Estimation of vineyard leaf area by linear regression

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Abstract

Vineyard leaf area is a variable that must be determined when assessing the productive potential of a vineyard and for characterizing the light and thermal microenvironments of grapevine plants. The aim of the present work was to validate the Lopes and Pinto method for determining vineyard leaf area in the vineyards of central Spain and with the area's cultivars. The results obtained were compared to those provided by a traditional and accurate —but much more laborious— non-destructive direct method. Experiments were performed over three years in six vineyards growing either cvs. Syrah, Cabernet Sauvignon, Cabernet franc, Merlot or Tempranillo. Good agreement was found between the two methods both in the determination of primary and lateral shoot leaf areas for all cultivars and vineyards. The simplicity of the Lopes and Pinto method means much larger sample sizes can be examined in the same period of time, increasing the accuracy of final vineyard leaf area values. In fact, regression analysis of the data collected for the Lopes and Pinto method showed that only three field-measured variables need to be recorded for the inspected shoots of either type: the area of the largest leaf, the area of the smallest leaf, and the number of leaves.

Additional key words: Cabernet franc; Cabernet Sauvignon; destructive methods; Merlot; non-destructive methods; Syrah; Tempranillo; *Vitis vinifera*.

Resumen

Estimación del área foliar del viñedo mediante regresión lineal

En viticultura, el área foliar del viñedo es una variable que debe ser determinada para evaluar el potencial productivo del viñedo, para caracterizar el microclima luminoso y térmico de la vid. El objetivo del presente trabajo fue validar el método propuesto por Lopes y Pinto para determinar el área foliar de viñedos del centro de España y con cultivares de la zona. Los resultados obtenidos fueron comparados con los obtenidos por un método directo no destructivo y preciso, más tradicional pero mucho más laborioso. Los ensayos se llevaron a cabo con los cultivares Syrah, Cabernet Sauvignon, Cabernet franc, Merlot y Tempranillo en seis viñedos durante tres años. Entre ambos métodos se observó un buen ajuste, tanto para la determinación del área foliar de principal como para la de nietos, en todos los cultivares y viñedos. La simplicidad del método de Lopes y Pinto permite incrementar el tamaño de muestra para un mismo tiempo de muestreo, incrementando así la precisión del valor final del área foliar del viñedo. Efectivamente, el análisis estadístico de los datos recogidos para el método de Lopes y Pinto mostró que sólo es necesario medir tres variables en campo: el área de la hoja más grande, el área de la hoja más pequeña y el número de hojas, para principal y para nietos separadamente.

Palabras clave adicionales: Cabernet franc; Cabernet Sauvignon; método destructivo; método no destructivo; Merlot; Syrah; Tempranillo; *Vitis vinifera*.

Abbreviations used: ILA (individual leaf area), L (area of the largest leaf), L_1 (area of the largest primary leaf), L_2 (area of the largest lateral leaf), LAI (leaf area index), LA_{sh} (total leaf area per shoot), LA_{sh1} (primary leaf area per shoot), LA_{sh2} (lateral leaf area per shoot), M (mean leaf area), M_1 (mean primary leaf area), M_2 (mean lateral leaf area), MAE (mean absolute error), MAPE (mean absolute percentage error), MLA (mean leaf area per shoot), MLA_1 (mean primary leaf area per shoot), MLA_2 (mean lateral leaf area per shoot), MVL (main vein length), NL (number of leaves), NL_1 (number of primary leaves), NL_2 (number of lateral leaves), S (area of the smallest leaf), S_1 (area of the smallest primary leaf), S_2 (area of the smallest lateral leaf).

Introduction

The leaf area produced by grapevines is a determining factor for their productivity since the leaves are the main site of both photosynthesis and transpiration. The distribution of leaf area and its density, *i.e.,* the shape of the canopy, determines the interception and distribution of solar radiation around the plant, and therefore its light, thermal and moisture microenvironments (Smart, 1985; Smart and Robinson, 1991; Schultz, 1995; Zufferey *et al*., 1998; Kliewer and Dokoozlian, 2000). Thus, leaf surface area necessarily influences the quantity and quality of the must produced by the grapes (Petrie *et al.,* 2000a,b; Howell, 2001; Ollat *et al*., 2002; Kliewer and Dokoozlian, 2005). In viticulture, equilibrium must be reached between leaf surface area and yield if good fruit ripening is to achieved; a figure of 7-14 cm^2 of leaf area per gram of fruit is deemed appropriate (Kliewer and Dokoozlian, 2005). However, this value is influenced by space and time. For example, the same leaf area/yield ratio can give very different results depending on the reigning environmental conditions, the training systems used and the canopy management followed, all of which affect the microenvironment of the leaves and fruit (Smart, 1985; Williams, 1987; Jackson and Lombard, 1993; Reynolds and Heuvel, 2009), and the phenological stage of the plants, which influences the sap flow between sources and sinks (Candolfi-Vasconcelos and Koblet, 1990).

