Shoot Development and Non-Destructive Determination of Grapevine (*Vitis vinifera* L.) Leaf Area

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A non-destructive method for determination of grapevine total leaf area is described. It is based on a highly significant correlation found between shoot leaf area and shoot length of Syrah (*Vitis vinifera* L.). Total leaf area per vine may be determined by using the equations described, by choosing a representative number of primary and secondary shoots and by knowing the total number of shoots of the plant considered. The equation seemed independent of vigour and terroir and reasonably sensitive to changes in leaf area that occurred independent of altered shoot length. It also allowed for recognition of compensation as a result of canopy manipulation. It would therefore be particularly useful for easy comparison of vines in different situations. A significant correlation between leaf fresh and dry mass and leaf area was also found. Dry matter partitioning, investigated to determine the relative importance of the main organs (primary and secondary shoot, leaf, cluster) during the growth period and in relation to primary shoot length, showed that the highest portion of carbon initially accumulated in the leaves, after which the primary shoot had the highest priority, followed by the clusters from véraison to harvest. Under the conditions of the experiment, secondary shoots and clusters were close in dry mass until véraison, after which berry dry mass increased significantly.

The concept of exposed leaf area is well known and the ratio between exposed leaf area and crop mass (per vine or square metre) has been established in order to obtain the desired berry sugar content (Kliewer & Weaver,1971; Hunter & Visser, 1990; Carbonneau, 1995; Murisier, 1996). Nevertheless, the total leaf area (TLA) per vine is also an important factor in relation to carbohydrate production and it directly or indirectly determines the berry composition for primary and secondary metabolism. In addition, TLA is critical for bunch microclimate (Hunter & Visser, 1990; Katerji *et al.*, 1994; Hunter, 2000) and is dependent on vine vigour, notably in relation to plant water status and nitrogen fertilisation (Conradie, 1980; Hardie & Martin, 2000; Lebon, 2001).

The non-destructive determination of grapevine leaf area had been the subject of many studies (Sepúlveda & Kliewer, 1983; Smith & Kliewer, 1984; Elsner & Jubb, 1988; Oliveira & Santos, 1995; Barbagallo *et al.*, 1996; Barbagallo *et al.*, 2000). However, cumbersomeness of leaf measurements and leaf mass determinations, inaccuracy of calculations and regressions, the effect of changing growth conditions and continuing seasonal development of the canopy were concerns.

In this investigation we have developed a non-destructive method to establish a relationship between shoot length and leaf area. The method has been developed for primary and secondary shoots of the variety Syrah. We have also investigated the dry matter partitioning between shoots, leaves and clusters at different growth periods and in relation to the primary shoot length.

MATERIALS AND METHODS

Experimental vineyards

Five-year-old Syrah vines, grafted onto SO4, and trained onto a vertical (Espalier) trellis system, were grown in France in the Agro Montpellier experimental vineyard under fertigation. Vines received a daily water equivalent of 90-100% of the transpirational water loss. Vines have therefore not been water stressed. These vines were used to develop and test a method for the establishment of a relationship between shoot length and TLA.

For leaf area and shoot length measurements, four to six shoots were taken at different growth periods and on different vines for each sampling. The shoot length classes were between 20 cm and 200 cm for the primary shoots and between 5 cm and 60 cm for the secondary shoots. For each shoot, all leaves were removed and the total surface measured. Fresh and dry mass of the primary shoot were also measured at different growth stages, between 30 cm and 200 cm length. For each sample, three replicates were used. Fresh and dry mass of the following organs were determined separately: primary shoot without cluster; leaves (with petioles

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intact) of the primary shoot; inflorescence/clusters of the primary shoot; and the entire secondary shoot (with leaves and tendrils).

