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Author: Y. Guisard and C. Birch

Title: Towards Developing Reliable Models of Leaf Area on Grapevines (*Vitis vinifera* L.)

Editor: V. r. BELLON-MAUREL

Conference Name: Information and technology for sustainable fruit and vegetable production. Frutic 05, 7th Fruit, Nut and Vegetable Production Engineering Symposium

Conference Location: Montpellier, France

Publisher: Cemagref

Year: 2005

Pages: 305-314

Date: 12-16 September 2005

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Towards Developing Reliable Models of Leaf Area on Grapevines (*Vitis vinifera* L.)

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Keywords: Shoot Leaf Area, Allometric Relationships, Digital Image Analysis.

Abstract

A field study in three vineyards in southern Queensland (Australia) was carried out to develop predictive models for individual leaf area and shoot leaf area of two cultivars (Cabernet Sauvignon and Shiraz) of grapevines (*Vitis Vinifera* L.). Digital image analysis was used to measure leaf vein length and leaf area. Stepwise regressions of untransformed and transformed models consisting of up to six predictor variables for leaf area and three predictor variables for shoot leaf area were carried out to obtain the most efficient models. High correlation coefficients were found for log₁₀ transformed individual leaf and shoot leaf area models. The significance of predictor variables in the models varied across vineyards and cultivars, demonstrating the discontinuous and heterogeneous nature of vineyards. The application of this work in a grapevine modeling environment and in a dynamic vineyard management context are discussed. Sample sizes for quantification of individual leaf areas and areas of leaves on shoots are proposed based on target margins of errors of sampled data.

INTRODUCTION

The quantification of leaf area in agricultural systems is an important component of crop models that focus on yield, competition with weeds, and modeling of heat, energy and water exchanges in the soil plant atmosphere continuum (Yin *et al.*, 2003). Leaf Area Index (LAI) has been used as a predictor for nitrogen partitioning in canopies (Yin *et al.*, 2003), and in the study of Normalized Difference Vegetation Index (NDVI) sensitivity in remote sensing studies of American vineyards (Johnson, 2003). Most published literature focuses on LAI, the main assumption being that the soil area concerned is uniformly covered by the crop canopy. In Australia, Smart and Robinson (1998) have correlated shading in the canopy with lower fruit quality, higher incidence of fungal disease and lower yield. They proposed optimal values of leaf area, shoot length, and leaf area/crop weight for vineyards varying in vigor, and argue that canopy microclimate is important to achieving high fruit yield and quality. Unfortunately LAI does not represent the discontinuous and heterogeneous nature of grapevine canopies (Mabrouk and Sinoquet, 1998). The diversity of training and trellising systems and vine management complicates the use of indices based on homogenous canopy structure. Canopy architecture modeling therefore has the potential to provide predictions of fruit yield and quality, and be useful in decision support systems and educational tools.

The use of allometric relationships to relate physical parameters to leaf area in viticulture was proposed by Galet (1998), who argued that using leaf length (L) x leaf width (W) allowed classification of leaves according to their size (9 classes in 50cm² increments to 450cm²). Galet also proposed the naming of the leaf veins (Figure 1). Carbonneau (1976) showed that L was highly correlated to leaf area (LA) ($r^2=0.91$). Carbonneau (1996, cited in Montero *et al.*, 2000) later showed that a combination of measurements of leaf veins was even more strongly correlated with LA ($r^2=0.95$). Montero *et al.* (2000) proposed that the distribution of LA and thus LAI within canopies could explain the distribution of radiation interception. Using digital analysis, they established: $LA = 0.58LxW$ ($r^2=0.99$) over two sampling seasons. Pire and Valenzuela (1993) found similar results, even when phenological stages were taken in account. Both dry and fresh leaf weight were highly correlated with LA (Winler *et al.*, 1956; Payne *et al.*, 1984; cited in Montero and al., 2000). However, these relationships varied with phenological stage and environmental conditions, and have not been used widely. Deterministic equations for leaf appearance and leaf area development using the plastochron concept have been proposed by Schultz (1992), and while the approach accurately predicted leaf emergence and LA on grapevines up to 100 days from leaf appearance, it did not account for lateral growth or water stress. Most models of grapevines consider them to grow continuously from budburst to harvest (Williams, 2001; Wermelinger and Baumgartner, 1990; Bindi *et al.*, 1997a; Ortega-Farias *et al.*, 2000; Lasko White and Tustin, 2001).