The leaf area index (LAI) is the variable most used for characterizing the development of grapevine foliage in a vineyard. It is defined as the relationship between the surface area of the leaves and that of the soil (Champagnol, 1984; Carbonneau, 1989); its units are m^2 m^{-2}. Both direct and indirect methods exist for estimating the LAI. The direct methods can be either destructive or non-destructive. The indirect methods have been the object of numerous studies (Sommer and Lang, 1994; Oliveira and Santos, 1995; Barbagallo *et al*., 1996; Ollat *et al*., 1998; Patakas and Noitsakis, 1999). These methods include the measurement of light extinction through the canopy, the use of empirical models in which leaf area development is defined according to temperature (Schultz, 1992), or remote imaging of the canopy (Dobrowski *et al*., 2002). Although these methods are rapid and non-destructive they are costly and usually require calibration and specific sampling protocols (Ollat *et al*., 1998). Further, in vineyards with very dense canopies, leaf area is often underestimated due to overlapping (Cohen *et al*.,

2000). Smart and Robinson (1991) indicate these methods to have the serious disadvantage of not distinguishing between the area of leaves on primary and lateral shoots. Estimating these areas separately is important since their physiological activity is different. Lateral leaves are younger since they emerge later than the primary leaves. Their youth can be an advantage during fruit ripening; the assimilation rates of young, fully developed leaves are higher than those of primary leaves, which are closer to senescence (Candolfi-Vasconcelos and Koblet, 1990; Palliotti *et al*., 2000). In addition, the development of the lateral leaves affects the density of the canopy and the light-thermal-moisture microenvironment of the leaves and grape clusters (Smart, 1985). The leaf area of the lateral shoots depends on the variety, the growth environment and cultivation practices. This leaf area may form a relatively important part of the total leaf area, with values of between 22 and 44% (Palliotti *et al*., 2000), a larger proportion being more important in highly vigorous vineyards (Huglin and Schneider, 1998; Palliotti *et al*., 2000).

The destructive direct methods for measuring leaf area require the collection of leaf and/or shoot samples in the field and their transfer to the laboratory for analysis. Although accurate, these methods destroy the photosynthetic area of the plant and are time-consuming and laborious. Further, they do not allow the change in the photosynthetic surface of the plant to be followed over the year. The *in situ* measurement of leaf area using portable devices provides a direct, non-destructive method, but unfortunately, this is very expensive and difficult to manage. Other non-destructive direct methods are based on the empirical relationships between leaf area and other leaf or shoot variables. Although they are quicker, a calibration curve is required before they can be used, and the results obtained are less accurate. In fact, the necessary calibration is established via the use of generally destructive direct methods, although it need not be performed every year once the statistical model for the leaf area-leaf/shoot variables is known. Generally, the precision of these methods is proportional to the time invested in measuring (Ollat *et al*., 1998). The leaf and shoot variables that can be recorded include leaf weight, the distance between the mucrons of the upper lateral veins, the length of the main vein, the length of the main lateral veins, or the sum of the lengths of different veins (Carbonneau, 1976; Lopes and Pinto, 2000). These methods are accurate and simple, but again are laborious since they require the inspection of all the primary and

Table 1. Location of the study plots, the cultivar (and rootstock) contained in each, years and effective temperature accumulated from budburst to harvest (degree day, °day) of data collection

Location	Cultivar/Roostock	Data collection	Degree day (°day)
Prov. of Toledo (Castilla-La Mancha Region)	Syrah / 110R	2005-2006-2007	2349 - 2405 - 1979
Madrid Region	Syrah / 140R	2005	1928
Madrid Region	Cabernet Sauvignon / SO4	2003-2004-2005	1924 - 1736 - 1928
Madrid Region	Cabernet franc / 140R	2006-2007	2006 - 1589
Madrid Region	Merlot / 140R	2006-2007	2006 - 1589
Madrid Region	Tempranillo / 110R	2005-2006-2007	1928 - 2006 - 1589

lateral leaves. However, these methods do not obtain the lateral shoot leaf area.

