ESTIMATION OF LEAF AREA IN GRAPEVINE CV. SYRAH USING EMPIRICAL MODELS

ESTIMATION DE LA SURFACE FOLIAIRE DU CÉPAGE SYRAH AVEC DES MODÈLES EMPIRIQUES

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Abstract

Aiming to obtain empirical models for the estimation of Syrah leaf area a set of 210 fruiting shoots was randomly collected during the 2013 growing season in an adult experimental vineyard, located in Lisbon, Portugal. Samples of 30 fruiting shoots were taken periodically from the stage of inflorescences visible to veraison (7 sampling dates). At the lab, from each shoot, primary and lateral leaves were separated and numbered according to node insertion. For each leaf, the length of the central and lateral veins was recorded and then the leaf area was measured by a leaf area meter. For single leaf area estimation the best statistical models uses as explanatory variable the sum of the lengths of the two lateral leaf veins. For the estimation of leaf area per shoot it was followed the approach of Lopes & Pinto (2005), based on 3 explanatory variables: number of primary leaves and area of the largest and smallest leaves. The best statistical model for estimation of primary leaf area per shoot uses a calculated variable obtained from the average of the largest and smallest primary leaf area multiplied by the number of primary leaves. For lateral leaf area estimation another model using the same type of calculated variable is also presented. All models explain a very high proportion of variability in leaf area. Our results confirm the already reported strong importance of the three measured variables (number of leaves and area of the largest and smallest leaf) as predictors of the shoot leaf area. The proposed models can be used to accurately predict Syrah primary and secondary leaf area per shoot in any phase of the growing cycle. They are inexpensive, practical, non-destructive methods which do not require specialized staff or expensive equipment.

Keywords : *Leaf area estimation, grapevine, statistical model, Syrah*

Résumé

Visant à obtenir des modèles empiriques pour l'estimation de la surface foliaire principale et secondaire d'un rameau de vigne de la varieté Syrah, un ensemble de 210 sarments ont été prélevés au hasard pendant la saison végétative 2013 dans un vignoble expérimental adulte, situé à Lisbonne, au Portugal. Des échantillons de 30 rameaux fructifères ont été prélevés périodiquement du stade des inflorescences visibles à la véraison (7 dates d'échantillonnage). Au laboratoire, les feuilles principales et des entrecoeurs de chaque rameau ont été séparées et numérotées en fonction de leur insertion sur le rameau. Pour chaque feuille, la longueur des nervures centrales et latérales a été enregistrée ainsi que la surface réelle des feuilles mesurée au moyen d'un analyseur de surface foliaire (planimètre). Pour l´estimation de la surface d'une feuille individuelle les meilleurs modèles statistiques utilisent comme variable explicative la somme des longueurs des deux nervures latérales. Pour l'estimation de la surface foliaire d'un rameau, nous avons suivi la methodologie de Lopes et Pinto (2005), basée sur 3 variables explicatives: le nombre de feuilles principales et la surface foliaire de la feuille la plus grande et la plus petite. Le meilleur modèle statistique pour l'estimation de la surface foliaire principale d'un rameau utilise une variable calculée par multiplication de la surface foliaire de la feuille principale moyenne (moyenne entre la surface foliaire de la feuille la plus grande et de la plus petite) par le nombre de feuilles principales. Pour l'estimation de la surface foliaire secondaire d'un rameau, un autre modèle a été utilisé avec le même type de variable calculée. Tous les modèles utilisés expliquent la très forte variabilité de la surface foliaire. Nos résultats confirment l' importance des trois variables mesurées (nombre de feuilles et surface foliaire de la plus grande et de la plus petite feuille) comme variables explicatives de la surface foliaire d'un rameau de vigne. Les modèles proposés peuvent être utilisés pour prédire avec précision la surface foliaire principale et secondaire d'un rameau de la varieté Syrah dans toutes les phases du cycle vegetatif. Ce sont des méthodes non destructives, pratiques et peu coûteuses, qui ne nécessitent pas de personnel spécialisé ou des équipements coûteux.

