

ESTIMATION OF VINEYARD WATER STATUS (*VITIS VINIFERA* L. CV. TEMPRANILLO) FROM THE DEVELOPMENTAL STAGE OF THE SHOOT TIPS

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Abstract

Aims: An experiment was set up to evaluate the visual assessment of « shoot tip stage » as a method to estimate the water status of vineyards and its utility in vineyard management. The specific objectives were (1) determining the functional relationship of shoot tip stage (S) with leaf water potential (Ψ_l) at solar noon, a more traditional method for evaluating vine water stress, and (2) testing its performance and simplicity in field conditions.

Materials and results: A field study was first conducted in 2009 in a vineyard located in the La Rioja wine producing region (Spain). Ψ_l and S measurements were performed at four different phenological stages and the data were collected in a completely randomized design. The relationship between the two parameters was established by multiple linear regression analyses and was validated with data collected in another vineyard during the next year.

Conclusion: The calculated shoot tip stage can be used reliably to estimate vineyard water status and to manage vineyard irrigation.

Significance and impact of the study: The shoot tip method is fast and non-destructive, it does not require any special skill or equipment, it is independent of weather conditions, and the calculations are simple.

Key words: grapevine, *Vitis vinifera*, shoot tip stage, leaf water potential

Résumé

Objectifs: Une expérimentation a été mise en place pour évaluer une méthode visuelle basée sur l'état des apex afin d'estimer de manière opérationnelle l'état hydrique du vignoble. Les objectifs spécifiques sont (i) la détermination d'une relation fonctionnelle entre l'état de l'extrémité des rameaux et le potentiel hydrique foliaire au midi solaire, une méthode plus traditionnelle pour évaluer le stress hydrique de la vigne, et (ii) tester ses performances et la simplicité de sa mise en œuvre sur le terrain.

Méthodes et résultats: La parcelle expérimentale est située dans la région viticole de La Rioja (Espagne). Les mesures Ψ_l et S ont été réalisées à quatre différents stades phénologiques. La relation a été établie par régression linéaire multiple avec les données recueillies dans un dispositif randomisé. La relation a été testée avec les données recueillies dans un autre vignoble au cours de la saison suivante.

Conclusion: En conclusion, l'évaluation visuelle de l'état des apex peut être utilisée de manière fiable pour estimer l'état hydrique de la vigne et pour gérer l'irrigation des vignobles.

Signification et impact de l'étude: La méthode des apex est rapide et non destructive, elle ne nécessite aucune compétence ou équipements particuliers et sa mise en œuvre est indépendante des conditions météorologiques. Le traitement des données obtenu est particulièrement simple.

Mots-clés: grappe, *Vitis vinifera*, extrémité des rameaux, potentiel hydrique foliaire

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INTRODUCTION

The grapevine (*Vitis vinifera* L.) is a horticultural crop of great importance in Spain, where soil water content during summer is a major limiting factor to vine growth and production. Grapevines must undergo a moderate water stress to produce quality wine (van Leeuwen and Seguin, 1994; Cifre *et al.*, 2005) but severely stressed plants yield very few berries of poor composition and can even die (Behboudian and Singh, 2001; Cortell *et al.*, 2005).

There are several methods to evaluate water stress in plants. The most common ones can be grouped in three distinctive sets (Naor, 2006): (1) those that estimate soil moisture availability such as soil water stress indicators and predawn leaf water potential, (2) those that estimate the maximum water stress on a given day, including midday stem water potential and midday leaf water potential, and (3) those that estimate parameters related to the average daily water stress like daily plant transpiration and daily growth of plant tissues. The methods grouped in the second set are widely used because their concept is simple, they directly relate to the water stress condition of plants and they integrate environmental and cropping factors. The use of the pressure chamber (Scholander *et al.*, 1965) to measure stem or leaf water potential is considered a reliable method for determining the water status of field-grown vines (Choné *et al.*, 2001). Leaf water potential measured at solar noon has been successfully used to estimate grapevine water status and relate these measurements to soil water content (Girona *et al.*, 2006; Sousa *et al.*, 2006; Williams and Baeza 2007; Keller *et al.*, 2008).

Most methods to evaluate plant water stress, including leaf water potential, are labor and time intensive, require the use of expensive equipment operated by experienced personnel, and can only sample a very small number of plants out of a large vineyard population and only at a certain time on clear sky days. Because of that, they cannot be used routinely by winegrowers who have to decide at any given moment what action must be taken to alleviate a water-stressed vineyard. The visual appraisal of vine growth named shoot tip method was presented by Rodriguez Lovelle *et al.* (2009) and, according to the authors, it relates to the water consumption of plants. The simplicity and non-destructiveness of the method is appealing for field use and for these reasons, we set up an experiment to a) determine its functional relationship with leaf water potential at solar noon, a more traditional method of evaluation of vine water stress, and b) test its performance and simplicity in field conditions.