Lopes and Pinto (2005) proposed an empirical model for estimating primary shoot leaf area based on the number of leaves on the primary shoot and the leaf area of the largest and smallest leaves. In the same work a similar model was proposed for leaves on lateral shoots. These models substituted earlier proposals made by the same authors (Lopes and Pinto, 2000); these earlier proposals included primary shoot length, but this was problematic since this variable is dependent on internode length, which can change over the year and can be altered by trimming, leaf removal practices and even the natural defoliation that occurs at the end of fruit ripening (Lopes and Pinto, 2005). Beslic *et al*. (2010) validated this method using cv. Blaufränkisch. In several fruit tree species (citrus, almond, pecan olive, walnut and asian pear) Spann and Heerema (2010) found a linear relationship between the biggest leaf length multiply by shoot leaf number and shoot leaf area. One of its advantages is that it allows the primary and lateral leaf area to be estimated separately. This method is easy, accurate and non-destructive; it also reduces measuring time, allowing larger sample sizes, which is an important advantage given the intrinsic heterogeneity of grapevines.

The aim of the present work was to validate the empirical model proposed by Lopes and Pinto (2005) under the environmental conditions of central Spain and for the grapevine cultivars grown in this area.

Material and methods

This three-year study involved six experimental vineyards; five at the El Socorro Viticulture Research Centre (belonging to the *Instituto Madrileño de Desarrollo Rural Agrario y Alimentario*) in the southwest of the Madrid Region (40° 8' N, 3° 23' W, alt. 730 m), and a commercial vineyard in the Province of Toledo (Castilla La Mancha Region) (44° 15' N, 3° 59' W, alt. 488 m). The vineyards had different areas and grapevine cultivars: Syrah, Cabernet Sauvignon, Cabernet franc, Merlot and Tempranillo. The spacing between the vines, the training systems employed, and the soils also differed (Tables 1 and 2). The vineyards contained three or four plots of dimensions sufficient to include two test rows containing at least 10 vines, with one row

Table 2. Grapevine material, soil type (USDA-Soil Taxonomy classification), vine spacing, training system, and shoot density for each of the experimental vineyards

Cultivar/Rootstock	Soil[1]	Vine spacing (m × m)	Training system	Shot density (shoot m^{-1})
Syrah / 110R	Typic Palexeralf	2.7 × 1.2	VSP[2], Sprawl	12-18
Syrah / 140R	Calcixerolic Xerochrept	2.4 × 1.1	VSP	13
Cabernet Sauvignon / SO4	Calcic Haploxeralf	2.5 × 1.1	VSP	11-13
Cabernet franc / 140R	Calcixerolic Xerochrept	2.5 × 1.5	VSP	10
Merlot / 140R	Calcixerolic Xerochrept	2.2 × 1.5	VSP	11-12
Tempranillo / 110R	Calcixerolic Xerochrept	2 × 1.25		
		2.5 × 1.25		
		3 × 1.25	VSP	8-12

[1] Soil Survey Staff (2006). [2] VSP: vertical shoot position.

of border vines on each side. Data were collected only from these test vines and in the years indicated in Table 1 (data were available for different vineyards in different years, taking advantage of those recorded in different studies).

Determination of leaf area by a traditional non-destructive method

A non-destructive, direct, statistical method requiring calibration was used to determine leaf area, against which the results obtained by the method of Lopes and Pinto (2005) could be compared. For the calibration step the relationship between the length of the main vein and the leaf area was established for each of the five cultivars studied. For this, 25 leaves of all sizes (near the end of the growth cycle) were randomly sampled from primary and lateral shoots (one leaf per shoot; the total number of leaves collected was therefore 50). In the laboratory, the length of the main vein and leaf blade area were measured using a WinDias image analysis system (Delta-T Devices, Cambridge, UK). Microsoft Excel v.2000 software was then used to perform linear or curvilinear regressions between individual leaf area (ILA, cm^2) and main vein length (MVL, cm), thereby obtaining the equation required to calculate the leaf area, as well as the correlation coefficient between these variables. The following calibration equations were obtained for the different cultivars ($p < 0.001$):

Cabernet franc $ILA = 1.9077 \times MVL^{1.803}$
                     $(R^2 = 0.93)$ [1]

Cabernet Sauvignon $ILA = 0.38 + 1.21 \times MVL^2$
                     $(R^2 = 0.93)$ [2]

Merlot $ILA = 18.291 \times MVL - 58.452$
                     $(R^2 = 0.86)$ [3]

Syrah $ILA = 21.41 \times MVL - 75.409$
                     $(R^2 = 0.94)$ [4]

Tempranillo $ILA = 20.306 \times MVL - 69.302$
                     $(R^2 = 0.93)$ [5]