Mots-clés : surface foliaire, vigne, modèles empiriques, Syrah

1. Introduction

Vine leaf area is a parameter which has gained significant importance during the past years, as it can provide viticulturists and researchers with important indications regarding the vineyard's environment, potential leaf gas exchange and productivity. Leaf area (LA) is a fundamental parameter for characterizing vine vigor, balance (Kliewer and Dokoozlian, 2005) and canopy density, allowing to understanding plant responses to environment, training systems and cultural practices, especially those related to canopy management and training systems (Smart, 1995). Yet, the monitoring of grapevine leaf area is not an easy task. There are several methods of determining LA, which can be categorized as direct or indirect. Direct methods can be destructive or non-destructive, while the indirect methods are usually non-destructive (Mabrouk and Carbonneau, 1996). Within the non-destructive methods the use of empirical models for the estimation of a single leaf area (e.g. Carbonneau, 1976, Schultz, 1992, Lopes and Pinto, 2000; Guisard *et al*., 2010) and of all the shoot leaf area (e.g. Barbagallo *et al*, 1996; Mabrouk and Carbonneau, 1996; Lopes et al., 2004; Lopes and Pinto, 2000, 2005) have been developed in recent years.

The models proposed by Lopes and Pinto (2005) to estimate shoot leaf area (SLA), which are based on three variables (number of leaves and area of the largest and smallest leave) have been well accepted by the researcher community as proved by the recent positive validations made with several varieties (Beslic et al.; 2010; Sánchez-de-Miguel et al. 2011; Döring et al., 2013). The simplicity and accuracy of those models makes them appropriate for common use, however it's still necessary to validate the methodology with independent datasets from other varieties, regions and training systems.

The aim of this paper is to develop the approach proposed by Lopes and Pinto (2005) using a set of shoots from cv. Syrah, collected at different growth stages, in order to provide a new and improved empirical model for the estimation of the area of a single leaf and of the shoot primary and lateral leaf area of the red grapevine variety Syrah.

2. Material and Methods

Field conditions and plant material

The study was conducted on the variety Syrah (*Vitis vinifera* L.) grafted on 140 Ru rootstocks. Shoot sampling was performed destructively at the experimental vineyard of the Instituto Superior de Agronomia, in Tapada da Ajuda, Lisbon, Portugal (38°42' N; 9°11' W), between April and July 2013. The vines were planted in 1998 and pruning in a double Royat cordon with vertical shoot positioning. The vines have an average of 4-5 spurs with 3 buds each and are planted at an interrow spacing of 2.5 meters and 1.2 meters between the plants (3333 vines/ha). The plot has a deep and fertile clay soil and a small slope to south. The vineyard was equipped with drip irrigation, but irrigation was not necessary during this season.

Leaf area measurements

Samples of 30 fruiting shoots were taken periodically from the phenological stage inflorescences visible to veraison, (middle April to middle of July - 7 sampling dates).The sampling was done at two weeks intervals; the shoots were randomly collected, immediately placed into plastic bags and transferred to the lab. After being numbered, the leaves were removed from the plant and measured. As a rule of the thumb, basal leaves were disregarded, as they usually have an abnormal shape and are too small. The smallest leaves considered were those whose primary vein length was at least 30 mm. Leaf area (LA) was measured with a leaf area meter (Delta – T devices, England) to the accuracy of 1 cm^2. The following observations were made: primary shoot length (cm) from the base to the apex (SL_1) and to the last measurable leaf (SL_{1L}); length (mm) of the central (V1) and lateral veins (left -V2L; right- V2R) and corresponding leaf area (cm^2) (LA) of each primary (LA_1) and lateral leaf (LA_2). For lateral shoots the measurements began at the 4^{th} sampling occasion (berry set - end of May) when the lateral leaves and shoots reached a considerable size and number.

From these measurements, the variables needed for the Lopes and Pinto (2005) methodology were computed for each primary shoot (subscripts 1): number of primary leaves (NL_1), area (cm^2) of the smallest (S_1) and largest primary (L_1) leaves and the total primary leaf area per shoot (SLA_1). From this data, two further variables were calculated (Eq. 1 and 2):

- the mean primary leaf area: $M_1 = (L_1 + S_1)/2$ (Equation 1);
- the mean primary leaf area per shoot: $MLA_1 = M_1 * NL_1$ (Equation 2);

For the lateral shoots the same type of variables (subscript 2) were computed.