MATERIALS AND METHODS

1. First year of the study

a. Experimental site

The experiment was first conducted in 2009 in a field located in a 4 hectare commercial vineyard in Laguardia (42,33 N, 2,35 W, 620 m above sea level), in the La Rioja wine producing region, Spain. The 20 years old *Vitis vinifera* cv. Tempranillo vines were grafted on 110-R rootstock, planted in East-West oriented rows on a 5 % sloping land, and trellised by double cordon. The vineyard was rain fed and subjected to the common agricultural practices of the region, with the exception that the fifteen randomly chosen plants were never tipped.

b. Determination of leaf water potential

Sampling took place on four clear sky days at different phenological stages: June 29 corresponding to the pea-size berry stage, July 16 corresponding to bunch closure, August 13 at veraison, and September 10 when the must had reached 11.5° of probable alcohol. Two fully developed and well-lit leaves located in the middle of shoots were collected per plant at solar noon to determine leaf water potential (ψ_l). A total of 15 plants were sampled at each stage and ψ_l was measured with a pressure chamber (PMS Instrument Company, USA, model 600). The ψ_l measurements were averaged to obtain a single mean ψ_l value per stage.

c. Determination of shoot tip stage

Each vine plant was also monitored for shoot tip development. The observations were made independently by three different operators and the average number of tips in each stage was recorded. The defined stages were (Rodriguez Lovelle *et al.*, 2009): stage 3 when the tip is undergoing active organogenesis and the shoot is growing, stage 2 when organogenesis is reduced and shoot growth is very slow, and stage 1 when the tip is dead and shoot growth has stopped. We propose the overall shoot tip stage (S) of a given plant as:

$$S = w_1S_1 + w_2S_2 + w_3S_3 \quad [1]$$

where w_x represents the proportion of tips in stage S_x in relation to the total number of tips and S_x represents the stage number (1 to 3). An S value of 1 means that all tips are dead and an S value of 3 means that all tips are undergoing active organogenesis.

2. Second year of the study

In 2010, a vineyard located about 8 km from the first vineyard, planted with the same grape variety and rootstock, trellised on the same system and lying also on a gently sloping land was chosen to measure leaf water

potential (Ψ_l) and to determine the shoot tip stage. Twenty randomly selected plants were sampled at harvest (September 15), on a clear sky day, when the must had reached 12° Brix. The measured Ψ_l was used to predict the value of shoot tip stage in 2010 (S_p) using the linear regression found in 2009. The value of shoot tip stage observed in 2010 (S_o) was regressed on the predicted value for the same year (S_p) and the fitness of the regression was used to validate the Ψ_l/S relationship.

The experimental layout was a completely randomized design, and the SPSS statistical package (SPSS for Windows release 11.5.0, SPSS Inc. 2002, Chicago) was used to perform the statistical analysis.

RESULTS

The mean values for leaf water potential (Ψ_l) and shoot tip stage (S) on each observation date can be seen in table 1.

Veraison marks a shift in Ψ_l . Indeed, Ψ_l was significantly ($P \leq 0.05$) higher before veraison (Jun 29 and Jul 16), ranging from -1.50 to -0.91 MPa, than after veraison (Aug 13 and Sep 10) when values between -1.88 and -1.37 MPa were recorded. The shoot tip stage values show that early in the season (Jun 29) there was active

organogenesis and all shoots were growing fast. At a later period (Jul 16), organogenesis was reduced and a few shoots with an S value lower than 2.0 stopped growing and some shoots tips started desiccating. After veraison, no active organogenesis was recorded and by September 10, most of the shoot tips were dead.

A highly significant ($P \leq 0.01$) correlation (2-tailed Pearson's) of 0.888 ($n = 60$) was found between leaf water potential and shoot tip stage. The functional relationship was established by multiple linear regression using shoot tip stage as dependent variable and leaf water potential and date as predictors. As shown in table 2, the regression was highly significant with adjusted R^2 of 0.901.

The regression coefficients shown in table 3 were all highly significant, and figure 1 graphically shows the agreement between the shoot tip stage and its standardized predicted value with R^2 of 0.904. The divergence between predicted and observed values was larger when the shoot tip was either in active organogenesis or already dead.

The simple linear regression of shoot tip stage against leaf water potential (figure 2) shows a good agreement between these variables ($R^2 = 0.789$). When the overall developmental stage of shoot tips is 1.9 or lower, very few shoots show active organogenesis and a number of

Table 1 - Mean, minimum and maximum values of Leaf Water Potential (Ψ_l) and Shoot Tip Stage (S) on each observation date. La Rioja 2009.

