AN ABSTRACT OF THE THESIS OF

Alejandra Navarrete for the degree of Master of Science in Horticulture presented on March 4, 2015.

Title: Characterizing Grapevine Canopy Architecture

Abstract approved:

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Abstract:

Vertically shoot positioned (VSP) training systems are common in Oregon's Willamette Valley, where deep fertile soils and high regional precipitation task growers with curbing vegetative vigor within this system. Management strategies, such as canopy hedging and cluster-zone leaf removal, are used to improve microclimate within the canopy and around the fruit. These cultural practices employed in commercial vineyards make it difficult to quantify canopy architecture and vine growth using currently established methods.

Given the importance of vine leaf surface area to productivity of the vine, a study was conducted to determine how to best quantify leaf area in the highly managed VSP canopies. A regression model was developed from various linear leaf measures compared to leaf area measures on primary and lateral leaves of Pinot noir vines in the north Willamette Valley of Oregon. Maximum leaf length, maximum leaf width, mid-vein length and the distance between the central and interior lateral lobe tips were positively associated with total leaf area. Leaf width at the petiole junction was not a suitable measure.

A second study was conducted to evaluate methods for quantifying vine leaf area and leaf distribution in moderate and high vigor VSP canopies, where dense foliage and interlacing shoots and tendrils can make vine measurements difficult. Traditional point quadrat analysis, digital photography, and a template leaf area method were compared to

leaf areas determined by destructive sampling. Results show that point quadrat analysis severely overestimated the number of shaded canopy leaves in dense VSP systems. Results from the digital photography pixel recognition program correlated green pixels with leaf exposure but was not in good agreement with exterior canopy leaf area. The template leaf area method results confirm that it can accurately estimate total vine leaf area.

The third study was developed to integrate these canopy quantification techniques with understanding how the leaf area: yield relationship affects fruit composition at harvest in cool climate Pinot noir grapes. A range of leaf area to yield ratios was created by cluster thinning vines to two crop levels. The study was replicated across four commercial vineyards with varying levels of moderate and high vigor. Results indicate that crop thinning had no impact on canopy leaf area, and there was limited impact of yield on fruit composition over two seasons.

The studies included herein aimed to develop and evaluate methods for estimating leaf area within VSP-trained canopies in the Willamette Valley. Results of this work will provide improved methods by which viticulture researchers and whole-plant physiologists can employ to determine leaf area as a measure of vine productivity, and better understanding of source-sink relationships in managed canopies.

Characterizing Grapevine Canopy Architecture

by
Alejandra Navarrete

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APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Alejandra Navarrete, Author

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Chapter 1:

EVALUATING LINEAR ALLOMETRIC METHODS FOR ESTIMATING LEAF AREA IN PINOT NOIR

1.1 Abstract

Estimation of leaf area is an important measure of vine growth and productivity. Various allometric models have been developed to evaluate leaf area in grapevine canopies; however, few have been developed for use in high density, hedged canopies trained to Vertically shoot positioned (VSP) systems. A study was conducted to determine accurate measures for leaf area estimates in moderate and high vigor Pinot noir grapevines during véraison in VSP canopies. Five linear leaf measurements, including two measures of length, two measures of width and one bisect measure, were assessed for their potential to estimate individual leaf area of primary and lateral leaves. Leaves were sampled from canopies in three zones, including lower, middle and upper. The relative location (exposed or internal) within the canopy was noted at sampling. Regression equations were developed using leaf area as the dependent variable and linear leaf measurements as the independent variables. One-variable models were found more suitable than two variable models. The overall model equations were not affected by experimental site or percent leaf shading. Leaf position along the shoot influenced model equations for both primary and lateral leaves. For each leaf type, slope and intercept values were calculated to develop a single linear regression model for each of the three horizontal canopy zones, using maximum leaf length or mid-vein length as the independent variables. Polynomial regression had greater fitting of the data when data was analyzed across all zones. Maximum leaf length had the tightest polynomial relationship for both primary and lateral leaves compared to other measures. Leaf width at the petiole junction was not a good predictor of leaf area. This study suggests that key measures need to be considered when conducting leaf area estimation on Pinot noir vines.

Keywords: grapes, leaf area, Pinot noir, VSP canopies

1.2 Introduction

The distribution of leaves within a plant canopy define plant atmospheric interactions. Leaf characteristics such as size, age (Kriedemann 1968; Kriedeman et al. 1970; Poni et al. 1994), position along the shoot (Hunter and Visser 1988) and location within the canopy (Cartechini and Palliotti 1995; Schultz et al. 1996) affect individual leaf and whole plant productivity (Petrie et al. 2000). Additionally, leaf characteristics change over time in relation to plant processes involved in CO_2 fixation (Poni et al. 2000), evapotranspiration (Williams and Ayars 2005, Williams et al. 2010), sap flow regulation (Candolfi – Vasconcelos and Koblet 1990) and light interception (Schultz 1995; Mabrouk et al. 1997). These plant processes are also influenced by cultural practices and canopy microclimate. Grapevine canopy leaf area quantification is often required in research to understand vine growth responses to the environment and cultural practices.

Direct methods for quantifying leaf area are based on measurements of individual leaves. Area meters, which use electronic rectangular approximation for measurements of two-dimensional objects, have been developed for a wide variety of leaves. Lab area meters require destructive leaf removal which prevents monitoring changes in plant growth over time and influences plant health and function. Portable area meters can take non-destructive measures but are limited to a finite measuring capacity incompatible with large grapevine leaves. Natural wrinkling and rolling of leaves can also affect the accuracy of such measurements.

Indirect allometric methods for estimating leaf area are based on the established relationships between leaf area and other canopy parameters. The relationship between leaf area and leaf weight has been evaluated for many plant species including grapevines (Sepúlveda and Kliewer 1983; Costanza et al. 2004). Gravimetric methods require the removal of foliage to determine mass. Studies have shown that leaf thickness and corresponding dry weight can vary between cultivars (Monteiro et al. 2013), solar exposure (Queiroz-Voltan et al. 2011; Palliotti et al. 2000), tropospheric ozone level (Ljubešić and Britvec 2006), leaf age (Wermelinger and Koblet 1990) and plant stress (Salem-Fnayou et al. 2011).

Several non-destructive allometric methods for primary leaf area estimation have been developed based on the relationships between individual leaf areas and other linear leaf measurements (Table 1.1). These methods are based on one- and two-variable models using various linear measures of leaf lamina or leaf veins and have been developed for use in both wine and table grape cultivars (Manivel and Weaver 1974; Carbonneau 1976; Sepúlveda and Kliewer 1983; Smith and Kliewer 1984; Elsner and Jubb 1988; Montero et al. 2000; Williams and Martinson 2003; Borghezan et al. 2010). The product of leaf blade length, defined as the linear length between petiole attachment and the central lobe tip, and leaf blade maximum width, have been used to estimate leaf area (Manivel and Weaver 1974; Sepúlveda and Kliewer 1983; Elsner and Jubb 1988; Williams and Martinson 2003). The product of the maximum leaf length, defined as the linear length between the central lobe leaf tip to the margin of the basal lobe, and maximum leaf width, defined as the distance between lateral lobe tips has also yielded models for estimating individual leaf area (Smith and Kliewer 1984; Montero et al. 2000; Williams and Martinson 2003). Additionally, the sum of squared leaf length and squared leaf width has been used as an estimate of leaf area (Elsner and Jubb 1988). Attempts have been made to correlate petiole length to leaf area; however, petiole parameters were found to be less accurate than with other leaf parameters at estimating leaf area (Manivel and Weaver 1974; Montero et al. 2000). Differences in the results of various allometric models for estimating single leaf area are influenced by cultivar due to differences in leaf ampelographic formula and cultural practices.

Leaf area estimation models have used shoot measures rather than individual leaves to estimate leaf area. Blom and Tarara (2007) used shoot length to estimate leaf area in hanging systems with little canopy manipulation. This allows for leaf area estimation to be scaled up to estimate total vine leaf area. However, cultural practices, such as hedging or leaf pulling that are common in Vertically Shoot Positioned trained vines, alter the natural relationship between shoot length and shoot leaf area making this method obsolete in such systems. Additionally, evidence of cultivar and climatic influences discredit the accuracy of the regression models for estimating leaf area based solely on shoot length and leaf number (Barbagallo et al. 1996).

Whole vine leaf measurements using leaf allometry becomes nearly impossible to quantify efficiently in high vigor vines with dense canopies, many leaf layers and excessive lateral shoot growth. Sampling techniques and statistical models have been established to reduce the number of measures required for estimating whole vine leaf area (Carbonneau 1976; Barbagallo et al. 1996; Lopes and Pinto 2005). Current allometric models for estimating leaf area were developed on canopies with minimal lateral growth or were based on leaf and shoot parameters unaltered by canopy management (Manivel and Weaver 1974; Carbonneau 1976; Sepúlveda and Kliewer 1983; Smith and Kliewer 1984; Elsner and Jubb 1988; Montero et al. 2000; Williams and Martinson 2003; Borghezan et al. 2010; Blom and Tarara 2007).

Statistical models developed for the estimation of leaf area based on electronically scanned leaf area data have determined that leaf blade length and width are the most important variables (Guisard et al. 2010). Stepwise regression used to evaluate models indicates that cultivar, site and the phenological stage of the vine must be considered. (Guisard et al. 2010). Various dimensional measures of leaves have been used to develop models for estimating leaf area; however, a precise regression equation requires a sufficient sample of leaves to accurately estimate the surface area of a single leaf when using non-destructive methods. Subsampling within vines is an imperfect method of estimating whole vine leaf area as it assumes that canopies are homogenous. Additional sampling is required with increasing vineyard size and vine heterogeneity. Extrapolating measured leaf parameters from several locations in a vine row to determine leaf area of individual vines lacks precision when canopy variability is not accounted in the model.

Currently, there are no non-destructive models for estimating leaf area of *Vitis vinifera* L. Pinot noir, nor models that have been developed in dense canopies trained to VSP and under heavy canopy manipulation. Our objective was to develop a model equation for accurate and efficient estimation of individual leaf areas of both primary and lateral shoot leaves under these conditions. Evaluating linear leaf measurements and developing appropriate sampling protocols is important for researchers who wish to conduct non-destructive in-field estimation of leaf area.

1.3 Materials and methods

1.3.1 Vineyard sites and experimental layout

Two commercial *Vitis vinifera* L. Pinot noir vineyards located in the Eola-Amity Hills American Viticultural Area (AVA) of the Willamette Valley of Oregon were used for leaf area quantification studies during the growing season of 2014. The vineyards were selected as representative of the range of vegetative vigor typically found in the Willamette Valley by evaluating historical pruning weight data and by visual observation. Sites EA1 and EA2 are both located in Salem, OR (45° 02' 02.98"N, 123° 08' 58.93"W; 153 m asl and 44° 57' 35.97N, 123° 10' 02.15"W; 96 m asl). The first Eola-Amity site, EA1, was planted in 1999 to Dijon clone 114 grafted to Schwarzman rootstock. Vines were spaced 2.3 m between row and 0.9 m between vines. The second site, EA2, was planted in 2001 to Pommard clone grafted to 3309 rootstock. Vines were spaced 2.7 m between row and 1.8 m between vines. Primary soil type at each site was Nekia silty clay loam. In both vineyards, rows were oriented north-south. Vines were cane pruned and trained to a unilateral Guyot system at site EA1 and a bilateral Guyot system at site EA2, both with vertical shoot positioning. Canopies were managed according to standard commercial practices for the region; EA1 was hedged twice, and EA2 was hedged once prior to véraison to maintain the structure of a typical VSP canopy and reduce shading. Leaves were removed in the cluster zone on the east side of the canopy before bunch closure at both sites. Vines were managed per standard practices of the region for disease control.

1.3.2 Sampling

Sixteen single vine plots were randomly selected within a plot of ~0.5 hectare at each site for evaluation. Vines were marked with plastic flagging tape placed vertically between the highest catch wire and the fruiting cane to demarcate the sampling boundary from vine to vine. Additional plastic tape was used to partition each single vine plot into 3 horizontal zones: upper, middle and lower. The lower zone was 1.2 m length by 0.3 m height, and centered on the fruiting zone at both sites. The remaining canopy above the fruit zone was divided into two equal sections of 1.2 m length by 0.6 m height. The

volume of the lower zone was smaller than other zones to allow quantification of only the fruiting zone (leaves and cluster).

To determine the position of individual leaves within the canopy, vines were sprayed with a 95% kaolin solution (Surround WP, NovaSource, Tessenderlo Kerley, Inc., Phoenix, AZ). Applications were made on both sides of the canopy in the early morning, between 6:00 and 6:30 AM, when wind speed was minimal. All leaves within each section were removed simultaneously from both sides of the canopy. The most interior leaves were removed first, followed by leaves exterior to those that were just removed. Fully exposed exterior leaves were removed last. Leaves with 100% spray coverage were designated as exterior leaves. Leaves with 0% spray coverage were designated as interior leaves. Leaves with partial spray coverage were assigned to one of three categories, 25%, 50% or 75%, based on visual assessment. Five levels of spray coverage was the maximum by which someone could reasonably categorize leaves into groups during visual field assessment. Leaves were placed in separate bags according to their percent spray coverage (0%, 25%, 50%, 75%, or 100%) and leaf type (primary or lateral). This process was repeated in each of the three zones.

Bagged leaves were immediately placed in coolers until transported back to the lab where they were kept in cold storage (4ºC) until area measurements could be completed, within five days after sampling. Sampling occurred when vines were at ~50% and ~80% véraison on 14 Aug and 2 Sept for site EA1 and EA2, respectively. The data reflect leaves sampled when primary leaves were in the secondary or tertiary phase of leaf development. Leaf unfolding dates were not recorded and exact leaf age and developmental phase was not determined.

Individual lamina areas were recorded using an area meter (model LI-3000, LiCor Inc., Lincoln, Nebraska, USA). Every fifth leaf was randomly selected upon area measurements and placed in a plastic bag and moved to cold storage (4°C) until linear leaf measurements could be made, within 24 hours. Linear leaf measurements were made using a straight-edge ruler, and measured to the nearest 0.1 cm, the accuracy at which in-field measurements can be replicated. One measure of lamina length, one measure of mid-vein length (Elsner and Jubb 1988; Williams and Martinson 2003; Tsialtas et al.

2008), two measures of lamina width (Manivel and Weaver 1974; Sepúlveda and Kliewer 1983; Smith and Kliewer 1984), and one lamina bisect leaf were chosen for this study due to their in-field reproducibility (Figure 1.1). The upper lateral lobes, L2 and L'2, were also measured using a straight-edge ruler to determine leaf symmetry. Leaves with severe damage were not used for linear leaf measurements.

1.3.3 Statistical Analysis

Data were analyzed by using SAS 9.3 (SAS Institute Inc., Cary, NC). A paired t-test was used to compare leaf symmetry of the L2 and L'2 lateral veins (Figure 1.1) to validate the use of asymmetrical linear leaf measures. Residual analysis was used to determine if transformations were necessary, and square root and power transformations were applied as needed. Regression analysis was to evaluate the relationships between linear leaf measurements and lamina areas. Linear and polynomial regression were performed using GLM and REG procedures and analyzed to determine the best fit model for estimating single leaf area (SLA). Slope and intercept values were calculated to develop regression equations for estimating leaf area. A test of equal slopes was used to compare regression coefficients across leaf type, site, canopy zone and percent leaf exposure. Means were separated using Tukey's Honestly Significant Differences ($p < 0.05$).

1.4 Results

Total vine leaf area at each site was determined by defoliating vines and measuring all defoliated leaves with a leaf area meter to determine differences in vine growth between sites at véraison. Leaf area per vine was 3.5 m^2 (±0.5) at site EA1 and 4.3 m^2 (±0.8) at site EA2. Dissimilar vine vigor levels and planting densities prevent statistical comparisons of total vine leaf area across sites; the planting density at site EA1 was two-fold greater than at site EA2. Leaf area density, defined as leaf area per length of canopy row, was higher at EA1 than EA2 with 3.8 m^2/m (±0.6) and 2.4 m^2/m (±0.9m), respectively. As expected, the less dense canopy at EA2 had a higher percentage of all leaves being primary leaves (73%) compared to EA1 (55%).

Observations from a frequency histogram of SLA of all leaves revealed the data was distributed about two means (Figure 1.2). The variation in mean leaf size between

primary and lateral leaves resulted in a bimodal distribution where each mode was associated with a leaf type. For this reason, primary and lateral leaves were analyzed independently. At the time of sampling, primary and lateral leaves were at different stages of leaf development. Leaf developmental phase is important because it determines rate of leaf expansion (Wermelinger and Koblet 1990). Young leaves developing from the apical meristems were removed prior to sampling as a result of hedging. Lateral leaves were in the earlier stages of leaf development as lateral shoot growth was induced mid-season by hedging.

1.4.1 Primary Leaves

A difference in mean primary SLA was observed between experimental sites. Site EA1 had a smaller SLA of primary leaves when compared to site EA2, with means of 121 cm^2 (±2) and 127 cm^2 (±2), respectively ($p<0.0001$). This difference in leaf size was expressed by linear leaf measures L, L1, W, Wp and B (Table 1.2). A test of equal slopes indicated that the difference in SLA between sites had no effect on the relationship between SLA and linear leaf measurements L ($p=0.2122$), L1 ($p=0.1985$), W ($p=0.0731$) and B ($p=0.2708$). Therefore, data from both sites were combined for model development.