Statistical analysis

Statistical analysis was done using the R version 3.0.0 statistical software (© 2013 The R Foundation for Statistical Computing). Simple and multiple linear regression analyses were performed between the above mentioned independent variables and the dependent variables LA_1, LA_2, SLA1 and SLA_2. Models were fitted using the least squares method and variable selection was done with a forward stepwise regression with 0.05 critical F statistic.

3. Results and Discussion

Single leaf area

The correlation matrix between the area of a single primary leaf (LA_1) and the length of the central (V1) and lateral veins (V2) shows that the highest correlation coefficient was obtained with the variable V2S (r= 0.95) while the smallest one was obtained with the variable V1 (r=0.93). Using the V2S as independent variable in a linear regression analysis we obtained a very good fit with actual leaf area (Fig. 1A).

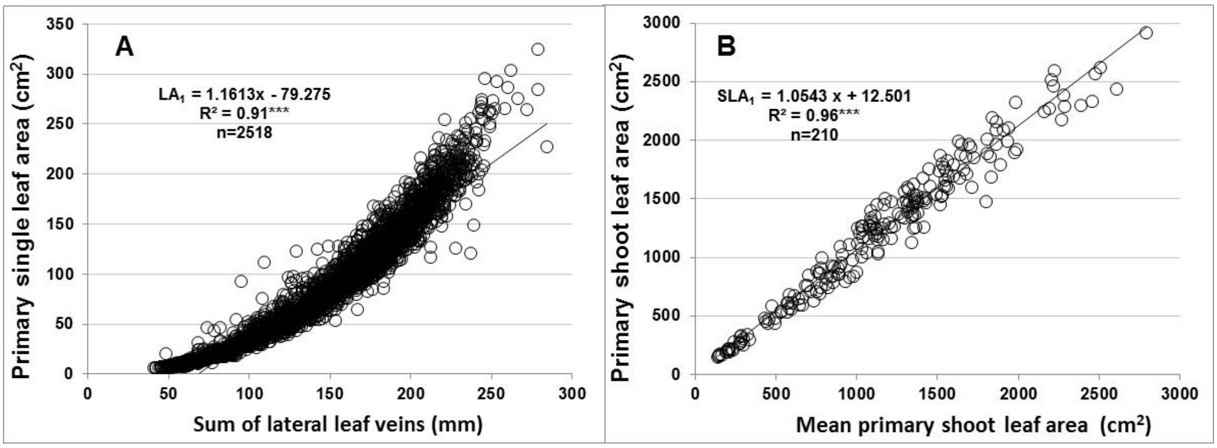

***Figure 1. A**- Relationship between the sum of lateral leaf veins (independent variable) and actual primary single leaf area (dependent variable); **B** - Relationship between mean primary shoot leaf area (average of the largest and smallest primary leaf area multiplied by the number of primary leaves) (independent variable) and actual primary shoot leaf area (dependent variable).*

Figure 1. **A**- Relation entre la somme des longueurs des deux nervures latérales (variable indépendante) et la surface réelle d'une feuille individuelle principale (variable dépendante); **B** - Relation entre la variable surface foliaire principale moyenne d'un rameau (moyenne entre la surface foliaire de la feuille la plus grande et de la plus petite multipliée par le nombre de feuilles principales) (variable indépendante) et la surface foliaire réelle principale d'un sarment (variable dépendante).

However, given the evident curvilinearity in the relation, alternative non-linear models were fitted. The best results were obtained with a logarithmic transformation of both variables, which linearizes a power function relation between LA_1 and V2S. The final linearized model shows that the logarithm of the variable V2S explains a very high proportion of the logarithm of the LA_1variability (Eq. 3):

$$\ln(LA_1)= -6.436 + 2.167 * \ln(V2S) <=> LA_1 = e^{-6.436} * V2S2.167 \ (R2 = 0.97; p < 0.001; n = 2518) \quad \text{(Equation 3);}$$

where LA_1 is the primary single leaf area (cm^2) and V2S the sum of the two lateral leaf veins (mm). In the original variables the computed mean square error is 13.6 cm^2.

