Research Note

Rapid and Nondestructive Estimation of Leaf Area on Field-Grown Concord (*Vitis labruscana*) Grapevines

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Abstract: Three potential variables, shoot basal diameter, leaf count per shoot, and shoot length, were examined as potential rapid, nondestructive methods for estimating leaf area per shoot, a frequent component of estimates of leaf area per vine. The metrics were recorded in large field-grown vines over five years. Shoot basal diameter, the most rapid method, was not a good predictor of leaf area per shoot. After transformation, shoot length and leaf count per shoot had relatively tight linear relationships with the square root of leaf area per shoot ($R^2 = 0.90$ and $R^2 = 0.85$, respectively). Some of the variation in the relationships due to between-year and within-season variability can be reduced by expressing the relationships as a function of thermal time. Furthermore, nonlinear models can be fit to the ratio of leaf area per shoot to the rapidly obtained metrics. Using this ratio approach accounts for the dynamics of canopy development and should increase the accuracy of leaf area estimates during early-season rapid shoot growth. Early in the season the length and count measurements can be made at ~0.5 min per shoot, but as the canopy develops and shoots intertwine, the sampling rate progressively slows to ~2.5 min per shoot.

Key words: leaf area, degree days, thermal time, grapes

An estimate of leaf area per plant often is required in field-based studies of canopy management, photosynthesis, transpiration, or other physiological processes in grapevines. Many experiments encompass few vines in established replicated research plots, thereby restricting destructive sampling. The scientific literature documents a number of approaches to developing relationships between some measured variable and leaf area so as to minimize loss of experimental material. At one end of the spectrum, remote sensing can be used to estimate canopy leaf area in a segment of row, a block, or a vineyard (Grantz and Williams 1993, Sommer and Lang 1994, Schultz 1995, Mabrouk and Sinoquet 1998). Several methods and sensors that might have application in viticulture have been compared and reviewed (Welles 1990, Weiss et al. 2004). The remote-sensing approach has limitations, including leaf clumping and variation in color of the vegetative material (including leaves) within the canopy. It also may be difficult to isolate estimates for an individual vine within a row. At the smallest scale, estimation of individual leaf area from maximum leaf width and midvein or maximal length are used most often among the nondestructive approaches (Manivel and Weaver 1974, Sepúlveda and Kliewer 1983, Smith and Kliewer 1984, Elsner and Jubb 1988, Williams and Martinson 2003). Given an adequate sample, the estimated area from individual leaves can be used to scale up to the shoot or vine.

Several researchers have used measurements of shoot length to estimate leaf area per shoot, and one study found a strong relationship between these two variables in Merlot (Mabrouk and Carbonneau 1996). Sparks and Larsen (1966) fit a polynomial to the relationship and used it together with independent estimates of average shoot length and shoots per vine to estimate leaf area per vine. Measuring shoot length is nondestructive, rapid, and reasonably accurate. Conversely, methods that require extensive hand sampling, such as measuring individual leaf dimensions, are more laborious and thus more costly, a legitimate consideration for most researchers. Working with large, well-watered vines (e.g., Thompson Seedless or Concord) in the field only exacerbates cost issues.

Our objective was to compare three potential variables that might be used to develop a rapid, nondestructive method from which to extrapolate total leaf area per vine. Because of the size of the research block and the limitations inherent in removing vegetation from perennial experimental units, destructive sampling was limited to that needed for establishing the correlational relationship between the measured variable and total leaf area at the shoot level. At several dates during the growing season, we estimated the number of hours required to record these measurements to facilitate calculation of cost estimates for the collection of similar data. Such information is useful in developing research protocols, calculating grant budgets, and assisting extension personnel and industry members in planning on-farm cooperative projects that require the collection of horticultural data.