Regression equations were developed using SLA as the dependent variable and linear leaf measurements as the independent variables. The following leaf parameters were regressed with SLA of primary leaves to determine which variable yielded the highest correlation coefficient: L, L1, W, Wp and B. When data were analyzed across all canopy zones, all variables except width at the petiole junction (Wp), resulted in a quadratic polynomial relationship. Maximum leaf length had the tightest polynomial relationship, followed by the distance between the central and interior lateral lobe tip, mid-vein length and maximum width (Table 1.4).

Multiple linear regressions showed percent leaf exposure, and vine-to-vine variability did not affect the relationship between SLA and linear leaf measurements. Canopy zone was found to impact model equations (L, $p<0.0001$; L1, $p=0.0072$). Mean slope and intercept values were calculated to develop a single linear regression model for

each of the three horizontal canopy zones, using maximum leaf length or length of the mid-vein as the independent variable. Maximum length resulted in a tighter linear fit compared to mid-vein length when regressed against SLA (Table 1.3). Site, percent exposure and canopy zone effects on linear regression equations were not analyzed for W, Wp and B due to unequal variance.

1.4.2 Lateral leaves

Mean lateral SLA, L, L1, W, Wp and B did not differ between sites (Table1.2). Lateral SLA at sites EA1 and EA2 was 39.1 cm^2 (±1) and 39.8 cm^2 (±0.5), respectively.

Variables L, W and B, resulted in a second order polynomial relationship when data were analyzed across all canopy zones. Maximum leaf length was found to have the tightest second order polynomial relationship, followed closely by maximum width and by the distance between the central and interior lateral lobe tip (Table 1.4). Mid-vein length, L1, was the only linear measure that yielded a third order polynomial equation (L1, R^2=0.85). Leaf width at the petiole junction, Wp, was not a good predictor of leaf area.

For lateral leaf measurements, site and percent leaf exposure had no effect on the overall model equations. However, canopy zone was found to impact model equations (L1, p=0.0013). Intercept values were calculated to develop a single linear regression model for each of the three horizontal canopy zones using length of the mid-vein as the independent variable. Experimental site, percent exposure and canopy zone effects on linear regression equations were also not evaluated for W, Wp and B due to unequal variance.

1.5 Discussion

Leaf measures were taken at a singular time point, véraison, after the rate of primary leaf expansion slowed. Earlier phenological time points were not evaluated because other more efficient allometric measures, such as the relationship between unaltered primary shoot length and total leaf area per shoot (Costanza et al. 2004, Mabrouk and Carbonneau 1996; Blom and Tarara 2007), can be used to estimate shoot

and vine leaf area during those times. Though shoot length is an effective tool for estimating leaf area, it is not applicable for hedged VSP canopies where shoots have been cut repeatedly. Differences in leaf area between sampling dates have been noted in some studies (Montero et al. 2000; Blom and Tarara 2007; Tsialtas et al. 2008), and are likely due to the changes in leaf development between sampling points. Conversely, Smith and Kliewer (1984) found no differences between estimation equations developed from bloom and véraison in head-trained and cane-pruned Thompson Seedless grapevines. They also found no difference in estimation equations developed at bloom across two years. Similar observations were made by Tsialtas et al. (2008), where a two year study using Cabernet Sauvignon found that year had no effect on leaf morphology of leaves sampled at bunch closure, véraison and ripeness.

1.5.1 Primary leaves

Two-variable models were examined in this study using the product of maximum L and W, and L1 and W. The resulting models were third order polynomial equations, which required transformations and the removal of more observations than single variable models. Additionally, two-variable models did not consistently result in higher correlation coefficients when compared to single variable models. Studies comparing both one- and two-variable models often find two-variable models to be the best fit. Two-variable models are typically built on a measure of length and width. Smith and Kliewer (1984) found the product of maximum length and width to result in the highest correlation coefficients for estimating SLA in Thompson Seedless. Using cultivars Chardonnay and Pinot noir, Sepúlveda and Kliewer (1983) found the maximum length and width at the petiolar junction resulted in the highest correlation coefficient. Elsner and Jubb (1988) utilized the product of mid-vein length and maximum leaf width in Concord to estimate SLA. The product of leaf length and width also resulted in the highest correlation coefficient for Cencibel, according to results from Montero et al. (2000). The additional information used by two-variable models can potentially improve accuracy when compared to single variable models. It is likely that two-variable models were not as effective in this study due to leaf variability caused by vertical shoot positioning, and hedging. These practices can damage leaves and alter the natural leaf

shape, thus additional information has the potential to inflate errors if either or both variables are altered. Additionally, models predicated on linearity to estimate SLA from singular measures of leaf length or width without any transformations often result in large negative intercepts (Sepúlveda and Kliewer 1983; Smith and Kliewer, 1984; Elsner and Jubb, 1988). When linear models were used to estimate SLA from our data, large negative intercept values were also observed (data not shown).

Single variable models for estimating SLA offer several advantages over two variable models. Single variable models are more time efficient because they necessitate a single measurement, reducing sampling time. Furthermore, they avoid issues of collinearity. Models developed herein, using a singular linear leaf measure, resulted in coefficients equal to or greater than 0.80. Similarly, Montero et al. (2000) found single measures of length to be more accurate than models based on width. Conversely, the power models produced by Williams and Martinson (2003) found that single measures of width were more valuable than using single measures of length.

In agreement with the results of this study, several authors have suggested single variable models for estimating SLA of primary leaves from linear leaf measures (Manivel and Weaver, 1974; Montero et al. 2000; Williams and Martinson, 2003). Only one study (Manivel and Weaver, 1974) suggested a single variable, second order polynomial model for use in Grenache. Of the single variable models evaluated by Montero et al. (2000), the most accurate was a function using leaf length. Williams and Martinson (2003) showed that a single variable power model accurately estimated leaf area of the cultivar Niagara and the interspecific hybrid cultivar DeChaunac; however, the equation was derived from a measure of leaf width rather than leaf length. The diversity of the single variable models is likely tied to the differences in leaf shape found between cultivars.

The use of the interspecific hybrid cultivar DeChanunac may be the cause of the converse finding between our data and Williams and Martinson (2003). DeChaunac has deeper lateral sinuses which can increase the tendency for overlapping lobes (Galet 1979). The two cultivars have different leaf shapes. DeChaunac has cunefo-truncate leaf shape which has smaller lateral vein lengths when compared to the orbicular leaf shape of Pinot noir, (Galet 1979). Leaf shape is indicative of evolutionary age; ancient leaf shapes

have shorter lateral veins relative to their more modern counterparts (Galet 1979). Leaf shape should be considered when applying allometric methods to different cultivars.

Of the leaf length variables used in this study, variable L resulted in a higher correlation coefficients than variable L1 (Table 1.4). This is likely due to the wider range of values for L, which in Pinot noir, is longer than L1. Additionally, variable L encompasses two of the five leaf lobes, the central lobe and a lower lateral lobe, whereas L1 only measures the central lobe. This is particularly applicable to leaves with a naked base of the petiolar sinus which result in smaller lateral lobes.

Variable Wp was the only linear measure evaluated in this study that did not relate to SLA. The lack of relationship may suggest a high degree of variability in leaf width at the petiole junction. This was expected given the various depths of the inferior sinuses observed. A degree of subjectivity was also noted by the personnel conducting linear measures. Variable Wp was made perpendicular to the base of the mid-vein to improve consistency; however, the linearity of the mid-vein is variable. The aid of a grid placed under the leaf for perspective, or the use of additional tools such as a protractor may be used to improve this measure; however, efficiency is compromised. The difficulty of this measure in a lab setting suggests greater inaccuracy if applied in-field. For this reason, Wp was not further pursued as a potential efficient measure of estimating individual leaf area.

The lack of relationship between Wp and SLA in our study contrasts those found by Manivel and Weaver (1974) and Sepúlveda and Kliewer (1983), where the authors use leaf width at the petiole junction to established second order polynomial and linear relationships, respectively. The findings from Manivel and Weaver (1974) may differ from our results due to the disparate leaf shape associated with the cultivars studied. In cuneiform grapevine leaves, like Grenache, the petiole junction is collinear with the inferior lateral L3 and L`3 veins, while the orbicular leaves of Pinot noir used in this study have the petiolar junction directly between the superior lateral lobes (Galet 1979). The cultivars examined by Sepúlveda and Kliewer (1983), Chardonnay and Chenin Blanc, have the same orbicular leaf shape and lateral sinus depth as Pinot noir, suggesting that the leaf shape is not likely the cause of different results. Vine size may be the source

of differences between our data and the study by Sepúlveda and Kliewer (1983) where potted vines were trained to a single shoot. Our leaf population is derived from commercially planted vines with significantly more shoots per vine. Between 12 and 23 shoots per meter length of row can be found at site EA1 and between 12 and 18 shoots per section canopy vine were present at EA2.

We do not know if shoot-to-shoot variability has an effect on the relationships between linear leaf measures and SLA. The presence of infertile shoots arising from the head, known as watershoots, is one example of an added source of variation in our study, as leaves developed on these shoots tend to be deeply lobbed (Galet 1979). Further studies need to be conducted to examine shoot-to-shoot variability within a vine, and vine-to-vine variability. Vine-to-vine variability was not revealed in the results from our data. One study noted vine-to-vine variability to linear models, but only in one year of the two years studied by Smith and Kliewer (1984).

Leaf photosynthesis, transpiration, stomatal conductance and leaf area are known to change along the shoot, suggesting that leaf position and thus leaf age are primary factors affecting leaf growth and development (Kriedemann et al. 1970). Canopy zone was found to affect the relationship between the leaf measurements of L and L1 and SLA in primary leaves. It was expected to find differences between the three zones. All primary leaves follow the same temporal pattern of growth, regardless of the start of formation (Wermelinger and Koblet, 1990; Schultz, 1992). Primary leaves were expanding at different rates because of their relative age. In a study of the seasonal growth of grapevine leaves in Pinot noir, it was observed that the first two leaves to emerge from a shoot did not reach the same size of their younger counterparts on the same shoot (Wermelinger and Koblet 1990). A reduction in final leaf size relative to leaf age was also documented by Schultz (1992). This could explain why the relationship between linear leaf measures and SLA varied between the lower zone and the middle and upper zones given that leaf age decreases along the shoot. Additionally, young primary leaves developing from the apical meristems of the primary shoots were likely removed at the time of the first hedging. A difference in canopy zones could be even more pronounced in canopies which are not managed with hedging due to the presence of younger primary leaves developing from the shoot tips in the upper canopy zone.

Leaf pulling in the fruit zone and hedging also contributed to variation between the three zones. Leaf pulling is centered on the area around the developing clusters affecting the lower zone. Hedging primarily affects the middle and upper zones where greater lateral shoot growth can be found. Variables W and B were not applicable for all zones of the canopy due to unequal variance.

A test for heterogeneity of slope indicated no difference in slope of the regressions between leaves with different positions in the vine canopy (inner versus outer leaves). Outer leaves would be exposed to full sun whereas inner leaves would be found shaded at varying degrees within the canopy interior. Our results do not agree with Schultz (1992) who found that leaf development was related to a leaf's shade or sun position. It is likely that no effect of full or partial shading was observed on individual leaves due to sunflecks, which result from variable incident light on individual leaves or partial areas of leaves (Kriedemann et. al.1973). The duration of sunflecks vary with time but have the ability to supply between 20-80% of the canopy's total daily photon flux (Pearcy 1990).

1.5.2 Lateral Leaves

Vineyard site had no effect on the relationship between all five linear leaf variables (L, L1, W, Wp, B) and SLA of lateral leaves. This is likely related to a lack of difference in mean lateral SLA between sites.

It is important to include lateral leaf area in models for estimating SLA and extrapolated to estimate leaf area per shoot and leaf area per vine, as lateral shoot leaf area can represent a significant portion of total vine leaf area. In this study lateral leaf area accounted for 27% and 35% of total vine leaf area at sites EA1 and EA2, respectively. A marginally wider range of lateral leaf area to total leaf area ratios was described by Dokoozlian and Kliewer (1995). Lateral leaf area amounted to 25% of total leaf area in low density vines and 50% for high density vines. Mabrouk et al. (1997) found analogous lateral leaf area percentages of 29% to 39%. Shultz (1992) observed 50% lateral leaf area relative to total vine leaf area. The lowest report of lateral shoot leaf area, 27%, 22%, and 12%, relative to total vine leaf area was by Williams (1987) in a three year study. The lateral shoot leaf area to total vine leaf area is important because

lateral leaves can act as a sink or a source depending on the developmental stage. Our model was developed using lateral leaves in all stages of growth to create a range of leaf area values; however, it is necessary to account for the percentage of the lateral leaves acting as a source or a sink, as this factor can affect the measure of photosynthetic capability of the plant. Lateral leaves with a mid-vein length of less than 4.5 cm, or less than 30 to 50 percent of their final size do not contribute to vine growth as a source of energy (Hale and Weaver 1962). Likewise, lateral shoots with less than two fully expanded leaves are also considered sinks to vine growth (Hale and Weaver 1962). Lateral leaves developed beyond these stages have the ability to export photosynthates and act as a source to vine growth (Hale and Weaver 1962; Zufferey et al. 2000; Intrieri et al.1992).

Of the variables examined, L1 and L, were most useful at estimating leaf area. They were the most versatile as they could be applied to both primary and lateral leaves, across sites, and across canopy zones. Variable L often yielded a higher coefficient and greater accuracy; however L1, is easier to measure in the field. Only a slight increase in accuracy (1%) was found when canopy zone was accounted for in the model, and this may not warrant the measurement by canopy zone due to lack of efficiency.

1.6 Conclusion

To our knowledge, this is the first study evaluating the suitability of linear leaf measurements SLA of both primary and lateral leaves of hedged VSP-trained Pinot noir grapevines. It is important to develop linear leaf measurements under different growing conditions, site, cultivar, leaf age and vine phenology as these factors have been found to influence models for estimating leaf area. The relationship between the two measures of leaf length (L1 and L) and SLA were the only linear measures consistent across both vineyards evaluated in this study despite a difference in mean SLA of primary leaves between the two locations. With data gathered from two different vineyards, our models may be applicable to other cool climate Pinot noir vineyards. This information can be used by researchers to develop appropriate leaf area estimation methods for vine physiology research. Both measures of width (W and Wp), and the bisect measure (B) had unequal variance therefore were not used to develop single variable models.

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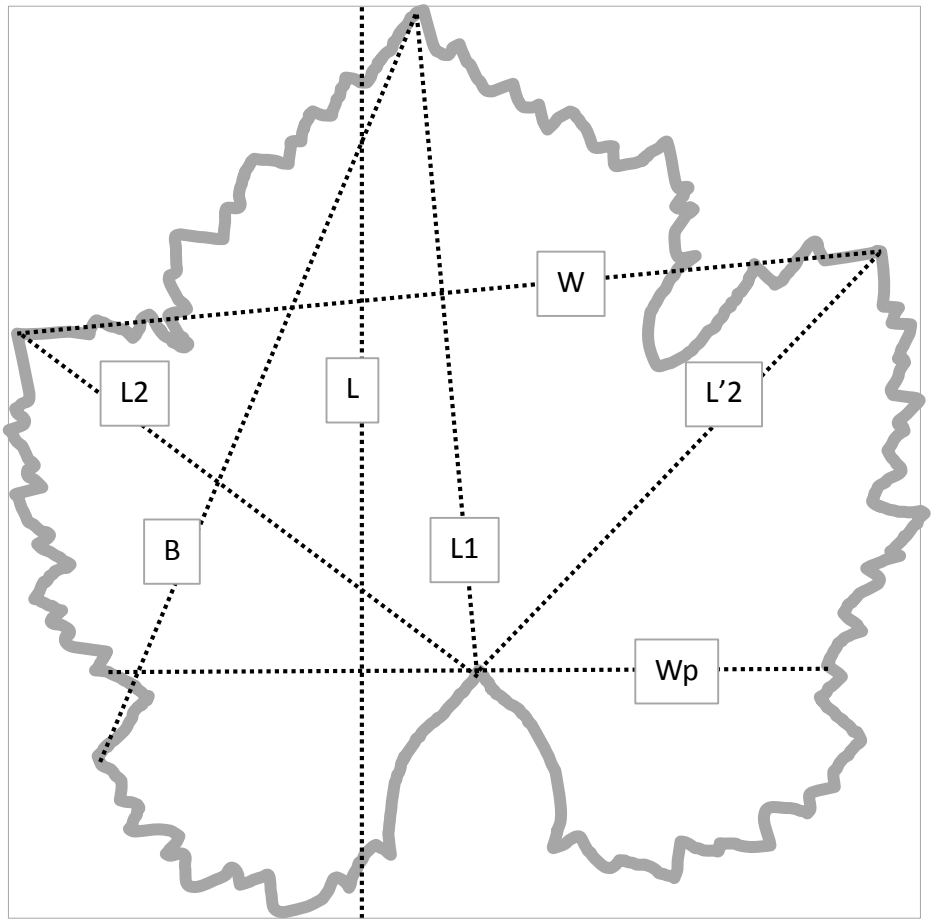

Figure 1.1. Schematic of leaf measurements made on randomly selected leaves sampled from whole vine defoliation. Maximum leaf length (L); mid-vein length, (L1) the length between the central lobe tip and the petiole attachment; leaf bisect (B), the distance between the central lobe tip and the inferior lateral lobe tip; leaf blade width (W), leaf width between superior lateral lobe tips; and leaf width at petiole (Wp), the width of the leaf perpendicular to the mid-vein at the point of the petiole attachment.