The total leaf area per primary and lateral shoot (LA_{sh1} and LA_{sh2} respectively; herein the use of subscripts 1 and 2 with any variable refers to leaves on primary shoots and lateral shoots, respectively) was then determined using a modification of the method of Carbonneau (1976) based on the field measurement of MVL for one of every three leaves on four representa-

tive fruiting shoots (4-5 plants per row). Over the years (see Table 1), the total number of grapevine shoots (*i.e.,* primary shoots plus their branching lateral shoots) measured for cv. Syrah in Toledo reached 1229; this figure was 215 for cv. Syrah in Madrid, 257 for cv. Cabernet franc, 1,689 for cv. Cabernet Sauvignon, 547 for cv. Merlot, and 919 for cv. Tempranillo. Measurements were made from the end of April (beginning of flowering) until the beginning of October (after harvest); the largest number of sampling dates fell between full bloom and harvest [growth stages according to Lorenz *et al.* (1995)]. The MVL data for the leaves of both primary and lateral shoots were converted into ILA values (ILA_1 and ILA_2) using equations 1-5. LA_{sh1} and LA_{sh2} values were obtained by multiplying the sum of the ILA_1 and ILA_2 values by three (since only one in every three leaves of each shoot type was sampled). These values, for each cultivar in each vineyard, were recorded for later comparison against the results provided by the Lopes and Pinto method.

Determination of leaf area by the Lopes and Pinto method

To determine LA_{sh1} and LA_{sh2} using the method suggested by Lopes and Pinto (2005), five variables were measured for both the primary and lateral shoots: area of the largest leaf (L), area of the smallest leaf (S), mean leaf area [$M = (L + S) / 2$], number of leaves (NL), and the mean leaf area per shoot ($MLA = M \times NL$). All measurements were taken for leaves on fruiting shoots representative of the studied vineyards (see above for numbers of shoots sampled), and recorded with respect to primary or lateral position.

To obtain the two equations required by the Lopes and Pinto method to determine the LA_{sh1} and LA_{sh2} values, the variable(s) which best explain(s) the variation in these values must be identified. For this, a correlation matrix was constructed including all five variables plus the LA_{sh1} and LA_{sh2} values obtained by the traditional method (the only LA_{sh1} and LA_{sh2} values available at this point). All values were $\log_{10}$-transformed to increase the linearity and variance stability (Table 3). Stepwise regression with a critical value of $F = 0.10$ was then performed to determine the most explicative variable(s) for the primary and lateral shoot areas. The resulting regression equations were then used to determine the LA_{sh1} and LA_{sh2} values by the Lopes and Pinto method.

Table 3. Representative correlation matrix (cv. Syrah, n = 1,229 shoots) for primary shoot leaf area ($\log_{10}$-transformed) (LA_{sh1}) and $\log_{10}L_1$ (largest primary leaf area), $\log_{10}M_1$ (mean primary leaf area), $\log_{10}NL_1$ (number of primary leaves) and $\log_{10}MLA_1$ (mean primary leaf area per shoot). All correlations were highly significant ($p < 0.01$)

	$\log_{10}LA_{sh1}$	$\log_{10}L_1$	$\log_{10}M_1$	$\log_{10}NL_1$	$\log_{10}MA_1$
$\log_{10}LA_{sh1}$	1				
$\log_{10}L_1$	0.92	1			
$\log_{10}M_1$	0.85	0.93	1		
$\log_{10}NL_1$	0.89	0.70	0.54	1	
$\log_{10}MLA_1$	0.99	0.93	0.87	0.89	1

Comparison of the LA_{sh1} and LA_{sh2} values provided by the traditional and Lopes and Pinto methods

The observed (traditional method) and estimated (Lopes and Pinto method) LA_{sh1} and LA_{sh2} data for the combination of all five cultivars in their different vineyards were plotted separately against one another (master curves) and the overall R^2 values calculated. Similar curves were also plotted for each cultivar in their separate locations and their deviation from the master curve examined. Deviation analysis was used to calculate the mean absolute error [$MAE = (\Sigma|y_i - \ddot{y}_i|)/n$] and the mean absolute percentage error [$MAPE = 100\ (\Sigma\ (|y_i - \ddot{y}_i|\ /\ |y_i|))/n$] for each of these lines, where y_i represents the observed values, $\ddot{y}_i$ represents the estimated values, and n the number of pairs. T-statistics were used to determine the equation for these curves, their adjusted correlation coefficients (R^2), the mean of the residuals, and the Durban-Watson coefficients.

All analyses were performed using SPSS v.14.0 software.