These results agree with those obtained with other varieties (Carbonneau, 1976; Lopes et al., 2000; Silvestre and Eiras-Dias, 2001) showing that, despite the duplication of the measurements, the sum of the two lateral veins allow a better accuracy as compared to the use of the main vein length. Similar results were obtained for the area of a single lateral leaf (LA_2) however the final statistical models were different from those of the primary leaf (Phinopoulos, 2013; data not showed).

Primary shoot leaf area

The correlation matrix between primary shoot leaf area (SLA_1) and the selected variables show that, with the exception of the variable S_1,all the other variables are significantly and positively correlated with SLA_1 being the highest correlation coefficient obtained with the variable $MSLA_1$ (0.98) and the lowest one with the variable S_1 (0.11). In order to find an appropriate set of independent variables to predict the values of the SLA_1, a forward stepwise regression analysis between SLA_1 (dependent variable) and 6 of the above mentioned independent variables was performed. To avoid collinearity problems the calculated variable M_1 was excluded as it is a linear combination of the two measured variables L_1 and S_1. The first variable entered into the model was $MSLA_1$, explaining a very high proportion of SLA_1 variability (R^2 = 0.96). The second and last variable entering into the model was the variable SL_{1L} but with a very low contribution to explain SLA_1 variability (partial R^2=0.01) enabling us to consider that the variable $MSLA_1$ can be used alone as a very good predictor of SLA_1, (Figure 1B), as already reported by Lopes and Pinto (2005) for the variety Tempranillo. As the constant variance assumption was violated a logarithmic transformation of both variables was applied. The final model shows that the logarithm of the variable $MSLA_1$ explains a very high proportion of the logarithm of the SLA_1variability (Eq. 4):

$$\ln(SLA_1) = -0.067 + 1.018 * \ln(MSLA_1) \ <=> \ SLA_1 = e^{-0.067} * MSLA^{1.018} \ (R2 = 0.98; p < 0.001; n = 210) \ \text{(Equation 4);}$$

where SLA_1 is the primary leaf area per shoot (cm^2) and $MSLA_1$ the mean primary leaf area per shoot (cm^2). In the original variables the computed mean square error is 121.9 cm^2.

Lateral shoot leaf area

For the estimation of lateral shoot leaf area (SLA_2) a similar statistic procedure was used. It was used the same type of variables reported for primary leaves except for lateral shoot length (not measured). As for primary shoots, the highest correlation coefficient was obtained with the calculated variable MLA_2 (r= 0.97) followed by NL_2 (r=0.95), L_2 (r=0.74) and M_2 (r= 0.71).

The variable S_2 presented a very low and non-significant correlation coefficient with lateral shoot leaf area. Likewise, $MSLA_2$ was the first variable selected by the stepwise regression to enter the model, explaining a very high proportion of lateral leaf area variability (partial R^2=0.94). In the second and third steps the variable NL_2 and S_2 were chosen but with a very low contribution to explain the SLA_2 variability (partial R^2=0.01 and 0.002 respectively). After the logarithmic transformation of all the variables the elected model is given by the following equation (Eq. 5):

$$\ln SLA_2 = 0.491 + 0.802 * \ln(MSLA_2) + 0.207 * \ln(NL_2) + 0.048 * \ln(S_2) \quad (R^2 = 0.98;\ p < 0.001;\ n = 120) \text{ (Equation 5);}$$

where SLA_2 is the lateral leaf area per shoot (cm^2), $MSLA_2$ the mean lateral leaf area per shoot (cm^2), NL_2 the number of lateral leaves and S_2 the area of the smallest lateral leaf in cm^2. In the original variables the computed mean square error is 104.9 cm^2.

4. Conclusion

Our results show that the single leaf area of the variety Syrah can be accurately predicted in a non-destructive way by measuring the length of the two lateral leaf veins. Moreover, these results confirm that the Lopes and Pinto (2005) approach is a valid and reliable method for Syrah leaf area estimation, and reinforces the importance of the three measured variables (number of leaves and area of the largest and smallest leaf) as predictors of the shoot leaf area. The proposed empirical models can be used to accurately predict Syrah primary and secondary leaf area per shoot in any phase of the growing cycle. They are inexpensive, practical, non-destructive methods which do not require specialized staff or expensive equipment.

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