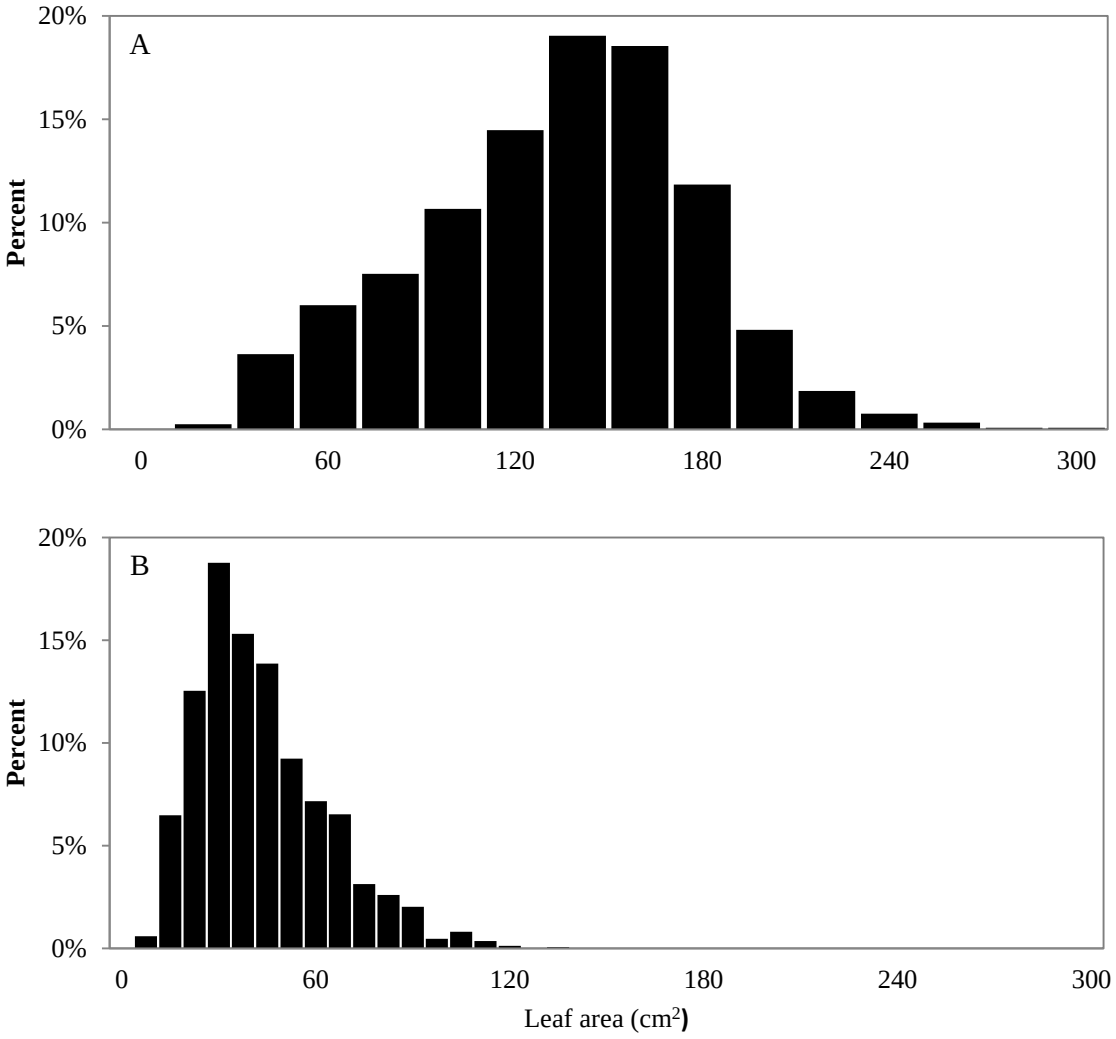

Figure 1.2. Distribution of (A) primary leaf areas (n=1181) and (B) lateral leaf areas (n=1731) sampled from 32 vines across two experimental sites.

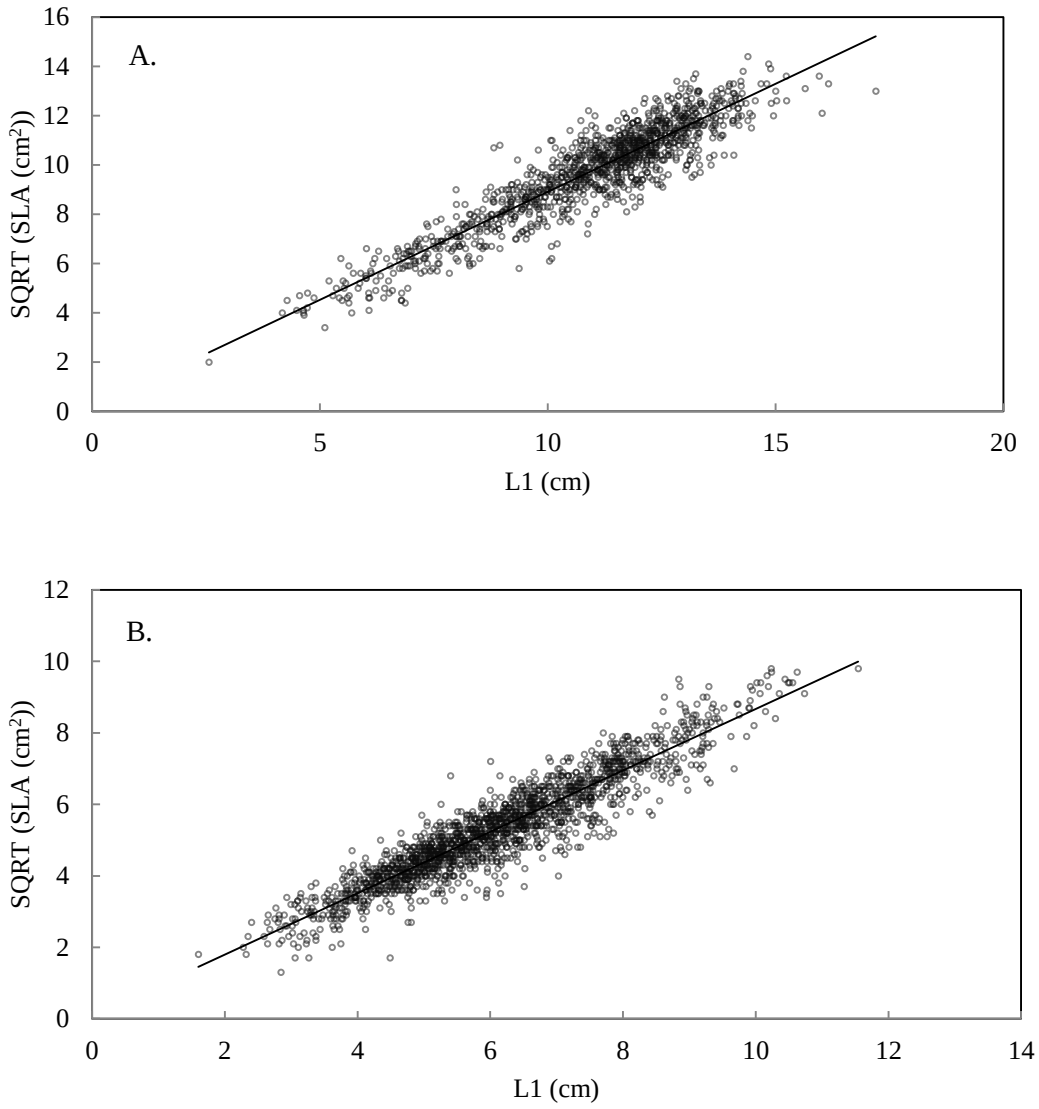

Figure 1.3. Linear relationship between square root of leaf area, SQRT(SLA), and mid-vein length, L1, in (A) primary leaves (df =1, P>F<0.0001, R^2 = 0.85, y = 1.537 + 0.966x, n=1171) and (B) lateral leaves (d=1, P>F<0.0001, R^2 = 0.87, y = 0.719 + 1.011x, n = 1715). The line represents the fitted linear regression analysis (n = 1715).

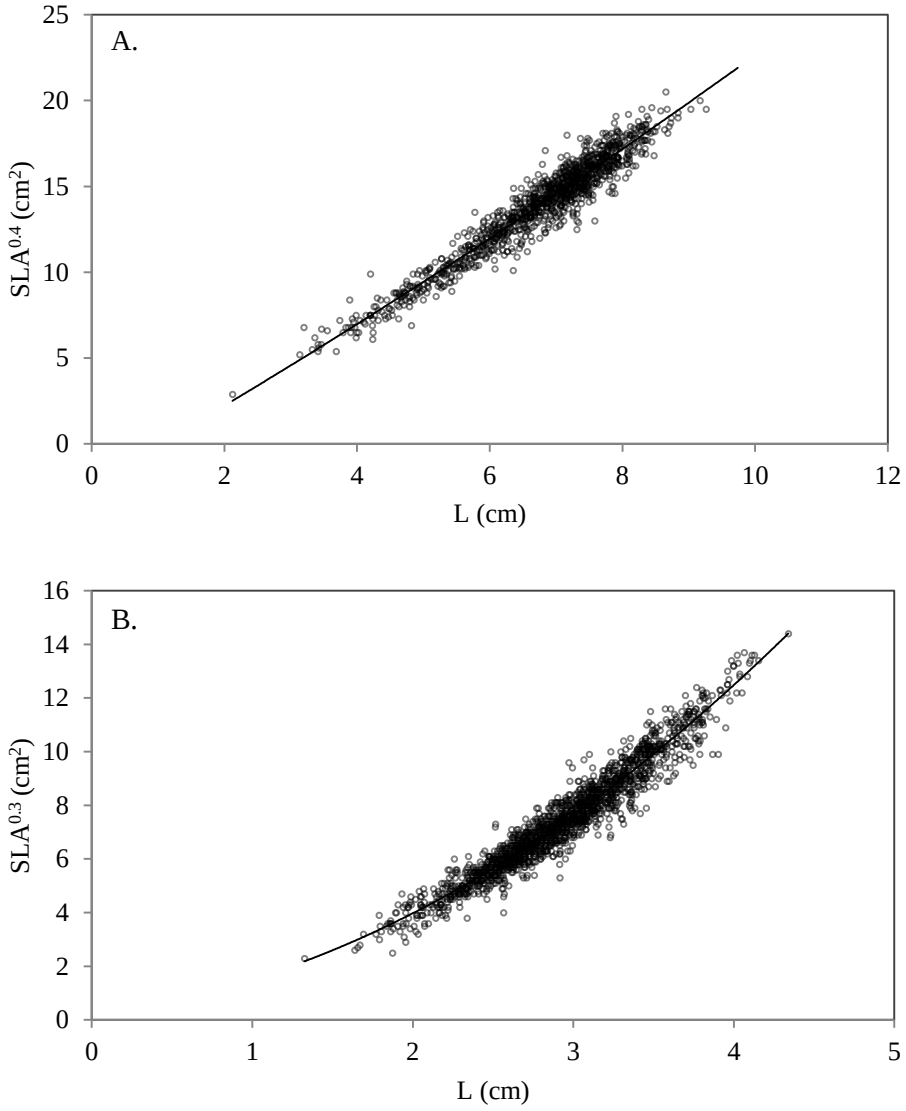

Figure 1.4. Relationship between leaf area $(SLA)^{0.4}$ and leaf maximum length L in (A) primary leaves (df =1, P>F<0.0001, R^2 = 0.93, y = 0.679 + 0.532x – 0.007 x^2, n=1171) and (B) lateral leaves (d=1, P>F<0.0001, R^2 = 0.93, y = 0. 831 + 0.343x – 0.008x^2, n = 1715). The line represents the polynomial fit of data from regression analysis.

Table 1.1. Summary of leaf area prediction models developed for *Vitis* species.

Author	Year	Cultivar	Regression equation	R^2	Variable description
Manivel and Weaver	1974	Grenache	$LA = 1.162\ L^2 – L + 1.051$	0.96	L – leaf length
			$LA = 0.644\ W^2 + 0.469\ W+0.109$	0.97	W – width at petiole junction
Carbonneau	1976	Cabernet Sauvignon	$LA = -6.885 + 1.605\ x + 0.305\ x^2$	0.97	X – sum of L + L'2
Sepúlveda and Kliewer	1983	Chardonnay	LA = 0.69 (LxW) + 3.17	0.99	L – maximum leaf length W – width at petiole
		Chenin Blanc	LA = 0.68 (LxW) + 2.49	0.97	
Smith and Kliewer	1984	Thompson Seedless	No equation presented	0.98	L – maximum leaf length W– maximum leaf width
Elsner and Jubb	1988	Concord	LA = -3.07 + 0.85 (LxW)	0.98	L – length of mid-vein W– maximum leaf width
			$LA = -1.41 + 0.527\ W^2 + 0.254\ L^2$	0.99	
Schultz	1992	White Riesling	LA = 1.18 (L-2.6) x (L+8.75)	0.97	Not defined
Montero et al.	2000	Cencibel	LA = 0.587 (LxW)	0.99	LW – leaf length x maximum width
			LA = 0.588 (LxW)	0.99	
Williams and Martinson	2003	Niagara	$LA = 0.637\ W^{1.995}$	0.98	W – maximum leaf width
		DeChaunac	$LA = 0.672\ W^{1.963}$	0.96	
Tsialtas et al.	2008	Cabernet Sauvignon	LA= 18.379 L -151.41	0.97	L – maximum mid vein length

Table 1.2. Mean leaf area and linear measurements for primary (n=1181) and lateral leaves (n=1731) sampled from 32 vines across two experimental sites.

Leaf type	Site	Leaf measurements					
		SLA (cm^2)[a]	L (cm)[b]	L1 (cm)[c]	W (cm)[d]	Wp (cm)[e]	B (cm)[f]
Primary	EA1	121.0	13.6	9.5	12.5	10.1	12.6
	EA2	127.3	14.2	9.9	13.1	10.5	13.1
	p	**0.0121**	**0.0006**	**0.0016**	**<0.0001**	**0.0094**	**0.0040**
Lateral	EA1	39.1	7.4	5.3	7.1	6.2	6.8
	EA2	39.8	7.4	5.3	7.2	6.2	6.9
	p	0.4015	0.3984	0.4247	0.8293	0.8295	0.3641

[a] SLA leaf area of individual leaves.
[b] maximum leaf length
[c] mid-vein length
[d] distance between the central lobe tip and the inferior lateral lobe tip
[e] width between superior lateral lobe tips
[f] width at the point of the petiole attachment.

Table 1.3. Linear regression analysis of single leaf area (SLA), length of the mid-vein (L1), and maximum leaf length (L) of leaves from three zones of the vine canopy (upper, middle and lower) sampled from 32 vines across two experimental sites. Combined indicates analysis across all zones.

Leaf Type	Zone	Regression Equation		R^2	p
Primary	Combined	SQRT(SLA) =	1.537 + 0.966 L1	0.85	**<0.0001**
		SQRT(SLA) =	1.004 + 0.713 L	0.92	**<0.0001**
	Lower	$SLA^{0.6}$ =	0.729 + 1.688 L1	0.86	**<0.0001**
		SQRT(SLA) =	1.180 + 0.687 L	0.93	**<0.0001**
	Middle	$SLA^{0.6}$ =	-0.679 + 1.892 L1	0.86	**<0.0001**
		SQRT(SLA) =	0.952 + 0.716 L	0.93	**<0.0001**
	Upper	$SLA^{0.6}$ =	-0.624 + 1.946 L1	0.86	**<0.0001**
		SQRT(SLA) =	0.917 + 0.732 L	0.93	**<0.0001**
Lateral	Combined	SQRT(SLA) =	0.719 + 1.011 L1	0.87	**<0.0001**
	Lower	SQRT(SLA) =	0.705 + 1.009 L1	0.87	**<0.0001**
	Middle	SQRT(SLA) =	0.683 + 1.009 L1	0.87	**<0.0001**
	Upper	SQRT(SLA) =	0.788 + 1.009 L1	0.87	**<0.0001**

Table 1.4. Polynomial relationship between square root of leaf area (SLA) and maximum leaf length (L), mid-vein length (L1), leaf width between the upper lateral lobe tips (W), and distance between the central lobe tip and lower lateral lobe (B) sampled from 32 vines across two experimental sites.