Hunt and Hodson (1999) showed that low resolution (75 Dots per inch (dpi)) scanning and image processing was as accurate and required less calibration than traditional planimetry methods for analysis of area of leaves of varying complexity. In an attempt to predict above ground vegetative biomass, Castelan-Estrada, Vivin and Gaudillere (2002) used allometric relationships to relate total shoot leaf area (SA) to shoot diameter (SD, at the mid point of the length of shoots) and shoot length (SL). Indirect methods of assessing canopy characteristics, principally LAI, have also been tested in vineyards, but are costly and require extensive calibration.

The objectives of this research were:

- To propose allometric models of LA and SA .
- To identify and discuss sampling sizes and margins of errors for selected models.

MATERIALS AND METHODS

Three vineyards located in the Stanthorpe wine region, extending from 25 km North to 25 km South of Stanthorpe, Queensland, Australia (Latitude -25.6653°S, Longitude 151.9333°E) were used for data collection during the 2002-03 season. The vineyards were spread across the region, site 1 in the north east, site 2 in the center, and site 3 in the southern end. Characteristics of the vineyards are presented in Table 1. The region is cool, being 871 m above sea level. Soils are duplex, with a sandy A-horizon of varying depth overlying a heavier clay B-horizon. The region is prone to frost during winter and spring, and water stress, as summer rainfall is limited and soils have low water holding capacity. The 2002-2003 season was unusually dry, with rainfall in the lowest 20% of seasons (www.bom.gov.au/climate/averages/). The vines were, drip irrigated as per vineyard managers normal practice. Hail and frost occurred in 2004, with site 1 being the most

severely affected, followed by site 2 and then site 3. Site 1 was also affected by high wind velocity.

Scanning Procedures

A flat bed scanner (Cannon Lide 30), using grey scale scan mode at a resolution of 75 dpi was used, and image analysis completed using Image Tool 3 (University of Texas Health Science Centre, San Antonio, Texas, USA). Two procedures are available for measuring an area, both being used in this study:

- The Black and White pixel method which returns %Black pixels present in the image.
- Blob objects method, which displays the area of objects found in the image (in cm²).

Single Leaf Area (LA)

Fifty leaves per sample were harvested randomly in 6 samplings of the three sites and two cultivars (Cabernet Sauvignon (CS) and Shiraz (SH)). Individual leaves without petioles were placed in separate plastic bags and refrigerated to minimize deterioration from sampling to scanning (up to 4 days). Leaves were scanned using the ‘Blob’ method. Length of leaf veins (L1, L2, L3 and L4), L and W, named (Galet 1998) (Figure 1), were measured with the ‘distance’ tool of Image Tool 3.

Overlapping Lobes

To evaluate the impact of these overlapping leaf lobes in leaves of CS on scanned leaf area, 15 leaves were sampled per site in each of 4 samplings) and scanned using both the ‘Blob’ and ‘%Black’ methods. Leaves were then dissected into parts (‘Parts’) so that no overlaps would occur, and ‘Parts’ scanned using the ‘Blob’ method only.

Shoot Characteristics and Leaf Area (SA)

Shoots of CS vines were sampled to quantify leaf area distribution on shoots and SA. Five random samples of 1 linear meter of vine canopy were destructively sampled as close as possible to flowering, pea size, veraison, and harvest for sites 1 and 2. Each sample was bagged individually, refrigerated and processed as soon as possible. In some cases, leaf abscission occurred in the bag due to the time from sampling to processing, and for these, individual shoot data was collected but leaf area was not used in the computation of the models. Measurements were SA (cm²), shoot diameter (SD, mm) at the shoot base, shoot length (SL, mm) leaf number (LN) and LA (cm², using the %Black method). SD, SL, LN and SL were included to quantify vineyard variability.

