SOIL AND TOPOGRAPHY EFFECTS ON WATER STATUS AND MUST COMPOSITION OF CHARDONNAY IN BURGUNDY & A MINI META-ANALYSIS OF THE δ ^{13}C/WATER POTENTIALS CORRELATION

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Abstract:

Context and purpose of the study: The measurement of carbon isotopic discrimination in grape sugars at harvest (δ ^{13}C) is an integrated assessment of water status during ripening. It is an efficient alternative to assess variability in the field and discriminate between management zones in precision viticulture, but further work is needed to completely understand the signal.

Material and methods: This work, spanning over 3 years, performed in in 8 different plots in a hillslope toposequence in Burgundy, delineates the relationships between main soil properties (gravel amount, slope, texture) and the grapevine water status assessed by δ^{13}C and predawn leaf water potentials (Ψ_{pd}). Brix, tartaric and malic acids were also measured.

Results: The highest δ^{13}C, indicating most severe water deficit, was recorded in gravelly soils on steep slopes. The amount of sugars and malic and tartaric acids was also related to δ ^{13}C. The relationship between δ 13C and Ψpd was also investigated, because the absolute values of measured δ ^{13}C were lower than the values currently found in the literature. A mini-meta-analysis was performed, which showed that the slope of the relationships between minimum Ψ_{pd} and δ ^{13}C was stable across studies (a change of 1‰ in δ ^{13}C corresponded to a change of −0.2 MPa in the minimum Ψ_{pd}), while the intercept of the comparison δ 13C/Ψ_{pd} changed, probably because of genetic variations between varieties, or environmental differences.

Keywords: carbon isotopic discrimination; water stress; terroir; slope; organic acids

1. Introduction

Water supply varies greatly between growing sites at very short distances, because of differences in soil propertiesor climatic parameters.In rainfed farming, water availability is amongst the main causes of variations in grapevine physiology across growing sites (van Leeuwen *et al.*, 2004).Although common for red wine grapes, the scientific literature generally lacks studies on the effect of water deficit on must composition of white wine grapes. The current opinion is that water stress will have a negative effect because (i) the increase in phenolic compounds with water stress is not favorable to this type of wine (Sadras and Schultz, 2012) (ii) some aromatic compounds are less abundant under moderate to high water deficit (des Gachons *et al.*, 2005) and (iii) white wines require acidity, which is rapidly degraded in water deficit conditions, often joint to heat (Sweetman *et al.,* 2014).

Grapevine water status is generally assessed by leaf water potentials, while the use of carbon isotope composition to have a continuous integrator of the water status throughout the ripening period has been introduced more recently in grapevine (Gaudillère *et al.*, 2002). Basically, stable carbon isotope uptake is discriminated by ribulose 1,5-diphosphate, which preferentially fixes ^{12}C, the most abundant carbon isotope. When stomata are closed this kinetic preference is reduced, because the ^{13}C/^{12}C ratio increases in the substomatal chamber, and the primary photosynthetic products are enriched in ^{13}C. Water deficit is the main factor affecting this ratio (Farquhar *et al.*, 1989).

This recently published work (Brillante *et al.*, 2018) had the aims: (i) to assess the effectiveness of berry $\delta^{13}C$ to evaluate plant water status in Burgundy conditions, a cool climate for grapevine; (ii) to relate variability of $\delta^{13}C$to the main soil properties driving grapevine water status throughout the ripening season in a characteristic Burgundy hillslope; and (iii) to understand the relationships of $\delta^{13}C$ on white must composition.

2. Material and methods

Experimental field site and plant material – The studywas carried out over 3 years (2011-2013) in a commercial vineyard (Aloxe-Corton, Burgundy, FR). Eight experimental plots were selected and labeled in alphabetical order (A–H) from the top (325 m) to the bottom of the hill (267 m) in order to characterize the natural variability occurring in a Burgundy toposequence. Plots were 7 m × 7 m squares containing 49 grapevines in seven rows (*Vitis vinifera* L. cv. Chardonnay B.) grafted on to SO4 rootstock (interspecific cross between *Vitis riparia*, Michx. and *Vitis berlandieri*, Planch.). Vines were Guyot pruned and trained in a vertical-shoot-position trellis system with the first training wire at 0.5 m and the fruiting cane hedged at 1.20 m. At the beginning of the study, soil samples were collected at 0.1 m intervals down to 1 m depth in a trench located in the middle of each plot and analyzed to determine soil texture and gravel content. Soil properties averaged over 0–1 m depth are presented in Table 1; a detailed description including a larger set of soil properties can be found in Brillante *et al.*, 2014.