Leaf Type		Regression Equations	R^2	p
Primary	$SLA^{0.4}$ =	$0.679 + 0.532\ L - 0.007\ L^2$	0.93	**<0.0001**
	SQRT(SLA) =	$-0.488 + 1.442\ L1 - 0.026\ L1^2$	0.85	**<0.0001**
	$SLA^{0.7}$ =	$-9.188 + 3.600\ W - 0.048\ W^2$	0.80	**<0.0001**
	SQRT(SLA) =	$-0.474 + 1.128\ B - 0.018\ B^2$	0.86	**<0.0001**
Lateral	$SLA^{0.3}$ =	$0.831 + 0.343\ L - 0.008\ L^2$	0.93	**<0.0001**
	SQRT (SLA) =	$1.529 + 0.495\ L1 + 0.103\ L1^2 - 0.006\ L1^3$	0.87	**<0.0001**
	$SLA^{0.3}$ =	$0.705 + 0.386\ W - 0.010\ W^2$	0.92	**<0.0001**
	$SLA^{0.3}$ =	$0.907 + 0.360\ B - 0.009\ B^2$	0.87	**<0.0001**

Chapter 2:

TECHNIQUES FOR QUANTIFYING GRAPEVINE LEAF AREA AND LEAF SPATIAL DISTIBUTION IN VERTICALLY SHOOT POSITIONED CANOPIES

2.1 Abstract

Allometric methods for estimating leaf area are difficult to apply in moderate to high vigor vertically shoot positioned (VSP) systems due to heavy canopy management required to improve the canopy microclimate. However, leaf area and canopy density measures are still important for estimating vine health and productivity in whole plant physiology research. A study was designed to evaluate various methods of leaf area analysis within VSP canopies with the following objectives: 1) evaluate a rapid template method for estimating total primary and lateral leaf area, 2) determine the accuracy of a modified point quadrat analysis (PQA) in VSP canopies, and 3) test a digital image analysis program for its ability to quickly estimate vine canopy surface area. All techniques were applied over a period of 3 to 4 days during véraison in two commercial Pinot noir vineyards of moderate to high vigor. Methods were compared to leaf area determined by a leaf area meter using leaves removed from the vine by defoliation. The template method was accurate at estimating total leaf area, but it was not a good predictor of lateral shoot leaf area. Overall, PQA underestimated the number of interior canopy leaves. There was a relationship between green pixels and exterior leaf area determined from 2 dimensional analysis. This work provides an evaluation of rapid methods for mapping canopy leaf distributions, determining canopy density and estimating exterior leaf area in VSP canopies where heavy management, dense canopies with intertwining shoots and tendrils complicate traditional measures for evaluating grapevine canopies. Methods outlined may help researchers increase accuracy in quantifying canopy metrics under such conditions.

Key words: point quadrat analysis, leaf area, canopy density, leaf exposure.

2.2 Introduction

The leaf is a vital organ for carbon assimilation and energy production, and thus its area is an important metric used to evaluate plant growth and productivity in physiological studies. Leaf area has been quantified in a range of studies including those relating to light interception and absorption (Dokoozlian and Kliewer 1995), photosynthesis (Bernizzoni et al. 2011), vine water status (Gómez-del-Campo et al. 2004; Tarara et al. 2011), vineyard irrigation (Intrigiolo and Castel 2011), vine nutrition (Schreiner et al. 2013), crop growth (Koblet et al. 1994; King et al. 2012), yield potential (Palliotti et al. 2011) and disease management (Siedfried et al. 2007; Valdéz-Gomez et al. 2008). The proportion of exposed leaf area relative to total vine leaf area is critical for defining the relationship between plant atmospheric interactions and biophysical processes that relate to vine productivity.

Whole vine leaf area alone is not representative of the potential vine capacity for carbon assimilation. The rate of carbon assimilation is defined by individual leaf characteristics, such as size (Hale and Weaver 1962; Zufferey et al. 2000), age (Williams 1987), position along the shoot (Kriedmann et al., 1970; Candolfi-Vasconcuelos and Koblet 1990; Intrieri et al. 1992; Palliotti et al. 2000) and light microclimate (Iacono and Sommer 2000). Leaves influence light microclimate through their size, location, and distribution within the canopy. Distinguishing between direct and diffuse light on leaves is important for understanding how energy derived from solar radiation will drive photosynthesis, stomatal conductance, transpiration, and respiration (Welles and Norman 1991; Dokoozlian and Kliewer 1995).

Furthermore, the relationship between canopy architecture, defined as the distribution of leaves and stems within the canopy, and vine growth responses has motivated plant scientists to develop methods for quantifying the architecture. This includes methods for estimating leaf area, identifying leaf characteristics and evaluating the spatial distribution of leaves within the canopy.

Leaf area is often measured using scanning leaf area meters. These allow for measurement of individual leaf area and were designed for use in annual cropping

systems where destructive sampling is feasible. Although precise, they are either destructive or too small for use in many large broadleaf species and can influence plant health in perennial systems. To avoid the destructive sampling issues, nondestructive allometric methods have been developed to estimate leaf area (Manivel and Weaver 1974; Sepúlveda and Kliewer 1983; Blom and Tarara 2007). However, these methods require significant sampling to obtain strong relationships with actual leaf area, and thus are time consuming, particularly in large field-based research trials.

To spatially define plant canopies, point quadrat analysis (PQA) methods have been developed using spatial point pattern analysis. Smart and Robinson (1991), were the first to broadly promote the use of a PQA method in grapevines to determine canopy density, leaf and cluster exposure and canopy homogeneity. PQA, as described by Smart and Robinson (1991), determines the following metrics: leaf layer number, percent interior leaves, percent interior clusters and percent gaps. The PQA technique is low cost, not limited by weather conditions, light environment or region of canopy, though it is often applied in the fruit zone. The versatility of the technique has led to its use in defining canopy metrics under various training systems and as a means to evaluate canopy density in vineyard management research. Though PQA has been widely used in viticulture research since its introduction by Smart and Robinson (1991), the method has never been standardized in grapevines. The number of insertions per length of canopy has varied across studies and certain metrics, such as percent interior leaves, are loosely defined.

In recent decades technologies have been developed to evaluate plant health on larger scales for application to production agriculture. The most common use of technology is to measure leaf area index (LAI), the ratio of total one-sided leaf area per unit of ground surface area, which allows the technology to be used at various spatial scales. Leaf area index has been the focus of many studies (Somner and Lang 1994; Ollat et al. 1998; Johnson et. al. 2003; Johnson and Pierce 2004; Jonckheere et al. 2004; Drissi et al. 2009; Mathews and Jensen 2013), but others have used technology to quantify plant biomass (Keightly and Bawden 2010), and canopy density (Hill et al. 2011) and vineyard spatial variability (Johnson et al. 2003; Hall et al. 2008;). Applications of this technology in vineyards have been able to capture intra-vine variability; however, these technologies

are unable to evaluate the architecture of a singular vine given the continuity of vine rows in most vineyards. Additionally, this technology can be expensive, often requires complex or destructive calibrations and tedious data processing, and can be limited by environmental conditions such as rain, bright light and overcast skies.

A lower-cost alternative has been RGB image analysis from digital photography (Jonckheere et al. 2004). Few studies have used RGB image analysis to classify pixels and estimate leaf area. A basic camera with the capacity to capture red-green-blue (RGB) images has proven a useful tool to assess grapevine canopy components such as leaf area and yields (Dunn and Martin 2004; Diago et al. 2012). It is also an alternative to PQA for determining canopy density, in terms of leaf layer number (Hill et al. 2011). Advantages of digital imagery based methods include robustness to changes in illumination, variation in point of focus and reduced labor.

Viticulture and vine physiology researchers are often seeking better methods to efficiently and accurately quantify canopy architecture and vine leaf area to apply to field research trials. To improve upon current methods available to researchers, a study of various grape vine canopy quantification methods was conducted during the growing season of 2014 in field trials across two commercial vineyards. The goal was to determine if current methods were sufficient for accurately measuring vine leaf area and leaf spatial distribution, and whether they could be altered to increase both accuracy and efficiency in managed vertically shoot positioned canopies.

2.3 Materials and methods

2.3.1 Vineyard sites and experimental layout

Two commercial Pinot noir vineyards (*Vitis vinifera* L.) located in the Eola-Amity Hills region of the Willamette Valley of Oregon were studied for canopy quantification during the growing season of 2014. The two vineyards were of different vine vigor classifications, one considered of high vegetative vigor and the other considered of moderate vegetative vigor, as determined by dormant pruning weights and visual assessment of vine growth from research conducted onsite during 2012 and 2013. One Eola-Amity site (EA1) was planted in 1999 to Pinot noir Djion clone 114 grafted to

Schwarzmann rootstock. Vines were spaced 2.3 m between row and 0.9 m between vines. The second site (EA2) was planted in 2001 to the Pommard clone of Pinot noir grafted to 3309 rootstock. Vines were spaced 2.7 m between row and 1.8 m between vines. Both EA1 and EA2 were located in Salem, OR (45° 02' 02.98"N, 123° 08' 58.93"W 153 m asl and 44° 57' 35.97"N, 123° 10' 02.15"W 96 m asl). The primary soil type at each site was classified as Nekia silty clay loam (NRCS Web Soil Survey).

Both vineyards had north-south oriented rows. Site EA1 was pruned to unilateral canes and EA2 was pruned to bilateral canes, and both trained to a Guyot system with vertical shoot positioning. Canopies were managed according to standard commercial practices, including hedging from July to August as needed to maintain the structure of a typical VSP canopy and prevent shading. Site EA1 was hedged twice, and site EA2 was hedged once. Cluster zone leaf removal was conducted on the east side of the canopy at the pea-size berry stage. Vines were managed for diseases and pests per normal production practices for the region. Canopy measures and sampling occurred on 25 August to 28 August in EA1, when vines were at ~50% véraison. Sampling at site EA2 was conducted from 8 September to 10 September when vines were at ~80% véraison.

Sixteen plots were selected within each vineyard block (~0.5 hectares) for canopy measures. Each plot was selected at random and consisted of a 1.2 m length of vine row. Plastic flagging tape was placed vertically between the highest catch wire and the fruiting cane to demarcate the plot boundary.

2.3.2 Photography method

Plots were photographed using an 18.00 megapixel digital SLR camera (Canon EOS Rebel T2i, JPN). A backdrop was placed behind the vine plot in the alley opposite the camera to eliminate background vegetation. The backdrop consisted of a panel of navy blue matte cotton fabric lined by a heavy duty white poly tarpaulin to reduce translucency. The backdrop was attached to a 2.3 m (height) by 1.7 m (length) frame built from 1.25 cm schedule 40 polyvinyl chloride (PVC) and four 1.25 cm 90˚ PVC couplings. The navy blue backdrop color was selected for its low green and red hue index which differs from the hue index of the vine foliage. Two 25 cm rubber pneumatic tires

were attached to the base of the frame via a metal axel for mobility. The camera was mounted on a tripod (Ravelli, Humacao, PRI) and placed perpendicular to the vine row at a distance of 1.5 m. The digital image resolution was set at 3456 x 2304 pixels (72 psi).

Images were imported into MATLAB® R2013b (The Mathworks, Natick, Massachusetts, USA) and processed using a custom-written program which characterized each pixel as 'foliage' or 'non-foliage' based on the relative red (R), green (G) and blue (B) values. The program calculated the total number of 'foliage' pixels relative to the total number of pixels per picture. The area of each pixel was defined by the distance from the vine and the number of pixels per photo. The one-sided canopy surface area was calculated using the pixels identified as 'foliage.'

Once all photographs were taken, plastic flagging tape was used to partition the section of canopy into 3 horizontal zones: upper, middle, and lower for additional measures. The lower zone was 1.2 m (length) by 0.3 m (width) and centered on the fruiting zone. The remaining canopy above the fruit zone was divided into two equal sections of 1.2 m (length) by 0.6 m (width). The area of the lower zone was smaller than the middle and upper zones to concentrate on the fruiting zone which was the area where leaf removal occurred as part of the commercial vineyard management.

2.3.3 Point quadrat

A modification of the point quadrat analysis (Smart and Robinson 1991) was performed in each canopy section. Measurements were collected by inserting a 3.2 mm diameter metal rod perpendicular to the vine row along a designated transect of the canopy face within each canopy zone. To enhance precision, a wooden beam with 1 cm holes drilled at 5 cm intervals was used to guide the insertions. Twenty-four insertions were made per 1.2 m zone. As the rod passed through the canopy, contacts were identified as leaves or clusters and recorded; a gap was recorded if no contact was made. From these data percent canopy gaps, percent interior leaves and leaf layer number were calculated. To obtain percent canopy gaps, the total number of gaps was divided by the number of insertions and multiplied by 100. Percent interior leaves was determined by dividing the number of interior leaves by the total number of leaf contacts and multiplied

by 100. Leaf layer number was calculated by dividing the number of leaves by the number of insertions.

2.3.4 Template method

To evaluate leaf area, two methods were conducted: 1) estimation of leaf area using a template method and 2) destructive leaf sampling followed by measurement by a leaf area meter. The non-destructive in-field leaf area estimation template method was conducted after PQA was completed. The template method was described by Skinkis and Schreiner (2013) and utilized a template that consisted of six known size categories: (1) 241.19 cm^2, (2) 177.30 cm^2, (3) 129.37 cm^2, (4) 79.28cm^2, (5) 46.48 cm^2 and (6) 18.63cm^2. The template for each leaf class was developed from 240 Pinot noir leaves obtained from two vineyards in the Willamette Valley. Leaves were classified into one of six sizes and their numbers were recorded. Leaves from each size class were scanned using a leaf area meter (model LI3000, LiCor Inc., Lincoln, Nebraska, USA) to determine the mean leaf area per class. Vine leaf area was determined by measuring two randomly selected shoots per vine using the template and multiplying the mean shoot leaf area by the number of shoots per vine.

Actual leaf area was determined by destructive sampling and analysis by a leaf area meter. This was done in a manner to allow strategic defoliation based on leaf position in the canopy (interior or exterior). Since the photographic method, PQA and template leaf area measures do not specifically quantify the exterior canopy, this allowed us to determine actual proportions of interior and exterior leaf area. A 95% kaolin clay solution of Surround WP (NovaSource, Tessenderlo Kerley, Inc., Phoenix, AZ) was applied using a backpack sprayer to both sides of the canopy in the early morning, between 6:00 and 6:30 PST, when wind speed was minimal. Once the spray had dried, leaves were removed and classified based on spray coverage. Five levels of spray coverage was the maximum number of levels by which the operator could group leaves in the field. Leaves with 100% spray coverage were designated as exterior leaves. Leaves with 0% spray coverage were identified as interior leaves. Leaves with partial spray coverage, were assigned to one of 3 categories, 25%, 50% or 75% spray coverage depending on the relative spray coverage and were considered partially shaded. All

leaves were simultaneously removed by multiple people from both sides of the canopy. The most interior leaves were removed first, followed by leaves increasing in proximity to the alley. Complete exterior leaves were removed last. Leaves were removed and placed in separate bags according to their percent spray coverage (0%, 25%, 50%, 75% or 100%), and leaf type (primary or lateral). This process was repeated in each of the three zones (lower, middle and upper) established for point quadrat analysis.

After classification, bagged leaves were stored in coolers until field collection was completed and during transport to the laboratory. Leaves were kept in cold storage (4ºC) until area measurements could be completed. Individual leaf areas were quantified using a leaf area meter (model LI3000, LiCor Inc., Lincoln, Nebraska, USA) within five days of sampling. To determine total exterior leaf area, the leaf area of all leaves with Surround WP coverage at the time of defoliation were multiplied by their respective percent coverage, 0%, 25%, 50%, 75% or 100% and summed. The remaining percentage was considered interior leaf area.

2.3.5 Statistical Analysis

Data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). Simple linear regression was used to evaluate the relationships between leaf area meter measurements and leaf area determined from picture analysis. Transformations were applied as needed. A two-sample t-test was used to compare like parameters between the template method and leaf area meter measurements, and percent shaded leaves determined from point quadrat analysis and spray analysis. Means were separated using Tukey's Least Significant Differences ($p < 0.05$).

2.3 Results

Of the two vineyards evaluated, EA1 had a 1.6 times more leaf area with 3.9 m^2/m compared to 2.4 m^2 /m found in site EA2.

2.3.1 PQA v. Actual interior canopy leaf area

Canopy density, determined by point quadrat analysis and expressed as leaf layer number was consistent across the three canopy zones at site EA1. Conversely, at site EA2

leaf layer number differed between canopy zones. The middle zone was found to have the highest leaf layer number, followed by the upper and lower zones with decreasing leaf layers, respectively. Percent canopy gaps in the middle zone of site EA1 was similar to the lower and upper zones; however, the upper and lower zones differed from each other, with more canopy gaps in the upper zone. At site EA2, the upper zone had increased percent gaps when compared to the middle and lower zones (Table 2.1) while the lower zone had a reduced percent interior leaves relative to the middle and upper zones.

Actual percent leaf shaded area analysis resulted in higher measures of partially or fully shaded leaves in all canopy zones compared to the estimates determined by PQA. The percentage of leaves in each zone that were completely shaded was found to be most similar to percent interior leaves determined by PQA at both sites (Table 2.2).

2.3.2 Template method versus area meter

The total vine leaf area, primary shoot leaf area per vine and lateral shoot leaf area per vine determined by destructive sampling was 3.5m^2, 2.5 m^2 and 0.9m^2 for EA1, and 4.3 m^2, 2.8 m^2 and 1.6 m^2, for EA2, respectively. Total leaf area per vine, estimated by the template method, was comparable to total vine leaf area determined via the destructive sampling method (Table 2.3). Primary shoot leaf area measured by the template differed from actual primary shoot leaf area at EA2 but did not differ at EA1. Total lateral leaf area per vine differed between methods at both sites. The ratio of primary shoot leaf area to total vine leaf area was 0.73 at site EA1 and 0.64 at site EA2. The ratio of primary shoot leaf area to whole vine leaf area measured by the template methods was greater than the ratio of primary shoot leaf area to total vine leaf area measured using the leaf area meter.

2.3.3 Digital pixel analysis v. Actual exterior canopy surface area

When comparing the digital pixel image analysis to the exterior canopy leaf area determined by destructive sampling, it severely underestimated ($p < 0.0001$) the canopy surface area per meter (data not shown). Using data across both sites, a positive linear relationship was found between the percent pixels identified as green foliage and the percent exterior leaf surface area ($y=0.029x+1.6001$, $R^2=0.30$ $p=0.02$).