Broken Shoots

Broken shoots (CS and SH) were recorded for analysis, but were not included where they would introduce bias by having an atypical length for a given diameter, or atypical number of leaves and associated shoot area. The impact of broken shoots on canopy structure was examined for canopy modeling purposes.

Statistical Methods

The impact of the scanning procedure on measured leaf area was assessed using a paired t test (%Black – ‘Parts’) and (Blob – ‘Parts’). Descriptive statistics and paired t tests were calculated using Microsoft Excel 2003’s Analysis Add on Tool. Linear regressions, data transformations and plotting were carried out with SPLUS Professional Release 2 (Lucent Technologies Inc.). All inferences and significance levels are reported at P=0.05. LA and

SA models were developed using stepwise regression (Neter *et al.*, 1996). A stepwise regression in SPLUS requires the user to specify a regression with the maximum number of predictor variables (‘upper formula’). The following upper formulae were used:

LA~L1 + L2 + L3 + L4 + W + L and;
SA~SD+SL+LN

The number of models for testing (determined as in Neter *et al.* (1996)) was 64 for LA and 16 for SA. To satisfy assumptions of linear regression, data were transformed (using x^2 , $1/x$, $\log_{10}x$, $\log_e x$, or \sqrt{x}) when necessary. Using SA as the response variable, graphs used to examine the suitability of the model included response vs. fitted values, residuals vs. fitted values and residuals normal probability plots to assess assumptions of linearity, normality and spread of residuals, and Cook’s distances (Neter *et al.*, 1996), to assess the impact of outlying residuals on the regression. Sample sizes for nominated margins of error were computed as in Neter *et al.* (1996):

$m = z^* \sigma / \sqrt{n}$ where m is the margin of error, z^* is the critical value for a chosen confidence level, σ estimated from the standard deviation, and n the sample size.

RESULTS AND DISCUSSION

Scanning Procedures

The scanning procedure (%Black or Blob method) produced smaller areas when measuring a full rather than dissected leaf (Parts) of leaves with overlapping lobes (as in CS). The ratio %Black: Parts and Blob: Parts was computed for each sampled leaf in order to quantify this impact (Table 2), showing that scanning of entire leaves requires correction factor for actual area but the method of scanning had no impact on the correction factor. Correction factors ranged between 1.02 and 1.042, factors similar to those in Hunt and Hodson (1999) using ‘blob’ scanning method. Digital image analysis requires extensive scanning time when large numbers of leaves are involved, but the ability to reuse the images for further studies and to share data with others justifies the time invested and storage of electronic files. Also, it is not necessary to calibrate the scanning method to measure actual leaf area.

Single Leaf Area

A summary of all LA and SA models is presented in Table 3. The sample margin of error for the LA models were computed using the sample standard deviation (sd) as estimator of the true σ (Table 4). The sd of samples increased as LA increased (site 1 had the smallest leaves and site 3 the largest). The margin of error (m) increased accordingly. The models relating LA to vein length generally agreed with Galet (1998) and Carbonneau (1976), and thus allometric relationships can be used to predict LA. The stepwise regression confirmed the importance of L and W as predictor variables of LA but showed improved R^2 by the inclusion of further leaf veins lengths in all models. However, the relationship varied with cultivar and across sites rendering generic equations less accurate, in contrast to the findings of Carbonneau (1976), Galet (1998), Montero *et al.*, (2000) and Pire and Valezuea, 1993). Therefore we propose that site specific relationships be preferred, taking samples varying between 148 and 339 leaves depending on the site and the cultivar, aiming to obtain a 5cm^2 margin of error for a sd in leaf area models of about 35cm^2 .

