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Leaf area estimation by simple measurements and evaluation of leaf area prediction models in Cabernet-Sauvignon grapevine leaves

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Abstract

For two growing seasons (2005 and 2006), leaves of grapevine cv. Cabernet-Sauvignon were collected at three growth stages (bunch closure, veraison, and ripeness) from 10-year-old vines grafted on 1103 Paulsen and SO4 rootstocks and subjected to three watering regimes in a commercial vineyard in central Greece. Leaf shape parameters (leaf area − LA, perimeter − Per, maximum midvein length − L, maximum width − W, and average radial − AR) were determined using an image analysis system. Leaf morphology was affected by sampling time but not by year, rootstock, or irrigation treatment. The rootstock×irrigation×sampling time interaction was significant for all the leaf shape parameters (LA, Per, L, W, and AR) and the means of the interaction were used to establish relationships between them. A highly significant linear function between L and LA could be used as a non-destructive LA prediction model for Cabernet-Sauvignon. Eleven models proposed for the non-destructive LA estimation in various grapevine cultivars were evaluated for their accuracy in predicting LA in this cultivar. For all the models, highly significant linear functions were found between calculated and measured LA. Based on r^2 and the mean square deviation (MSD), the model proposed for LA estimation in cv. Cencibel [LA = 0.587(L×W)] was the most appropriate.

Additional key words: leaf length; leaf width; non-destructive methods; *Vitis vinifera.*

Introduction

Organ shape determination is useful for many studies in genetics, ecology, and taxonomy (Iwata and Ukai 2002). Leaf area (LA) estimation is often necessary in field studies related with radiation interception, photosynthesis, transpiration, and growth analysis (Goudriaan and van Laar 1994). In olive, LA estimations are useful criterion for fast growing and early flowering genotype selection (Pritsa *et al.* 2003).

Repeated measurements such as in leaf expansion studies demand non-destructive determination of LA which is feasible only by using portable and expensive instruments or by developing LA prediction models (Serdar and Demirsoy 2006). Till now, non-destructive models for LA prediction have been developed for many shrub and tree species such as banana (Potdar and Pawar 1991), cherry (Demirsoy and Demirsoy 2003), chestnut (Serdar and Demirsoy 2006), cocoa (Asomaning and Lockard 1963), hazelnut (Cristofori *et al.* 2007), kiwi (Mendoza-de Gyves *et al.* 2007), peach (Demirsoy *et al.* 2004), pecan (Whitworth *et al.* 1992), rabbiteye blueberries (NeSmith 1991), and sago palm (*Metroxylon sagu* Rottb.) (Nakamura *et al.* 2005). These models are based on leaf length (L) and width (W) measurements, which are used individually or in combination in order to establish linear, quadratic, or exponential functions and the best-fitted curve to be chosen. Obviously, linear models based on only one dimension are preferable due to their simple application especially in the field (Lu *et al.* 2004, Tsialtas and Maslaris 2005).

Carbonneau (1976) used leaf dimension measurements in order to describe grapevine cultivars and proposed an LA prediction model based on the sum of the

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Abbreviations: ANOVA – analysis of variance; AR – average radial; CV – coefficient of variation; DI – deficit irrigation; ET_c – evapotranspiration; FI – full irrigation; L – midvein length; LA – leaf area; LSD – least significant difference; MSD – mean square deviation; NI – not irrigated; Per – perimeter; RCB – Randomized Complete Block; W – maximum leaf width.
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length of the primary lateral nerves. Since then, LA prediction models based on L and W measurements have been established for cvs. Grenache (Manivel and Weaver 1974), Chardonnay and Chenin blanc (Sepúlveda and Kliewer 1983), Thompson Seedless (Smith and Kliewer 1984), Concord (Elsner and Jubb 1988), White Riesling (Schultz 1992), Cencibel (Montero *et al.* 2000), Niagara and DeChaunac (Williams and Martinson 2003). We are unaware of any model based on leaf dimensions for non-destructive LA estimation in Cabernet-Sauvignon, one of the most widely-planted grape cultivars in the world and possibly the most renowned cultivar for making red wine. Any proposed model for non-destructive LA predictions in Cabernet-Sauvignon should be tested for its accuracy because leaf shape formation is strongly affected by genetic (Iwata *et al.* 2002, Kessler and Sinha 2004) and environmental factors (Njoku 1957, Bhatt and Chanda 2003, Tsialtas and Maslaris 2007).

