohydric definitions, but instead vary across a continuum from anisohydric to isohydric (Chaves et al., 2010b; Klein, 2014) and this is also true across Vitis vinifera varieties, for which differences across genotypes mainly occur at medium levels of water deficit (Levin et al., 2019). In addition, growth conditions (e.g., greenhouse vs field grown) and the climatic conditions which impact the range of
water potentials can influence isohydric versus anisohydric behaviour even within the same genotype (Chaves et al., 2010; Charrier et al., 2018, Hochberg et al., 2018).

Recently the “hydroscapes” approach was proposed to visualize how plants regulate their water potential by means of stomatal regulation and to classify genotypes according to their behaviour under drought stress (Martínez-Vilalta et al., 2014). The area of the hydroscapen plot defines the range over which a plant regulates its $\Psi_s$ as a function of $\Psi_{rd}$ with the larger the range, the less strict (i.e., more anisohydric) the genotype is at controlling its stomatal conductance under water deficit (Meinzer et al., 2016). Conversely, a small hydroscapes indicates a genotype with more strict stomatal control (i.e., more isohydric). This methodology has shown to be effective in classifying drought-responses of different species (Li et al., 2019, Meinzer et al., 2016, Alvarez-Maldini et al., 2021). Hydroscapes, however, are time-consuming to produce, requiring multiple measurements of $\Psi_{rd}$ and $\Psi_L$ over a wide range of water deficits.

This study evaluates the drought stress responses of several grapevine varieties by means of $\delta^{13}$C measurements in berry juice sugars and by hydroscapes. Some apparent relationships between the results obtained by both methods are also discussed.

**MATERIALS AND METHODS**

1. VitAdapt vineyard

Data for this study were obtained in the VitAdapt experimental vineyard located at the INRAE (Institut National de Recherche pour l’Agriculture et l’Alimentation) research center of Bordeaux, in France (44°47’23.8”N, 0°34’39.3”W) (Destrac-Irvine and van Leeuwen, 2017). The soil is composed mainly of gravel and sand, representative of the Pessac-Leognan AOP (Bordeaux, France). The vineyard was planted in 2009 with 52 different varieties, including 4 hybrids and 48 Vitis vinifera L. cultivars on a 0.7 ha parcel with 46 rows of 75 vines each on a density of 5,555 vines/ha (row spacing of 1.8 m and inter vine spacing of 1.0 m). The trunk height is 0.5 m and grapevines are pruned to a double guyot and topped during the growing season at 1.5 m. Each variety was planted on Selection Oppenheim 4 (SO4) rootstock, clone 761. Each variety was tested for major virus diseases and only clean material was planted. There is a mowed cover crop in between each vine row with mechanical tillage under the vine row. The vineyard is dry-farmed and fertilisation is adjusted based on petiole analyses carried out at mid-veraison. To account for soil variability, the VitAdapt experimental design was laid out in randomized blocks with each block containing two rows of five vines per variety. For this study, four blocks were sampled.

2. Carbon isotope discrimination ($\delta^{13}$C)

Measurements of $\delta^{13}$C were taken from berry juice sugar collected each year at maturity from 10 vines of each variety in each of the four blocks. Measurements of grape sugars and acids were carried out weekly starting at mid-véraison and measured after initial planting in 2009, and continued each year through 2020. Berries were harvested from the 10 vines in each block and juice was extracted and centrifuged at 13,500 rpm for 10 minutes (Sigma 13 6K15, SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The juice was then analyzed on a Vario Micro Cube elemental analyzer coupled in a continuous flow mode to an isotopic ratio mass spectrometer (IsotopePrime, Elementar). Glutamic acid USGS40 and Caffeine IAEA-600 were used as international standards during the analyses. All results are expressed in delta notation ($\delta^{13}$C) and reported relative to the Vienna Pee Dee Belemnite (VPDB) international reference. Over the seven years a total of 17 to 28 $\delta^{13}$C measurements were obtained for each variety.