Shoot Leaf Area

The models benefited from data transformation to reduce the influence of observations with large Cook's distances. In all cases a single transformation was sufficient. Models relating SA to SD, SL and LN were similar to those of Castellan-Estrada, Vivin and Gaudillere (2002), so it appears that allometric relationships can also be used to predict SA. Table 4 shows the margins of errors and sd of the field samples. Targeting margins of errors of 80cm², it is proposed that samples of 212 and 400 shoots be taken in site 1 and 2 respectively in future studies. These large proposed samples are due to the large sample standard deviation reflecting the heterogeneity of the vine canopies. Using a recommended 900 mm for SL, 12 cm² of leaf area per gram of berry fresh weight (Smart and Robinson, 1998), and the appropriate SA model, it should be possible to design optimum vine structure to achieve target yield or quality key parameters.

Broken Shoots

Descriptive statistics for broken shoots showed that it is necessary to account for potential shoot loss in site 1, but not in site 2 (data not shown). It is therefore commended that simulated sites be surveyed for the presence of broken shoots and the simulated SA appropriately corrected.

CONCLUSION

This work supports the use of allometric relationships to predict LA and SA. The proposed methodology allows for the rapid determination of LA and SA as a "one step method". Recent interest in architectural modeling of canopies of crops has demonstrated that models of canopy architecture can support studies in plant physiology, and provide predictive tools for crop parameters. The most important variables to include in such model are LA, SA, and LN but variations due to site specific micro climate and vineyard design need consideration in modeling grapevine canopies. Increased stability of the models is achieved by the inclusion various vein length measurements, varying on a site and cultivar basis. This work demonstrates the heterogeneous and discontinuous nature of grapevine canopies, leading to variable recommended sample sizes.

Acknowledgement

The assistance of Mr A. Comino, Mr G. Casley and Mr E. Macpherson for allowing data collection on their properties and to Dr Peter Carberry (CSIRO Sustainable Ecosystems) for his support and advice is gratefully acknowledged.

Literature Cited

- Bindi M., Miglietta F., Gozzoni B., Orlandini S. and Seghi L. (1997) A simple model for simulation of growth and development in grapevine (*Vitis vinifera* L.). I. Model description. *Vitis* 36, 67-71.
- Carbonneau A. (1976) Analyse de la croissance des feuilles du sarment de vigne: estimation de sa surface foliaire par echantillonnage. *Connaissance vigne vin* 10, 141-159.
- Carbonneau A. (1996) General relationship within the whole plant: examples of the influence of vigour status, crop load, and canopy exposure of the sink "berry

- maturation" for the grapevine. *ISHS Acta horticulturae* 427.
- Castelan-Estrada M., Vivin P. and Gaudillere J.P. (2002) Allometric relationships to estimate seasonal above ground vegetative and reproductive biomass of *Vitis vinifera* L. *Annals of Botany* 89, 401-408.
- Galet P. (1998) *Precis d'ampelographie pratique*, Imprimerie JF Impression.
- Hunt M.A. and Hodson S.L. (1999) Accurate area determination of complex leaves using digital image analysis. *Australian Journal of Plant Physiology* 26, 767-772.
- Johnson L.F., Pierce L., DeMartino J., Youkhana S., Nemani R., and Bosh D. (2003) Image based decision tools for vineyard management. ASAE Annual International Meeting 27-30 July 2003, Las Vegas, USA
- Lasko A.N., White M.D., Tustin D.S. (2001) Simulation modelling of the effects of short and long term climatic variations on carbon balance of apple trees. *ISHS Acta Horticulturae* 557.
- Mabrouk H. and Sinoquet H. (1998) Indices of light microclimate and canopy structure of grapevines determined by 3D digitising and image analysis, and their relationships to grape quality. *Australian Journal of Grape and Wine Research* 4, 2-13.
- Montero F.J., de Juan J.A., Cuesta A. and Brasa A. (2000) Nondestructive methods to estimate leaf area in *Vitis vinifera* L. *HortScience* 34, 696-698.
- Neter J., Kutner M.H., Nachtsheim C.J., and Wasserman W. (1996) Applied linear statistical models. Mc Graw Hill (Eds). Third Edition. ISBN 0256-11736-5
- Ortega -Farias S.O., Lozano P., Moreno Y. and Leon L. (2002) Desarrollo de medeleos predictivos de fenologia y evolucion de madurez en vid para vino cv Cabernet Sauvignon y Chardonay. *Agricultura Tecnica* 62, 27-37.
- Payne W.A., Wendt C.W., Hossner L.R. and Gates C.E. (1991) Estimating pearl millet leaf area and specific leaf area. *Agronomy Journal*. 83, 937-941
- Pire R. and Valenzuela I. (1993) Estimacion del area foliar en *Vitis vinifera* L. "French Colombar" a partir de medicion de lineales en las hojas. *Revista de Agrinimia* 10
- Schultz H.R. (1992) 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 Horticulturae* 52, 179-200.
- Smart, R. and Robinson M. (1998) *Sunlight into Wine*, Hyde Park Press edn. Adelaide, Australia: Winetitles. ISBN 1 875130 10 1
- Wermelinger B. and Baumgärtner J. (1990) Application of a demographic crop growth model: an explorative study on the influence of nitrogen on grapevine performance. *ISHS Acta Horticulturae* 276
- Williams L.E. (2001) Irrigation of winegrape in California. *Practical Winery and Vineyard*. Nov-Dec 2001, pp42-45.
<http://www.practicalwinery.com/novdec01p42.html>
- Winter E.J., Salter P.J., Stanhill G. and Bleasdale J.K. (1956) Some methods of measuring leaf area. In: F.L. Milthorpe (ed.) *The growth of leaves*. Butterworths, London.
- Winter F. (1986) Foreword. *ISHS Acta horticulturae* 184
- Yin X., Lantinga E.A., Schapendonk H.C.M and Zhong X. (2003) Some quantitative relationships between leaf area index and canopy nitrogen content and distribution. *Annals of Botany* 91, 893-9

