SPIDERδ: AN EMPIRICAL METHOD TO EXTRAPOLATE GRAPEVINE (*VITIS VINIFERA* L.) WATER STATUS AT THE WHOLE DENOMINATION SCALE USING $\delta^{13}C$ AS ANCILLARY DATA

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Abstract

Aims: The aim of this study is to test a method to extrapolate vine water status (estimated by the water potential; Ψ) over a whole appellation (protected geographical indication). The spatial extrapolation is based on an empirical approach that combines a reference site (baseline measurements) and carbon isotope discrimination ($\delta^{13}C$) values as ancillary data (AD).

Methods and results: Experiments were conducted on the whole Tavel appellation (Gard, France). The study focused on the dominant variety: Grenache. Ψ was measured as predawn leaf water potential and was monitored over three consecutive years, 2008, 2009 and 2010, on 10, 24 and 24 sites, respectively. $\delta^{13}C$ measurements were made at harvest in 2010 on the 24 sites. The spatial model (SPIDERδ) was calibrated using Ψdata from 2009 and 2010 and $\delta^{13}C$ data from 2010. The quality of prediction was tested on the 2008 data, considered as an independent data set. The results show that SPIDERδ was relevant in estimating Ψ at the whole appellation scale. The extrapolation model significantly improves the prediction ($R^2 = 0.88$) compared to a conventional method based on Ψ averages across the appellation ($R^2 = 0.66$).

Conclusion: Based on a single measurement taken at time «*t*» on a reference site, SPIDERδ makes it possible to estimate Ψ on all sites where a $\delta^{13}C$ value is available. The use of AD like $\delta^{13}C$ makes it possible to consider the spatial extrapolation of Ψ with higher spatial resolution than when only direct measurements are used to calibrate the model.

Significance and impact of the study: This work demonstrates the value of using an AD like $\delta^{13}C$ to assess Ψ at a scale larger than the single field. This significant result opens the door to the practical use of spatial extrapolation models with higher spatial resolution.

Key words: predawn leaf water potential, spatial variability, spatial extrapolation, carbon isotope discrimination

Résumé

Objectif : L'objectif de ce travail est de tester une méthode d'extrapolation de l'état hydrique de la vigne (estimé par le potentiel hydrique ; Ψ) à l'échelle de toute une appellation. Le modèle d'extrapolation est basé sur une approche empirique qui combine une mesure de référence sur un site défini et des mesures de discrimination isotopique du carbone ($\delta^{13}C$) considérées comme données auxiliaires (DA).

Méthodes et résultats : Les expérimentations ont été conduites sur l'appellation de Tavel (Gard, France). L'étude s'est concentrée sur le cépage dominant de l'appellation, le Grenache. Des mesures de potentiel hydrique foliaire de base (utilisé pour estimer Ψ) ont été réalisées sur trois années consécutives, 2008, 2009 et 2010, sur respectivement 10, 24 et 24 sites de mesure. En 2010, des mesures de $\delta^{13}C$ ont été effectuées à la vendange sur les 24 sites. Le modèle spatial (SPIDERδ) a été étalonné grâce aux données Ψ de 2009 et 2010 et aux données $\delta^{13}C$ de 2010. La qualité de la prédiction a été testée sur les données de 2008, considérées comme un ensemble de données indépendantes. Les résultats montrent que l'approche est tout à fait transférable à l'échelle d'une appellation. Le modèle d'extrapolation améliore significativement la prédiction ($R^2 = 0,88$) comparée à une méthode conventionnelle basée sur la moyenne de Ψ sur l'ensemble de l'appellation ($R^2 = 0,66$).

Conclusion : Sur la base d'une seule mesure prise à un temps «*t*» sur un site de référence, SPIDERδ rend possible l'estimation de Ψ sur tous les sites où une mesure de $\delta^{13}C$ a été effectuée. L'utilisation de DA telles que le $\delta^{13}C$ permet d'envisager l'extrapolation spatiale de Ψ avec une résolution spatiale plus importante que lorsque les mesures directes de Ψ sont utilisées seules pour étalonner le modèle.

Importance et impact de l'étude : Cette étude représente une avancée importante puisqu'elle démontre l'intérêt d'utiliser une DA comme le $\delta^{13}C$ pour extrapoler Ψ à l'échelle d'un bassin de production viticole. Cette connaissance permet d'envisager l'utilisation pratique des modèles d'extrapolation spatiale avec une plus grande résolution spatiale.

Mots clés : potentiel hydrique foliaire de base, variabilité spatiale, extrapolation spatiale, discrimination isotopique du carbone

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INTRODUCTION

Vine water status can be highly variable at the within-field level (van Leeuwen *et al.,* 2006), at the vineyard level (Taylor *et al.*, 2010) and, of course, at the whole appellation level (Baralon *et al.*, 2012). Variability in vine water status induces a great variability of vine response in terms of vigour, yield, precocity and grape composition (Tisseyre *et al.*, 2008). Hence, characterizing the spatial variability of the vine water status is a key issue for terroir study (Seguin 1983, van Leeuwen *et al.*, 2009), as it provides important information to manage and/or assess grape quality (van Leeuwen *et al.*, 2009, Hakimi Rezaei and Reynolds, 2010).

In a review paper, Acevedo-Opazo *et al.* (2008) discussed the importance of methods for spatial monitoring of vine water status. Furthermore, the same authors proposed an empirical spatial model (Equation 1) to predict the vine water status (estimated with the water potential ; Ψ) across a given domain (vineyard block, vineyard, region, etc.).

$$\Psi(s,t) = a_s.\Psi(s_{re},t) \text{ [eq.1]}$$

The principle of the model is to extrapolate a reference Ψ value $\Psi(s_{re},t)$ measured at a reference site s_{re} and time t. The extrapolation is based on a linear relationship defined by the coefficients a_s whose values are specific to site s. The model provides an estimate $\Psi(s,t)$ of Ψ values at any site s where a coefficient a_s is available. This spatial model has been successfully tested at the within-field level (Acevedo-Opazo *et al.*, 2010a) and at a vineyard level constituted of several blocks (Taylor *et al.*, 2011). More recently, the model has been successfully tested at the whole denomination scale by Baralon *et al.* (2012). At this scale, the approach was called SPIDER (SPatial extrapolation of the vIne water status at the whole DEnomination scale from a Reference site). Note that in Baralon *et al.* (2012), the term denomination refers to an appellation (a legally defined and protected geographical indication). The scale at which these authors have actually tested the approach is a small appellation of the south of France (Tavel) where climatic conditions where assumed homogeneous. For consistency, the term denomination was kept in this paper.