The aim of this work was to study the effect of rootstock and irrigation regime on leaf shape of Cabernet-Sauvignon leaves, to establish an LA prediction model based on L and W measurements, and to test the accuracy of already proposed models on the LA estimation.

Materials and methods

The experiments took place during the 2005 and 2006 growing seasons in a ten-year-old commercial vineyard in Thessaly, central Greece (39º48´N, 22º27´E, 190 m a.s.l.) laid on a deep, clay-loamy soil. Cabernet-Sauvignon grapevines (*Vitis vinifera* L.) were grafted on 1103 Paulsen (*V. rupestris*×*V. berlandieri*) and SO4 (*V. riparia*×*V. berlandieri*) rootstocks. The vineyard grows under a typical Mediterranean climate with 22 ºC mean temperature and 45 mm average rainfall during summer. Three irrigation treatments were applied: FI corresponding to 100 % evapotranspiration (ET_c), DI corresponding to 50 % ET_c, and NI corresponding to no irrigation.

Each year, three leaf samplings took place corresponding to three growth stages (bunch closure, veraison, and ripeness). In each plot, three fully expanded leaves of the outside part of the canopy, used for water potential measurements (Koundouras *et al.* 2006), were sealed in plastic bags, put on a portable refrigerator, and transferred to the Physiology Laboratory of Larissa factory, Hellenic Sugar Industry SA, for LA determinations. Leaf dimension parameters (LA, Per, L, W, and AR) were determined using *WinDias* image analysis system (*Delta-T Devices*, Cambridge, UK). The AR is the average of all the distances measured from the centroid to each perimeter point. For the two years of experimentation, a total of 324 leaves were measured.

The data were subjected to ANOVA as a four-factor RCB design with the rootstocks, irrigation treatments, and samplings as split plots on years. The *M-STAT* statistical package (version *1.41*, Crop and Soil Sciences Department, Michigan State University) was used for the analysis and the means were separated with LSD test at $p<0.05$. Figures were displayed using *Excel 98* software (*MSOffice*, *Microsoft*) and the significance of the best-fitted curves was determined by *SPSS* (version *14.0*, *SPSS*, IL, USA).

The following models proposed for various grapevine cultivars were evaluated for LA prediction in Cabernet-Sauvignon: Manivel and Weaver (1974): (*1*) $LA = 1.162\,L^2 - 0.802\,L + 1.051$; (*2*) $LA = 0.644\,W^2 + 0.469\,W + 0.109$; Sepúlveda and Kliewer (1983): (*3*) $LA = 0.69\,(L\times W) + 3.17$; (*4*) $LA = 0.68\,(L\times W) + 2.49$; Elsner and Jubb (1988): (*5*) $LA = -3.01 + 0.85\,(L\times W)$; (*6*) $LA = -1.41 + 0.527\,W^2 + 0.254\,L^2$; Schultz (1992): (*7*) $LA = 1.18\,(L-2.6)\times(L + 8.75)$; Montero *et al.* (2000): (*8*) $LA = 0.587\,(L\times W)$; (*9*) $LA = 0.647\,L^{1.956}$; Williams and Martinson (2003): (*10*) $LA = 0.637\,W^{1.995}$; (*11*) $LA = 0.672\,W^{1.963}$.

For model validation, measured and calculated LA were correlated and MSD was estimated as $MSD = \Sigma\,(X_n - Y_n)^2/N$, where X and Y are the model-based and measured values, respectively, and N is the number of observations (Kobayashi and Salam 2000, Gauch *et al.* 2003).