From 2017 through 2020 $\delta^{13}$C was also measured on eight individual vines of six varieties (Cabernet-Sauvignon, Merlot, Grenache, Tempranillo, Semillon, Ugni blanc), two of each from the four blocks, upon which water potential measurements were also taken periodically over the season. Of these individual vines, some started to lose too many leaves due to disease pressure or multiple water potential measurements, in which case water potential was taken on an adjacent vine, upon which $\delta^{13}$C was also measured with the values being averaged.

3. Water Potential

Water potential measurements were taken periodically over the seasons 2017 through 2020 on two vines each in the four blocks of Cabernet-Sauvignon, Grenache, Merlot, Semillon, Tempranillo and Ugni blanc. These varieties are some of the most widely planted varieties worldwide (Anderson and Aryal, 2013). Merlot, Tempranillo and Semillon (Rodgers et al., 2009) are known for being sensitive to drought stress, while Cabernet-Sauvignon and Grenache are renown for being less sensitive (Santesteban et al., 2009). No data were obtained on Grenache in 2018 because of downy mildew damage and measurements on Ugni blanc did not start until 2018. Measurements were collected at seven timepoints in 2017 and 2018 and six timepoints in 2019 and 2020.

From each vine on each sampling date, three types of water potential were measured with leaf water potential ($\Psi_s$) and stem water potential ($\Psi_{rd}$) measurements beginning at 2:00 p.m. on a given day and predawn water potential ($\Psi_{rd}$) started around 3 a.m. the morning after, with all measurements completed within two hours. For midday $\Psi_s$ measurements the leaves were sealed in foil covered bags while on the plant one hour prior (Choné et al., 2001). Measurements were taken by the method of Scholander et al. (1965) using a pressure chamber with digital manometer (DG MECA, 33175 Gradignan, France).

4. Hydroscapes

The method for preparing hydroscapes was adapted from the concepts presented by Meinzer et al. (2016), Charrier et al. (2018), and Hochberg et al. (2018). First, following the procedure of Meinzer et al., (2016), a scatter plot is
made of $\Psi_L$ on the vertical axis and corresponding $\Psi_{PD}$ on the horizontal axis using only the minimum values of $\Psi_{PD}$ and $\Psi_L$ measured from replicate vines on each date. By using minimum $\Psi_L$ values, data that was more influenced by non-limiting conditions for other variables, such as vapour pressure deficit, were removed. Then a 1:1 line is added representing the point at which $\Psi_{PD} = \Psi_L$, a theoretical condition when stomata are completely closed and the whole vine is in equilibrium with soil matric potential.

Then following the method of Charrier et al. (2018), bins of $\Psi_{PD}$ for every 0.05 MPa were made and the average of all the minimum $\Psi_L$ measurements within each bin was calculated. Then starting with the bin-averaged $\Psi_L$ at zero $\Psi_{PD}$ (the intercept), the bin averages for progressively more negative $\Psi_{PD}$ are sequentially compiled into a linear regression using bin averages at all previous, less negative $\Psi_{PD}$. This is done iteratively until the coefficient of determination ($r^2$) starts to decrease, suggestive of a breakpoint (called critical leaf water potential, $\Psi_{Lcrit}$) in the linear relationship between $\Psi_L$ and $\Psi_{PD}$ (Hochberg et al., 2018). From this point, the data suggests the bin-average $\Psi_L$ at more negative $\Psi_{PD}$ will have similar values that form a horizontal line of slope zero at $\Psi_{Lcrit}$. This represents the lowest $\Psi_L$ against which the plant will regulate its stomatal conductance at increasingly negative $\Psi_{PD}$. In uncontrolled field conditions, the condition of $\Psi_{PD} = \Psi_L$ is rarely achieved, so the horizontal line at $\Psi_{Lcrit}$ must be extended to meet the 1:1 line in order to complete the enclosure of the hydroscape. The area of the hydroscape is then bounded by the horizontal line at $\Psi_{Lcrit}$ (a in Figure 1), the 1:1 line (b in Figure 1), the vertical line at $\Psi_{PD} = 0$ (c in Figure 1), and the regressed line between the

**FIGURE 1.** A conceptual hydroscape bordered by the line representing the $\Psi_{Lcrit}$ (a), the 1:1 line (b), the vertical line at $\Psi_{PD} = 0$ (c) and the regressed line between the intercept at $\Psi_{PD} = 0$ and $\Psi_{Lcrit}$ (d).