Date	Number of observations	Ψ_l (MPa)			S		
		Mean	Minimum	Maximum	Mean	Minimum	Maximum
10 Sep	15	-1.626 ^a	-1.88	-1.30	1.08 ^a	1.00	1.55
13 Aug	15	-1.604 ^a	-1.80	-1.37	1.30 ^a	1.00	1.91
16 Jul	15	-1.289 ^b	-1.50	-1.15	2.32 ^b	1.50	3.00
29 Jun	15	-1.147 ^b	-1.40	-0.91	2.86 ^c	2.56	3.00

Different superscript letters indicate significant differences at $\alpha \leq 0.05$ (Tukey HSD)

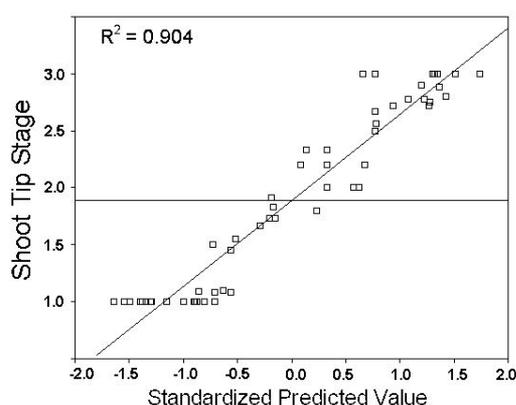


Figure 1 – Regression of observed Shoot Tip Stage on its Standardized Predicted Value. La Rioja 2009.

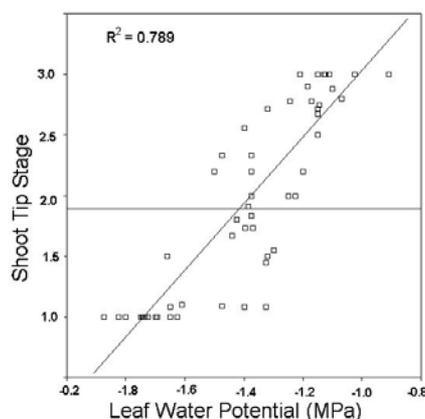


Figure 2 – Regression of shoot tip stage on leaf water potential. La Rioja 2009.

them are dead. Consequently, when S was close to 1 there was a poor agreement with ψ .

The sampling of 15 plants for leaf water potential measurements (two leaves per plant) took over one hour. A single operator could monitored the shoot tip stages of the same plants in about 10 minutes and there were no significant differences among the observations made by distinct operators (data not shown).

The residual statistics of leaf water potential and predicted shoot tip stage from the 2010 data can be seen in table 4. Based on measured ψ_p , the predicted S_p values covered the full range of shoot tip development from tips in active organogenesis ($S = 2.95$) to a high number of dead ones ($S = 0.91$).

A highly significant correlation (2-tailed Pearson's) of 0.958 ($n = 20$) was found between predicted (S_p) and observed shoot tip stage (S_o) and the ANOVA of the simple regression of S_p (predictor) versus S_o (dependent) showed that the regression is highly significant and the variables have a regression coefficient of 0.917 (table 5). The graphical representation of this regression is shown in figure 3, where it is clear that S_o fits closely to S_p when the shoot tips are actively growing or growing slowly, but that the fitness between the two variables is poorer when the shoot tips are dead.

DISCUSSION

Midday leaf water potential above -1.2 MPa and fast growing shoots are reported for well watered grapevines, while ψ_l between -1.2 and -1.3 MPa is considered a moderate stress, shoot growth stops at $\psi_l = -1.4$ MPa, and at ψ_l below -1.5 MPa the plant is severely stressed (Choné *et al.*, 2001; Williams and Araujo, 2002; Myburgh, 2005; Girona *et al.*, 2006).

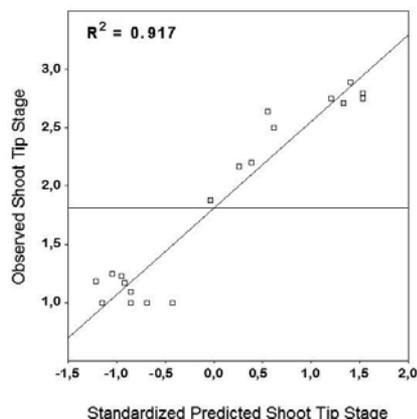


Figure 3 – Regression of observed shoot tip stage against standardized predicted shoot tip stage. La Rioja 2010

Table 2 - ANOVA and R² of regression of leaf water potential and date (predictors) on shoot tip stage (dependent). La Rioja 2009.