For further testing of the method, data obtained from eight-year-old Syrah/99 Richter vines grown in South Africa and trained onto a vertical trellis system with low-intensity (supplementary) irrigation at pea size and véraison, were also used. Vines in this experiment were subjected to two treatments, namely a control and a canopy management treatment comprising the application of seasonal practices, such as suckering of infertile shoots, topping and leaf thinning (approximately 33% at berry set in the bunch zone and at pea size in the rest of the lower half of the canopy – Hunter, 2000). The experiment consisted of a randomized block design with three block replications and 10 or 15 vines per replicate. Primary and secondary shoot length and total leaf area (by means of a LICOR Model 3100 area meter) of seven shoots were measured at each sampling date (16 in all, starting at berry set and ending at full ripeness) and expressed on a per primary shoot basis.

Methods for leaf area measurement

Image capture

The leaf area was measured by image analysis. A very simple, low-cost device was used. Image capture was performed with a webcam (Quickcam VC logitech). The output of the webcam is an image sampled into 320*240 pixels. The lens had a 46° angle of view (focal information was not provided by the manufacturer). The aspect ratio of the span of the image was standard (2/3). The output of the webcam was directly connected to a personal computer (USB port). Image acquisition, camera tuning, image display as well as image processing were driven from software written in Matlab (Mathworks, Inc.).

Image processing

The webcam was mounted at a distance of about 37 cm over the leaf, pointing vertically. The leaves were manually placed on a white background. A real-time preview system allowed the operator to be sure that the leaf to be measured is in the span of the image. Once the image is captured, it is segmented by a threshold on grey level. Pixels belonging to leaves were set to 1 and pixels belonging to the white background were set to 0. The threshold operation is performed directly by the operator by clicking on the grey level histogram plotted on the screen. The leaf area corresponds to the number of pixels set to 1. A calibration factor area (R_a:pixel.cm^{-2}) is used to compute the leaf area in cm^2. A pixel label algorithm allowed measurement of the area of several leaves simultaneously. The area was then computed for each leaf.

Calibration factor determination

The theoretical calibration factor of the device was given by the following equation (Russ, 1999):

$$R = \frac{N}{L \times 2 \times \tan\left[\frac{\alpha}{2}\right]} \quad [1]$$

where

R = the linear calibration factor in pixel.cm-^{1},

N = the number of pixels sampled by the webcam CCD sensor in the x direction,

L = the distance between the sensor and the leaf to be measured (in cm), and

α = the angle of view of the lens.

From [1], it may be calculated that the span of the image in the x direction was 31.4 cm. The aspect ratio (the ratio of width to height) of the image was 2/3, implying that the span is 20.9 cm in the y direction. Theoretical calibration factor in x (320 pixels width) and y (240 pixels height) of our image processing device should be: 10.2 pixel.cm^{-1} in x and 11.5 pixel.cm^{-1} in y. The theoretical area calibration factor is then R_a = 10.2 × 11.5 = 117.3 pixel.cm^{-2}.

Since this is a very low-cost device, it was important to check the calibration factor and its robustness. This step is needed because the imaging conditions were not precisely controlled (natural light, height of the camera, threshold determination by the operator, lens distortions).

Calibration factor estimation

R_a was estimated by imaging a green piece of paper of 10 × 10 cm. The image processing allowed assessing the paper area in pixels. Knowing the paper area in cm^2, it was possible to assess the area calibration factor. Two hundred measurements were carried out in order to take into account the variability due to light, threshold operation, paper orientation and paper location in the image. The mean area calibration factor (X_{ac}) obtained over 200 measurements was of 117.0 pixel.cm^{-2} with a standard deviation (σac) of 2.1 pixel.cm^{-2}. The population is Gaussian (Chi-2 test). X_{ac} is very close to the theoretical calibration factor.

Calibration protocol

The aim of the device was to be able to measure a standard leaf area of 100 cm^2 with a relative accuracy of 1%. Errors due to calibration factor estimation have to be minimised. Measurements were spread over several weeks and it was very important to determine the number of repetitions needed to assess as accurately as possible the calibration factor before each experiment.