![Graph showing conceptual hydroscape](image)

**FIGURE 2.** Individual vine $\delta^{13}$C as a function of $\Psi_{PD}$, averaged over the data collected between 20 days before until 60 days after veraison, for six varieties in 2017 (except for Ugni blanc), 2018 (except for Grenache), 2019 and 2020 in Villenave d’Ornon, France.
intercept at $\Psi_{\text{ro}} = 0$ and $\Psi_{\text{end}}$ (d in Figure 1). In theory, the greater the area enclosed by the hydroscapes, the more anisohydric the variety (Meinzer et al., 2016).

Filtering, graphing, and linear regression analysis was performed in the R software environment using several functions from the `dplyr` package (Wickham et al., 2021a) and the `ggplot2` package (Wickham et al., 2021b) with hierarchical clustering analyses and dendrograms being done using the `cluster` package.

**RESULTS AND DISCUSSION**

1. Climate conditions

The two years with the greatest water deficits were 2016 followed by 2018. Some years showed particular climatic conditions. In 2015 (globally a dry year), August was very rainy (109.5 mm). In 2017, evaporative demand was low in May and rainfall was high in September (82 mm), which explains why water deficit was low in this year despite a dry July and August. 2020 was one of the earliest vintages ever in Bordeaux, with high rainfall in May and June, while July was very dry (2.5 mm) and rainfall in August was close to average (46.5 mm). More information regarding vine water status during these years is presented in Figures S1 and S2.

2. Carbon isotope discrimination ($\delta^{13}$C)

2.1 Relationship of berry juice $\delta^{13}$C to leaf water status

Figure 2 presents plots of $\delta^{13}$C on an individual vine basis for six varieties from 2017 through 2020 versus the corresponding average $\Psi_{\text{ro}}$ measured during berry ripening (i.e., from 20 days before mid-veraison until 60 days after veraison). The $\delta^{13}$C and average $\Psi_{\text{ro}}$ were well correlated for Cabernet-Sauvignon, Grenache, Semillon and Tempranillo, while not as well correlated for Merlot and Ugni blanc (Note, only three years of data were collected for Ugni blanc and Grenache). Plots of $\delta^{13}$C versus $\Psi_s$ (not shown) showed similar relationships. These results are consistent with previous studies showing $\delta^{13}$C as being well correlated to $\Psi_{\text{ro}}$ (Gaudillère et al., 2002) and to midday $\Psi_s$ (Santesteban et al., 2012) suggesting increased water use efficiency in response to increasing vine water deficit.

2.2 Varietal differences in $\delta^{13}$C

Figure 3 presents the range of $\delta^{13}$C values for each of the 48 varieties across the seven years of measurement, with the thickness of the violin plot indicative of the distribution of values and the black dot representing the mean of all measurements. As discussed in the next section, the average $\delta^{13}$C measured in the year with the most negative values is an important metric of drought responses for a given variety. The red dots on the violin plots represent this minimum mean value for each variety and are the values upon which the varieties are ordered along the vertical axis in Figure 3.

$\delta^{13}$C is variety dependent, with Tinto Cão showing the most negative mean values across all years (black dots) and Colombard showing the least negative mean values. The difference in these mean $\delta^{13}$C values across the 48 varieties is up to 2.8 ‰, representing a 44 % variation across the full range of varieties relative to Tinto Cão. Some varieties (e.g., Liliorita and Ugni blanc) appear to operate in a narrower range of $\delta^{13}$C values across the years, while others (e.g., Touriga nacional and Saperavi) operate across a wider range of $\delta^{13}$C. As discussed in the next section, the range of $\delta^{13}$C provides an indication of the plasticity of a variety to adapt its WUE to dry vs. wet conditions.