At the denomination scale, SPIDER proved to be more accurate in estimating Ψ values than a classical approach based on the average of Ψ values sampled over the domain. At this scale, SPIDER may be of particular interest as a decision support tool for field selection based on the water restriction experienced by the vines (Reynard *et al.*, 2011) or for identifying zones where irrigation is most needed. However, as outlined by Baralon *et al.* (2012), applying SPIDER at a large scale raised some practical limitations, of which the most important is the spatial resolution that is possible with such an approach. Indeed, the spatial resolution of the model is limited by the number of sites where a_s coefficients are known. The determination of a_s values needs Ψ to be monitored over several dates on each site s. Considering Ψ measurements are cumbersome and cost prohibitive, this practical constraint necessarily limits the number of measurements and the resulting spatial resolution of the model. One possibility to overcome this limitation is to use ancillary data (AD) related to the water status of the vines and available at a high or medium spatial resolution. At the within-field level and in Mediterranean conditions, Acevedo-Opazo *et al.* (2010b) successfully proposed the use of AD like exposed leaf area or normalized difference vegetation index (NDVI) (Rouse *et al.*, 1973) derived from multispectral airborne images to improve the spatial resolution of the model. However, at the denomination scale, these AD cannot be implemented since they may be affected by field characteristics like planting density, trellising, training system, etc. (Tisseyre *et al.*, 2011). Therefore, at large scale, the variability of these AD may not only be related to spatial variability of vine water status and hence may be irrelevant to perform Ψ extrapolation.

More recently, Herrero-Langreo *et al.* (2013) successfully proposed the use of carbon isotope discrimination (called hereafter $\delta^{13}C$) as AD to extrapolate Ψ values at the within-field level. $\delta^{13}C$ provides an integrative measure of water restriction during grape ripening (Gaudillère *et al.* 2002). It is based on the following principle : ambient atmospheric CO_2 contains 98.9 % of ^{12}C isotope and 1.1 % of ^{13}C isotope. Given that ^{12}C is more easily incorporated in hexoses during photosynthesis, the sugar produced by photosynthesis contains a higher proportion of ^{12}C isotope than ambient CO_2. This process is called "isotope discrimination". When plants face water deficit conditions, isotope discrimination is reduced because of stomatal closure (Farquhar *et al.*, 1989). Therefore, the $^{12}C/^{13}C$ ratio in photo assimilates provides a signature of plant water status over the period in which they were synthesised. The greatest sensitivity of $\delta^{13}C$ response to water restriction occurs during two critical periods of the berry ripening process : around veraison and 4-6 weeks before harvest (Santesteban *et al.*, 2012). The empirical model proposed by Herrero-Langreo *et al.* (2013) is derived from the approach proposed by Acevedo-Opazo *et al.* (2010a) (Equation 2).

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$$\Psi(s,t) = [b_0 + b_1.\delta^{13}C(s)].\Psi(s_{re},t) \quad [eq.2]$$

Similarly to Equation 1, the principle of the model is to extrapolate a Ψ value, $\Psi(s_{re},t)$, measured at a reference site s_{re} and time t. The extrapolation is performed using a linear function defined by two coefficients (b_0 and b_1) that relate Ψ to the $\delta^{13}C$ values over each site (s) ($\delta^{13}Cs$) of the spatial domain under consideration. Therefore, this approach provides an estimate of Ψ values, $\Psi(s,t)$, at any site s where a $\delta^{13}C$ value is available.

Compared to SPIDER, the approach proposed by Herrero-Langreo *et al.* (2013) presents two major practical advantages. First, its calibration is less cumbersome and less cost prohibitive because the model requires the determination of only two parameters (b_0 and b_1) at the whole denomination scale. Therefore, the calibration hypothetically needs to monitor Ψ on only two sites (in addition to the reference site). This reduces drastically the number of Ψ measurements required to calibrate the model. Second, the spatial resolution of Ψ estimation may be improved very easily since it only depends on the spatial resolution at which the $\delta^{13}C$ values are available. Vine water status assessment with $\delta^{13}C$ is carried out on grape juice sampled just before ripeness. Samples can then be frozen for later analysis. Unlike direct Ψ measurement, $\delta^{13}C$ measurement is less subject to time constraints. The only factor limiting the number of measurements is the cost of the analysis and the time required to collect the samples. Therefore, $\delta^{13}C$ makes it possible to collect a significant number of samples over a large area. At the denomination scale, $\delta^{13}C$ could be used as a medium spatial resolution AD for Ψ spatial extrapolation.

Considering the practical interest of such an approach to map Ψ, the objective of the current work is to test the methodology of Herrero-Langreo *et al.* (2013) at the whole denomination scale. This refinement of the SPIDER approach is called hereafter SPIDERδ. To our knowledge, such an approach has never been used at this scale. It is very likely that $\delta^{13}C$ is not influenced by production systems (rootstock, vine age, planting density, training system, etc.) in another way than their effect on vine water status. However, at this scale, its use as an AD to perform Ψ extrapolation needs to be verified. The present work uses the same data base as Baralon *et al.* (2012). It aims at testing the possibility and the value of using $\delta^{13}C$ to calibrate an empirical spatial model (Equation 2) to extrapolate Ψ values at a whole denomination scale.

MATERIALS AND METHODS

The experimental site and the measurements were described in detail in a previous paper (Baralon *et al.,* 2012). The main features of the experiment are summarised hereafter.