It is known from statistics (Saporta, 1990) that:

$$I_{(\bar{x}_n, p)} = m \pm \frac{t_p \times \sigma}{\sqrt{n}} \quad [2]$$

where

I = the interval of values around the mean (m) in which the average (x_n) of n measurements is included with a probability p,

m = the mean of the population,

σ = standard deviation of the population,

n = number of measurements,

p = probability of observing the average x_n in the interval I, and

t_p = coefficient which depends on the probability p in the case of a Gaussian population ($t_{95} \approx 2$, $t_{99.8} \approx 3$, $t_{99.9} \approx 4$).

Knowing that the error of the calibration factor estimation had to be less than 1%, it was possible to compute the number (n) of measurements needed according to the equation [2]. A probability (p) of 99.9 % was fixed:

$$\left|0.01 \times X_{ac} \pm X_{ac}\right| \geq \left|R_{mes} \pm X_{ac}\right| \Leftrightarrow 0.01 \times X_{ac} \geq \frac{t_{99.9} \times \sigma_{ac}}{\sqrt{n}} \quad [3]$$

where

X_{ac} = average of the calibration factor estimated over 200 measurements,

σ_{ac} = standard deviation of the population (over 200 measurements),

R_{mes} = mean calibration factor estimated over n measurements (n<200),

n = number of measurements,

$t_{99.8}$ = coefficient (Gaussian distribution:$t_{99.9}$ = 4).

From equation [3], the number of measurements (n) needed to have a relative error of calibration less than 1% was computed. The protocol needed the mean of six calibration factors to assess the expected accuracy.

Accuracy of the area measurement

Validation of our device and protocol was performed by the measurement of 10 green papers with a known area. This validation was performed with different conditions and different calibration factors. Results showed that the relative error of our device never exceeded 1% of the true area.

Fresh and dry mass measurements

Fresh and dry mass of the primary shoot without leaves; secondary shoot with leaves; and leaves, inflorescences and clusters of the primary shoot was determined from one month after bud break to harvest on the Syrah vines grown at Agro Montpellier. The shoots, leaves, inflorescences and clusters sampled before véraison were oven dried at 65°C for 24 – 48 hours, whereas the clusters sampled after véraison were dried for 4 days. Anthesis was observed when the primary shoots were 50 – 60 cm in length (15 May in our experimental vineyard). Véraison was taken as 30% to 50% of coloured berries (20 July in our experimental vineyard).

RESULTS AND DISCUSSION

Total leaf area and shoot length

A good correlation between shoot length and total leaf area was found for primary shoots (Fig. 1) and secondary shoots (Fig. 2). The equations can be used to calculate the Syrah total leaf area in other situations, simply by measuring the shoot length, which is a non-destructive technique. The interest of such an indirect method is to follow the evolution of leaf area during growth of the vine and also to compare different growth situations, e.g. in relation to terroir, trellising system, irrigation, fertilisation, etc. Similar research has been done on Merlot by Mabrouk & Carbonneau (1996). These authors found no effect of different vigour situations and training systems on the equation. Similar results were also found for Syrah, Grenache noir and Carignan (unpublished data). In contrast, Barbagallo *et al.* (2000) found that cultivar and climatic and cultural factors affected linear and/or multiple regressions (using shoot length and leaf number as independent variables) to such an extent that it could not be used to accurately estimate leaf area per shoot. In another study good estimations of leaf area were found by using a model based on leaves in selected positions on the shoot (Barbagallo *et al.*, 1996). It is possible that the original method of leaf area measurement presented by us may also be used to establish the equation of the relationship between leaf area and shoot length for other grapevine varieties.