An effect is also observed across the years with the lowest and highest mean $\delta^{13}$C values for each variety. For years with the most negative $\delta^{13}$C values, as associated with wetter conditions and lower WUE during ripening, a difference of up to 3.1 ‰, or a 50 % variation was observed across all varieties relative to the variety with the most negative corresponding $\delta^{13}$C value (Saperavi). For years with the least negative $\delta^{13}$C values, as associated with drier conditions and higher WUE during ripening, a difference of up to 4.0 ‰, or a 63 % variation was observed across all varieties relative to the variety with the least negative corresponding $\delta^{13}$C values (Xynomavro).

In addition to apparent varietal differences, measured $\delta^{13}$C may also be impacted by the different phenology of each variety, with later ripening varieties being more likely to experience water deficits during the sugar loading period than earlier varieties. Thus, in attempting to characterize genetically determined varietal differences in $\delta^{13}$C, differences in the phenology of the berry ripening period in relationship to the driest times of the season must also be considered.

2.3 Categorization based on key traits

Several combinations of key $\delta^{13}$C and other physiological traits were used in a hierarchical clustering analysis (HCA) to identify groupings of varieties with similar characteristics. The most consistent groupings were obtained when HCA was applied using the following three traits: “veraison date”, “minimum $\delta^{13}$C” (i.e., $\delta^{13}$C in non-limiting conditions) and “range of $\delta^{13}$C” (i.e., the difference between $\delta^{13}$C in a wet vintage compared to a dry vintage). The HCA in Figure 4 suggests a range and groupings of varieties based on the above three key traits. All varieties defined traditionally as drought-tolerant (blue arrows) and all varieties defined traditionally as non-drought-tolerant (green arrows) were separated between the two distinct groups. Moreover, varieties generally grown in dry climates were separated from varieties generally grown in more humid climates. While it is very complex to define drought tolerance quantitatively, a consensus could possibly be reached by considering the yield and quality data of the different varieties in the scientific literature and from the wine industry.

Interestingly, the classification based on HCA provided more consistent results with the minimum value of $\delta^{13}$C (i.e., non-limiting conditions) compared to the maximum value of $\delta^{13}$C (i.e., limiting conditions). Varieties with a more negative $\delta^{13}$C in non-limiting conditions may have a greater stomatal conductance and thus greater transpiration and water use early in the season which could more quickly deplete available

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soil water (Lebon et al., 2003) and increase the likelihood of water deficits during berry ripening. This hypothesis is supported by the ΨPD data (Figure 2) where Tempranillo and Semillon, varieties with very relatively negative δ13C in non-limiting conditions are the two varieties that reached the most negative ΨPD. The other metric of interest in this HCA classification is the difference between the minimum value of δ13C (i.e., the δ13C under non-limiting conditions) and the maximum value of δ13C (i.e., the δ13C under limiting conditions). This trait would characterize the plasticity of the variety to adapt to the drought conditions of the vintage.

One confounding factor in assessing the δ13C characteristics of different varieties is the timing of phenological stages. Of particular concern with δ13C in berry juice sugars is the vine water status during the period of sugar loading in the berries, roughly starting one week to ten days before...
mid-veraison and ending three or four weeks after mid-veraison (Suter et al., 2021). In most years, water deficit increases as the season progresses, which means that an early variety faces less water deficit during berry ripening compared to late varieties. Interestingly, adding average mid-veraison dates in the HCA classification improves the separation of drought sensitive versus drought tolerant varieties, as defined by hydroscapes in section 3 below. The relationship between the characteristics “late ripening” and “drought tolerant” may be due to the fact that late ripening varieties were selected to grow in warm climates, which are also most often dry climates. In the HCA presented in Figure 4, most varieties grown around Mediterranean basin are classified in the left panel, such as Assyrtiko, Sangiovese, Mourvèdre, Grenache, Carignan, and Rkatsiteli.