1. Experimental site

The study was carried out in the Tavel denomination (44°0'46.34"N, 4°41'52.84"E, Gard, France). The study site has an area of 950 ha (Figure 1) and

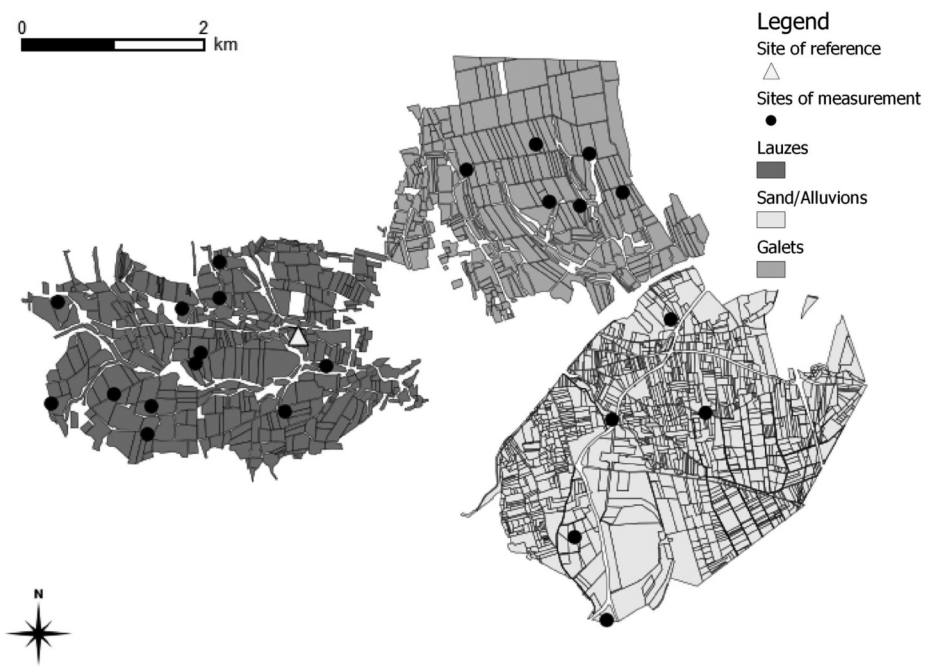


Figure 1. Location of the three soil units and measurement sites in 2009 and 2010 over the whole Tavel denomination (from Baralon *et al.* 2012).

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exhibits significant variability in soil composition, elevation (average of 80 to 200 m), field area, and production systems (vine age, rootstock, planting density, trellising system, soil management, etc.). It has a Mediterranean climate characterized by high summer evapotranspiration rates, its pedo-climatic context leads to strong spatial variability in Ψ, and the vine fields are not irrigated. The Tavel denomination has three main varieties, of which Grenache is dominant and accounts for around 60 % of the planted area. Since the objective was to focus on the spatial model over the whole denomination, the analysis was simplified by taking into account only Grenache. This choice removed any potential varietal effect from the analysis. Furthermore, it allowed taking into account a significant proportion of the spatial variability due to environmental factors since areas planted to Grenache variety remained large enough and were spread over the whole study site. The Tavel denomination presents a large diversity of rootstocks, of which 110R, Rupestris du Lot, 140Ru and 41B are the most common. Rootstock information is hardly known by the growers. Therefore, our experiment may encompass several rootstocks representative of the area. Three soil types are present in the denomination (Figure 1): i) Lauzes (stony soil on marly calcareous soil with argillaceous seams), ii) Galets (upper terraces with rounded quartzite galets and red clay) and iii) Sand/Alluvions (light soil on Pliocene sands with low water holding capacity).

2. Seasonal climatic characterization

During the three years of the experiment (2008, 2009 and 2010), climatic data were recorded by a weather station located approximately in the centre of the study site. The dryness index (DI) was derived from the climatic data as proposed by Tonietto and Carbonneau (2004). It was used to characterize the seasonal potential soil water balance and its potential effect on Ψ. DI is an indicator of the dryness level calculated over a 6-month period (April 1st to September 30th). Based on the DI values, four classes are usually considered (Tonietto and Carbonneau, 2004): humid (wet climate with DI >150 mm), sub-humid (50 < DI ≤ 150 mm), moderately dry (-100 < DI ≤ 50 mm) and very dry (DI ≤ -100 mm). The last two classes represent conditions of medium to high levels of water restriction for the vine.

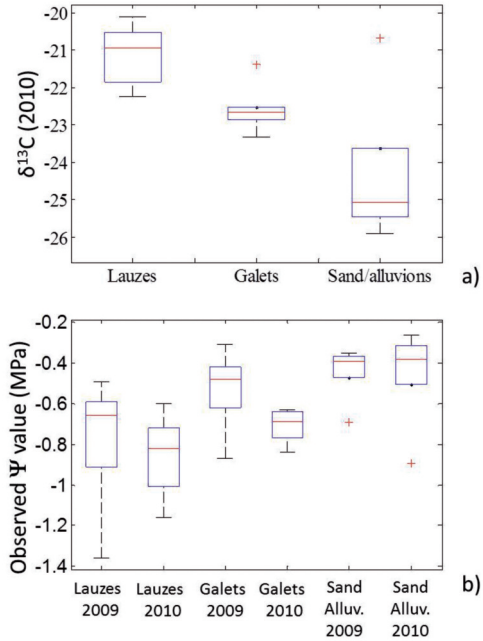


Figure 2. Distribution of the $\delta^{13}C$ (a) and Ψ values (b) observed on the different soil units. The distribution of Ψ values corresponds to one date of measurement close to veraison for 2009 and 2010.

Table 1 shows accumulated precipitation (C.Pp), accumulated reference evapotranspiration ($C.ET_0$) and DI values for each year of the experiment. Season 2008 had the highest DI (DI = 85.4 mm), corresponding to sub-humid climate; seasons 2009 and 2010 had DI values corresponding to a moderately dry climate.