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Materials and Methods

Vines were sampled destructively from border rows of a 0.34-ha vineyard (*Vitis labruscana* L. cv. Concord) near Prosser, Washington (46.30°N, 119.75°W). Other ongoing research in this block prohibited destructive sampling from the central rows. The plot was bordered to the west, approximately the direction of the prevailing wind, by several rows of *V. vinifera*. Planted in 1981, own-rooted Concord vines had been grown with a single trunk trained to a bilateral cordon ~1.7 m aboveground and were pruned annually to six- or seven-node spurs, a standard practice among Concord growers in the region. Rows were oriented north-south with 2.4 m between vines and 3.0 m between rows. Two vines were selected at random from each row approximately biweekly during the 2002 through 2006 growing seasons. Shoot length was measured to the nearest 0.5 cm with a cloth sewing tape on 10 shoots per vine (n = 40 shoots per sampling date). Basal shoot diameter was recorded midway between the first and second nodes with an electronic digital caliper (±0.02 mm; model ProMax, Fowler, Newton, MA). Leaves per shoot were counted. Lateral shoots, which typically are few and shorter than three nodes in this vineyard, were not included in measurements. Sample shoots were removed from vines, immediately placed in plastic bags, and transported to the lab on ice. Each leaf was removed from the shoot and its area determined by an area meter (model 3100; LI-COR, Lincoln, NE). Leaf area for each shoot was derived by summing individual leaf areas. Leaf area per vine was computed from average leaf area per shoot for that sampling date and the number of shoots per sample vine. Because leaf area per vine is a scalar quantity and the emphasis of this report is on shoot-level methodology, data are reported on a per shoot basis.

Univariate analysis showed leaf area per shoot, shoot length, and shoot count per vine to require transformation to normalize their distributions. Square root and logarithmic transformations were applied as appropriate. Linear regressions and tests for heterogeneity of slope were performed in SAS (version 9.1, SAS Inc., Cary, NC) using REG and GLM procedures, respectively. Within-season nonlinear relationships were fit with the model

$$y = A + B\left(1 - e^{-x/C^2}\right) \quad (1)$$

using the NLIN procedure.

Thermal time, or degree days (DD), was computed using a trapezoidal method of integration (Tobin et al. 2001) of 15-min average air temperatures (2-m reference height) obtained from the on-site Washington State University Public Agricultural Weather System (PAWS) station. Degree day accumulation was initiated on 1 Jan using a 10°C lower threshold and no upper threshold. We departed from the local convention of initiating a degree day accumulation from 1 Apr to standardize the thermal time index and ensure the inclusion of budbreak regardless of seasonal variation in its calendar date.

Results and Discussion

Data over five field seasons showed significant linear relationships for all three field measurements (basal shoot diameter, shoot length, number of leaves per shoot) to the square root of leaf area per shoot (Figure 1). The relationship between shoot basal diameter and leaf area per shoot had the greatest variability ($R^2 = 0.58$). That was not unexpected because (in addition to variation in diameter associated with leaf area) precise measurement of shoot diameter, even with a sensitive caliper, can be physically difficult for the operator working in thick canopies with intertwined shoots and leaves and lignified tendrils. The range in diameter was narrow (~1.5 to 10 mm), making it sensitive to minor operator-associated measurement error. Furthermore, shoot or cane shape was assumed to be circular. Departures toward an ellipse were not considered.

The log of leaf count per shoot and square root of shoot length had much tighter relationships to the square root of shoot leaf area ($R^2 = 0.85$ for leaf count; $R^2 = 0.90$ for shoot length), ostensibly favoring a measurement of shoot length over leaf count. However, depending on resources, available time, and experimental conditions, leaf count might be preferred. By midsummer, when a well-watered canopy has filled out and shoots are tangled, leaf count may be more rapid and accurate than an attempt at disentangling shoots to measure length. This limitation may be inconsequential in canopies that are shoot-thinned and/or managed under deficit irrigation. In nondestructive sampling for another data set in well-watered vines, repeated measurements were taken on sentinel shoots and this repeated handling kept them from extensive entanglement. What is not known is the frequency with which sentinel shoots can be handled without inducing a thigmomorphogenetic response (e.g., increase in shoot diameter, decrease in shoot length) (Braam 2005). For example, thigmomorphogenesis from mechanical stimulus of frequent wind has been demonstrated recently in the scientific literature (Tarara et al. 2005) and previously in nonreviewed articles (Bettiga et al. 1996, Kliewer and Gates 1987). Another option is to use both leaf count and shoot length in the regression-based model. Including both variables improved the leaf area estimate ($R^2 = 0.92$, df = 1, $P > F < 0.0001$, $R^2 = 0.92$, $y = -9.15 + 8.09LC + 2.76SL$). With relatively little experience, our observers could count leaves as they extended the measuring tape along the shoot, thus capturing both measurements simultaneously.