Results

Calculation of the regression equation for determining the estimated (Lopes and Pinto) LA_{sh1} value

The correlation matrix showed the strongest R^2 between the observed and estimated data for LA_{sh1} to be provided by $\log_{10}MLA_1$, followed by $\log_{10}NL_1$, $\log_{10}L_1$ and $\log_{10}M_1$. The weakest coefficient was obtained with $\log_{10}S_1$ (Table 3). $\log_{10}S_1$ was therefore excluded from further analysis. To avoid colinearity, $\log_{10}M_1$ was also excluded since it is the result of the linear combination of two measured variables ($\log_{10}L_1$ and $\log_{10}S_1$). In stepwise regression, the first variable introduced into the model was therefore $\log_{10}MLA_1$, which provided the regression equation: $LA_{sh1} = 10 \times [0.012 + 0.996 \times \log_{10}(MLA_1)]$ (Eq. [6]) ($R^2 = 0.98$; $p < 0.001$) When the other variables were introduced into the model, the correlation coefficient was not improved. This equation was therefore used to calculate the estimated (Lopes and Pinto) LA_{sh1} values.

Calculation of the regression equation for determining the estimated (Lopes and Pinto) LA_{sh2} values

The correlation matrix showed the strongest R^2 between the observed and estimated data for LA_{sh2} to be provided by $\log_{10}MLA_2$, followed by $\log_{10}NL_2$, $\log_{10}L_2$ and $\log_{10}M_2$ (Table 4). The weakest coefficient was obtained with $\log_{10}S_2$, which was therefore excluded from further analysis. To avoid colinearity, $\log_{10}M_2$ was also excluded since it is the result of the linear combination of two measured variables ($\log_{10}L_2$ and $\log_{10}S_2$). In stepwise regression, the first variable introduced into the model was therefore $\log_{10}MLA_2$, which provided the regression equation: $LA_{sh2} = 10 \times [0.036 + 0.982 \times \log_{10}(MLA_2)]$ (Eq. [7]) ($R^2 = 0.99$; $p < 0.001$). When the other variables were introduced into the model, the correlation coefficient was not improved. This equation was therefore used to calculate the estimated (Lopes and Pinto) LA_{sh2} value.

Comparison of the LA_{sh1} and LA_{sh2} results obtained by the traditional and Lopes and Pinto methods

A good fit was found between the observed and estimated LA_{sh1} and LA_{sh2} values for all cultivars and

Table 4. Representative correlation matrix (cv. Syrah, n = 1,229 shoots) for lateral shoot leaf area ($\log_{10}$-transformed) (LA_{sh2}) and $\log_{10}L_2$ (largest lateral leaf area), $\log_{10}M_2$ (mean lateral leaf area), $\log_{10}NL_2$ (number of lateral leaves) and $\log_{10}MLA_2$ (mean lateral leaf area per shoot). All correlations were highly significant ($p < 0.01$)

	$\log_{10}LA_{sh2}$	$\log_{10}L_2$	$\log_{10}M_2$	$\log_{10}NL_2$	$\log_{10}MA_2$
$\log_{10}LA_{sh2}$	1				
$\log_{10}L_2$	0.93	1			
$\log_{10}M_2$	0.87	0.96	1		
$\log_{10}NL_2$	0.90	0.71	0.60	1	
$\log_{10}MLA_2$	0.99	0.93	0.89	0.90	1

vineyards when plotted in combination (master curves) (Fig. 1); the slopes were close to 1 and had high R^2 values (0.945 for LA_{sh1} and 0.948 for LA_{sh2}).

Lines for LA_{sh1} and LA_{sh2} were also plotted for each cultivar in their separate locations to observe their deviation from the master curves (not shown; see Tables 5 and 6 respectively for data). The Durbin-Watson coefficients were always higher than the R^2 value from the curves for cultivar in their separate locations. The MAPE values ranged between 8 and 11%. The mean of the residuals was 0 for all these curves.

Table 7 shows ILA of primary and lateral leaves and number of leaves per shoot for each cultivar at flowering and ripening at the experimental vineyards, and Figure 2 shows the mean distribution of ILA along primary shoots of the five studied cultivars over the growing season.

Discussion

The present results show that the Lopes and Pinto method is a valid, reliable means of determining vineyard leaf area. The master curves for both LA_{sh1} and LA_{sh2} comparing the two methods had slopes of almost 1 and R^2 values of 0.945 and 0.948 ($p < 0.001$).