3. Measurements

Vine water status was assessed with the predawn leaf water potential. Hereafter, Ψ will refer exclusively to predawn leaf water potential. Ψ was measured over three consecutive years (2008, 2009 and 2010) in 10,

Table 1. Summary of the main climatic variables (April-September period) characterizing the growing conditions during the three years of the experiment.

Year	C.Pp (mm)	$C.ET_0$ (mm)	DI (mm)
2008	346.8	941.0	85.4
2009	354.1	1068.7	27.0
2010	136.0	860.7	-46.8

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Table 2. Summary of predawn leaf water potential (Ψ) and carbon isotope discrimination ($\delta^{13}C$) measurements for investigated years and soil units (adapted from Baralon *et al.* 2012).

Soil Unit	Number of Ψ and $\delta^{13}C$ measurements						
	Years						
	2008		2009		2010		
	sites	dates	sites	dates	sites	dates	$\delta^{13}C$
Sand/Alluvions	2	7	5	10	5	9	5
Lauzes	6	7	13	10	13	9	13
Galets	2	7	6	10	6	9	6
Total Ψ	70		240		216		
Total $\delta^{13}C$	-		-		-		24

24 and 24 sites, respectively, located throughout the denomination (Figure 1). Other information like elevation, slope, and aspects were used to select the sites in order to encompass a large diversity of situations at the denomination scale. At the within-field level, the sites were chosen after a survey performed by two viticulturists and local growers. The objective was to provide sites as representative as possible of the field within which they were located, avoiding too low or too high vigour zones and unhealthy vines.

We acknowledge that the use of 24 sites is far from being sufficient to provide a decision map at the denomination scale. However, the goal of the study was not to provide an exhaustive map of Ψ but to test the relevance of the SPIDER model (Baralon *et al.,* 2012) calibrated with $\delta^{13}C$ measurements for a large diversity of situations. Ψ monitoring consisted, respectively, of 7, 10 and 9 measurement dates for the years 2008, 2009 and 2010 (Table 2). Measurements were carried out between 04:00 and 06:00 using a pressure chamber (Scholander *et al.,* 1965). Five adjacent vines were measured at each site and values were averaged to obtain a site value. $\delta^{13}C$ was measured in 2010 at each of the 24 sites following the protocol outlined by van Leeuwen *et al.* (2010). Briefly, grape samples were taken prior to harvest. For each site, 200 berries were sampled on five adjacent vines, from various parts of the clusters. Samples were placed in plastic bags and grape juice was extracted by crushing the berries inside the bag. The grape juice was then frozen and sent to a laboratory (Dubernet, Montredon-des-Corbières, France) for $\delta^{13}C$ analysis by isotope-ratio mass spectrometry (IRMS) (van Leeuwen *et al.,* 2010).

The sampling sites were geo-referenced with a stand-alone eTrex GPS receiver (Garmin International Inc., Olathe, Kansas). In addition to soil units, the sampling sites encompassed a large diversity of situations. For the 24 samples of years 2009-2010, elevation ranged from 79 to 202 m (above sea level), planting density ranged from 3000 to 4500 vines per hectare, and different training systems were taken into account (no trellising and one, two, or three levels of wires for trellising system). Note, however, that for the three years of the study all the sites presented north-south orientated rows and inter-row weed control was performed either chemically or mechanically.

4. Computation of the spatial model

Model computation (Equation 2) required several steps, as follows:

1. Choice of the reference site (s_{re})

The choice of the reference site can affect the accuracy of prediction from the proposed model, particularly in high water restriction conditions (non-irrigated) when there is a wide range of variation in Ψ over the whole denomination. In a previous analysis, Baralon *et al.* (2012) showed that the random selection of the reference sites did not adversely affect the model output. The reference site was therefore chosen randomly.

Still, a sensitivity analysis was performed to confirm the validity of the method when using $\delta^{13}C$ as AD to calibrate the model. This sensitivity analysis aimed to test the quality of the model generated after calibration. It was carried out on the 2009 and 2010 measurements, using each of the 24 measurement sites available (Table 2) as reference site. This procedure generated 24 different models. For each of them, the standard error of calibration (SEC) (see next section) was used to assess its ability to predict Ψ on all the 23 remaining sites.

2. Model calibration

The calibration of the model was implemented using the 2010 $\delta^{13}C$ data as AD. It was carried out across the 2009 and 2010 Ψ measurement dates. For these two

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years, the accuracy of the calibration was estimated across all sites and dates using the SEC and the proportion of variance (R^2) explained by the model. The SEC was computed as follows:

$$SEC = \sqrt{\frac{\sum_{1}^{n}\sum_{1}^{m}(E(s,t))^2}{(n \times m)-1}} \text{ , with } E(s,t) = \left(\hat{\Psi}(s,t) - \Psi(s,t)\right) \text{ [eq.3]}$$

Where *n* is the number of sites and *m* is the number of available dates.

3. Model prediction

The ability of the model to predict Ψ values was assessed using the 2008 data set. As the 2008 data set was not used to calibrate the model, it allowed to test the prediction of the model on an independent data set. Two standard errors of prediction (SEP) were then computed:

$$SEPt = \sqrt{\frac{\sum_{1}^{n}(E(s,t))^2}{n-1}} \quad \text{and} \quad SEPs = \sqrt{\frac{\sum_{1}^{m}(E(s,t))^2}{m-1}} \quad \text{[eq.4]}$$

The temporal standard error of prediction (SEPt) indicated how well the model on average predicts across the domain at a given date when a reference measurement was taken, while the spatial standard error of prediction (SEPs) indicated how well each point was predicted and identified areas in the domain that were correctly or poorly predicted. Thus, the first indicated whether the model is valid temporally and the second indicated the spatial pattern of prediction in the domain and allowed identifying areas where larger prediction errors occurred.