To further test the equations found in this study, they were applied to data obtained from differently grown Syrah vines (in South Africa) that were not manipulated and vines to which seasonal canopy management practices were applied (Figs. 3 & 4). From this it is evident that leaf area on both primary and secondary shoots of control vines was largely under-predicted, whereas in the case of canopy management vines, leaf area on primary shoots was largely over-predicted and that on secondary shoots also under-predicted. In the case of canopy management vines, primary shoot growth was restricted by topping, whereas leaves were also removed (Hunter, 2000). An over-prediction of primary shoot leaf area in this case can therefore be expected and is evidence of the sensitivity of the method. The method, however, largely allowed for the detection of compensation in the form of slightly manipulated secondary shoots. Interestingly, the method also clearly over-predicted the last three sampling dates of the control primary shoot leaf area. During this time, these relatively dense canopies had already started losing leaves, particularly from the interior. This again points to the sensitivity of the method. Stress factors and manipulations affecting leaf area, but not shoot length, would therefore have an effect on the accuracy of the predicted values. The method may, however, under such circumstances even be useful as an indicator of stress symptoms or manipulation. Nevertheless, the general 7 – 10% average deviation (from the measured values) when leaf area was predicted by the original calculated equation seems reasonable and acceptable, at least under field conditions for monitoring growth and for the purpose of comparison.

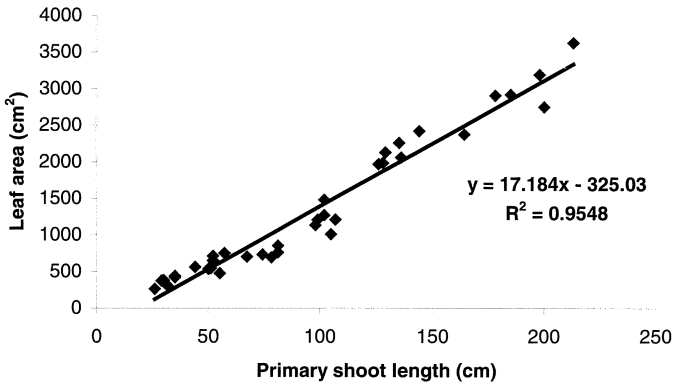

FIGURE 1
Relationship between the primary shoot length and leaf area.

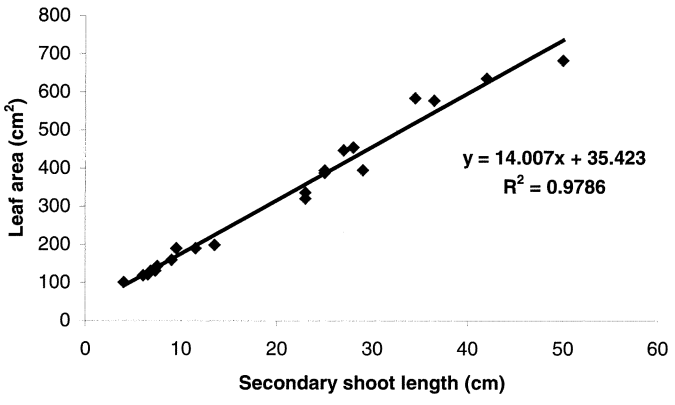

FIGURE 2
Relationship between the secondary shoot length and leaf area.

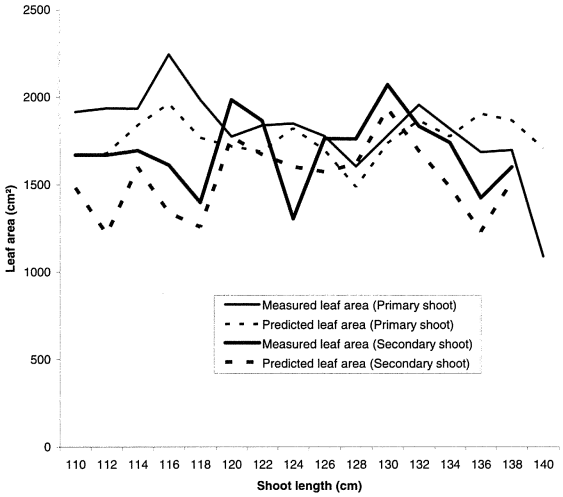

FIGURE 3

Relationship between measured and predicted primary and secondary shoot length and respective leaf areas of non-manipulated vines.