2.3.4 Labor requirements

Manual labor time was determined for each procedure applied in this study and reported based on individual laborer time (man hours). To conduct PQA in each of three canopy zones it took a total of 16 minutes per vine. This time included set up of the PQA in each zone and movement of equipment between sample plots. Additional time was required to enter data. For the digital photography component, it took an average of 14 minutes to photograph each vine. This time includes set up, deconstruction of the backdrop assembly, and moving equipment across the vineyard block to each sample vine. Computer processing time for image analysis was ~1 minute per photo. For the template leaf area method used, a total of 4 minutes were required to measure all leaves on two shoots per vine and count the number of shoots per vine. Additional time was required to enter data and compute results. Destructive leaf area measures were the most time consuming of all measures evaluated in this study. It took 3 hours 4 minutes (man hours) to measure all leaves on a single vine using a leaf area meter. This time does not include the time required to destructively sample the vine. Using the template method to estimate leaf area reduced the amount of measurement time required to determine vine leaf area by 98%.

2.4 Discussion

2.4.1 PQA

To our knowledge, there have been no published validation of the PQA method for accuracy in grapevines, yet it has been frequently used to quantify canopy density differences within viticulture research related to training systems (Gladstone and Dokoozlian 2005; Bordelon et al. 2008; Bavougian et al. 2013), shoot density (Smart 1988; Reynolds 2005; Sun et al. 2011; Sun et al. 2012), leaf removal (Percival et al. 1994; Zoecklein et al. 1992; DiProfio et al. 2011; Coniberti et al. 2012) crop level management (DiProfio et al. 2011; Sun et al. 2012; Geller and Kurtural 2013), disease management (Austin et al. 2011), and vineyard floor management (Tesic et al. 2007). The somewhat popular use of PQA has likely been due to the lack of more efficient, low-cost methods available in the literature.

There are several underlying problems related to PQA. First, the PQA method does not have standardized insertion intervals. In general, point pattern analysis has no defined scale because quadrat size, or distance between insertions in the case of point quadrat, is arbitrary. It is important to standardize insertion intervals because point pattern analysis, which is the foundation of PQA, is affected by scale. Preliminary work for this study using PQA across 3 canopy zones indicated that results from insertion intervals of 5 cm, 10 cm, 20 cm, and 25 cm, were not different. Studies have used insertion intervals ranging from 2 cm (Smart 1988) to 20 cm (Meyers and Vanden Heuvel 2008). It is common for studies to reference a given number of insertions per replicate (Zoecklein et al. 1992; Bordelon et al. 2008; DiProfio et al. 2011; Coniberti et al. 2012) rather than insertion intervals. PQA can still be applied at various scales; however, it is necessary to ensure that results do not vary with changes in scale. A larger range of insertion intervals in different canopy regions needs to be evaluated to determine the appropriate distance between insertions. It is difficult to draw conclusions across studies using PQA given the variation of insertions.

A second problem with PQA is that it the method is a measure of dispersion. The PQA method quantifies the density of points, but it does not indicate the relationship between points. Our results indicate that PQA severely underestimates the number of interior leaves in all canopy zones of VSP canopies, with insertions every 5 cm and in three zones in the canopy, typically more than applied in studies utilizing PQA (Table 2.2). Using PQA, leaves located in the canopy interior can be considered exterior leaves, despite being completely shaded by surrounding leaves.

Lastly, PQA results in a single value representative of the whole distribution. This can lead to variations within regions going unrecognized as well as different point patterns resulting in the same frequency distributions.

2.4.2 Digital pixel analysis

The RGB image analysis method described herein was designed as a simple, inexpensive alternative method for rapid, nondestructive estimation of canopy leaf surface area. A relationship was found between measured exterior leaf area and the percent of pixels categorized as foliage; however, the image analysis program severely

underestimated exterior leaf surface area. The method was not as successful as other RGB image analysis methods designed to estimate various aspects of a vine canopy (Diago et al. 2012) and requires further investigation to improve accuracy. The thresholds used were developed for mature green leaves, which are characteristically darker than younger, developing leaves. Lateral leaves comprised 27% of vine leaf area at site EA1 and 45% of vine leaf area at site EA2, suggesting that a significant portion of the plant canopies were younger leaves. One RGB image analysis method that yielded better accuracy (Diago et al. 2012) used multiple classifications levels for leaf age. Establishing multiple classification levels is complex, requiring multiple defoliation stages and image captures per vine as well as manual pixel validation. The categories used by Diago et al. (2012) successfully identified fruit, wood, background (gaps), and leaves. Leaves were further categorized into four leaf classes according to leaf age. Sampling time is pivotal to defining pixel thresholds for each classification level. Vine components change color over the course of the season, including shoot lignification, cluster ripening, leaf darkening with age and yellowing with senescence.

Using processed images instead of raw images is one possible tool which could lead to improving the RGB method developed in this study. Using several plant species Easlon and Bloom (2014) were able to successfully estimate leaf area by recoloring the plant foliage to a known RGB value. This method was developed using small plant species relative to large broadleaf grapevine and has not been tested at large scales. Additionally, this method and requires calibration to determine the percent of foliar overlap, and as such It may not be applicable for use in dense VSP canopies with multiple leaf layers.

2.4.3 Template Method

Leaf area per vine estimated using a circular template method did not differ from leaf area measured by destructively leaf sampling, suggesting that shoot-level methodology is appropriate for combined primary and lateral shoot leaf area in managed VSP canopies. This method is a scalar method used to estimate whole vine leaf area. The method was suitable for primary shoot leaf area at site EA1 but was not suitable for site EA2. The discrepancy between sites may be result of different primary to total leaf area

ratios. A relationship between primary shoot length and shoot leaf area has been established by Blom and Tarara (2007); however, this method was developed in an arid climate on vines with minimal lateral leaf area. Their shoot length method (Blom and Tarara 2007) for estimating leaf area has not been demonstrated as an applicable method for estimation of lateral shoot leaf area. The template method was not found to be an appropriate estimate total lateral leaf area, as leaves smaller than the smallest templates were not measured, and this is likely the cause of the underestimation. The template method may not be a suited method for estimating lateral leaf area for vines with excessive lateral leaf growth. However, one advantage of the template method is that is discriminates against small lateral leaves which are likely vegetative sinks.

A rapid estimation of shoot leaf area was developed for both primary and lateral shoots by Lopes and Pinto (2005). Using the area of smallest and largest leaves on a given shoot as well as the number of leaves per shoot, the authors were able estimate shoot leaf area. Their method was developed using multiple cultivars grown in the warm and arid regions of southern Portugal and trained to a bilateral spur pruned cordon system with vertical shoot positioning. Though this method requires fewer overall leaf measurements, it is difficult and time consuming to identify the largest and smallest leaves on a shoot. It is particularly difficult to assess leaf size in dense canopies that have been hedged, such as those grown in Oregon's cool-climate Willamette Valley. The method developed by Lopes and Pinot (2005) has been validated in other cultivars (Beslic et al. 2009) but it has not been tested as a scalar quantity and it is unknown as to whether it has the ability to estimate whole vine leaf area.

2.6 Conclusion

Of the various canopy methods evaluated and compared to destructive leaf area assessments, the template method was the most rapid and accurate technique for estimating whole vine leaf area. Of the methods used to evaluate canopy exterior and interior leaves, the point quadrat method severely underestimated the percent of interior leaves within the canopy. Though, a relationship was established between percent green 'foliage' pixels and total exterior leaf area in digital photography, further calibrations would be required to improve the relationship. Further work will be required to improve upon point quadrat analysis and other methods by which to quantify canopy surface area.

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Table 2.1. Mean leaf layer number, percent interior canopy, and percent canopy gap parameters from point quadrat analysis parameters applied in three horizontal canopy zones at two vineyard sites during véraison.

Site	Canopy Zone	Leaf layer number	Percent interior leaves	Percent exterior leaves	Percent gaps
EA1	Lower	1.5 a	15.5 a	84.5 b	8.3 b
	Middle	1.6 a	15.1 a	84.9 b	13.5 ab
	Upper	1.5 a	12.9 a	87.1 b	23.2 a
	p	0.4004	0.5252	0.5252	**0.0023**
EA2	Lower	0.9 c	6.3 b	93.7 a	15.1 b
	Middle	1.9 a	19.3 a	80.7 b	10.4 b
	Upper	1.5 b	18.0 a	89.2 b	26.6 a
	p	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Means followed by different letters are considered different according to Tukey's Least Significant Difference test ($p < 0.05$).

Table 2.2. Percent of interior leaves as determined by point quadrat analysis compared to actual leaf position within the canopy.

Site	Canopy Zone	PQA Percent interior leaves[a]	Percent of leaves in full or partial shade[b]											
			100%	p value	Δ[c]	75%	p value	Δ[c]	50%	p value	Δ[c]	25%	p value	Δ[c]
EA1	Lower	15.5	17.4	0.0730	+1.9	35.5	**<0.0001**	+20.0	55.4	**<0.0001**	+39.9	77.5	**<0.0001**	+62.0
	Middle	15.1	24.3	**0.0002**	+9.2	42.7	**<0.0001**	+27.6	63.0	**<0.0001**	+47.9	81.6	**<0.0001**	+66.5
	Upper	12.9	22.7	**0.0027**	+9.8	44.0	**<0.0001**	+31.1	66.2	**<0.0001**	+53.3	81.6	**<0.0001**	+68.7
EA2	Lower	6.3	16.7	**<0.0001**	+10.4	34.0	**<0.0001**	+27.7	53.9	**<0.0001**	+47.6	78.9	**<0.0001**	+72.6
	Middle	19.3	25.5	**0.0059**	+6.2	43.7	**<0.0001**	+24.4	63.4	**<0.0001**	+44.1	83.0	**<0.0001**	+63.7
	Upper	18.0	27.0	**0.0002**	+9.0	47.2	**<0.0001**	+29.2	68.8	**<0.0001**	+50.8	84.3	**<0.0001**	+66.3

[a]Mean percent interior leaves determined by point quadrat analysis

[b]Percent of canopy leaves under different shade levels determined from Surround coverage. 100% - fully exposed exterior leaves, 50% and 25% partial shaded, interior leaves.

[c]Percent interior leaves was subtracted from levels of percent of leaves in full or partial shade to calculate delta.

Table 2.3. Comparison of leaf area and template leaf area measured across two vineyard sites in 2014.

Site	Method	Leaf area (m^2)	Primary leaf area (m^2)	Lateral leaf area (m^2)
EA1	Template	3.0	2.4	0.5
	Leaf area meter	3.5	2.5	0.9
	p	0.1060	0.5824	0.0169
EA2	Template	4.2	3.3	0.9
	Leaf area meter	4.3	2.8	1.6
	p	0.5640	**0.0159**	**0.0006**

Means are presented (n=16).

Chapter 3:

COMPARING COMPONENTS OF VINE BALANCE TO FRUIT COMPOSITION AT HARVEST IN OREGON PINOT NOIR VINEYARDS

3.1 Abstract

Cluster thinning is a common management strategy used to reach target yields and maintain vine balance. The objective of this study was to determine if cluster thinning resulted in differences in fruit composition of cool climate Pinot noir. A field study was conducted in 2013 and 2014 across four commercial *Vitis vinifera* L Pinot noir vineyards in Oregon's Willamette Valley. Vines were cluster thinned to one cluster/shoot and compared to vines with full crop (no cluster thinning). Crop levels were imposed in two vineyards of moderate vegetative vigor and two vineyards of high vegetative vigor. Cluster thinning effectively reduced yields and created a range of leaf area to yield ratios and pruning weight to yield ratios. Cluster thinning had no effect on vine vegetative growth at any site in either year. Grape maturity parameters and free-form volatile compounds varied between years and consistent trends of cluster thinning were not observed across vineyard sites. Regression analysis using data across sites indicated a relationship between the leaf area to yield ratio and C_{13} norisoprenoids, and pruning weight and yeast assimilable nitrogen in only one year. This work suggests that cluster thinning has limited impact in Pinot noir fruit composition at harvest.

Keywords: Pinot noir, vine balance, crop load, cluster thinning, fruit composition

3.2 Introduction

Vine balance is integral to the production of quality winegrapes. A grapevine is considered balanced when the canopy has sufficient leaf area to mature the vine's yield to a desired ripeness while maintaining vine health and long term productivity. Vines with high or low vegetative vigor are considered unbalanced and can be a result of site conditions or management practices. Vine vegetative vigor level as affected by various vineyard management practices, has been found to affect fruit composition and resulting wine quality (Reynolds and Wardle 1989; Kliewer and Dokoozlian 2005; Song et al. 2012).

Vine balance is often defined by the ratio of leaf area per unit weight of fruit or by the yield to pruning weight ratio (Winkler et al. 1974; Bravdo et al. 1984; Kliewer and Dokoozlian 2005). For several *V. vinifera* cultivars studied in California, Kliewer and Dokoozlian (2005) define the leaf area required to ripen fruit to maturity in a single curtain canopy to be between 0.8 and 1.2 m^2 per kg of fruit. They contend that the ratio of yield to pruning weight of 5 to 10 is conducive to balance *V. vinifera* vines in warm climates, while a range of 3 to 5 has been proposed for varieties with small clusters, such as Pinot noir, grown in cool climates (Kliewer and Casteel 2003; Kliewer and Dokoozlian 2005).

Cultural practices can be employed in the vineyard to physically alter vegetative and reproductive growth to favor a balanced vine. Vegetative growth can be manipulated through pruning, shoot thinning, hedging, leaf removal, irrigation and fertilization. These practices can have direct effects on canopy architecture and indirect effects on shoot and root growth, fruit development, and both can influence carbohydrate reserves (Reynolds et al. 1994; Howell et al. 1994; Hunter et al. 2004). Reproductive growth can be adjusted using shoot and cluster thinning, and adjusting yield to attain a more optimum vine balance as defined by both leaf area to yield ratio and yield to pruning weight ratio (Kliewer and Dokoozlian 2005; Terry and Kurtural 2011).

Cluster thinning is a practice employed by many commercial winegrape growers across the world. It is commonly used to obtain high quality fruit and ensure maturity; however, it is a costly management practice for Pinot noir producers in Oregon (Julian et al. 2008), where as much as 25-50% of vine yield can be removed in one growing season (Skinkis, in progress). Cluster thinning is conducted manually throughout most regions both nationally and internationally and can be performed at various berry developmental stages. In Oregon, cluster thinning is typically conducted at lag phase (Skinkis and Uzes, submitted). Changes in grape maturity resulting from cluster thinning have been observed in many studies conducted in warm and arid climates with varying results (Pallioti and Cartechini, 2000; Keller et al. 2005; Tardaguila et al. 2008; Gatti et al. 2012; Tardaguila et al. 2012). Cluster thinning levels should be determined by appropriate target vine balance metrics. However, the majority of Oregon producers use a standard yield target across varied climates and vineyard with variable productivity (Skinkis, in progress). While there has been research conducted in warm climates with heavier yielding cultivars to define such metrics, there have been few published studies to suggest specific vine balance metrics for cool climate cultivars such as Pinot noir (Reynolds et al. 1994; Vance 2012; Brasher 2002; Feng 2014). It is important to understand the effects of crop load on vine productivity and fruit composition in order to make better economic and vineyard management decisions. Research is underway to develop vine balance metrics for Oregon Pinot noir growers to target yields that ensures quality wine production (Skinkis, in progress).

We hypothesized that the balance of yield to vine size is more important than a standardized yield target to obtain good fruit ripening and quality. By investigating crop load and fruit composition in commercial vineyards with varying vine vigor in the Willamette Valley, we began to explore the impact of vine size and yield on fruit quality.

3.3 Materials and methods

3.3.1 Vineyard locations and experimental layout.

Four commercial *Vitis vinifera* L. Pinot noir vineyards located in the Eola-Amity Hills and Yamhill-Carlton American Viticultural Areas of Oregon were evaluated during 2013 and 2014 (Table 3.1). They were selected as representing a range of vineyard vigor commonly found in the Willamette Valley. One high vigor and one moderate vigor site from each AVA were chosen based on historical pruning weight data and visual inspection. The high vigor Eola-Amity site (EA1) was planted in 1999 to Pinot noir Djion clone 114 grafted to Schwarzmann rootstock. Vines were spaced 2.3 m between row and 0.9 m between vines. The moderate vigor Eola-Amity site (EA2) was planted in 2001 to Pinot noir clone 667 grafted to Riparia Gloire rootstock. Vines were spaced 1.6 m between row and 1.0 m between vines. Sites EA1 and EA2 are both located west of Salem, OR (45° 02' 02.21"N; 123° 08' 57.90"W, 497 m asl and 44° 58' 46.93"N; 123° 06' 50.30"W, 203 m asl). The high vigor Yamhill-Carlton site (YC 1) was located in Yamhill, OR (45° 21’ 23.75"N; 123° 08' 11.21"W 137 m asl.). Site YC1 was planted in 1995 to Pinot noir clone 115 grafted to 3309 rootstock. Vines were spaced 2.3 m between row and 1.5 m between vines. The moderate vigor Yamhill-Carlton site (YC 2) was located in Carlton, OR (45° 19' 30.89"N; 123° 08' 29.69"W, 137 m asl). Site YC 2 was planted in 2001 to Pinot noir clone 777 grafted to 3309 rootstock. Vines were spaced 2.0 m between row and 1.0 m between vines.

All vineyards in this study had similar design and training per the region. Rows were oriented north-south. Vines were cane pruned to a Guyot system and vertically shoot positioned. Canopies were managed according to standard commercial practices, including hedging of the top and sides of canopies from July to August as needed to maintain the structure of a typical VSP canopy and reduce shading. Leaf removal in the cluster zone was conducted on the east side of the canopy at the pea-size berry stage. Vines were managed for powdery mildew and Botrytis bunch rot with appropriately timed fungicide sprays per normal production practices for the region.