Plant measurements – Predawn and stem water potentials were monitored weekly from bunch closure to harvest on eight leaves per experimental unit. Carbon isotope composition of grape musts was measured on sugars in mature grapes, following the protocol described in (Gaudillère *et al.*, 2002).For sampling, the plots were subdivided into three groups of 14 grapevines in two rows, and from each group one composite sample of 100 berries was collected (3 samples × 8plots × 3 years) and isotopic analyses were performed in triplicate on a Vario Micro Cube elemental analyzer coupled in a continuous flow mode to an isotope ratio mass spectrometer, using USGS40 as internal standard and reporting values in delta notation relative to the VPDB international reference.

Grape composition – At harvest, a composite sample of 500 berries was sampled in each plot, ground and analyzed using a specifically calibrated Fourier transform infrared spectroscopy (FTIR, Winescan Flex Auto, FOSS Analytical) for measurement of malic and tartaric acids. Sugars were measured by electronic refractometry (Mettler-Toledo, Inc.), and expressed as °Brix. Measurements were made for all plots in each year (8 plots × 3 years).

Statistical analysis - Effects of time-invariant (at the experiment time scale) factors – slope, gravel and texture factors – on $\delta^{13}C$ were analyzed using mixed-effect analysis of variance (ANOVA), with either slope and gravel, plot and year as nested random intercepts, to control for the associated intraplot correlation without data aggregation, and because the effect of the year is not of explicit interest (considered as a random). Furthermore, the effect of year on individual plot $\delta^{13}C$ is considered variable across plots, being dependent on their soils. These models also tolerate the unequal number of responses per factor (slope and gravel). Continuous grape composition variables were analyzed using multiple linear regression to predict water status ($\delta^{13}C$ as dependent variable) based on grape quality composition (sugars and organic acids, as independent variables). The $\delta^{13}C$ samples were averaged at the plot level. Independent variables were checked for collinearity, and in such cases the choice of the variable to include was assessed based on performance in a cross-validation routine using the same subsets for unbiased comparison. Twenty-five repetitions of six fold cross-validation was used (with n = 24). Residual diagnostics were performed in order to assess important deviations from basic assumptions of the ordinary least square regression, and the inclusion of a bilinear term (for sugars) was taken as corrective action, to solve nonlinearity issues. The statistical analysis was run in R using the packages lme4, 31 and multcomp.32. Unless otherwise specified, significance is used to indicate a P-value lower than 0.05

3. Results and discussion

3.1. *Carbon isotope discrimination of grape sugars at ripeness and effect of soil properties*

The minimum Ψ_{pd} ranged from −0.64 MPa (plot B, 2012) to −0.20 MPa (plot F 2011), and compared to those data the variability of $\delta^{13}C$ was lower than expected, ranging from −27.95‰ to − 26.30‰. The correlation between Ψ_{pd} (taking the minimum value recorded for the period between veraison, and harvest) and $\delta^{13}C$ was then checked, as in the work by Gaudillère *et al.*, 2002. The correlation with minimum Ψ_{pd} was significant (P-value < 1e−04, r=−0.72) and is shown in Fig. 1. The correlation with Ψ_{pd} averaged over the season was not significant (data not shown). Figure 1 also shows that data group in separate sections of the graphic area, according to soil properties. This was confirmed by the linear mixed model analysis presented in Table 2. The ANOVA performed on the fixed-effect part of the model revealed that the contribution of slope was highly significant (P-value <0.01), as well as the effect of gravel (P-value < 0.01). When the slope was steeper and the gravel content higher the $\delta^{13}C$ was less negative, indicating more water stress. The analysis of the random factor, showed that the 2013 vintage was the least water stressed, and was significantly different from the 2012 and 2011.

Soil texture and gravel content directly affect grapevine water status because of the effect on the soil water-holding capacity, sandy and gravelly soil having less capacity than clayey soils without gravels (Brillante *et al.*, 2015). The slope, inducing runoff, reduces the amount of water that can penetrate the soil (Celette *et al.*, 2008). Therefore, soils in steep slopes and rich in gravel cause an increase in grapevine water deficit, compared to soils on flat ground and without gravels (van Leeuwen *et al.*, 2004; Brillante *et al.*, 2016). The effect of texture in this study was not significant, as conversely found in previous studies (Tramontini *et al.*, 2013) because once averaged over a depth of 1 m soil texture varied very little, and only ranged from loamy to clay-loamy. Moreover, a possible effect of soil texture on total transpirable soil water can be counterbalanced by differences in rooting depth.
Results of the effect of slope, gravel amount and texture on $\delta^{13}C$ confirm the outcome of a previously published machine-learning model to predict leaf water potentials from climate and soil data that was developed in this same experimental site (Brillante *et al.*, 2016). These results show that well-trained machine-learning models are not only suitable to accurately predict grapevine water status but also to capture the essential relationships between plant physiology and the environment.