3. Hydroscapes

3.1. Constructed hydroscapes

Hydroscapes are presented in Figure 5 for the six varieties using individual vine data for which there was both δ13C and corresponding water potential data. Grenache has the smallest hydroscopic surface and is known for being very strict in its stomatal regulation (Schultz, 2003). Semillon, the variety with the largest hydroscopic surface, is known for its anisohydric behaviour, due to its poor control over stomatal conductance, leading to substantial nighttime transpiration (Rogiers et al., 2009; Dayer et al., 2020; Dayer et al., 2021). In the same study of Rogiers et al., (2009), Cabernet-Sauvignon and Merlot were not significantly different in terms of stomatal conductance which corresponds with our finding of the two varieties having a similar hydroscopic surface (10.7 MPa2 and 10.9 MPa2, respectively).

These results are also consistent with empirical knowledge gathered in production areas. In La Rioja (Spain), Grenache is planted in the driest parts of the production area around the town of Logroño, while Tempranillo dominates in the cooler locations at higher altitude, where drought- and heat-stress are less frequent. Semillon thrives in the Atlantic climate of Bordeaux (France) and is the major variety in the Hunter Valley (Australia), one of the wettest production areas in the world (Johnson, 2013). The drought responses of Ugni blanc (Trebbiano) is less well documented, but these results suggest that it may be well adapted to dry conditions. The hydroscopic approach, however, only refers to stomatal behaviour of plants under various levels of water deficit. Drought responses involve other mechanisms like hydraulic conductance, osmotic adjustment, leaf versus root development, duration of the crop cycle, and timing of phenology to ensure development in the wettest part of the season (Tardieu et al., 2018).

3.2. The metrics of the hydroscapes

The areas of the hydroscapes presented in Figure 5 provide an insight into the stomatal behaviour of plants under drought conditions, but other metrics can also be extracted (see also Figure S3 in supplementary materials). For example, the intercept of the hydroscopic represents the least negative Ψ_L observed under non-limiting water availability (least negative ΨPD). Interestingly, this intercept is very different for Grenache compared to Semillon (-0.96 MPa of ΨL and -1.4 MPa of ΨPD, respectively). This may be explained by differences in stomatal conductance, which was observed as being higher in Semillon than Grenache (Rogiers et al., 2009). The slope of the hydroscopic also differs among varieties, with Grenache having the steepest slope compared to the other varieties.

A distinct critical midday leaf water potential (ΨLCrit) is more evident for Grenache, Ugni blanc, Cabernet-Sauvignon and Merlot, but less so for Tempranillo and Semillon.

FIGURE 4. Hierarchical Clustering Analyses (HCA) performed with the Ward method of the 48 varieties as a function of “Average Veraison (DOY)”, “Minimum δ13C” and “Range of δ13C”.

Traditionally grown in limiting conditions Traditionally grown in non-limiting conditions

Drought tolerant Non-Drought tolerant

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The variation observed for Tempranillo and Semillon could be due to a variety of factors, including differences in vapour pressure deficit during the precise time of measure, and/or measurement artifacts (e.g., severe levels of stress causing petiole embolisms, hydraulically disconnecting the measured leaf from the vine). There are also differences in the $\Psi_{PD}$ at which $\Psi_{L_{crit}}$ is reached. For example, Merlot reaches $\Psi_{L_{crit}}$ at $\Psi_{PD} = -0.3$ MPa, while Tempranillo reaches $\Psi_{L_{crit}}$ at $\Psi_{PD} = -0.7$ MPa.

The $\Psi_{L_{crit}}$ is a very important metric related to the mortality thresholds of various plant species under drought (Choat et al., 2018). Cultivars with high stomatal conductance in well-watered conditions will deplete soil water earlier in the season. Depending on the degree to which stomatal conductance is adjusted under decreasing $\Psi_{PD}$ these cultivars could face more severe water deficits. For example in this study, Tempranillo had one of the largest hydroscapes and most negative critical $\Psi_{L_{crit}}$. It was also observed that among the 48 varieties, Tempranillo was one of the earliest varieties to show leaf wilting and leaf abscission.