A range of leaf area per unit weight of fruit ratios was obtained by cluster thinning. Cluster thinning treatments were applied to whole rows of vines in a randomized complete block design with four replicates in each site. For both years, treatments for all sites were (1) no cluster thinning and (2) thinned to 1 cluster per shoot at lag phase. Due to low fruitfulness at site YC2 in 2013, vines were thinned, removing 50% and 75% of the clusters per vine at fruit set. All vine data were collected from 10 contiguous sample vines located within each vineyard plot.

3.3.2 Weather Data

Daily precipitation and daily temperature data were obtained for each growing season from the Aurora (ARAO), OR weather station of the Pacific Northwest Cooperative Agriculture Weather Network (Agrimet). Growing Degree Day (GDD_{10}) units were calculated using $(T_{max} + T_{min})/2$ - 10ºC with no upper temperature limit (Table 3.2).

3.3.3 Vine growth

Leaf area, yield, and dormant pruning weight data were obtained each season. Shoot leaf area was estimated non-destructively at véraison by measuring leaves on two randomly selected shoots per vine using a template method described by Skinkis and Schreiner (2013). The mean leaf area per shoot was then multiplied by the number of shoots per vine to estimate whole vine leaf area. Whole vine leaf area per unit weight of fruit ratios (m^2/kg) were calculated from whole vine leaf area measured at véraison and vine yield at harvest. Pruning weights were collected during the dormant season within two weeks of each other at all four sites, typically during late January or early February each year. Pruning weights were measured per vine within each plot. Yield to pruning weight ratio was calculated by dividing the yield per vine by the pruning weight per vine. Cane weights were obtained by dividing vine pruning weight by the total number of shoots removed per vine during pruning.

3.3.4 Harvest date, fruit sampling and analysis

Cluster counts and whole vine yields were collected from each plot by each vineyard collaborator following outlined protocols. All treatments within each site were harvested on the same day. Fruit samples were collected just prior to commercial harvest and consisted of 40 clusters randomly selected from the fruit harvested from each plot. In 2013, harvest sampling occurred on 2 October for site YC1, 3 October for both sites EA2 and YC1, and 9 October for site EA1. In 2014, commercial harvest was 19 September, 23 September, 26 September, and 29 September for sites YC1, EA2, EA1 and YC2, respectively. Samples were collected in large plastic bags, stored in coolers, packed with ice packs and transported via overnight shipping to the laboratory (Modesto, CA) for next day analysis. Brix, malic acid, tartaric acid, total acidity and pH, ammonia and free primary amino acids were determined using FTIR: Fourier-transform infrared spectroscopy (Versari et al. 2008). The concentration of yeast assimilable nitrogen (YAN) was calculated by summing the concentration of ammonia and primary free amino acids.

Analysis of volatile compounds important to wine quality was conducted on fresh fruit following harvest. To quantify volatile compounds whole berries were ground fresh for 4 minutes using a GENO grinder model 2000 (SPEX, NJ, USA) at 1400 revolutions per minute. Total C_6 compounds were determined from the sum concentration of C_6 alcohols (hexenol, trans-2-hexenol, trans-3-hexenol, cis-3-hexenol) and aldehydes (hexenal, trans-3hexenal) using solid phase microextraction (SPME) and gas chromatography (GC-MS) using the method described by Sanchez-Palomo et al. (2005). Terpinoids, including linalool, geraniol and 1-octen-3-ol, were determined using solid phase microextraction (SPME) and gas chromatography (GC-MS). The C_{13}-norisoprenoids, β-damascenone and 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) were determined using solid phase micro-extraction (SPME) and gas chromatography (GC-MS).

3.3.5 Statistical Analysis

Data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). Two levels of statistical analysis were conducted. Plot means were used for within site analyses. Treatment means were used for analysis across sites. Regression was used to evaluate the relationships between measures of vine productivity and fruit composition at harvest. Transformations were applied as needed.

3.4 Results

3.4.1 Climate conditions

Climate conditions varied between the 2013 and 2014 growing seasons (Table 3.3). Growing degree days were similar between years with 1393 and 1628 GDD_{10} in 2013 and 2014, respectively. Total precipitation between April 1 and November 1 was similar each season with nearly 50% of the growing season precipitation occurred during the months of September and October. However, large rain events in 2013 during the month of September distinguished 2013 from 2014. There was a total of 191 mm of precipitation in September of 2013, 65% of which occurred during two 48 hour rain events. The first rain event (51 mm) occurred on September 5 and 6, and was followed by a two week dry period. The second larger rain event (73 mm) occurred on September 28 and 29. This rain event occurred in the midst of a 2 week period of daily precipitation prior to harvest at all sites that year. By contrast, a single rain event occurred in 2014, wherein 24mm of precipitation fell during another 48 hours period during the week prior to harvest at sites EA1 and YC2. The remaining sites, EA2 and YC1, were subject to less than 1 mm post-véraison rain in 2014. The differences in pre-harvest precipitation influenced fruit integrity between the seasons and may have influenced fruit composition.

3.4.2 Vine growth and yields

There were no differences observed in leaf area measured at véraison with respect to cluster thinning. In 2013, site YC2 had the lowest average leaf area with 1.8 m^2/m followed by sites EA2 with 2.8 m^2/m, EA1 with 3.8 m^2/m and YC1 with 5.3 m^2/m (Table

3.4). Leaf area in 2014 averaged 3.2 m^2/m at both high vigor sites, EA1 and YC1, while the moderate vigor sites, EA2 and YC2, had leaf areas of 1.5 m^2/m and 1.9 m^2/m, respectively. Shoots per meter of canopy row length did not differ between treatments in either year with the exception of site YC1 in 2014 (Table 3.5).

Similar early season climatic conditions between 2013 and 2014 resulted in similar phenology progression between bud break and véraison. Therefore, lag phase cluster thinning was imposed the same approximate date each year (Table 3.2). As expected, cluster thinning reduced yield in all vineyards across the two-year period. Yield was reduced by an average of 41% across sites in both 2013 and 2014. In 2013, yields across all sites ranged from 0.5 kg/m to 1.5 kg/m meter in 2013 with yield reduction from 27% to 53% in Thinned compared to Control (Table 3.4). In the following year, yields ranged from 0.6 kg/m to 2.8 kg/m and slightly narrower yield reduction range of 33% to 54% was observed (Table 3.5). Overall, crop levels were higher in 2014 compared to 2013 at sites EA1, YC1 and YC2.

This difference in yield resulted in a difference in leaf area to crop weight ratios which resulted in treatment differences between Thinned and Control for all sites except YC2 in 2013 (Table 3.4). Leaf area to yield ratios of treatment means across sites ranged from1.6 m^2/kg to 4.7 m^2/kg in 2013. A narrower range of leaf area to crop weight ratios, 1.2 m^2/kg to 2.5 m^2/kg, was observed in 2014 (Table 3.5).

Pruning weights per vine and cane weights did not differ between the Control and Thinned treatments at any site in 2013 (Table 3.4) or 2014 (Table 3.5). However, the yield to pruning weight ratio was lower in the Thinned vines when compared to the control in all sites and years except EA1 in 2013 (Tables 3.4 and 3.5). Differences in yield to pruning weight ratios were expected between treatments due to changes in yield by cluster thinning.

3.4.3 Grape maturity parameters

Total soluble solids (TSS), pH and acidity levels are common metrics used to track the grape ripening process and assist in determining harvest. Few consistent differences were observed in the composition between fruit from the Control and Thinned vines in either 2013 or 2014, despite a consistent reduction in yield across sites and years. Total soluble solids were lower overall in 2013, ranging from 19.6 to 21.9 °Brix (Table 3.6), compared to 2014, which had a range of 22.8 to 25.5°Brix (Table 3.7). The only site to show a difference in TSS between treatments was EA1. All other sites did not have differences in TSS with cluster thinning. Total acidity levels were higher in 2013 when compared to 2014. In both years, total acidity was lower in fruit from vines thinned to one cluster per shoot at site EA2 only. There were no differences in total acidity between treatments in the remaining sites in either year.

There were few clear and consistent results with respect to crop level for pH, total acidity, tartaric and malic acids during the two-year study. Site EA2 was the only vineyard to show a difference in pH in 2013 (Table 3.6). No differences in pH were observed between Control and Thinned vines in 2014 at any site (Table 3.7). Total acidity levels were higher in 2013 when compared to 2014. In both years, total acidity was lower in Thinned vines thinned at site EA2 only. There were no differences in total acidity between treatments in the remaining sites in either year. Lower levels of tartaric acid were quantified in 2013 compared to 2014. Tartaric acid levels varied across sites in 2013 (Table 3.6). A higher concentration of tartaric acid was found in the Control fruit at site EA2, while a lower concentration was found in the control fruit at site YC1, and no differences were observed at sites EA1 and YC2. Treatment differences for were not observed for tartaric acid in 2014 at any site (Table 3.7). Malic acid was higher in 2013 than 2014 across all sites, except YC1. Cluster thinning did not result in different malic acid levels between the Control and Thinned treatments at any site in either year.

Ammonia and free primary amino acids are primary sources of nitrogen (N) containing compounds used by yeast during fermentation (Juhász and Törley 1985), the

total of which comprise yeast assimilable nitrogen (YAN) concentrations. Ammonia N did not differ between treatments in 2013 at any site, despite a 24% higher average across the four sites in Thinned compared to Control vines (Table 3.6). In 2014, ammonia N levels were also not different between treatments at any site (Table 3.7). Nitrogen from primary amino acids did not differ between treatments in any site during 2013 (Table 3.6). However, a higher quantity of primary amino acids was found in Thinned compared to Control vines at site EA2 only in 2014 (Table 3.7).

Yeast assimilable nitrogen (YAN), is important for yeast growth and metabolism during fermentation (Henschke and Jiranek 1993). In 2013, imposed thinning levels did not affect YAN concentrations (Table 3.6) though regional trends were observed between the Eola-Amity sites (EA1 and EA2) and the Yamhill-Carlton sites (YC1 and YC2). Results from 2014 indicate that treatments were not different despite a 5% higher average YAN concentration in Thinned fruit compared to Control across the four sites (Table 3.7).

3.4.4 Grape volatile composition

C_6 compounds are a main class of volatile compounds that contribute to the green and grassy aromas in grapes and wines (Ferreira et al. 2000). There were few differences between treatments in C_6 aldehydes and alcohols quantified in this study, and few differences between the treatments were observed. In 2013, a lower concentration of the C_6 alcohols, 1-hexanol and trans-3-hexenol, were found in the fruit from Control vines compared to Thinned vines at site EA2 only (Table 3.8). A higher concentration of cis-3-hexenol was observed in the Control fruit at site YC2 in 2014 (Table 3.9). No other differences were observed between treatments for any C_6 alcohols in 2014. Results from 2013 indicate that both Yamhill-Carlton sites had higher concentrations of C_6 aldehydes, 1-hexenal and trans-2-hexenal, in the Thinned fruit (Table 3.8). In the Eola-Amity sites, lower concentrations of C_6 aldehydes were observed at site EA2, and no treatments differences were observed at site EA1 in 2013. Treatment differences in C_6 aldehyde concentrations were not observed in 2014 at any site (Table 3.9).

Terpenoids and C_{13} norisopresnoids are classes of grape-derived volatile compounds that contribute to grape and wine aroma. Monoterpenes, a subgroup of terpenoids, are found at low levels relative Muscat related cultivars. However, even at low levels they can contribute to floral aroma in Pinot Noir wine (Fang and Qian 2005; Loscos et al. 2007). Free-forms of the monoterpenes linalool, geraniol, and 1-octen-3-ol were not different any site in either 2013 or 2014. C_{13} norisoprenoids, also contribute to the aroma profile in multiple capacities. Two of the most common C_{13} norisoprenoids, β-damascenone and TDN, were quantified in both 2013 and 2014. Site EA2 was the only site that showed differences between treatments. In 2013, a higher concentration of TDN was observed in the Thinned fruit (Table 3.8). Similarly, a higher concentration of β-damascenone was found in the Thinned fruit in 2014 (Table 3.9).

3.4.5 Vine balance metrics and fruit composition

The relationship between the two measures of vine balance, including leaf area to yield ratios and yield to pruning weight ratios, were examined to better understand the range of crop loads in the region and determine if there was good agreement between the two different metrics. A negative linear relationship was observed between leaf area to yield ratios and yield to pruning weight ratios across all sites and years except site EA1 in 2013 (Figure 3.1).

Regression analysis was used to assess how fruit yield relative to canopy size affects fruit composition at harvest for each of the compounds quantified. Year-to-year variability had a strong influence on regression analyses and resulted in a lack of consistency across multiple growing seasons. At only one site was the relationship between a vine growth parameter and fruit composition consistent across multiple years. At site YC1, a positive linear relationship was observed between pruning weight per vine and malic acid in 2013 (Figure 3.2) and 2014 (Figure 3.3). Using treatment means from across sites, a positive linear relationship was observed between leaf area to yield ratios and the concentration of C_{13} norisprenoids, β-damascenone (Figure 3.5) and TDN in 2013 (Figure 3.6).

3.5 Discussion

3.5.1 - Weather effects

By comparison with long-term weather data, the 2013 and 2014 seasons were both warm, dry years for the Willamette Valley. Despite similar precipitation between April 1 and November 1, the 2013 growing season was wetter in the days and weeks before all harvest dates compared to the 2014. Post-véraison rain is thought to increase berry size, and beyond a certain point maycause berries to crack. Widespread berry cracking was observed in the fruit samples at harvest in 2013 as a result of the pre-harvest rain events. Since Pinot noir is a tight-clustered variety, splitting may have been a result of swollen berries bearing pressure on one another. However, due to the heavy rainfall it is likely that water uptake by the berry exceeded water loss via transpiration forcing the berry cuticle to rupture (Considine and Kriedemann 1972; Considine 1982; Lang and Düring 1990). Clake et al. (2010) found that berry splitting was correlated with the degeneration of pericarp cells, suggesting that berry susceptibility to splitting decreases during ripening. The extensive berry cracking observed in 2013 may have been due to the first rain event in early September when fruit was less ripe. Additionally, the increased water uptake by the berry likely diluted the berry contents, such as sugars and organic acids. Overall, lower total soluble solids were observed across all sites in 2013; however, an inverse trend in total acidity in 2013 was not observed across sites.

3.5.2 *Vine growth response*

Vine vegetative growth characteristics have the capacity to affect canopy microclimate and fruit quality (Smart et al. 1985; Dokoozlian and Kliewer 1995; Gladstone and Dokoozlian 2003). Measures of vine growth such as leaf area and pruning weights did not differ by crop level in this study. Many cluster thinning studies also have not found a difference in vine growth parameters between treatments (Reynolds et al. 1994; Reynolds et al. 1996; Naor et al. 2002; DiProfio et al. 2011: Gatti et al. 2012). Some studies that have cited an effect of cluster thinning on stimulating vine vegetative growth have been conducted in high-yielding interspecific hybrid grape cultivars where

cluster thinning was likely required for sustaining vine health (Fisher et al. 1977; Dami et al. 2006).

Leaf area per unit weight of fruit was influenced by yield as a result of cluster thinning at most sites. The leaf area to yield ratios observed in this study were higher than the optimal ranges (0.8 to 1.2 m^2/kg) proposed by Kliewer and Dokoozlian (2005) for single canopy grapevines. Ratios ranged from 1.6 m^2/kg to 4.7 m^2/kg in 2013, and 1.2 m^2/kg to 2.5 m^2/kg in 2014. Vance (2012) found a slightly narrower range of leaf area to yield ratios improved overall fruit composition in a high vigor Pinot noir vineyard in one year of a two year study. The leaf area required to mature a unit weight of fruit varies according to cultivar and site conditions (May et al. 1969; Kliewer and Antcliff 1970; Kliewer and Weaver 1971; Kliewer and Dokoozlian 2005). Given the moderate to high vigor levels of vineyards evaluated in this study, larger ratios compared to studies conducted in warmer, arid regions is not surprising. Though cool climate regions may require higher total leaf area to maintain productivity when the season length and heat units are limiting, excessive leaf area may result in shading and a loss of productivity, particularly within a VSP trained Guyot system. Results from one shoot density study, conducted over three years in a cool climate region, indicated that Pinot noir can sustain both high shoot densities and yields without detriment to fruit composition (Reynolds et al. 1994).

Cluster thinning also influenced the yield to pruning weight ratios across most sites. In this study, yield to pruning weight ratios ranged from 1.5 to 4.5 in 2013 and 1.8 to 4.1 in 2014, well below the optimal yield to pruning weight range (5 to 10) proposed for vines grown in warm climates (Kliewer and Dokoozlian 2005). In both years, all Thinned vines had yield to pruning weight ratios which were also below the 3 to 5 range suggested for small-clustered varieties in cool climate regions (Kliewer and Casteel 2003; Kliewer and Dokoozlian 2005). In 2013, the Control vines from the two high vigor sites, EA1 and YC1, also had lower yield to pruning weight ratios than the suggested optimums cited in literature. This was not observed in 2014, where all Control vines had appropriate

yield to pruning weight ratios as defined by literature. This variability between years is due to differences in base yields, as 2013 was considered a more normal yield year while 2014 was a very high yield year due to high fruit set and cluster size. Similar yield to pruning weight ratios were observed in a two year study by Brasher (2002) replicated across two Pinot noir vineyards in Oregon's Willamette Valley. In a one year study by Vance (2012), conducted in single vineyard located in the northern Willamette Valley, yield to pruning weight ratios fell within the range observed in this study. In a study conducted by Reynolds et al. (1994) the four year mean yield to pruning weight ratio was 3.6 and 2.1 times higher than the two year means for non-thinned and thinned vines in our study. There are few studies currently published on the impacts of cluster thinning in cool climate Pinot noir vineyards, and further long-term research studies will help develop better vine balance metrics to guide yield management decisions.