In comparisons of the curves for the different cultivars and vineyards against the master curves, the Durbin-Watson coefficients and the MAPE indices again indicated the Lopes and Pinto method to be valid. The MAPE was <10% for all the present cultivars' LA_{sh1} values except for Cabernet franc (10%), and the MAPE of LA_{sh2} was <10% for all cultivars except Syrah and Cabernet Sauvignon (11%). Although Kleijnen (1987) proposed a threshold MAPE value of 10%, the present figures remain acceptable.
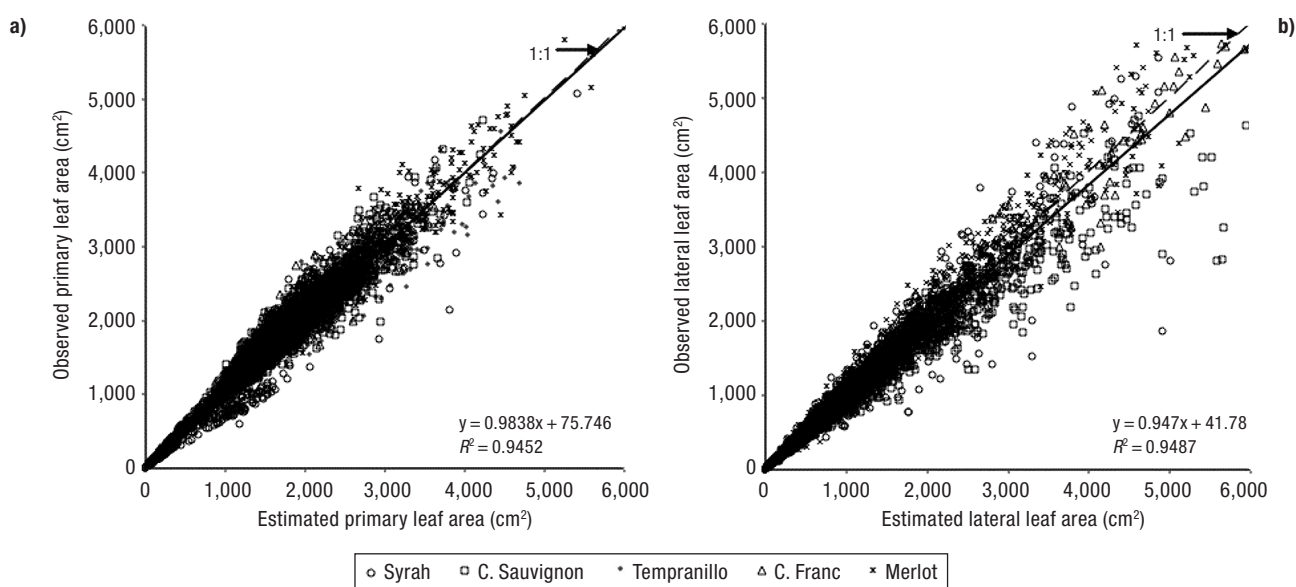

Figure 1. Relationship (master curves) between observed and estimated values of primary shoot leaf area (LA_{sh1}) (a) and lateral shoot leaf area (LA_{sh2}) (b) using the datasets for the five cultivars examined.

Table 5. Validation of the linear regression model ($LA_{sh1} = a + b \times$ estimated LA_{sh1}) for primary shoot leaf area (LA_{sh1})

Cultivar	Intercept		Slope		Adjusted R^2	Durbin-Watson	n	Mean of residuals	MAPE	MAE
	a	t	b	t						
C. franc	333.79	6	0.89	31	0.79***	1.77	257	0.00	10.3	208.9
C. Sauvignon	62.27	6	1.01	167	0.94***	1.79	1,689	0.00	8.4	138.5
Merlot	121.26	4	0.98	81	0.92***	1.68	547	0.00	7.3	188.4
Syrah Madrid	141.74	2	0.98	41	0.89***	1.40	215	0.00	7.8	213.8
Syrah Toledo	18.10	2	1.00	162	0.96***	1.29	1,229	0.00	8.7	120.7
Tempranillo	217.67	12	0.90	102	0.92***	1.33	919	0.00	6.3	136.8

t: absolute value of t-statistic. n: number of shoots. MAE: mean absolute error. MAPE: mean absolute percentage error. ***: $p < 0.001$.

Table 6. Value of the linear regression model ($LA_{sh2} = a + b \times$ estimated LA_{sh2}) for lateral shoot leaf area (LA_{sh2})

Cultivar	Intercept		Slope		Adjusted R^2	Durbin-Watson	n	Mean of residuals	MAPE	MAE
	a	t	b	t						
C. franc	7.33	0	0.99	80	0.96***	2.20	257	0.00	8.0	182.0
C. Sauvignon	143.87	15	0.81	143	0.93***	1.95	1,497	0.00	11.2	166.7
Merlot	71.16	5	0.99	158	0.98***	1.94	547	0.00	9.2	158.9
Syrah Madrid	−14.83	−1	1.06	76	0.97***	1.86	207	0.00	7.7	90.6
Syrah Toledo	38.80	3	0.97	95	0.92***	1.72	790	0.00	11.3	137.6
Tempranillo	−25.08	−4	1.08	209	0.98***	1.93	809	0.00	6.9	90.5

t: absolute value of t-statistic. n: number of shoots. MAE: mean absolute error. MAPE: mean absolute percentage error. ***: $p < 0.001$.