Results and discussion

Leaves of Cabernet-Sauvignon showed heteroblastic development, *i.e.* changes in leaf shape occurred during development (Byrne *et al.* 2001) but leaf morphology was not affected by the growing season, rootstock, and irrigation treatment (Table 1). The rootstock×irrigation×sampling time interaction was significant for all the leaf traits determined (LA, Per, L, W, AR) and the means of the interaction were used to establish relationships between measured LA and leaf dimensions (Table 2). A reliable LA prediction model should take into account the effects of growing conditions on leaf shape formation (Bhatt and Chanda 2003, Tsialtas and Maslaris 2007).

As Table 3 shows, significant correlations were found between the leaf dimension parameters with L-AR and W-Per relationships being the strongest. The LA increase was the product of the simultaneous increase of L and W as revealed by the significant correlation between the two parameters.

When accuracy is not a matter, linear functions should be preferred for establishing an LA prediction model due to its simplicity and applicability even under field conditions (Lu *et al.* 2004, Tsialtas and Maslaris 2007, 2008). A strong, linear relationship between L and LA [$LA = 18.379\,L - 151.41$, $r^2 = 0.97$, $p<0.001$, $n = 18$]

Table 1. *ANOVA* of the leaf parameters determined. ns – not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Source of variation	LA	Per	L	W	AR
Blocks	ns	**	ns	ns	ns
Years (Y)	ns	***	ns	ns	ns
Rootstocks (Rs)	ns	ns	ns	ns	ns
Y×St	ns	ns	ns	ns	ns
Irrigation (I)	ns	ns	ns	ns	ns
Y×I	ns	ns	ns	ns	ns
Rs×I	ns	ns	ns	ns	ns
Y×Rs×I	ns	ns	ns	ns	ns
Samplings (S)	**	***	**	***	***
Y×S	ns	*	ns	ns	ns
Rs×S	ns	ns	ns	ns	ns
Y×Rs×S	ns	ns	ns	ns	ns
I×S	ns	ns	ns	ns	ns
Rs×I×S	*	*	*	*	*
Y×Rs×I×S	ns	ns	ns	ns	ns
CV [%]	8.12	5.76	4.12	4.19	4.36

accomplishes the main criteria of a reliable, non-destructive LA prediction model for Cabernet-Sauvignon. On contrary to Williams and Martinson (2003), the relationship between W and LA had a lower r^2 than that of L-LA function and thus it was of lower accuracy (Fig. 1). LA prediction models, based only on L measurements, have already been proposed for cvs. Grenache (Manivel and Weaver 1974), White Riesling (Schultz 1992), and Cencibel (Montero *et al.* 2000). Often, dimension squares (L^2, W^2) or their product have been used for increasing accuracy in establishing LA prediction models (Sepúlveda and Kliewer 1983, Smith and Kliewer 1984, Elsner and Jubb 1988, Montero *et al.* 2000) but in Cabernet-Sauvignon the derived models were of equal or lower accuracy than those based on simple dimension measurements (data not shown). The strongest relationship found was that between AR and LA [$y = 43.409x - 143.26$, $r^2 = 0.98$, $p<0.001$, $n = 18$] but it could not be used as an LA prediction model due to the inconvenience of AR determination especially in the field (Tsialtas and Maslaris 2007).

Table 2. Comparison of means for the leaf traits showing a significant rootstock×irrigation×sampling interaction. For the same trait, means labelled with the same letter did not differ significantly at $p<0.05$.