Another metric of interest is the range of $\Psi_{PD}$ at which a variety normally operates, representing its ability to extract water at low soil water potential. As discussed above, this can be linked to less stringent stomatal control (e.g., Grenache operates over a small range of $\Psi_{PD}$ because of a very strict stomatal control). Some varieties appear able to extract water at very low $\Psi_{PD}$ which may be due to the root architecture associated with the cultivar x rootstock combination. It is impossible, however, to assess the rooting zone of the vines in field conditions, which may vary from one vine to the other in the VitAdapt parcel due to soil heterogeneity. It is also possible that the variety influences the ability of the rootstock to explore the soil more or less profoundly (Tandonnet et al., 2010). Vines having access to a greater volume of soil water due to a greater rooting depth are likely to experience less negative values of $\Psi_{PD}$.

The approach presented offers a possible way of classifying varieties according to their level of (an)isohydricity, an important criterion in drought tolerance (i.e., the larger the hydroscape, the more drought sensitive). Results are in line with the literature regarding stomatal behaviour, in particular for the well documented varieties Grenache and Semillon (Schultz, 2003; Rogiers et al., 2009). Results were obtained on six varieties only and need to be confirmed on a wider range of cultivars. It would also be interesting to assess whether, for a given variety, the hydroscapes have a different shape when the data are collected on different soil types, or with different rootstocks. One of the limitations of the hydroscape approach is that it requires time-consuming water potential field data collected, ideally over multiple years.

4. Comparison of $\delta^{13}C$ and hydroscapes

Both $\delta^{13}C$ and hydroscapes are mainly driven by the stomatal behaviour of plants (Farquhar, 1989; Meinzer et al., 2016). Hydroscrapes were constructed as described above for six varieties for which there were also measurements of $\delta^{13}C$. Several metrics were extracted from the analysis performed using these two tools and are listed in Table 1 along with the associated values for each variety.

A correlation analysis of the metrics presented in Table 1 across the different varieties is presented in Figure 6. It is observed that the size of the hydroscapes is strongly...
TABLE 1. Metrics of hydroscapes and δ¹³C for six grapevine varieties. Varieties are classified from the lowest to the highest hydroscape area as representative of (an)isohydricity. For each metric, values are classified with shades of green.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Intercept (MPa)</th>
<th>Slope (MPa)</th>
<th>Ψ_{Lcrit} (MPa)</th>
<th>Range of Ψ_{PD} (MPa)</th>
<th>Hydroscape (MPa²)</th>
<th>Range of Ψ_{L} (MPa)</th>
<th>Range of δ¹³C (‰)</th>
<th>Max δ¹³C (‰)</th>
<th>Min δ¹³C (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grenache</td>
<td>-0.96</td>
<td>1.80</td>
<td>-1.43</td>
<td>-0.89</td>
<td>7.07</td>
<td>0.47</td>
<td>4.08</td>
<td>-23.20</td>
<td>-27.28</td>
</tr>
<tr>
<td>Ugni blanc</td>
<td>-1.2</td>
<td>0.74</td>
<td>-1.57</td>
<td>-1.12</td>
<td>9.06</td>
<td>0.37</td>
<td>4.06</td>
<td>-23.34</td>
<td>-27.40</td>
</tr>
<tr>
<td>Cabernet-Sauvignon</td>
<td>-1.2</td>
<td>1.10</td>
<td>-1.73</td>
<td>-1.23</td>
<td>10.70</td>
<td>0.53</td>
<td>4.65</td>
<td>-23.11</td>
<td>-27.76</td>
</tr>
<tr>
<td>Merlot</td>
<td>-1.4</td>
<td>0.85</td>
<td>-1.60</td>
<td>-0.89</td>
<td>10.92</td>
<td>0.20</td>
<td>3.90</td>
<td>-23.70</td>
<td>-27.60</td>
</tr>
<tr>
<td>Tempranillo</td>
<td>-1.2</td>
<td>0.77</td>
<td>-1.78</td>
<td>-1.18</td>
<td>11.04</td>
<td>0.58</td>
<td>5.96</td>
<td>-22.35</td>
<td>-28.31</td>
</tr>
<tr>
<td>Semillon</td>
<td>-1.4</td>
<td>0.88</td>
<td>-1.80</td>
<td>-1.58</td>
<td>13.24</td>
<td>0.40</td>
<td>5.15</td>
<td>-23.30</td>
<td>-28.45</td>
</tr>
</tbody>
</table>

* Difference on the y-axis between the intercept of the hydroscope and the Ψ_{Lcrit}.