Tables

Table 1. Characteristics of vineyards at 3 sites and two cultivars (Cabernet Sauvignon (CS), Shiraz (SH)) used.

	Site 1		Site 2		Site 3	
	CS	SH	CS	SH	CS	SH
Cultivar clone	G9V3	PT23	G9V3	PT23	G9V3	PT23
Rootstock variety	Richter 109 Teleki 5A	Richter 109 Teleki 5A	Teleki 5A	Own Roots	Schwartzmann	Schwartzmann
Planting year	1996		1997	1979	1999	
Soil type	Sandy Loam		Sandy Loam		Fine Sandy Loam	
Trellis type	Vertical Shoot Positioning 3 wires		Vertical Shoot Positioning		Smart Dyson	
Pruning regime	2 bud spurs		4 to 6 canes		2 bud spurs + sacrificial canes	
Pruning weight (2003) (g/m canopy)	200	240	130	170	1250	1400

Table 2. Ratio of area by ‘%Black’ or ‘Blob’ and actual leaf area from ‘Parts’ of Cabernet Sauvignon leaves at three sites.

Site	% Black : Parts area	sd	n	Blob :Parts area	sd	n
#1	0.98	0.02	57	0.98	0.03	57
#2	0.97	0.02	58	0.97	0.02	58
#3	0.96	0.03	59	0.96	0.03	59

Table 3. Summary of transformed Leaf area (LA) and Shoot area (SA) models for three sites (S1, S2, S3) and two cultivars (Cabernet Sauvignon (CS) and Shiraz (SH)).