5. Computation of reference models

The results of SPIDERδ were compared to two reference models: the Non-Spatial model (NS) and the SPIDER model (Baralon *et al.,* 2012). In this work, NS was considered as the best possible estimation of Ψ in absence of a spatial model, while SPIDER was considered as the best possible estimation of Ψ with a spatial approach.

In NS, the reference measurement was considered as the mean Ψ over the whole denomination. Therefore, at each date and any location, the predicted Ψ was the mean. In SPIDER (Equation 1), spatial extrapolation required the calibration of the a_s coefficient which is site specific, using a data base of Ψ values. In this work, SPIDER is not considered as the best possible spatial model but as a reference spatial model since extrapolation relies on coefficients calibrated with Ψ values instead of AD. The comparison with SPIDER aims to quantify the relative loss of precision of the estimates when the spatial extrapolation is performed with SPIDERδ, where the extrapolation is based on a model defined at the whole denomination scale (Equation 2).

6. Data analysis and mapping

Mapping was done with Quantum GIS software (version Copiapo 1.7.3, under General Public Licence). Model computation and evaluation was carried out with Matlab (Mathworks, Natick, Massachusetts).

RESULTS

1. Distribution of $\delta^{13}C$ and Ψ values across the denomination

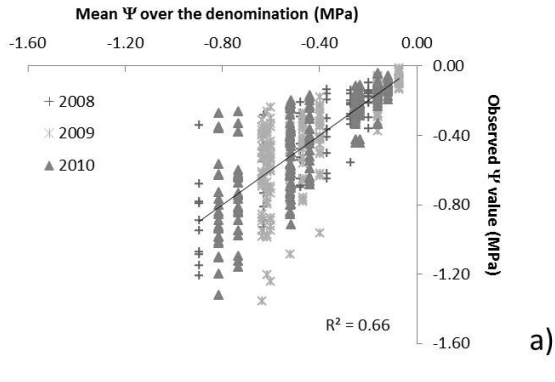
The $\delta^{13}C$ values observed in 2010 (Figure 2a) and the Ψ values observed at a date close to veraison in 2009 and 2010 (Figure 2b) show the large spatial variability of plant water status across the denomination. Although part of this variability is explained by differences between soil units, a large variability may be observed within soil units (Figure 2b). Whatever the year and/or the estimation method, the different soil units present the same water restriction pattern: Lauzes present the highest water restriction, Sand/Alluvions the lowest, and Galets an intermediary state. This trend is observed for the median, but the boxplots highlight the observed variability.

In accordance with computed climate indices (Table 1), water restriction was higher in 2010 than in 2009 for each soil unit. This inter-annual variability does not affect the rank of the median water restriction for each soil unit (Lauzes > Sand/Alluvions > Galets). Finally, both figures (2a and 2b) show the strong consistency between the $\delta^{13}C$ values measured at harvest and the Ψ values observed at veraison across the denomination. This result shows the

Table 3. Standard error of calibration (SEC) according to years and the different pedological units

	Years		Soil Units		
	2009	2010	Lauzes	Sand/Alluvions	Galets
SEC (MPa)	0.12	0.11	0.14	0.08	0.10

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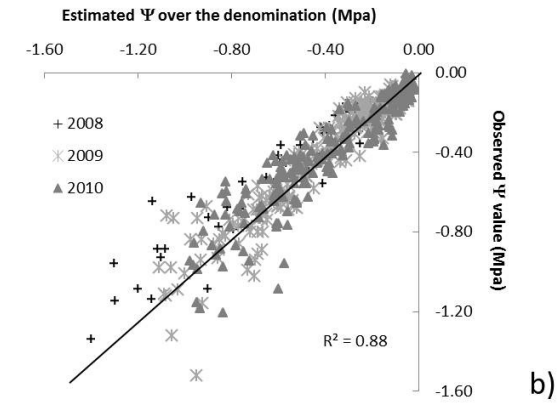


Figure 3. Prediction results of Ψ values with the reference models : (a) Non-Spatial model (NS) and (b) SPIDER model for 2008, 2009 and 2010. Total of 526 measurements (from Baralon *et al.*, 2012).

potential value of $\delta^{13}C$ as AD to perform Ψ extrapolation.

2. Results of the reference models (NS and SPIDER)

The results of the reference models are presented in Figures 3a and 3b. The fit of NS was $R^2 = 0.66$ (Figure 3a), while the fit of SPIDER was $R^2 = 0.88$ (Figure 3b). For the latter, results were thereafter obtained with a randomly chosen reference site that happens to be located on the Lauzes soil unit (Figure 1). In the next section, the results of SPIDERδ are compared to the results of both of these models.

As outlined by Baralon *et al.* (2012), SPIDER significantly improved the predictions, especially in limiting conditions (< -0.4 MPa) where $R^2 = 0.70$ was observed. For NS, a significant difference between non-limiting (> -0.4 MPa) and limiting water conditions (< -0.4 MPa) was observed. In non-limiting conditions, R^2 remained rather high ($R^2 = 0.70$), while in limiting conditions, NS resulted in a poorer fit ($R^2 = 0.33$). In Figure 3a, it is clear that the range (and

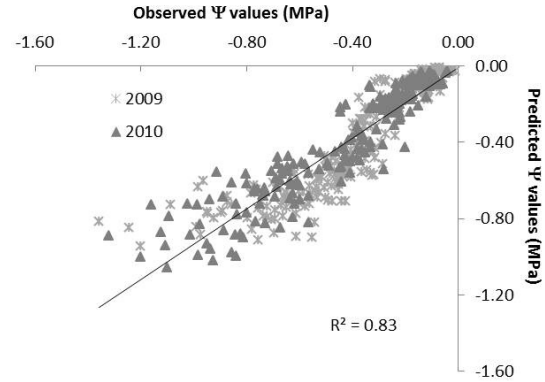


Figure 4. Prediction results of Ψ values from the spatial model (SPIDERδ) calibrated with $\delta^{13}C$ as ancillary data for the years 2009 and 2010 (data set used to calibrate the model).

variance) of measured Ψ increased as water restriction became more severe.