Regression analyses of the two crop load metrics, leaf area to pruning weight and yield to pruning weight, resulted in a negative linear relation. A similar relationship was demonstrated in studies with Cabernet Sauvignon grown in a warm climate (Kliewer and Dokoozlian, 2005). Using the optimal ranges suggested by Bravdo et al. (1984) and Kliewer and Dokoozlian (2005), our data falls below the proposed optimum range and suggests that the vines are under cropped; however, the optimal ranges are established using higher yielding, larger cluster cultivars grown in warm climates. Even our full crop Control vines were considered under-cropped by virtue of the low yields of Pinot noir. This suggests that newly defined crop load metrics are required to interpret quality impacts in Oregon's Pinot noir vineyards. Research on crop load will continue in future years to better inform metrics for the region (Skinkis, in progress).

5.2.2 Fruit composition

Grape maturity parameters such as TSS, pH and acidity levels which are used to determine harvest dates have had variable responses to cluster thinning. Overall, cluster thinning has been found to increase sugar content of berries at harvest (Keller et al. 2005; Tardaguila et al. 2012; Gatti et al. 2012; Sun et al. 2012). Higher total soluble solids were

found in fruit from Thinned compared to Control in only one year of this study. The inverse trend observed in 2013 may be attributed to heavy precipitation prior to and during harvest, and the lack of differences in 2014 is likely due to the warm, dry season leading to full ripeness despite differences in yield. Total acidity and pH levels observed in this study were variable; no consistent trends were observed in either 2013 or 2014. Titratable acidity was lower as a result of cluster thinning in a one year study in Pinot noir conducted in a cool growing season with limiting heat units (Vance 2012). Several authors have reported little to no effect of cluster thinning on pH or organic acids (Ough and Nagaoka 1984; Iacono et al. 1994; Reynolds et al. 1994).

C_6 alcohols and aldehydes can result in undesirable compounds associated with leafy and grassy aromas (Ferreira et al. 1995; Hatanaka 1996) when above sensory detection thresholds. The total concentration of C_6 compounds found in the grape samples during the two-year period were well below the sensory thresholds (Guth 1997), and any difference observed between treatments would not lead to a sensory difference in wines.

Monoterpenes, such as linalool, geraniol and 1-octen-3-ol, contribute to caramel apple, floral and mushroom aromas, respectively, depending on their concentrations. These aroma compounds are typically found below sensory thresholds in Pinot noir (Fang and Qian 2005; Fang and Qian 2006). Results from our study indicate that the concentration of monoterpenes observed are at sensory detection thresholds and may add to the overall floral and fruity aromas.

C_{13}-norisoprenoids, such as β-damascenone and TDN contribute to the aroma profile in grapes and subsequent wine in various capacities. In Pinot noir, the concentration of β-damascenone is typically found above sensory thresholds and is considered an important aroma compound. When concentrations are above sensory thresholds, β-damascenone can contribute to multiple aromas, specifically sweet and floral and aromas in Pinot noir (Fang and Qian 2005). The concentration of β-damascenone observed in this study was well above the sensory threshold (Ferreira et al. 2000; Guth 1997). Compared to β-damascenone, TDN has a higher sensory threshold.

Our results indicate that observed concentrations of TDN were below the level of detection and that the treatment difference observed at site EA2 in 2013 is not considered important for wine quality. Using pooled data from across sites, a positive linear relationship was observed between leaf area to yield ratios and the concentration of β-damascenone and TDN. A similar relationship between pruning weight and β-damascenone concentration was also observed in a three year study using Pinot noir grown in a cool climate (Feng 2014), wherein higher pruning weights led to higher concentrations of β-damascenone. These results may suggest that great pruning weight was indicative of larger canopy size resulting in an increase potential for shading. The relationship between C_{13}-norisoprenoids and canopy vigor has not been well defined. However, it is known that other classes of volatile compounds, such as terpenoids, are influenced the by canopy light environment and increased cluster exposure (Skinkis et al. 2010). Given these relationships it is not surprising that cluster thinning did not result in differences in the concentration on β-damascenone as it also did not alter vine vegetative parameters. The relationship between vine vigor and grape quality, especially volatile composition has not been fully understood. Regression analyses of vine parameters against fruit parameters were not consistent between years as a result of variable weather patterns and differences in base yields. At only one site was the relationship between a pruning weight per vine and malic acid, consistent across multiple years. At site YC1, a positive linear relationship was observed in both 2013 and in 2014. Site YC1 is the only site of the four evaluated to be trained bi-laterally, resulting in higher yield on a per vine basis. In order to better understand the relationships between crop load metrics and fruit quality, longer-term studies need to be conducted to span across such seasonal variability.

3.6 Conclusion

Vine balance parameters, such as leaf area to yield and pruning weight to yield were affected as a result of yield reduction, but there were no effects on vine growth parameters with differences in yield. High leaf area to crop weight ratio were observed across all vineyard sites. No consistent trends were observed in grape maturity

parameters or free volatile parameters across sites and years. This suggests that Pinot noir vines examined in the four commercial vineyards in the Willamette Valley were not over-cropped and have sufficient vine canopy to support full development of fruit to ripeness during a typical growing season. Additional research, over multiple growing seasons is needed to assess the long term impact of cluster thinning and to understand the effect of source to sink variation on vine physiology and fruit composition in moderate to high vigor vineyards in a cool climate region.

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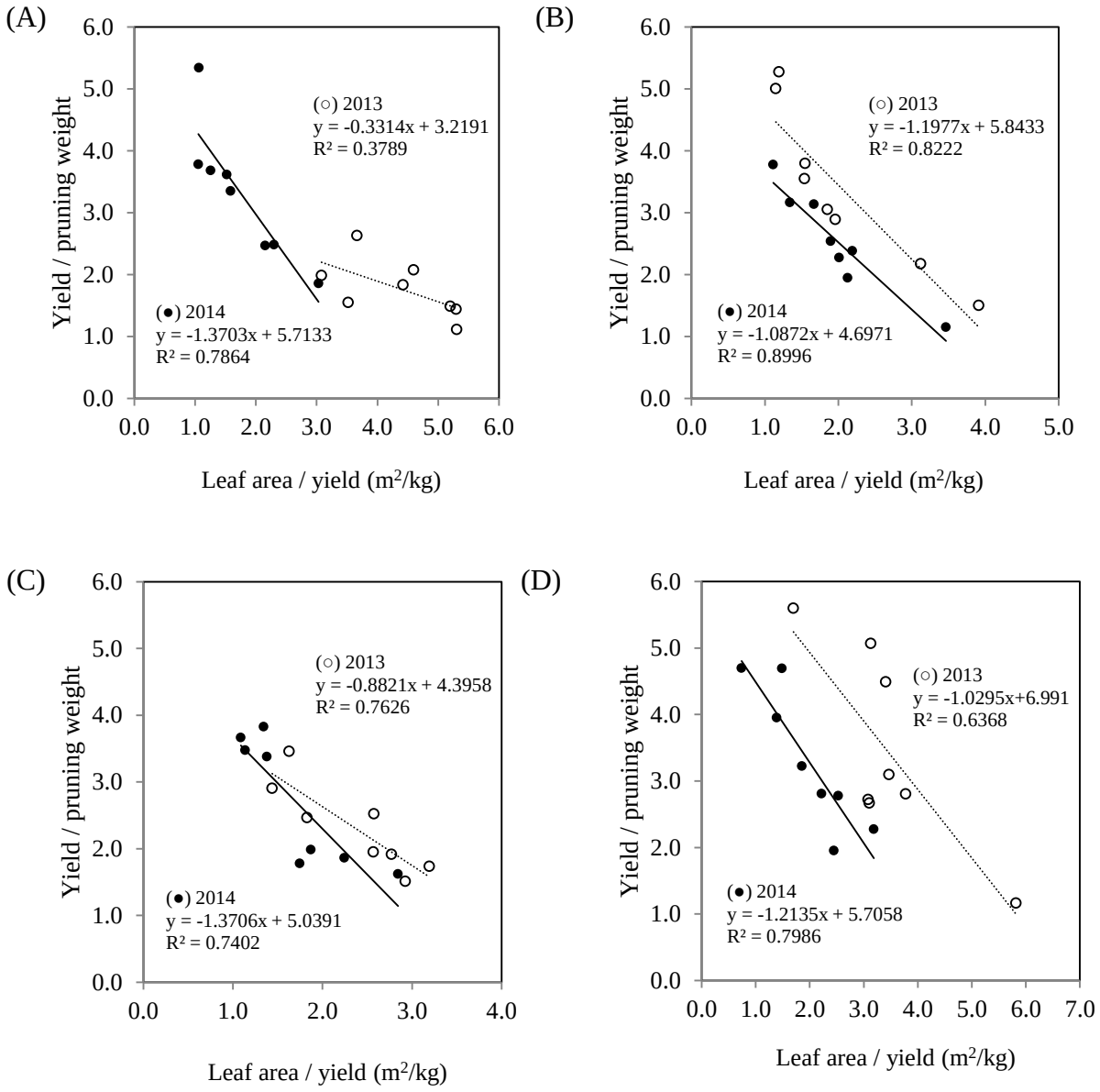

Figure 3.1. Relationships of leaf area to crop weight ratios (m^2/kg) and crop weight to pruning weight ratios in four vineyard sites across two years: (A) Eola-Amity1 in 2013, y=-0.3314x+3.2191 (R^2=0.3789, p=0.1043) and 2014, y=-1.3706x+5.0391 (R^2=0.7864, p-value=0.0033); (B) Eola-Amity2 in 2013, y=-1.1977x+5.8433 (R^2=0.8222, p=0.0019) and in 2014, y=-1.0872x+4.6971 (R^2=0.8996, p=0.0003); (C) Yamhill-Carlton1 in 2013, y=-0.8821x+4.3958 (R^2=0.7626, p=0.0046) and in 2014, y=-1.3706x+5.0391 (R^2=0.7402, p=0.0061); (D)Yamhill-Carlton2 in 2013 y=-1.0295x+6.991 (R^2=0.6368, p=0.0176), and in 2014 y=-1.2135x+5.7058 (R^2=0.7986, p=0.0028). Lines represent the fitted linear regression analysis. Each data point represents values from one plot (n=8).

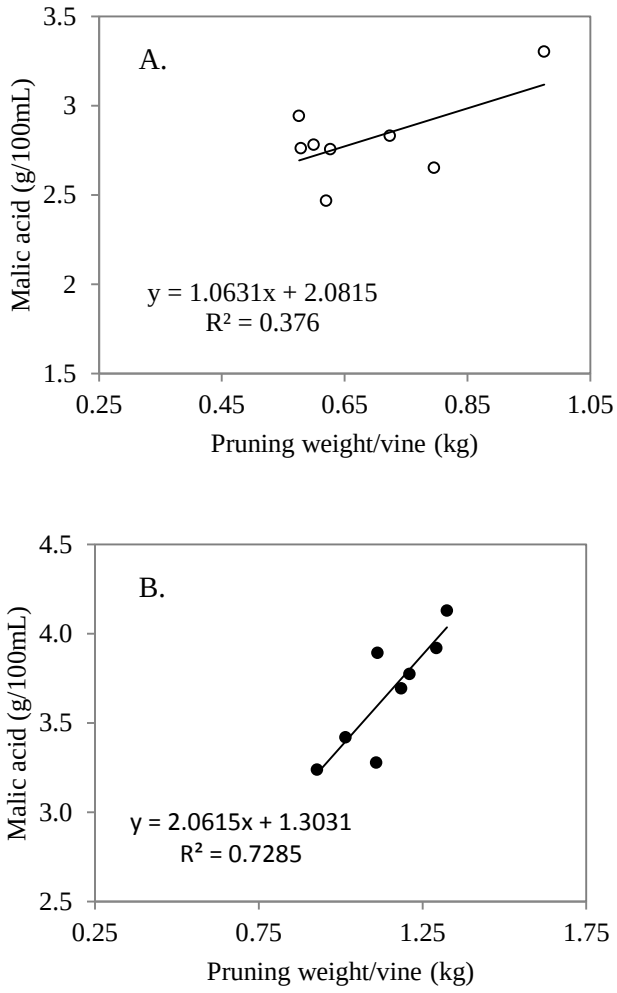

Figure 3.2. Malic acid (g/100mL) in Pinot noir grapes at harvest as a function of vine pruning weight at Yamhill-Carlton1 (YC1) in (A) 2013, y=1063.1x+2.0815 (R^2=0.376, p=00.51) and (B) 2014, y=2.0615x+1.3031 (R^2=0.7285, p=0.0007). Each data point represents plot means (n=8).

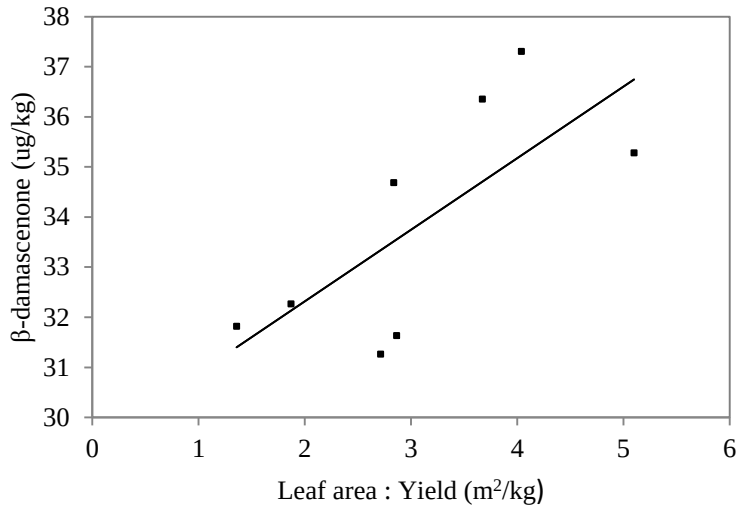

Figure 3.3. β-damascenone in Pinot noir fruit at harvest as a function of the ratio of leaf to yield (m^2/kg) across all experimental sites in 2013. Regression analysis indicated a linear relationship demonstrated by the equation y=1.4297x + 29.46 (R^2=0.5221, p=0.0429). Data points represent treatment means (n=8).

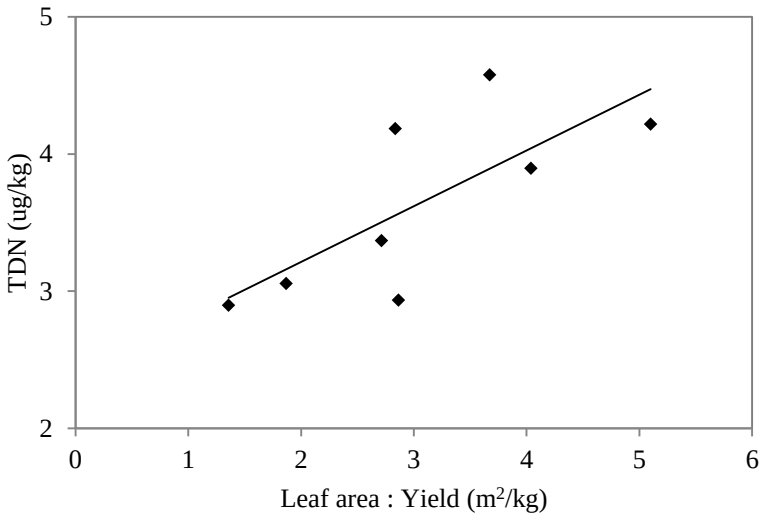

Figure 3.4. The concentration of 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) in Pinot noir fruit at harvest as a function of the ratio of leaf to yield (m^2/kg) across all experimental sites in 2013. Regression analysis indicated a linear relationship demonstrated by the equation y=0.4065x+2.3999 (R^2 =0.5450, p=0.0135). Data points represent treatment means (n=8).

Table 3.1. Site characteristics of Pinot noir vineyards used for crop thinning trial in 2013 and 2014.

Site[a]	Location[b]	Spacing (m) (vine x row)	Year planted	Clone	Rootstock	Soil type[c]	Soil depth (m)[d]
Eola-Amity 1(EA 1)	Salem (Polk)	0.9 x 2.3	1999	114	Schwarzmann	Nekia silty clay loam	>2.0
Eola-Amity 2 (EA 2)	Salem (Polk)	1.0 x 1.6	2004	667	Ripare Gloire	Jory silty clay loam	0.5-1.0
Yamhill-Carlton 1 (YC 1)	Yamhill (Yamhill)	1.5 x 2.3	1995	115	3309	WillaKenzie silty clay loam	0.5-1.0
Yamhill-Carlton 2 (YC 2)	Carlton (Yamhill)	1.0 x 2.0	2001	777	3309	WillaKenzie silty clay loam	0.5-1.0

[a]American Viticultural Area
[b]City (county)
[c]Primary soil type for each site was determined by the NRCS Web Soil Survey.
[d]Depth to restrictive feature was determined by the NRCS Web Soil Survey (shallow < 1.0, moderate 1.0 m to 2.0 m, deep > 2.0 m).