Irrigation	Sampling	LA [cm²] 2005	LA [cm²] 2006	Per [cm] 2005	Per [cm] 2006	L [cm] 2005	L [cm] 2006	W [cm] 2005	W [cm] 2006	AR [cm] 2005	AR [cm] 2006
NI	1st	161.9 c-f	173.6 a-d	111.2 a-e	113.9 a-c	17.03 c-f	17.63 a-d	15.97 b-f	16.41 a-c	7.04 b-e	7.32 a-c
	2nd	158.8 d-f	173.6 a-d	107.2 c-e	111.3 a-e	16.78 ef	17.54 a-e	15.61 d-f	16.15 a-f	6.97 c-e	7.31 a-c
	3rd	168.6 a-f	158.8 d-f	114.9 ab	105.1 e	17.34 a-f	16.80 ef	16.38 a-d	15.66 c-f	7.12 a-e	7.04 b-e
DI	1st	176.9 a-c	180.5 a	116.6 ab	117.6 a	17.88 ab	18.04 a	16.58 ab	16.76 a	7.39 ab	7.44 a
	2nd	175.8 a-c	171.6 a-e	112.8 a-d	111.6 a-e	17.44 a-c	17.66 a-d	16.37 a-d	16.24 a-e	7.32 a-c	7.23 a-d
	3rd	156.2 ef	163.4 b-f	107.0 c-e	109.2 b-e	16.73 ef	17.28 a-f	15.53 ef	15.79 c-f	6.88 de	7.06 b-e
FI	1st	178.4 ab	164.2 b-f	117.5 a	115.3 ab	17.99 ab	17.20 b-f	16.67 ab	16.25 a-e	7.43 a	7.10 a-e
	2nd	175.6 a-c	157.7 ef	112.3 a-e	106.4 de	17.82 a-c	16.85 d-f	16.26 a-e	15.43 f	7.32 a-c	6.91 de
	3rd	153.3 f	162.7 c-f	105.8 de	110.3 a-e	16.67 f	17.17 b-f	15.58 ef	15.98 b-f	6.83 e	7.07 b-e

Table 3. Correlations between leaf dimension parameters showing significant differences in the rootstock×irrigation×sampling time interaction. All functions were significant at $p<0.001$.

[x] \ [y]	W	Per	AR
L	$y = 0.8297x + 1.7023$, $r^2 = 0.86$	$y = 7.5225x - 19.008$, $r^2 = 0.74$	$y = 0.412x - 0.0095$, $r^2 = 0.94$
W		$y = 9.4087x - 39.941$, $r^2 = 0.93$	$y = 0.4384x + 0.1$, $r^2 = 0.86$
Per			$y = 0.0406x + 2.6271$, $r^2 = 0.70$

Although many models have been developed for non-destructive LA estimation in grapevine cultivars, we are unaware of any attempt to test these models for their accuracy in LA prediction in other cultivars. Montero *et al.* (2000) proposed a comparison of the vine parameters measured in Cencibel with the respective ones of other cultivars, including Cabernet-Sauvignon, in order to test model accuracy. Table 4 presents the linear functions between LA calculated by 11 proposed models for various cultivars and measured LA in Cabernet-Sauvignon. All the relationships were highly significant ($p<0.001$) with r^2 of 0.87–0.97. A high r^2 is a major

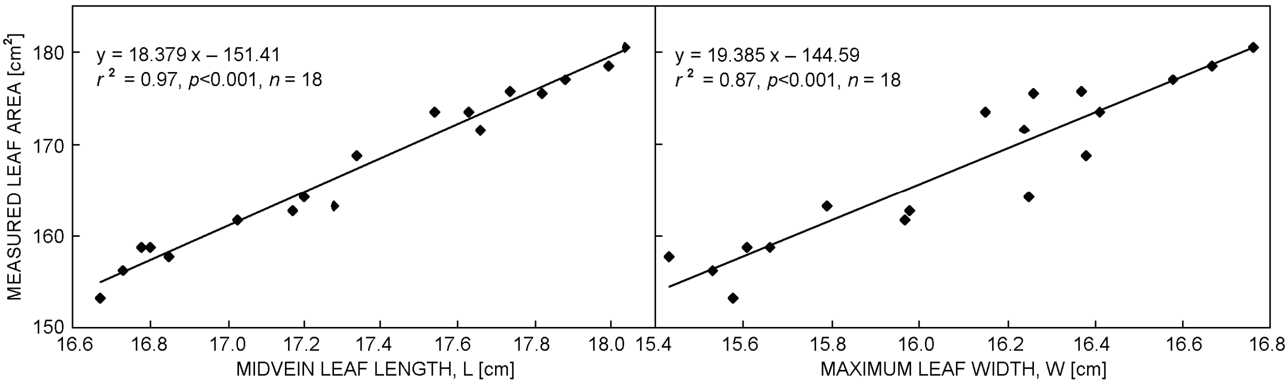

Fig. 1. Correlations between leaf dimensions, length and width (L, W), and measured leaf area (LA). $^{***}p<0.001$.