3. Results of the spatial model calibrated with $\delta^{13}C$ values (SPIDERδ)

The results of SPIDERδ were obtained with the same reference site as SPIDER (Figure 1). The plot of SPIDERδ predictions against observed values is shown in Figure 4 (compared to Figures 3a and 3b). Considering the calibration on all the available $\delta^{13}C$ measurements in 2010 to estimate Ψ values in 2010 and 2009, the fit of SPIDERδ is $R^2 = 0.83$ (0.65 if only limiting conditions corresponding to Ψ < -0.4 MPa are considered). Compared to NS, the addition of AD like $\delta^{13}C$ into the model considerably reduced the dispersion of the predicted values, particularly during periods of high water restriction. Compared to SPIDER, a slight decrease in R^2 was observed. This decrease is due to the higher dispersion of the predictions as well as a systematic over estimation of Ψ values observed for very high water restriction (Ψ < -1.1 MPa).

Figure 4 does not show any patterns related to either years or pedological units. A non-parametric analysis of variance (Kruskal-Wallis test, $p < 0.05$) was used to test any potential effect of the soil unit or year on the residuals of the model. The test does not show any statistical difference (results not shown). In addition, Table 3 shows the SEC for both years (2009 and 2010) and the three soil units. The SEC remains low (≤ 0.14 MPa) for all the years and all the soil units, showing that the prediction from a reference site located on the Lauzes soil unit may be relevant for the other soil units at all dates.

4. Results of the prediction of SPIDERδ

The plot of model predictions against observed values is shown in Figure 5. The predictions were performed

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Table 4. Spatial standard error of prediction (SEPs) according to the different pedological units.

	Soil Units		
	Lauzes	Sand/Alluvions	Galets
SEPs (MPa)	0.12	0.06	0.09

Table 5. Temporal standard error of prediction (SEPt), mean Ψ and standard deviation (Std) observed for each date (*t*) and phenological stage (*F*: flowering, *V*: veraison) in 2008.

	Dates						
	t_1	t_2	t_3	t_4	t_5	t_6	t_7
	06/26	07/10	07/24	07/30	08/07	08/21	08/30
Phenological stages (MPa)	F + 21 days	F+ 32 days	F + 46 days	F + 52 days	V	V +14 days	9 days before harvest
Mean	-0.16	-0.20	-0.27	-0.37	-0.48	-0.63	-0.90
Std	0.02	0.07	0.11	0.17	0.17	0.20	0.25
SEPt	0.04	0.05	0.11	0.13	0.09	0.08	0.10

with the 2008 data set, which was not used for the calibration. Figure 4 shows the predictions made from the Ψ measured on the reference site, which were extrapolated according to SPIDERδ with the 2010 $\delta^{13}C$ values and calibrated with the 2009 and 2010 Ψ values. The fit of the model is $R^2 = 0.87$ for the three soil units together (0.76 if only limiting conditions corresponding to Ψ < -0.4 MPa are considered). Surprisingly, the observed R^2 was higher for the independent data set (2008) than for the data set used for calibration (2009 and 2010).

Table 4 shows that SEPs does not change much compared to SEC (Table 3) whatever the soil unit. Further, it confirms that the model calibrated for 2009 and 2010 may be used on other years. Note that the Lauzes soil unit systematically presents the highest SEC and SEPs. A better estimation was expected since this is the soil unit on which the reference site is located. This point was already observed by Baralon *et al.* (2012) with SPIDER. The 2008 data set presents a higher water restriction compared to 2009 and 2010 (Figure 3a). Moreover, the spatial variability of Ψ was consistently larger on the Lauzes soil unit (Figure 2b). This feature may explain the larger spatial variability in 2008 on this soil unit than in 2009 and 2010. This observation points out a limitation due to our experimental design but not to $\delta^{13}C$ measurements used as AD to calibrate the model.

Table 5 shows the SEPt over the seven different measurement dates in 2008. The mean Ψ increased throughout the summer season from the first date of measurement (t_1) to the last (t_7). The standard deviation (Std) also increased, showing that the spatial variability was significant for high water restriction. For low water restriction (Ψ > -0.36 MPa), the SEPt remained very similar to the Std, showing that the spatial model does not improve the prediction. For high water restriction, the SEPt remained low compared to the Std, showing the improvement in prediction brought by the spatial model.

5. Results of the sensitivity to the choice of the reference site

The study of the sensitivity to the choice of the reference site shows that whatever the site considered, the SEC was between 0.10 and 0.15 MPa (Figure 6). The mean SEC was 0.13 MPa. It remains smaller than that observed with NS (SEC = 0.19 MPa) and is slightly higher than that observed with SPIDER (SEC = 0.12 MPa). The quality of the estimations remains always better with SPIDERδ than with NS, whatever the reference site. However, it varies slightly, showing that the choice of the reference site affected the quality of the model. The SEC observed was 0.11, 0.11 and 0.13 MPa respectively for Lauzes, Galets and Sand/Alluvions. The highest SEC observed for the Sand/Alluvions soil unit was not significantly different from the other values (results not shown).

The error map shown in Figure 6 was made to highlight any potential spatial patterns. It shows the spatial distribution of SEC in relation with the choice of the reference site. The reference sites leading to medium or high quality models (SEC < 0.12 MPa) can be located either in the centre or in the periphery of the denomination and can be found on all of the soil units. The same observation can be made for the

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reference sites providing models with a higher error (SEC > 0.13 MPa). The Sand/Alluvions soil unit had a higher proportion of sites that led to significant SEC. Despite this observation, the SEC related to the choice of the reference site can be considered randomly distributed.

Note that the site randomly chosen to conduct this study (Figure 1) results in SEC between 0.12 and 0.13 MPa. This reference site is among the good sites but is not the best.