Table 3.2. Phenology for growing seasons 2013 and 2014**.**

	Budbreak		Bloom		Véraison		Harvest	
Site	2013	2014	2013	2014	2013	2014	2013	2014
Eola Amity 1 (EA 1)	April 8	April 17	June 18	June 13	August 26	August 22	October 9	September 26
Eola Amity 2 (EA 2)	April 23	April 16	June 19	June 13	August 19	August 20	October 3	September 23
Yamhill-Carlton (YC 1)	April 23	April 17	June 13	June 11	August 16	August 19	October 3	September 19
Yamhill-Carlton (YC 2)	April 12	April 14	June 12	June 9	August 20	August 22	October 2	September 29

Table 3.3. Monthly growing degree days (GDD_{10}), daily average temperature, and precipitation in 2013 and 2014.

	GDD_{10} (°C)[a]		Daily average temperature (°C)[b]		Precipitation (mm) [b]	
Month	2013	2014	2013	2014	2013	2014
April	57	58	11.2	11.7	54	88
May	151	179	14.8	15.8	110	65
June	234	212	17.8	17.1	33	36
July	332	368	20.7	21.9	0	18
August	341	368	21.0	21.9	14	3
September	231	283	17.7	19.4	191	28
October	46	160	11.3	15.2	27	172
Cumulative	1393	1628	-	-	429	409

[a]Growing degree days (GDD) calculated using $(T_{max} + T_{min})/2 - 10^oC$ with no upper temperature limit. [b]Daily average temperature and precipitation was obtained from Aurora, OR station (ARAO) of the Pacific Northwest Cooperative Agriculture Weather Network (http://www.usbr.gov/pn/agrimet/index.html).

Table 3.4. Vine growth and crop yield in Pinot noir grapes with different crop levels in 2013.

	EA1			EA2			YC1			YC2		
Parameter	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
Leaf area (m^2)	3.6	3.9	0.1786	2.7	2.8	0.5740	5.6	5.0	0.1851	1.9	1.7	0.3468
Shoots/m canopy length	12	12	0.8147	12	12	0.1288	9	10	0.6411	9	8	0.5510
Yield (kg/m)	1.1	0.8	**0.0129**	1.7	0.9	**0.0014**	1.5	0.7	**0.0002**	0.8	0.5	**0.0415**
Pruning wt./m (kg)	0.59	0.56	0.7364	0.38	0.41	0.7065	0.48	0.44	0.5530	0.20	0.22	0.6314
Cane wt. (g)	62	59	0.6539	36	37	0.9432	59	53	0.3370	31	38	0.4394
Leaf area/yield (m^2/kg)	3.2	4.7	**0.0085**	1.6	3.1	**0.0054**	2.9	4.2	**0.0153**	2.4	3.6	0.1355
Yield/pruning wt.	2.0	1.5	0.1739	4.4	2.4	**0.0115**	2.8	1.8	**0.0055**	4.5	2.4	**0.0415**

[a]Means are presented (n=4). Crop levels include Control=no thinning and Thinned = 1 cluster/shoot.

Table 3.5. Vine growth and crop yield in Pinot noir grapes with different crop levels in 2014.

	EA1			EA2			YC1			YC2		
Parameter	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
Leaf area (m^2)	3.0	3.3	0.6859	1.4	1.5	0.6789	3.5	2.9	0.2078	1.7	2.0	0.5472
Shoots/m canopy length	14	14	0.3202	9	8	0.0952	13	12	0.3235	10	9	**0.0365**
Yield (kg/m)	2.6	1.5	**0.0006**	0.9	0.6	**0.0011**	2.8	1.3	**0.0013**	1.2	0.8	**0.0469**
Pruning wt./m (kg)	0.63	0.60	0.7513	0.30	0.34	0.4623	0.79	0.74	0.4522	0.31	0.33	0.7566
Cane wt. (g)	49	48	0.8932	36	47	0.0964	70	69	**<0.0001**	34	43	0.3459
Leaf area/yield (m^2/kg)	1.2	2.3	**0.0167**	1.50	2.45	**0.0484**	1.2	2.2	**0.0106**	1.46	2.51	**0.0426**
Yield/pruning wt.	4.1	2.5	**0.0229**	3.2	1.9	**0.0179**	3.5	1.8	**0.0001**	4.0	2.6	**0.0305**

[a] Means are presented (n=4). Crop levels include Control=no thinning and Thinned = 1 cluster/shoot.

Table 3.6. Fruit composition of Pinot noir grapes with different crop levels in 2013.

	EA1			EA2			YC1			YC2		
Parameter	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
TSS (°Brix)[b]	21.9	21.7	0.3867	20	19.6	0.1340	20.35	19.65	0.1790	20.53	20.18	0.1905
pH	3.4	3.4	0.5019	3.3	3.4	**0.0124**	3.4	3.4	0.7049	3.5	3.5	0.5945
Total acidity (g/L)	0.6775	0.6650	0.7389	0.7350	0.6650	**0.0012**	0.6375	0.6425	0.8793	0.5300	0.5675	0.0697
Malic acid (g/100ml)	3.25	3.20	0.7162	3.20	3.16	0.6091	2.92	2.71	0.2345	2.05	2.30	0.3292
Tartartic acid (g/100ml)	4.75	4.56	0.5398	3.53	2.62	**0.0086**	4.65	5.06	**0.0021**	4.60	4.86	0.1124
NH_3	59.25	57.00	0.8474	41.00	33.0	0.5079	25.25	16.75	0.4412	18.75	11.25	0.2625
Primary Amino Nitrogen	132.5	140.0	0.6652	87.00	95.75	0.2308	92.25	79.25	0.1962	76.75	75.75	0.8599
YAN	191.8	197.0	0.8540	117.0	128.8	0.1848	117.5	96.00	0.2797	95.50	87.00	0.4536

[a]Means are presented (n=4). Crop levels include Control=no thinning and Thinned = 1 cluster/shoot. TSS = Total soluble solids, and YAN=yeast assimilable nitrogen.

Table 3.7. Fruit composition of Pinot noir grapes with different crop levels in 2014.

	EA1			EA2			YC1			YC2		
Parameter	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
TSS (°Brix)[b]	22.8	23.8	**0.0478**	23.7	23.9	0.4274	23.7	24.3	0.2607	25.3	25.5	0.6115
pH	3.6	3.6	0.3611	3.335	3.375	0.0656	3.6	3.6	0.5584	3.7	3.7	0.1728
Total acidity (g/ml)	0.4925	0.4675	0.1738	0.5825	0.5600	**0.0388**	0.5575	0.5475	0.7002	0.4275	0.4275	1.000
Malic acid (g/100ml)	2.71	2.46	0.0585	2.82	2.71	0.5120	3.54	3.50	0.1427	2.03	2.15	0.3865
Tartartic acid (g/100ml)	5.19	5.12	0.3656	5.38	5.20	0.2837	4.18	4.25	0.5991	5.70	5.60	0.2026
NH_3	14.5	17.8	0.7350	15.7	9.5	0.2677	23.7	26.7	0.7687	39.5	28.8	0.9161
Primary Amino Nitrogen	85.8	88.8	0.7255	105.0	114.8	**0.0293**	146.5	152.3	0.7378	130.7	142.8	0.0659
YAN	100.3	106.5	0.7260	116.8	124.3	0.4400	164.3	172.3	0.7712	179.5	181.5	0.1090

[a]Means are presented (n=4). Crop levels include Control=no thinning and Thinned = 1 cluster/shoot. TSS = Total soluble solids, and YAN=yeast assimilable nitrogen.

Table 3.8. Composition of free-form volatile compounds in Pinot noir grapes with different crop levels in 2013 (μg/kg berry).

Compound	EA1			EA2			YC1			YC2		
	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
C_6 alcohols												
1-hexanol	498	558	0.5398	837	1186	**0.0450**	733	694	0.4213	584	537	0.4382
trans-3-hexenol	19	19	0.8758	22	33	**0.0096**	23	20	0.3789	14	14	1.000
cis-3-hexenol	5	7	0.6193	25	27	0.6193	25.6	21	0.4382	40	28	0.4391
trans-2-hexenol	129	125	0.9306	157	192	0.5054	280	340	0.2436	276	287	0.6023
C_6 aldehydes												
1-hexenal	734	802	0.6259	241	198	0.0543	385	438	**0.0409**	460	608	**0.0216**
trans-2-hexenal	798	780	0.8950	867	627	**0.0092**	1175	1337	**0.0119**	1405	1691	**0.0372**
Terpinoids												
linalool	2.2	2.2	0.9672	4.3	4.8	0.1419	3.8	3.4	0.0632	3.7	3.6	0.5338
geraniol	5.6	6.1	0.3734	5.7	7.1	0.4116	5.9	4.1	0.3008	5.0	4.5	0.5871
1-octen-3-ol	5.1	6.3	0.3476	4.4	5.5	0.0986	15.5	13.9	0.4817	12.6	13.1	0.7454
C_{13}-norisoprenoids												
β-damascenone	36.4	35.3	0.6801	31.8	31.27	0.6641	32.3	31.6	0.8223	34.7	37.3	0.5521
TDN	4.6	4.2	0.3098	2.9	3.4	**0.0501**	2.9	2.9	0.6317	4.2	3.9	0.06118

[a]Means are presented (n=4). Analysis was performed by ANOVA. Crop levels are defined as Control = no thinning and Thinned = 1 cluster/shoot. TDN=1,1,6-Trimethyl-1,2-dihydronaphthalene.

Table 3.9. Composition of free-form volatile compounds in Pinot Noir grapes with different crop levels in 2014 (μg/kg berry).

Compound	EA1			EA2			YC1			YC2		
	Control[a]	Thinned	p	Control	Thinned	p	Control	Thinned	p	Control	Thinned	p
C_6 alcohols												
1-hexanol	924	1148	0.1776	485	505	0.8063	651	470	0.1749	330	282	*0.5317*
trans-3-hexenol	32	35	0.4458	41	51	0.1693	25	24	0.7710	15.5	16	*0.7651*
cis-3-hexenol	51	54	0.6825	10	6	0.1127	-	-	-	81	67	***0.0470***
trans-2-hexenol	440	526	0.3480	298	289	0.8823	322	155	0.1074	197	154	*0.3853*
C_6 aldehydes												
1-hexenal	945	763	0.3659	1258	1468	0.1358	1035	1177	0.3020	1274	1345	*0.6681*
trans-2-hexenal	2938	2709	0.5100	2285	2559	0.1592	2295	1972	0.1868	620	599	*0.8200*
Terpenoids												
linalool	3.7	3.6	0.8542	4.3	4.6	0.1535	3.8	3.1	0.3920	2.7	2.4	*0.2485*
geraniol	-	-	-	2.1	2.5	0.3241	1.9	1.7	0.3924	0.9	1.3	*0.3108*
1-octen-3-ol	3.8	4.2	0.5501	9.6	10.2	0.7585	5.9	5.5	0.0630	5.2	6.2	*0.2711*
C_{13}-norisoprenoids												
β-damascenone	46.1	46.8	0.8716	41.8	43.8	0.0381	46.4	46.3	0.9184	44.9	44.2	*0.6897*
TDN	4.8	5.1	0.5779	4.9	5.5	0.1575	4.3	4.1	0.8538	5.2	4.9	*0.6213*

[a]Means are presented (n=4). Analysis was performed by ANOVA. Crop levels are defined as Control = no thinning and Thinned = 1 cluster/shoot. TDN=1,1,6-Trimethyl-1,2-dihydronaphthalene.

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APPENDIX A

Data collection and processing

1. Set up Data Acquisition subsystem (Section 3.4.1).
    a. Mount camera on tripod.
    b. Face camera parallel to grapevine row.
    c. Position the tripod such that the front of the camera lens is 5.0 ft. from the average/center plane of the camera-side grapevine foliage.
    d. Put camera on "CA" setting, and toggle the flash to remain on.
2. Acquire data.
    a. Move from left to right along a grapevine row (or any given set of grape plants) taking one photo per plant.
    b. Maintain settings listed in Step 1 above.
3. Set up Data Processing subsystem (Section 3.4.2).
    a. Remove SD card from camera in insert into computer.
    b. Select "open folder to view files" from "AutoPlay" popup. If popup does not display, find files in the file path *Computer\SD*.
    c. Select "DCIM" then "100CANON" to find the images taken in Step 2 (file path *Computer\SD\DCIM\100CANON*).
    d. Copy images of interest from Step 2, noting the initial image number and image quantity.
    e. Paste the images in the same folder as the provided MATLAB code (Appendix B).
    f. Open MATLAB and the provided code.
4. Process data.
    a. Select "Run".
    b. If the "MATLAB Editor" window pops up, select "Add to Path".
    c. In the popup prompts, insert initial image number and image quantity (from Step 3d) and click "OK".
    d. Copy list of "Foliage_Densities" and "Surface_Areas" from the MATLAB Command Window and paste into a new program (Microsoft Excel, for example. Further processing is outside of the scope of this project). Average foliage density and surface area for entire file set will also be given

Notes and Calibration Techniques

1. Note for subsequent runs.
    a. Ensure that only files that are to be sent through the MATLAB program are in the same file as the program.

   b. If you have questions, please refer to the comments throughout the code, or contact Team Six.

2. (If necessary) Calibrate color thresholds.
   a. Using method listed in Steps 1-4, take picture(s) of a green sheet (or leaf collection) of *known area* against the backdrop.
   b. Send images through the MATLAB program, adjusting color thresholds ("threshold_r", "threshold_g", and "threshold_b". See code comments for more details) until the outputted surface area matches the expected value.
   c. Test new threshold values against a second green sheet (or leaf collection) of a different known area. If the outputted value is unexpected, redo test with readjusted threshold values.
   d. Note: You can use the software Paint's "Color picker" tool to select a pixel, then click "Edit colors" in order to see its rgb color value.
3. (If necessary) Calibrate pixel are to square inch area ratio (dependent on camera distance from grapevine. Default: 5 ft.)
   a. Using method listed in Steps 1-4, take picture(s) of a green sheet of *known area* against the backdrop.
   b. Using any image editing sotware, crop the green sheet. Note its pixel area.
   c. Divide the actual area of the green sheet by its pixel area from Step 7b.
   d. Replace the MATLAB variable "pix_to_in" with the resulting ratio from Step 7c.

APPENDIX B

```
clc
clear all

%% Variables defined in code
n = str2double (inputdlg ('Input quantity of images.'));
first_image = str2double (inputdlg ('Input initial image number.'));
% n = 4;
% first_image = 5881;

pix_to_in = 0.00043403;

threshold_r = 50;                % green_pix must be MORE red than this value.
threshold_g = 120;               % green_pix must be MORE green than this
value.
%%% Starting point for direct sunligh calibration: threshold_g = 180;
threshold_b = 255;               % green_pix must be LESS blue than this value.

%%% In the future, it may be good to replace threshold_r,g,b system with
%%% percent relationships. For example, to qualify as a "green_pix",
%%% the green value of the pixel must be at least 120% the blue value of the
pixel.
%%% To do this, replace "P(j,k,1) > threshold_r && P(j,k,2) > threshold_g &&
P(j,k,3) <
%%% threshold_b"
%%% With "P(j,k,2) > (1.2*P(j,k,3))".

Percent_Foliage = zeros(n, 1);  % nx1 matrix for percent foliage of every image
Surface_Area = zeros(n, 1);     % nx1 matrix for percent foliage of every image

green_pix_for_avg = 0;          % Initial value for "if" statement (total green
pixel count)

for i=1:n

   green_pix = 0;              % Initial value for "if" statement (individual
green pixel counts)

   %%% Convert number variables into strings for filename integration.
   text_i = num2str(i+(first_image-1));

   %%% Find all relavent files, and log pixel information.
   filename = ['IMG_',text_i,'.jpg'];

   %%% For each file, create a 3D matrix with (r,g,b) color info for every
pixel.
   P = imread(filename,'jpg');

   %%% Determine image size.
   width = size(P,1);
   length = size(P,2);

   %%% For every pixel, determine if it qualifies as a "green_pix".
   for j=1:width;
       for k = 1:length;
           if P(j,k,1) > threshold_r && P(j,k,2) > threshold_g && P(j,k,3) <
threshold_b;
               green_pix = green_pix +1;
               green_pix_for_avg = green_pix_for_avg +1;
           end
       end
```

```
   end

   %%% Display Image+n before each individual Percent_Foliage
   % disp(strcat('Image: ', text_i));
   % display(percent_foliage)

   total_pix = width*length;                          % Total pixels of an image
   foliage_density = (green_pix)/(total_pix);         % Green pixels per total
pixels
   percent_foliage = foliage_density*100;             % Foliage density as a
percent
   Percent_Foliage(i) = percent_foliage;              % Percent foliage for
every single image
   green_pix;

   real_area = total_pix*(pix_to_in);                 % Foliage surface area
[cm^2]
   Surface_Area(i) = foliage_density*real_area;       % Surface area for every
single image
end

%%% Average Foliage Density
avg_foliage_density = (green_pix_for_avg)/(total_pix*n);
AVERAGE_Percent_Foliage = (avg_foliage_density*100);

%%% Average Surface Area
AVERAGE_Surface_Area = sum(Surface_Area/n);

%%% Display output.
format long g                                         % avoid scientific
notation
display(Percent_Foliage)                              % *1.20 correction factor
(for surface area, too)
display(Surface_Area)
display(AVERAGE_Percent_Foliage)
display(AVERAGE_Surface_Area)
```