3.2. *Relationship between grape composition and water status a posteriori*

The water deficit experienced by grapevine throughout the season, as measured by $\delta^{13}C$, was estimated from the composition of grapes at harvest, as shown in Fig. 2. A multiple linear regression was used to model the relationships between $\delta^{13}C$, sugars and malate; tartaric acid was not included because of collinearity problems with sugar content. The model's R^2 was equal to 0.64 ± 0.03, and RMSE was equal to 0.26 ± 0.3‰, as evaluated by 25 repetitions of sixfold cross-validation. The multiple linear regression was highly significant. The significance of sugars and malate in the model was high (P < 0.01).

3.3. *A mini-meta analysis of $\delta^{13}C$ measured on grape sugars and its relationship with other point-type measurements of water status*

The $\delta^{13}C$ data measured in this study were low with respect to other data already reported in the literature (Gaudillere *et al.*, 2002; Guix-Hébrard et al., 2007) when considering the absolute values reached at equivalent levels of minimum Ψ_{pd}, as reported in Table 3. While the absolute values vary between the different studies, the slope of the relation does not, once including errors in measuring the slope from published data and the instrumental accuracy. A reduction of approximately −0.2 MPa in minimum Ψ_{pd} corresponds to an increase of 1‰ in $\delta^{13}C$. This result would allow future studies to directly interpret the relative differences in $\delta^{13}C$ between treatments as relative differences in water potentials, therefore taking advantage from the directcomparison with a measurement (the water potential) that has a longer history of interpretation from a physiological point of view, and is widespread in the viticulture industry. This result has great practical use in production contexts and in precision viticulture, where the number of experimental replicates is often too large to allow the measurement of water potentials within the limited time around noon or dawn.
This work does not allow a complete understanding of the reasons for such variability in the intercept of the relationship between $\delta^{13}C$ and Ψ, and further studies are needed on the subject to clarify and promote the use of $\delta^{13}C$, which is a very effective tool for both production and research. In our opinion, a valid hypothesis of such differences across studies is the use of the minimum Ψ_{pd} to fit the relationships, $\delta^{13}C$ being an integrative indicator of plant water status over a longer period of time (basically, the time of sugar accumulation in grape berries), while minimum Ψ_{pd} is very time specific.

4. Conclusions

This work shows soil properties affecting berry $\delta^{13}C$ in rainfed vineyards: gravel and slope were the statistically significant factors determining variations in the values. The effect of texture was not important under the conditions of this study because the variability was too low to affect grapevine physiology in a significant way. Water status assessed by $\delta^{13}C$ was related to berry composition, a linear increase in malic acid was related to a decrease in water stress, while an increase in sugars was related to an increase in water stress, although the relation was bilinear and significant only for low sugar content and water stress. Berry $\delta^{13}C$ was well related to the minimum Ψ_{pd}, and confirmed itself as a good integrator of grapevine water status. However, a comparison with previously published works showed that the relationship between $\delta^{13}C$ and Ψ_{pd} is not stable across varieties and grape-growing regions. Specifically, while the slope of the relationship between minimum Ψ pd and $\delta^{13}C$ was stable, and in all studies an increase of 1‰ in $\delta^{13}C$ corresponded to a decrease of 0.2 MPa in minimum Ψ_{pd}, the intercept and then the values of $\delta^{13}C$ at equivalent minimum Ψ_{pd} variedbetween studies. This result will allow a direct interpretation of differences in $\delta^{13}C$ as water potential differences, and therefore will simplify the physiological interpretation of relative differences in $\delta^{13}C$ between experimental units. This is particularly important for those conditions, as in precision viticulture, where the number of experimental units is too large to allow the measurement of water potentials within a limited time frame around noon or dawn. In future studies this variability of $\delta^{13}C/\Psi_{pd}$ needs to be better investigated, also considering genetic and environmental aspects.

For a full version of this work and for citations, readers are kindly referred to Brillante et al., 2018 *Water status and must composition in grapevine cv. Chardonnay with different soils and topography and a mini meta-analysis of the δ13C/water potentials correlation* Journal of the Science of Food and Agriculture 98 (2), 691-69, DOI: 10.1002/jsfa.8516

5. Literature cited

Brillante L., Bois B., Mathieu O., Bichet V., Michot D., Lévêque J., 2014. Monitoring soil volume wetness in heterogeneous soils by electrical resistivity: a field-based pedotransfer function. J. Hydrol. 516:56–66.