DISCUSSION

This study demonstrates the possibility to use $\delta^{13}C$ values as AD to extrapolate Ψ over a large region corresponding to a whole denomination. At this scale, $\delta^{13}C$ seems to be more affected by vine water status than by rootstock, vine age or training system. Therefore, it constitutes relevant information to extrapolate Ψ. This approach increases the informative value of $\delta^{13}C$ measurements. Until today, $\delta^{13}C$ measurements were only used to estimate plant water status spatial variation during a given season (van Leeuwen *et al.* 2010), providing one single integrative map per season. This study demonstrates the possibility to use the $\delta^{13}C$ of the previous year to run a spatial model the year to come.

Compared to a non-spatial approach, SPIDERδ significantly improves the quality of Ψ estimations. Note, however, that these results remain specific to the studied area. Although it encompasses three very different soil units, these present similar water regimes. This feature can explain why a reference site located on a given soil unit is relevant to provide an estimation on the other soil units. On a smaller area,

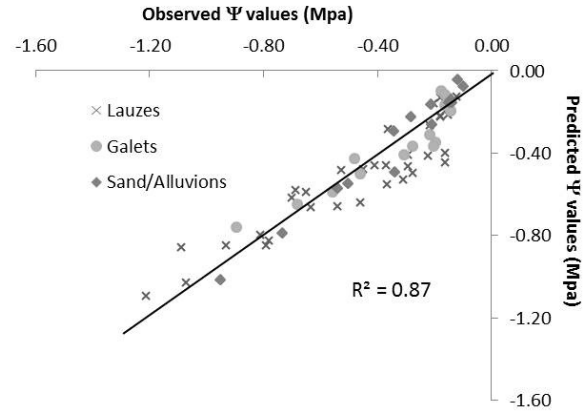


Figure 5. Prediction results of Ψ values from the spatial model (SPIDERδ) for the independent 2008 data set.

Taylor *et al.* (2011) showed that extrapolation with SPIDER was irrelevant between two soil units with very different water regimes. The application of SPIDERδ or SPIDER to a new area necessarily requires knowledge of the soil units. At least the first year, one reference site per soil unit should be considered.

Compared to SPIDER (calibrated with reference Ψ measurements), the estimates obtained with SPIDERδ proved to be slightly less accurate. This study did not identify the origin of this decrease in accuracy, but two assumptions may be stated. First, the sampling quality and the resulting measurement accuracy. Note that this aspect is also crucial with SPIDER, which requires Ψ to be monitored on specific sites. However, estimating the $\delta^{13}C$ from a sample of berries spread over five consecutive plants may have a more erratic

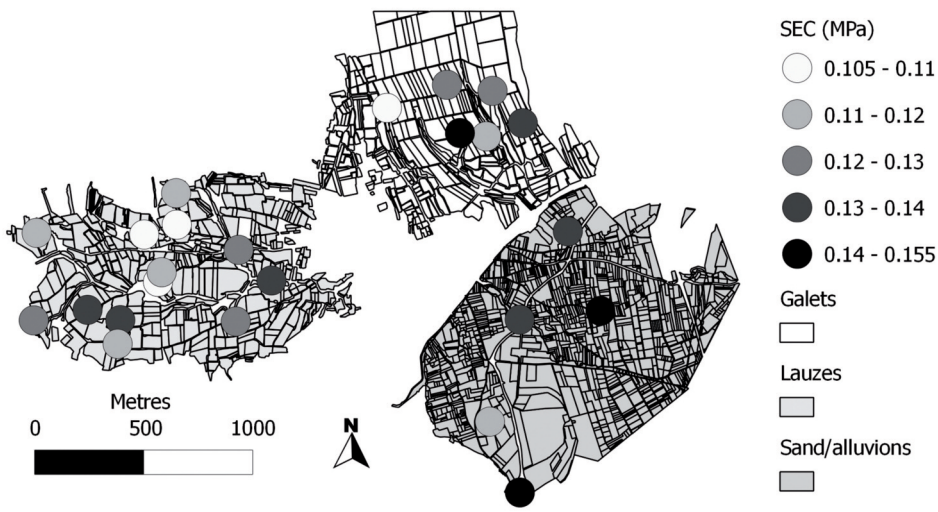


Figure 6. Map of the standard error of calibration (SEC) when each of the 24 available sites was used as reference site.

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behaviour than the site-specific coefficient a_s (Equation 1) estimated from several Ψ measurements at different dates on the same five plants. Second, the significance of water restriction in the year of $\delta^{13}C$ measurements. In that respect, Herrero-Langreo *et al.* (2013) showed that at the within-field level, the water restriction observed the year of $\delta^{13}C$ measurements could affect the quality of the model. Over the three years of their study, the $\delta^{13}C$ values of the year with the highest water restriction led to a better model. These authors assumed that the spatial variability of Ψ was better highlighted (higher signal/noise ratio) when the $\delta^{13}C$ magnitude of variation was the highest, leading to a better calibration of the spatial model. In the case of our experiment, $\delta^{13}C$ was measured in 2010, where significant water restriction was observed (Figure 3a). If available, $\delta^{13}C$ values observed in 2008 (higher water restriction than in 2010) would probably have led to a better model. This point is currently being investigated with new experiments. Indeed, the year of $\delta^{13}C$ measurements necessarily affects the quality of the model. Therefore, the practical implementation of SPIDERδ requires considering several years of $\delta^{13}C$ measurements. This will increase the possibility to have a year with a significant water restriction and to calibrate the best possible model. Note that this constraint also applies for the calibration of SPIDER (with Ψ data).

In this context and at the spatial scale investigated here, SPIDERδ may present several practical advantages : i) the use of AD such as $\delta^{13}C$ makes it possible to sample a large number of sites at harvest. This feature helps improve the potential spatial resolution of Ψ values estimated with the spatial model and addresses an important limitation of SPIDER. The spatial resolution of SPIDERδ is only limited by the number of samples that can be collected and the cost of the analysis ; ii) it relies solely on $\delta^{13}C$ and direct Ψ measurements, which can be performed by wine growers. Therefore, the calibration of the model can be implemented within a conventional monitoring of Ψ. The method does not require spatial estimates of other variables related to soil or other environmental factors that may be expensive and difficult to measure ; iii) the model can also be calibrated from previous measurements. Therefore, it makes it possible to use existing data bases of Ψ or $\delta^{13}C$ (historical data bases), provided that the data are geo-located ; and iv) the model is simple, and its calibration requires the determination of only two coefficients, b_1 and b_0 (Equation 2). In theory, the calibration of the model requires to monitor Ψ on only three sites : the reference site and any two sites over the considered area. This characteristic greatly reduces the number of field measurements and presents a significant practical advantage. However, in practice, we do not recommend using such a small number of calibration sites.