In the determination of the Lopes and Pinto regression equations for determining LA_{sh1} and LA_{sh2}, $\log_{10}MLA$ was the variable which best explained their variation. Indeed, $\log_{10}MLA$ explained 99% of the variation in $\log_{10}LA_{sh2}$ and 95% of that of $\log_{10}LA_{sh1}$. Lopes and Pinto (2005) indicated the importance of determining the MLA since it incorporates three field-measured variables: S, L and NL. In slight differences to the present

Table 7. Individual area (ILA, cm²) of primary (1) and lateral (2) leaves: maximum (ILA max), minimum (ILA min), mean (ILA mean) and mode (ILA mode), and number of leaves per shoot: mean (NL mean) and mode (NL mode), for each cultivar at flowering and ripening. Number of sampled shoots: 257 for Cabernet franc, 855 for Cabernet Sauvignon, 384 for Merlot, 385 for Syrah and 513 for Tempranillo

|		Flowering						Ripening					
	Leaf	ILA				NL		ILA				NL	
		max.	min.	mean	mode	mean	mode	max.	min.	mean	mode	mean	mode
C. franc	1							309	13.8	118	121	17	15
	2							186	13.8	56	48	41	33
C. Sauvig	1	244	6.8	96	121	20	18	273	1.6	98	98	17	15
	2	199	11.3	40	44	27	27	241	1.6	40	31	34	27
Merlot	1	600	1.9	118	143	17	15	252	3.7	114	88	25	27
	2	163	1.9	55	33	23	12	177	1.9	57	33	36	12
Syrah	1	325	1.7	183	224	12	12	346	1.7	173	181	12	9
	2	224	1.7	69	74	17	21	220	1.7	71	53	22	12
Temp	1	296	1.8	141	154	12	12	302	1.8	144	164	14	9
	2	144	1.8	50	32	7	3	199	1.8	68	73	17	6
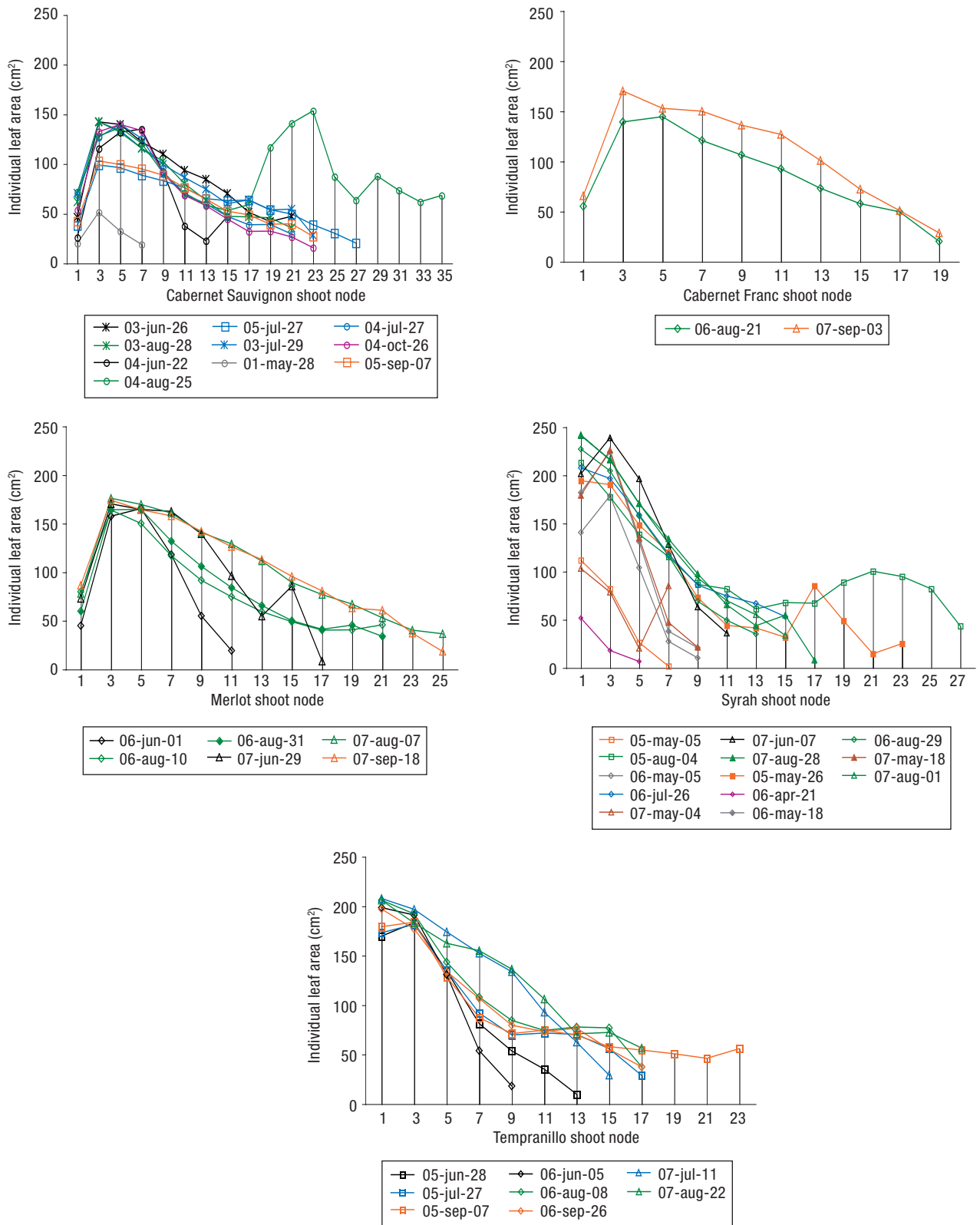
Figure 2. Mean distribution of individual primary leaf area (ILA, cm²) along primary shoots of the five studied cultivars (shoot node from base to apex) over the growing season.