A test for heterogeneity of slope indicated significant differences in the aforementioned regression relationships between seasons (Figure 1). The relationship between shoot basal diameter and leaf area per shoot had the largest between-year variability. Leaf count per shoot was the most stable variable of the three evaluated. Only 2003 differed from the other years in terms of the slope of this regression equation. Although there were statistically significant year-to-year differences in slope for the shoot length versus leaf area per shoot relationship, the absolute values of the differences were small.

Shoot basal diameter is a poor metric for estimating leaf area; consequently, estimates of sampling time for this technique were not made. Recording diameter measurement is generally rapid. Thus, at the start of the season, when shoots are neither entangled nor impeding access to the internode, caliper measurements were noticeably faster than either leaf counts or shoot-length measurements. However, as the shoots rapidly lengthened, it was time-consuming to gain access to the internode, in that the canopy of a well-watered vine often contains several layers of leaves and shoots. During this study, vines averaged from 99 to 160 shoots.

From our data it is difficult to compare directly the sampling rates for shoot length and leaf count. In most instances, observers recorded leaf counts while running the measuring tape along the length of the shoot, capturing both measurements simultaneously. Measurement of shoot length would be expected to require more time than measurement of leaf number, especially later in the season because it is necessary to interweave the long tape among intertwined shoots within the canopy. The required labor and thus the cost can be approximated for the combined metrics of shoot length and leaf count per shoot. Early-season (late April to May) labor averaged ~0.5 min per shoot, while late May to June labor averaged ~1.25 min per shoot. After shoot elongation and canopy development had reached their maxima, a measurement could take up to 2.5 min per shoot, resulting in ~2 hr to measure 50 shoots or 4 hr 10 min to measure 100 shoots, excluding time to move between sample vines. The destructive sampling (n = 40) to develop the correlational relationships required ~6 hr at the beginning of the season and ~16 hr at the end of the season.

Seasonal dynamics of canopy development were apparent from the steady increase in leaf area per shoot until after approximately day of year (DOY) 195, or mid-July in each year of the study (Figure 2A, Table 1). Between-