Indeed, our results showed that the approach remains sensitive to the choice of the reference site. Therefore, the quality of the model may be sensitive to the choice of the calibration sites too. Moreover, Lesch *et al.* (1995) showed that the calibration of spatial models requires a data set that includes the range of variation of the parameter to be estimated. In our case, the calibration sites should therefore include sites where water restriction is low, moderate and high. The random selection of a small number of calibration sites may not fit with these requirements and may result in a poor model. The following section details the proposals to overcome these constraints. It deals with the choice of the calibration sites and the choice of the reference site.

Regarding the choice of the calibration sites, a simple recommendation would be to calibrate the model over two years. Year 1 : i) measurement of $\delta^{13}C$ over the whole area with the desired spatial resolution, ii) identification of the spatial variability of Y, and iii) choice of calibration sites among the sites presenting low, moderate and strong water restriction. Methods to select automatically the best possible calibration sites are under investigation. They were successfully tested by Herrero-Langreo *et al.* (2012) for calibrating a similar spatial model at the within-field level. These methods are easily transferable to the scale investigated here. Year 2 : monitoring of Ψ on calibration sites and model determination. Note that if year 1 presents a low water restriction, $\delta^{13}C$ measurements may be repeated in year 2. If higher water restrictions are observed in year 2, the quality of the model may be improved (Herrero-Langreo *et al.* 2012).

The incidence of the reference site was observed with either SPIDER or SPIDERδ. Therefore, this effect may not be attributed to the use of $\delta^{13}C$ as AD but to the limitations of the approach on large areas. Regarding the choice of the reference site, at the within-field level, Acevedo-Opazo *et al.* (2013) showed that the consideration of simple rules improved the selection of relevant reference sites : avoid border effects and conditions (diseases, weed control, drainage problems, etc.) that affect Ψ. However, some of these conditions are difficult to identify since they depend on cultural practices during the year. A simple recommendation to overcome this constraint is to consider several potential reference sites (3-4 sites) and then select the best one. The drawback of this recommendation is that it increases the number of sites requiring Ψ monitoring.

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SPIDERδ has some common limitations with SPIDER. These limitations have been widely detailed by Baralon *et al.* (2012). A brief summary is recalled hereafter : i) it presents limitations on irrigated vineyards since the linear relationship with the reference site may be altered by irrigation ; ii) it assumes that climate is approximately the same over the considered area, otherwise, the linear relationship with the reference site could be affected. Determining a zone over which precipitations can be assumed homogeneous remains problematic : it can be based on a topographic survey or rely on the exploitation of data from sensor networks or weather station network (Matese *et al.,* 2009) ; and, finally, iii) it assumes that parameters that affect predominantly Ψ depend on stable environmental factors like soil. Other parameters like disease, weed control, etc. are supposed to have little to no effect on Ψ. This assumption may be true in Mediterranean climate, which results in particularly high water restriction. In this context, the spatial variability is mainly determined by stable environmental factors like soil. The effect of these factors prevails when water restriction is particularly important, resulting in a significant magnitude of variation of the plant water status and a significant spatial variability. Such an approach remains to be validated in moderate water restriction conditions (> -0.4 MPa). This point was investigated at the within-field level by Herrero-Langreo *et al.* (2013). In accordance with these authors, our results suggest that the spatial variability explained by SPIDERδ decreases with average water restrictions. Note, however, that even for low water restriction (> -0.3 MPa), SPIDERδ presents the advantage of producing an estimate with equivalent or better quality than a conventional approach with only one single measuring site (the reference site).

SPIDERδ presents, however, specific limitations due to the diversity of varieties and differences in precocity that may be encountered at the spatial scale investigated here. Indeed, Santesteban *et al.* (2012) showed that the greatest sensitivity of $\delta^{13}C$ response to Ψ occurs during two critical periods of the berry ripening process (around veraison and 4-6 weeks before harvest). This study focused on a single grape variety : Grenache. The phenology of this variety is assumed to be homogeneous at the investigated spatial scale. Therefore, observed $\delta^{13}C$ values correspond to Ψ experienced by the vines over the same period of time. Being comparable, they highlight the spatial variability of Ψ. If the model is calibrated with several varieties with different phenology, then $\delta^{13}C$ values correspond to Ψ experienced by the plants at different periods of time. Depending on the climatic conditions of the year, these values may not be comparable and are likely to be unsuitable for SPIDERδ calibration and use. This point deserves to be confirmed by further experiments. In the absence of results, two approaches can be proposed for a practical implementation of the model : i) work with the prevailing grape variety, provided that the number and the spatial distribution of fields planted with this variety fit with expected spatial resolution of the model or ii) work with several grape varieties and calibrate a model on a grape variety basis. This solution has the drawback of providing as many spatial models as grape varieties. Moreover, it may increase significantly the number of calibration sites and the resulting Ψ measurements.

CONCLUSIONS

This study represents a significant step in the prediction of grapevine water status, since it shows the possibility of using an AD like $\delta^{13}C$ to extrapolate Ψ at a scale larger than the single field. It has been demonstrated that at this scale a linear relationship between the Ψ of a reference site and ancillary $\delta^{13}C$ values provided a relevant estimation of Ψ. This linear relationship is simple to determine with a relatively small number of Ψ measurements, it is temporally stable, and it remains relevant over a large territory. This step opens the possibility to provide a spatial extrapolation model of Ψ with a medium to high spatial resolution.

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