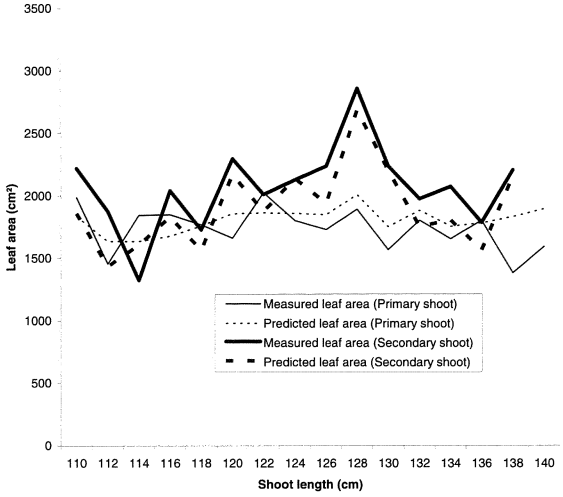

FIGURE 4

Relationship between measured and predicted primary and secondary shoot length and respective leaf areas of manipulated (canopy management) vines.

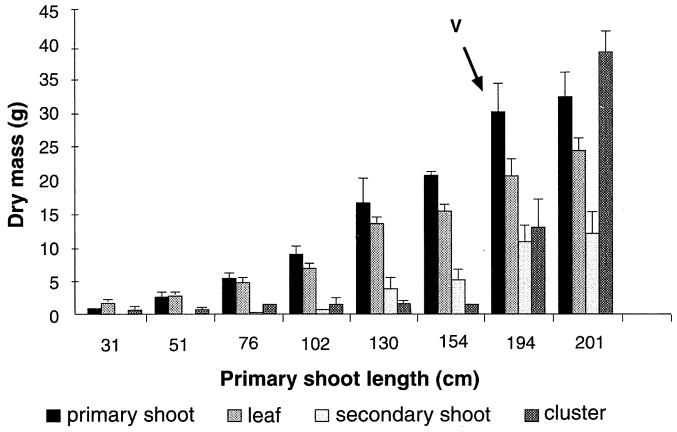

FIGURE 5

Dry mass of defoliated primary shoot, leaves, clusters and complete secondary shoot, at different growth stages of the primary shoot. V = véraison.

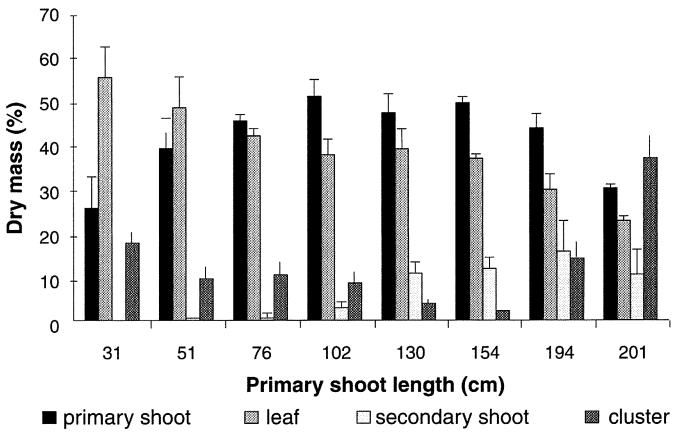

FIGURE 6

Percentage relative dry mass between defoliated primary shoot, leaves, clusters and complete secondary shoot, at different growth stages of the primary shoot.

Dry matter partitioning and shoot length

The dry mass in particular of the aerial organs (primary shoot without leaves; secondary shoot with leaves; leaves, inflorescences and clusters of the primary shoot) showed interesting results (Fig. 5). Dry mass of the primary shoots and leaves increased until véraison, i.e. when the primary shoot reached approximately 200 cm. The primary shoot dry mass exceeded that of their leaves when the shoot reached about 100 cm. Up to véraison, the primary shoot dry mass can increase by a factor of approximately 12 (for a factor of 4 in shoot length) and leaf dry mass by a factor of 6 – 7. Under the conditions of the experiment, secondary shoots began their growth when the primary shoot reached about 51 cm and slowed down or stopped their growth at véraison. Secondary shoots and clusters are close in dry mass until véraison, after which berry dry mass increased significantly. The dry berry mass can increase by a factor of approximately 3 from the end of the green growth stage to harvest. This is in accordance with the marked increase in berry sugar content, when the berry begins to soften and is applicable to the majority of grapevine varieties (Coombe, 1992).