work, these authors transformed their data into Naperian logarithms (which are somewhat harder to use than log_{10} transformations) and the regression equation for the determination of LA_{sh2} included L_2. Its inclusion in the regression equation for LA_{sh2} in the present work did not improve the R^2 value obtained.

Compared to the method proposed by Carbonneau (1976), the three physical variables included in MLA (L, S, NL) can be easily and rapidly measured in the field, which facilitates the eventual determination of LA_{sh1} and LA_{sh2}. While invested with the same precision, the unmodified Carbonneau method is based on the much more laborious measurement of the main vein length of every leaf on the primary and lateral shoots. Thus, the proposed method has the advantage that it allows a larger sample to be measured in the same space of time. This is of particular importance given the intrinsic heterogeneity of grapevine vegetative growth (Cloete *et al*., 2006) and physiological activity (Cloete *et al*., 2008). This heterogeneity is reflected in the coefficients of variation (CV) associated with the measurement of ILA and NL in the traditional method (Table 7). The ILA is a more stable measurement than NL, more so for the primary leaves (CV_{ILA} 15-25%; CV_{NL} 19-47%) than lateral leaves (CV_{ILA} 23-47%; CV_{NL} 45-77%). This is to be expected since the expansion of growing primary leaves ends when flowering approaches (Wermelinger and Koblet, 1990). In addition, between flowering and veraison, shoot growth ceases or may even be stopped by trimming. However, the development of lateral shoots and leaves is more irregular (Schultz, 1992). Thus, the CV values reflect the heterogeneity of grapevine leaf growth; its correct characterization therefore requires large sample sizes. Our own experience suggests that some 30% of the shoot load of at least 20% of a vineyard's plants need to be sampled, a figure that must be increased in line with the heterogeneity of the plots involved.

The most difficult variables to accurately obtain are L and S, especially for the lateral leaves. The largest primary shoot leaf (for the measurement of L_1) is usually found around the third node from the base of the shoot (Lopes and Pinto, 2005) (see Fig. 2). The smallest primary shoot leaf (for the measurement of S_1) can be more difficult to locate since its position varies with the growth stage, the length of the primary shoot, and whether the plant has been trimmed (Fig. 2). However, it is always located at the apex in non-trimmed, growing primary shoots. The largest and smallest secondary shoot leaves (for the measurement of L_2 and

S_2) are even harder to locate given the more heterogeneous development of lateral shoots. Lopes and Pinto (2005) recommend these leaves be identified when NL_2 is determined. However, when searching for any of the above leaves, not all need be inspected; leaves must have a main vein at least 4.5 cm long for them to be considered sources of photosynthetic products rather than sinks (Intrieri *et al*., 1992; Zufferey *et al*., 2000; Sanchez-de-Miguel *et al*., 2010). Thus, when selecting leaves for the measurement of NL, and when trying to locate the appropriate leaves for the measurement of L_1, L_2, S_1 or S_2, only those with a length of ≥ 4.5 cm need be inspected. Further, Hale and Weaver (1962) indicate that lateral shoots only become sources of photosynthetic products rather than sinks when they have two or more fully expanded leaves; thus, only those meeting this condition need be inspected.

In conclusion, the present work shows that the Lopes and Pinto method for measuring the leaf area of vineyards is valid for use in Central Spain with the cultivars grown there. The use of this method should help researchers and vine-growers in the assessment of vineyard potential productivity.

Acknowledgements

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