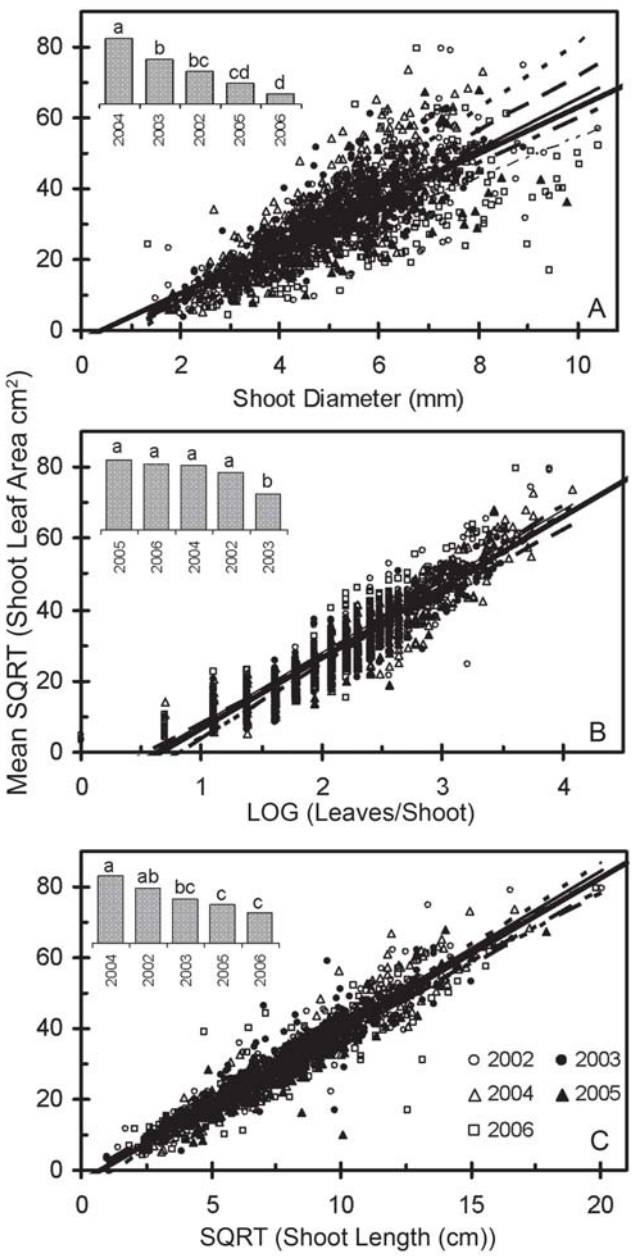

Figure 1 Relationship between square root of leaf area per shoot and (**A**) shoot basal diameter (df = 1, P > F <0.0001, R^2 = 0.58, y = -2.69 + 6.54x), (**B**) the log of number of leaves per shoot (df = 1, P > F <0.0001, R^2 = 0.85, y = -13.27 + 19.77x), and (**C**) the square root of shoot length (df = 1, P > F <0.0001, R^2 = 0.90, y = -3.14 + 4.27x), 2002–2006. The solid line reaching the right edge of each panel represents the linear fit to these data across all years. Broken lines represent linear fits for each year. Insets display results from the test for heterogeneity of slope between years. Bars represent relative magnitude of the slopes, and slopes sharing a letter are not significantly different.

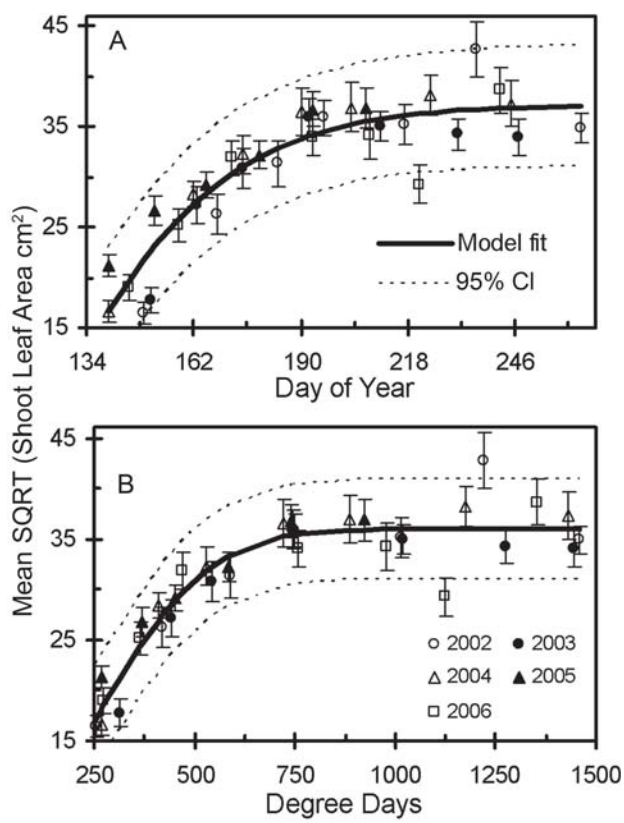

Figure 2 Mean (±SE) square root of leaf area per shoot from 2002–2006 by (**A**) calendar day and (**B**) degree days (start 1 Jan with 10°C lower threshold and no upper threshold).