Table 4. Linear functions, r^2, and MSD for the calculated by models and measured leaf area (LA). For all functions, $p<0.001$ and $n = 18$.

Model	Linear function	r^2	MSD
1	y = 0.4653 x + 10.586	0.97	1601.59
2	y = 0.9158 x + 7.5106	0.87	3.40
3	y = 0.8511 x + 0.6608	0.96	45.42
4	y = 0.8636 x + 1.2084	0.96	35.07
5	y = 0.6909 x + 5.4382	0.96	250.27
6	y = 0.7656 x + 5.3459	0.94	109.30
7	y = 0.3814 x − 5.9066	0.97	4579.62
8	y = 1.0004 x + 3.3587	0.96	0.82
9	y = 1.0771 x + 7.5584	0.87	20.58
10	y = 0.9625 x + 10.67	0.87	1.68
11	y = 1.0135 x + 8.126	0.87	6.34

criterion for selecting a model for its accuracy (Kobayashi and Salam 2000, Gauch *et al.* 2003). Based on that, many proposed models could be suitable for LA prediction in Cabernet-Sauvignon (Table 4). A low MSD shows that a calculated LA is close to the measured one and thus MSD should be the main criterion for selecting an LA prediction model when a precise estimation of the actual LA is necessary (Gauch *et al.* 2003). Thus, combining both criteria (high r^2 and low MSD), only model *8* [LA = 0.587(L×W)] proposed by Montero *et al.* (2000) for the LA estimation in Cencibel could be a substitute for the linear function [LA = 18.379 L – 151.41] we proposed for LA estimation in Cabernet-Sauvignon.

References

Asomaning, E.J.A., Lockard, R.G.: Note on estimation of leaf areas of cocoa from leaf length data. − Can. J. Plant Sci. **43**: 243-245, 1963.

Bhatt, M., Chanda, S.V.: Prediction of leaf area in *Phaseolus vulgaris* by non-destructive method. − Bulg. J. Plant Physiol. **29**: 96-100, 2003.

Byrne, M., Timmermans, M., Kidner, C., Martienssen, R.: Development of leaf shape. − Curr. Opin. Plant Biol. **4**: 38-43, 2001.

Carbonneau, A.: Principes et méthodes de mesure de la surface foliaire. Essai de caractérisation des types de feuilles dans le genre *Vitis*. − Ann. Amél. Plantes **26**: 327-343, 1976.

Cristofori, V., Rouphael, Y., Mendoza-de Gyves, E., Bignami, C.: A simple model for estimating leaf area of hazelnut from linear measurements. − Sci. Hort. **113**: 221-225, 2007.

Demirsoy, H., Demirsoy, L.: A validated leaf area prediction model for some cherry cultivars in Turkey. − Pakist. J. Bot. **35**: 361-367, 2003.

Demirsoy, H., Demirsoy, L., Uzun, S., Ersoy, B.: Non-destructive leaf area estimation in peach. − Eur. J. hort. Sci. **69**: 144-146, 2004.

Elsner, E.A., Jubb, G.L., Jr.: Leaf area estimation of Concord grape leaves from simple linear measurements. − Amer. J. Enol. Viticult. **39**: 95-97, 1988.

Gauch, H.G., Jr., Hwang, J.T.G., Fick, G.W.: Model evaluation by comparison of model-based predictions and measured values. − Agron. J. **95**: 1442-1446, 2003.

Goudriaan, J., van Laar, H.H.: Modelling Potential Crop Growth Processes. − Kluwer Academic Publ., Dordrecht 1994.

Iwata, H., Nesumi, H., Ninomiya, S., Takano, Y., Ukai, Y.: Diallel analysis of leaf shape variations of citrus varieties based on Elliptic Fourier descriptors. − Breed. Sci. **52**: 89-94, 2002.

Iwata, H., Ukai, Y.: SHAPE: a computer program package for quantitative evaluation of biological shapes based on Elliptic Fourier descriptors. − J. Hered. **93**: 384-385, 2002.

Kessler, S., Sinha, N.: Shaping up: the genetic control of leaf shape. − Curr. Opin. Plant Biol. **7**: 65-72, 2004.