Model	Sum of Squares	Degrees of freedom	Mean Square	F	P	R ²	Adjusted R ²
Regression	33.63	2	16.82	269.95	0.00	0.905	0.901
Residual	3.55	57	0.06				
Total	37.18	59					

Table 3 - Coefficient's values, standard errors and significances of the regression of leaf water potential and date (predictors) on shoot tip stage (dependent). La Rioja 2009.

Model	Coefficient	Standard error	t	P
Constant	4 928	0.210	23,509	0.00
Date	-0.374	0.045	-8,291	0.00
Leaf Water Potential	1 484	0.198	7,480	0.00

Table 4 - Residual statistics of Leaf Water Potential (Yl) and Predicted Shoot Tip Stage (Sp). La Rioja 2010.

	Number of observations	Mean	Minimum	Maximum	Standard deviation
S _p	20	1,81	0,91	2,95	0,74
Standard S _p	20	0,00	-1,21	1,53	1,00
S _p Standard residual	20	0,00	-2,15	1,83	0,97
ψ _l (Mpa)	20	-1,24	-1,51	-0,94	0,21

The results of this experiment slightly departed from the ones presented above. In average, pre-veraison plants showed very active organogenesis, corresponding to non-stressed vines, at ψ_l above -1.15 MPa. The shoots tips showed decreased organogenesis and slower growth when mean ψ_l was about -1.3 MPa, corresponding to moderately stressed vines. Veraison marked a shift in plant development: the shoots stopped growing and ψ_l reached values below -1.6 MPa, corresponding to severely stressed vines. The S value of 1.9 was about the upper threshold of severely stressed vines when ψ_l was about -1.45 MPa.

Shoot growth is a sensitive indicator of water deficit and some authors consider that it can reveal water deficit stress even before changes in leaf water potential can be detected (Grimplet *et al.*, 2007). This experiment demonstrated a significant relationship between shoot tip stage, related to shoot growth, and leaf water potential at solar noon. Once growth ceases, more severe deficit do not have an additional effect (Keller *et al.*, 2008). Accordingly, our results showed that S values close to 1 are practically independent of ψ_l .

The relationship between leaf water potential measured at solar noon and shoot tip stage was validated with data collected independently in 2010 in a different vineyard and showed that the functional relationship found in 2009 can be extrapolated to other vineyards planted with the same grape variety. The observed and predicted values of shoot tip stage showed a good fitness in particular for shoot tips that are growing, corresponding to the stage when the management of the water supply can still produce desirable results.

Photosynthesis is adversely affected by severe water stress (Medrano *et al.*, 2003) and decreases berry quality for wine production (Keller, 2005). Photosynthesis is reduced by water stress because of stomata closure, and

this reduction starts at ψ_l below -0.5 MPa and might cease completely at ψ_l between -1.2 -1.5 MPa (Kriedemann and Smart, 1971). Once shoot growth stops after veraison, vines should be only sufficiently stressed to prevent new shoot growth that may have a major and prejudicial effect on berry ripening and harvest quality (Lebon *et al.*, 2006). The results of this experiment showed that could be achieved with S values between 1.9 and 2.0.

In the specific case of Tempranillo variety, water stress applied from fruit set to veraison appears to reduce berry quality (soluble solids content, polyphenol and anthocyanin concentration), in particular for water stress levels above -0.95 MPa (Girona *et al.*, 2009). After veraison, berry quality increases linearly for light-to-mild levels of water stress, whereas it decreases above a certain water stress threshold ($\psi_l = -1.12$ MPa).

Winegrowers could randomly select a number of plants, left untipped, across the vineyard to use as control to determine the overall shoot tip stage. The counting is fast and non-destructive, it does not require any special skill or equipment, the calculations are simple, and it is largely independent of weather conditions. The results obtained in this work generally follow those presented by Rodriguez Lovelle *et al.* (2009) and they also do not address the issue of different interpretation for each grape variety. The calculated shoot tip stage can be used reliably to manage vineyard irrigation given that moderate water stress is primarily affected by soil water content (William and Baeza, 2007 ; Intrigliolo and Castel, 2008).

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Table 5 - ANOVA and R² of regression of predicted shoot tip stage (predictor) on observed shoot tip stage (dependent). La Rioja 2010.

Model	Sum of Squares	Degrees of freedom	Mean Square	F	P	R ²	Adjusted R ²
Regression	10,481	1	10,481	198,613	0,00	0,917	0,912
Residual	0,950	18	0,053				
Total	11,431	19					

Table 6 - Coefficient's values, standard errors and significances of the regression of predicted shoot tip stage (predictor) on observed shoot tip stage (dependent). La Rioja 2010.

Model	Coefficient	Standard error	t	P
Constant	-1,975	0,273	-7,222	0,00
Predicted shoot tip stage	2,425	0,172	14,093	0,00

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