Model for	Model retained	Models tested	n	r ²
LA, CS, S1	$\log_{10}(\text{LA}) = -0.06 + 0.08\log_{10}(\text{L2}) + 0.17\log_{10}(\text{L4}) + 0.82\log_{10}(\text{L}) + 0.91\log_{10}(\text{W})$	64	308	0.96
LA, SH, S1	$\log_{10}(\text{LA}) = -0.09 - 0.14 \log_{10}(\text{L1}) + 0.12\log_{10}(\text{L2}) + 0.08\log_{10}(\text{L3}) + 0.72\log_{10}(\text{L}) + 1.16\log_{10}(\text{W})$	64	276	0.97
LA, CS, S2	$\log_{10}(\text{LA}) = -0.1 + 0.06\log_{10}(\text{L2}) + 0.07\log_{10}(\text{L4}) + 0.86\log_{10}(\text{L}) + 0.98\log_{10}(\text{W})$	64	266	0.98
LA, SH, S2	$\log_{10}(\text{LA}) = -0.02 + 0.17\log_{10}(\text{L2}) + 0.12\log_{10}(\text{L4}) + 0.82\log_{10}(\text{L}) + 0.83\log_{10}(\text{W})$	64	362	0.96
LA, CS, S3	$\log_{10}(\text{LA}) = -0.11 + 0.08 \log_{10}(\text{L3}) + 0.06\log_{10}(\text{L4}) + 0.71\log_{10}(\text{L}) + 1.14\log_{10}(\text{W})$	64	315	0.99
LA, SH, S3	$\log_{10}(\text{LA}) = -0.13 - 0.44\log_{10}(\text{L1}) + 0.17\log_{10}(\text{L2}) + 0.11\log_{10}(\text{L3}) + 1.26\log_{10}(\text{L}) + 0.87\log_{10}(\text{W})$	64	321	0.98
SA, CS, S1	$\log_{10}(\text{SA}) = 0.88 + 0.3\log_{10}(\text{SD}) + 0.28\log_{10}(\text{SL}) + 0.75\log_{10}(\text{LN})$	16	242	0.9
SA, CS, S2	$\log_{10}(\text{SA}) = 0.92 + 0.38\log_{10}(\text{SL}) + 0.35\log_{10}(\text{LN})$	16	291	0.95

Table 4. Actual sampling margins of error and proposed target sample sizes and margins of error of LA samples for confidence level 0.05, for three sites (Cabernet Sauvignon (CS) and Shiraz (SH)).

	sd	Actual n	Actual margin of error (cm ²)	Proposed margin of error (cm ²)	Proposed n
LA – CS, Site 1	21	308	2.3	3	188
LA – SH, Site 1	31	276	3.7	5	148
LA – CS, Site 2	31	267	3.7	5	148
LA – SH, Site 2	37	362	3.8	5	210
LA – CS, Site 3	47	316	5.2	5	339
LA – SH, Site 3	60	321	6.6	7	282
SA – CS, site 1	595	242	74.9	80	212
SA – CS., site 2	817	291	93.9	80	400

Figures

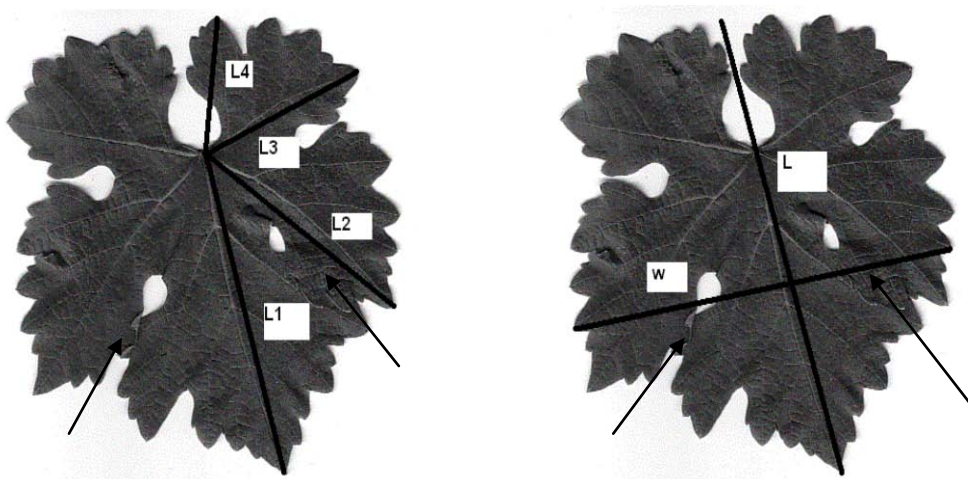


Figure 1. Distances measured on Cabernet Sauvignon leaves using digital analysis for the computation of leaf area (LA) models (L1, L2, L3, L4, L and W) as described by Galet (1998). Arrows show overlapping lobes of Cabernet Sauvignon leaves.