FIGURE 6. A correlation heatmap of the metrics extracted from the hydroscapes and from the δ¹³C (as shown in Table 1). Only significant correlations at P < 0.05 are indicated.

dependent on two variables, the intercept and the Ψ_{Lcrit}. While the intercept becomes more negative the size of the hydroscope increases, suggesting a generally anisohydric behaviour even at low Ψ_{PD}. A similar dynamic exists with a more negative Ψ_{Lcrit} also being associated with a larger hydroscope.

The minimum δ¹³C, which is the most negative δ¹³C obtained in non-limiting conditions, appears better correlated to hydraulic traits from the hydroscapes than does the maximum δ¹³C, which is the least negative δ¹³C obtained in dry conditions. Minimum δ¹³C is well correlated negatively with the area of the hydroscapes (Hydroscape) and positively with Ψ_{Lcrit}, suggesting the anisohydric behaviour of a larger hydroscope is somehow associated with a lower level of water use efficiency in well-watered conditions (Chaves et al., 2010). Increased water use in non-limiting conditions, as suggested by more negative Ψ_{Lcrit}, may lead to greater water deficits later in the season and hence less negative δ¹³C.

By integrating the δ¹³C metrics with the insights provided by the hydroscapes metrics, minimum δ¹³C appears to be a relatively easily accessible metric to classify grapevine varieties according to their drought responses. It should be noted that, although these metrics are easy to collect in field conditions, they need to be measured over multiple years in homogeneous soil conditions, or with enough replicates to rule-out possible variability in soil water holding...
capacity. These conditions were met in the VitAdapt setting (Destrac-Irvine and van Leeuwen, 2017).

**CONCLUSION**

The measured $\delta^{13}C$ in berry juice sugars provides an indication of vine water status during the period of sugar accumulation from veraison through maturity, as was confirmed by comparison against corresponding measurements of predawn water potential on six varieties over four seasons. Distinct differences in $\delta^{13}C$ measured in berry juice sugar collected in a vineyard setting over seven years of differing climatic conditions found significant differences across 48 varieties. The drought responses of these varieties were then ranked using a hierarchical cluster analysis based on: i) the mean $\delta^{13}C$ in years of relatively low water stress; ii) the range of $\delta^{13}C$ for a variety as measured in wet versus dry years; and iii) the day of year of mid-veraison. This ranking corresponded well with generally understood drought tolerance characteristics of the different varieties. Of particular importance were the highly variable mid-veraison dates for the different varieties affecting the water deficits experienced by each variety, and hence the corresponding measured $\delta^{13}C$ in a given year, independent of the genotype response.

The predawn measurements on the six varieties over four years, along with corresponding midday leaf water potential measurements were used to construct hydroscape, which allowed insight into the stomatal behaviour of these varieties. Among several hydroscape metrics, minimum critical leaf water potential ($\Psi_{Lcrit}$), and the closely related total area of the hydroscape were considered to provide a good indication of drought stress sensitivity. Less negative values of $\Psi_{Lcrit}$ and correspondingly smaller hydroscape areas suggest an isohydric variety, which regulates its stomata more strictly to maintain water potential. Conversely, more negative $\Psi_{Lcrit}$ and larger hydroscape are suggestive of a more anisohydric variety, which regulates its stomata less strictly, allowing for more negative water potentials.

Comparison of $\delta^{13}C$ and hydroscape metrics found minimum $\delta^{13}C$ (i.e., from years with less limiting conditions) to be correlated negatively with the area of the hydroscape and positively correlated with $\Psi_{Lcrit}$ suggesting the anisohydric behaviour of a larger hydroscape is somehow associated with a lower level of water use efficiency. This may be due to increased water use in non-limiting conditions as suggested by more negative $\Psi_{T}$, resulting in greater water deficits and more stress later in the season and hence less negative $\delta^{13}C$. This also suggests that this minimum $\delta^{13}C$ could provide a relatively easy way to classify grapevine varieties according to their drought stress responses.

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