Brillante L., Mathieu O., Bois B., van Leeuwen C., Lévêque J., 2015. The use ofsoil electrical resistivity to monitor plant and soil water relationships in vineyards. Soil 1:273–286.

Brillante L., Mathieu O., Lévêque J., Bois B., 2016. Ecophysiological modeling of grapevine water stress in Burgundy terroirs by a machine-learning approach. Front Plant Sci; doi: 10.3389/fpls.2016.00796

Brillante L., Mathieu O., Lévêque J., Van Leeuwen C., Bois B., 2018.Water status and must composition in grapevine cv. Chardonnay with different soils and topography and a mini meta-analysis of the δ13C/water potentials correlation J. Sci. Food Agric. 98 (2), 691-69.

Celette F., Gaudin R., Gary C., 2008. Spatial and temporal changes to the water regime of a Mediterranean vineyard due to the adoption of cover cropping. Eur. J. Agron. 29:153–162.

des Gachons C. P., van Leeuwen C., Tominaga T., Soyer J. P.,Gaudillère J. P., Dubourdieu D., 2005. Influence of water and nitrogen deficit on fruitripening and aroma potential ofVitis viniferaL. cv Sauvignon blancin field conditions. J. Sci. Food Agric. 85:73–85.

Farquhar G. D., Ehleringer J. R., Hubick K. T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503–537.

Gaudillère J.-P., van Leeuwen C., Ollat N., 2002. Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. J. Exp. Bot. 53:757–763.

Guix-Hébrard N., Voltz M., Trambouze W., Garnier F., Gaudillère J.P., Lagacherie P., 2007. Influence of watertable depths on the variation of grapevine water status at the landscape scale. Eur. J. Agr. 27:187–196.

Sadras V. O., Schultz H. R., 2012. Grapevine, in Crop Yield Response to Water, ed. by Steduto P., Hsiao T., Fereres E. and Raes D. FAO, Rome,pp. 460–187.

Sweetman C., Sadras V. O., Hancock R. D., Soole K. L. and Ford C. M., 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. J. Exp. Bot. 65:1–14.

van Leeuwen C., Friant P., Choné X., Tregoat O., Koundouras S., Dubourdieu D., 2004. Influence of climate, soil, and cultivar on terroir. Am. J. Enol. Vitic. 55:207–217.

Tramontini S., van Leeuwen C., Domec J. C., Destrac-Irvine A., Basteau C.,Vitali M., Mosbach-Schulz O., Lovisolo, C., 2013. Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. Plant Soil 368:215–230.

Table 1. Summary of soil properties in the experimental field site.

Plot	**Slope** (%)	**Gravel** (%)	**Texture** (USDA)	**Gravel** (class)	**Slope** (class)
A	20.6	24.2	Loam	high	steep
B	28.5	36.2	Loam	high	steep
C	22.1	14.5	Loam	low	steep
D	6.2	23.9	Clay-loam	high	mild
E	9.1	8.2	Clay-loam	low	mild
F	6.2	10.3	Clay-loam	low	mild
G	6.6	22.9	Clay-loam	high	mild
H	4.1	26.4	Loam	high	mild

Slope was measured with a differential GPS and expressed in percent (change in elevation over a 100-m distance). Texture and gravel content were computed by averaging data measured at approx. 0.1-m intervals over a 1-m depth in each plot. The USDA triangle was used to classify the texture. Gravel content is expressed in percent per volume. The right side of the table shows the data in the categorical classes used in the statistical analysis.

Table 2. Estimates of the linear model explaining $\delta^{13}C$ in function of slope and gravel amount.

	Estimates (‰)
Fixed effects	
(Intercept)	-27.17 (0.20)***
Slope (steep – mild)	0.33 (0.11)**
Gravel (low – high)	-0.36 (0.11)**
Random effects	
Var: plot:year (Intercept)	0.06
Var: year (Intercept)	0.10
Var: Residual	0.02

Fixed effect: asterisks indicate levels of significance forP-values; allterms are significant. Standard error of coefficients is reported in parentheses. Estimates are in the same unit of the response ($\delta^{13}C$, ‰). Random effects: the average variance is reported for the intercepts of plots in years, and years (Var: plot:year; Var: year). Variance of residuals is also reported. The largest variability is between years, followed by plots within years and finally the residuals. ***P<0.001;**P<0.01;*P<0.05.