Table 1 Results from nonlinear fits (equation 1) to within-season relationships.

Variables[a]					Asymptotic correlations	
Dependent	**Independent**	**Parameters**	**Estimate**	**Asymptotic SE**	**B**	**C**
SQRT leaf area per shoot	DOY	A	-145.30	80.62	-1.000	0.985
		B	182.40	79.90		-0.984
		C	94.74	9.21		
SQRT leaf area per shoot	DD	A	6.61	3.40	-0.985	0.875
		B	29.39	3.27		-0.813
		C	378.30	30.01		
Ratio LAS to SL	DD	A	4.49	1.08	-0.989	0.887
		B	12.01	1.05		-0.839
		C	355.40	20.42		
Ratio LAS to LC	DD	A	26.34	10.28	-0.969	-0.851
		B	82.75	9.81		0.745
		C	-432.60	43.30		
Ratio LAS to SL+LC	DD	A	3.84	0.78	-0.986	0.879
		B	10.34	0.75		-0.821
		C	371.80	18.84		

[a]SQRT: square root; LAS: leaf area per shoot; SL: shoot length (cm); LC: leaf count per shoot; DOY: day of year; DD: degree day.

season variability was reduced markedly by treating the increase in leaf area as a function of thermal time (Figure 2B). Leaf area per shoot increased until ~750 DD, suggesting that leaf area per shoot reaches its maximum again around DOY 190 to 196, or mid-July at our location (near the time of seed hardening—following bunch closure and before veraison). The ratio of leaf area per shoot to shoot length followed a similar pattern (Figure 3A), also reaching an asymptote at ~750 DD. This pattern suggests that the relationship between leaf area per shoot and shoot length is dynamic until midseason. Over the five years, vines were managed under both well-watered (2005–2006) and deficit conditions (2002–2004) as part of a separate experiment, yet as a function of degree day, canopy development essentially was complete by the same time each year.

The ratio of leaf area per shoot to shoot length as a function of DD (Figure 3A) shows less variability than the simple linear model (Figure 1C), both within and between seasons, and may provide a preferred approach to leaf area estimation because the simple linear model appears insensitive to the within-season dynamics of canopy development. The ratio of leaf area per shoot to leaf count had a seasonal pattern similar to the ratio of leaf area per shoot to shoot length, but reached an asymptote slightly later, ~880 DD (DOY 203 to 205; Figure 3B). If shoot length and leaf count data are combined by summing their numeric values, then the ratio of leaf area per shoot to the combined variable forms a similar relationship to thermal time as that found with shoot length alone (Figure 3C). Regressing the ratio of leaf area per shoot to the easily measured metric (e.g., shoot length or leaf count + shoot length) against an index of thermal time results in a unique nonlinear equation that can be used across all phenological stages. This equation allows for accuracy in estimating leaf area per shoot during the period of rapid shoot growth. Using the ratio of leaf area to the easily obtained metric and applying a nonlinear function result in precision that otherwise would be missed for early-season measurements under the classical linear approach.

Of the three metrics examined, shoot length and leaf count per shoot are useful for predicting leaf area per shoot. The linear relationship with shoot length has the highest R^2, but the relationship can be improved by including leaf count in the model. Shoot length and leaf count per shoot are both easy and rapid nondestructive measurements. Early in the season these measurements require less than 1 min per shoot, but can require ~2.5 min per shoot by the time of maximum canopy size. However, the variability in these linear models was increased by between-year and within-season dynamics, that is, the growth curve of the canopy. Some of the between-year variability can be reduced by developing relationships with degree days rather than calendar days. Within-season variability can be further reduced by expressing the relationship as a ratio of leaf area per shoot to one or a combination of the easily obtained metrics, then fitting a nonlinear model as a function of thermal time. This last approach is recommended as it is more sensitive to the dynamic period of shoot elongation and leaf development and thus can be expected to produce more accurate estimates of leaf area across the season.