Kobayashi, K., Salam, M.U.: Comparing simulated and measured values using mean squared deviation and its components. − Agron. J. **92**: 345-352, 2000.

Koundouras, S., Bakratsa, G., Zioziou, E, Nikolaou, N., Tsialtas, I.: Influence of irrigation and rootstock cultivar on gas exchange, growth and ripening of Cabernet-Sauvignon (*Vitis vinifera* L.) under the semi-arid conditions of central Greece. − In: Proceedings of the 2nd International Symposium Ampelos 2006. Pp. 29-34. Santorini 2006.

Lu, H.-Y., Lu, C.-T., Wei, M.-L., Chan, L.-F.: Comparison of different models for nondestructive leaf area estimation in

taro. − Agron. J. **96**: 448-453, 2004.
Manivel, L., Weaver, R.J.: Biometric correlations between leaf area and length measurements of 'Grenache' grape leaves. − HortScience **9**: 27-28, 1974.
Mendoza-de Gyves, E., Rouphael, Y., Cristofori, V., Mira, F.R.: A non-destructive, simple and accurate model for estimation the individual leaf area of kiwi (*Actinidia deliciosa*). − Fruits **62**: 1-7, 2007.
Montero, F.J., de Juan, J.A., Cuesta, A., Brasa, A.: Non-destructive methods to estimate leaf area in *Vitis vinifera* L. − HortScience **35**: 696-698, 2000.
Nakamura, S., Nitta, Y., Watanabe, M., Goto, Y.: Analysis of leaflet shape and area for improvement of leaf area estimation method for sago palm (*Metroxylon sagu* Rottb.). − Plant Prod. Sci. **8**: 27-31, 2005.
NeSmith, D.S.: Nondestructive leaf area estimation of rabbiteye blueberries. − HortScience **26**: 1332, 1991.
Njoku, E.: The effect of mineral nutrition and temperature on leaf shape in *Ipomoea caerulea*. − New Phytol. **56**: 154-171, 1957.
Potdar, M.V., Pawar, K.R.: Non-destructive leaf area estimation in banana. − Scientia Hort. **45**: 251-254, 1991.
Pritsa, T.S., Voyiatzis, D.G., Voyiatzis, C.J., Sotiriou, M.S.: Evaluation of vegetative growth traits and their relation to time to first flowering of olive seedlings. − Aust. J. agr. Res. **54**: 371-376, 2003.
Schultz, H.R.: An empirical model for the simulation of leaf appearance and leaf area development of primary shoots of several grapevine (*Vitis vinifera* L.) canopy-systems. − Scientia Hort. **52**: 179-200, 1992.
Sepúlveda, G.R., Kliewer, W.M.: Estimation of leaf area of two grapevine cultivars (*Vitis vinifera* L.) using laminae linear measurements and fresh weight. − Amer. J. Enol. Vitic. **34**: 221-226, 1983.
Serdar, Ü., Demirsoy, H.: Non-destructive leaf area estimation in chestnut. − Scientia Hort. **108**: 227-230, 2006.
Smith, R.J., Kliewer, W.M.: Estimation of Thompson Seedless grapevine leaf area. − Amer. J. Enol. Vitic. **35**: 16-22, 1984.
Tsialtas, J.T., Maslaris, N.: Leaf area estimation in a sugar beet cultivar by linear models. − Photosynthetica **43**: 477-479, 2005.
Tsialtas, J.T., Maslaris, N.: Leaf shape and its relationship with Leaf Area Index in a sugar beet (*Beta vulgaris* L.) cultivar. − Photosynthetica **45**: 527-532, 2007.
Tsialtas, J.T., Maslaris, N.: Leaf area prediction model for sugar beet (*Beta vulgaris* L.) cultivars. − Photosynthetica **46**: 291-293, 2008.
Whitworth, J.L., Mauromoustakos, A., Smith, M.W.: A non-destructive method for estimation of leaf area in pecan. − HortScience **27**: 851, 1992.
Williams, L., III, Martinson, T.E.: Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. − Sci. Hort. **98**: 493-498, 2003.