The percentage relative dry mass between the different organs studied, in relation to primary shoot length, is shown in Fig. 6. The highest portion of carbon accumulated in the leaves at 30 cm shoot length, in the primary shoot *per se* at 75 cm shoot length, and in the clusters at harvest. Clearly, from véraison until harvest, carbon was preferentially translocated to the clusters, whereas a reduction in carbon accumulation in the primary shoot occurred. In situations where a different pattern is found, excessive vigour may be experienced, e.g. if carbon accumulation in vegetative organs (primary and secondary shoots) continues at the same level until harvest. A strong correlation between leaf area and leaf fresh and dry mass occurred for both primary (Fig. 7) and secondary shoots (Fig. 8).

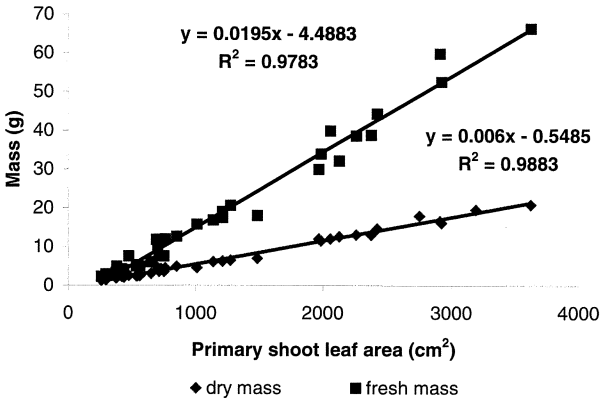

FIGURE 7
Relationship between primary shoot fresh and dry mass and leaf area.

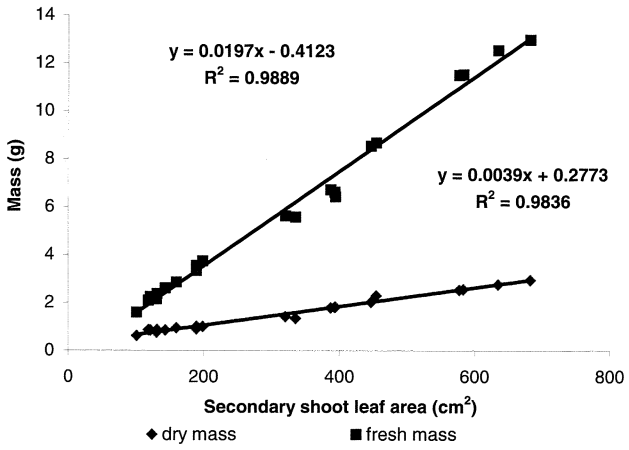

FIGURE 8
Relationship between secondary shoot fresh and dry mass and leaf area.

CONCLUSIONS

A clear relationship between Syrah shoot length and total leaf area per shoot was found. It seems reasonable that the method described in this paper could also be valuable in predicting or establishing leaf area and shoot length relationships in other grapevine varieties. It is possible to calculate for the considered variety, and in all situations, the vine total leaf area with this non-destructive method. A strong relationship between leaf area and leaf fresh and dry mass also occurred on the same shoot. This equation may be used to calculate the total leaf area, but in this case it is destructive. The equation seems particularly useful for comparitive purposes and is sensitive enough for detecting changes to the canopy, whether they result from leaf loss induced by external factors or by compensation. It is clear that carbon distribution (measured by means of dry mass comparison) may be a useful tool for comparing and monitoring the growth of the respective organs during the growth cycle of the vine. Abnormal distribution and growth patterns may be detected in such a way.

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