Conclusion

Shoot length, leaves per shoot, or their combination appear to be reasonable nondestructive metrics useful to the estimation of leaf area per vine. Because leaf area per shoot is particularly dynamic earlier in the season, using a ratio of the leaf area per shoot in these metrics improved

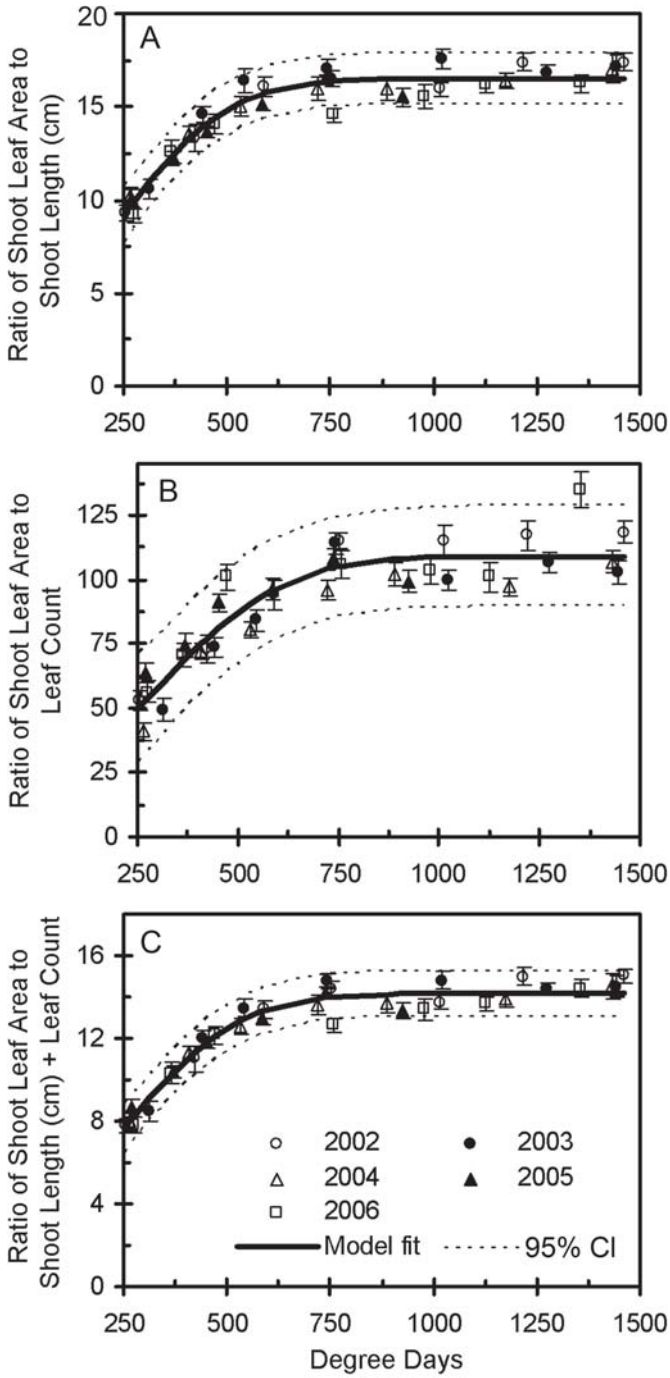

Figure 3 Means (±SE) of the ratio of shoot leaf area to one or a combination of easily obtained metrics as a function of degree days (start 1 Jan, 10°C lower threshold, no upper threshold). (**A**) Ratio of leaf area per shoot to shoot length; (**B**) ratio of leaf area per shoot to leaf count; (**C**) ratio of leaf area per shoot to the sum of numeric values of shoot length and leaf count.

model fit of the relationship across years. While our data set was drawn from years differing in degree days, pest pressure, and irrigation application, we nonetheless consider it important for local data to be gathered for parameter estimation. Local parameter estimates would be especially important if this approach were applied to more intensively managed canopies than those of Concord vines in the Washington state region.

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