Final Progress Report
Virginia Wine Board, 20 July 2016

Optimized wine quality potential through fruit-zone management practices in red varieties

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Type of Project: Research
Amount funded: $37,957

Objective: Evaluate both the severity and the timing of fruit-zone leaf and lateral shoot removal for impacts on grape aroma precursors and wine quality potential of two Bordeaux red varieties.

Summary: The rationale for fruit-zone leaf removal in a humid environment has been driven more by disease management than by documented changes in fruit composition. Though a common practice for several decades, leaf removal recommendations remain general and are not variety-, timing-, or magnitude-specific. We evaluated if the timing or magnitude of fruit-zone leaf and lateral removal would alter fruit composition and crop yield components of Cabernet Sauvignon, Cabernet franc and Petit Verdot. Wine Board funding requested and provided during the 2015-2016 fiscal year allowed us to finish this multi-year project. Wage support was principally used to help conduct laboratory analyses and data interpretation of carotenoids in Cabernet franc and Petit Verdot samples that had been collected and frozen from each of the 2012, 2013, and 2014 experiments. Carotenoids are pigments that perform photo-oxidative protection in plant systems, but are also precursors to some of the very important aroma compounds, including norisoprenoids, in grape varieties. Funding also provided graduate tuition and stipend awards for Fall, 2015 semester for graduate student Cain Hickey. Cain began a post-doctoral appointment with Dr. Terry Bates at Cornell University in February 2016, and successfully defended his dissertation in May. This year-end report principally provides details on what was done in the past fiscal year. In addition, abstracts of other aspects of the multi-year work are attached as appendices. Dr. Hickey is on tap to present his findings at the 2017 VVA Winter Technical meeting (23-25 February 2017), and we are working on an Extension Bulletin that translates his research findings into grower recommendations. Major findings and applications of the multi-year project include:

- Removal of up to 6 leaves per shoot post-fruit set increased grape phenolics and anthocyanins (both by about 22%), which is a positive outcome, and reduced botrytis bunch rot (76% compared with no leafing), without increasing risk of fruit sun-burning.
- Pre-bloom leaf removal resulted in some of the same trends in fruit quality improvement; however, crop yields were reduced by the more aggressive pre-bloom leaf removal (8 leaves per shoot) by nearly 60%.
- The more modest pre-bloom leaf removal (4 leaves/shoot) improved grape composition without having significant effects on crop yield reductions.

- The more aggressive leaf removal, whether conducted pre-bloom or post-fruit set, tended to increase berry carotenoids in the pre-veraison period. Certain carotenoids are precursors to aroma compounds found in Cabernet franc and Petit Verdot wines.
- Removal of up to 4 leaves/shoot pre-bloom, or as many as 6 leaves/shoot post-fruit set, appears to be an optimal canopy fruit zone management strategy to improve fruit composition, sustain an acceptable yield, and reduce disease incidence with Cabernet franc and Petit Verdot.
- Our findings illustrate that leaf removal can be more extensive, and be performed earlier (pre-bloom) than traditionally recommended, without risk of adversely impacting grapes.

Background: Research dating back to the mid-1980s has shown that exposing the fruit-zone of vine canopies is beneficial, as fungal disease incidence is reduced and fruit and wine quality are often improved. As such, fruit-zone leaf removal became a commonly recommended practice, with more extensive leaf removal often recommended. However, more recent research showed that too much of a "good thing" can actually be bad, when extreme radiant heating of fruit was shown to decrease grape anthocyanins in red varieties. Some of that research was conducted in the arid conditions of central Washington State and the Central Valley of California, where sky conditions were more conducive to sunburning of fruit. Thereafter, fruit-zone leaf removal became more conservative, even in humid growing regions where shaded fruit-zones exacerbate grape fungal disease infections. Though conventionally conducted after fruit set, fruit-zone leaf removal before bloom has many documented benefits, including improved juice soluble solids, grape phenolics and anthocyanins, and reduced cluster compactness and *Botrytis* bunch rot incidence. We questioned whether early (pre-bloom) and more extensive leaf/lateral removal had merit under the warm/hot conditions of Virginia, and on varieties for which little or no fruit exposure research had been conducted (Cab franc and P. Verdot). Our comprehensive objective was to evaluate the effects of aggressive pre-bloom and post-fruit set leaf removal on crop yield components and fruit-composition in three regionally important red Bordeaux varieties: Cabernet franc, Petit Verdot, and Cabernet Sauvignon.

Methods: Although presented in previous Wine Board reports, the following provides some background on the nature of treatments used in the project.

Project 1 was conducted during the 2013-2014 seasons in a commercial vineyard in Shenandoah County. Two separate experiments were conducted in adjacent Cabernet franc and Petit Verdot blocks of the vineyard. Canopy treatments in both varieties included <u>post-fruit set</u> removal of fruit-zone leaves to **no** (NO), **medium** (MED), or **high** (HIGH) levels of defoliation, as well as a <u>pre-bloom</u> removal of fruit-zone leaves to the high extent (P-B).
Project 2 was conducted for three seasons (2013-2015) with Cabernet Sauvignon grown at the AHS Jr. AREC vineyard. Treatments were designed to evaluate the effects of pre-bloom and post-fruit set leaf removal on several vine responses. The pre-bloom leaf removal experiment evaluated a no leaf removal-control ("PB-NO") and pre-bloom leaf removal of four (PB-4) or eight (PB-8) basal leaves and laterals from primary shoots. A second experiment evaluated a no leaf removal-control ("PFS-NO") and post-fruit set removal of six basal leaves and laterals ("PFS-6") from primary shoots. Additional data were only collected from *Project 2* in 2015, and consisted of measurement of: fruit-zone architecture measurement, berry temperature, berry weight over time, and crop yield components and primary juice chemistry at harvest. Further, berry temperature was logged on 1-minute intervals for the third consecutive season. The majority of lab work in 2015 consisted of extracting carotenoids from Petit Verdot and Cabernet franc grapes, and quantifying total grape anthocyanins and phenolics in all three varieties. Quantification of carotenoids with ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) was performed in the fall of 2015 and into early 2016 at the University of

Missouri's Grape and Wine Institute in Columbia, MO. In addition to completing the grape carotenoid and wine sensory data analysis, a grape temperature prediction model was developed using berry temperature data that had been collected over the 2013-2015 growing seasons (see Appendix C).

Canopy characterization and dormant cane pruning weight: Leaf removal resulted in a fruit-zone leaf layer number (LLN) of zero in all years (Table 1). Leaf removal resulted in at least a three- and, sometimes, four-fold increase in fruit-zone cluster exposure flux availability (CEFA) compared to removing no leaves, and there was no difference in CEFA between PB-4 and PB-8. Though differences between PFS-6 and PB-4 and PB-8 could not be statistically analyzed, their LLN and CEFA values were similar. As such, the pre-bloom and post-fruit set leaf removal experiments offered a platform to compare the impact of leaf removal *timing* without the confounding of fruit exposure extent. Leaf layer number is a commonly used index of canopy density used by informed growers. CEFA is not a common term in the grower's lexicon, but it's widely used in viticulture research; it basically defines the exposure of fruit clusters to the outside of the canopy and values range from complete occlusion/shading (~0.00) to complete exposure (~1.00). Pruning weight was reduced by PB-8 in 2013 and pruning weight tended to be further reduced over time when pre-bloom leaf removal was re-implemented in consecutive seasons, but only to a significant extent by PB-8 '13re compared to PB-NO in 2014, and both PB-NO and PB-4 in 2015. In other words, a grower could expect to see a reduction in vine capacity if he or she chose to remove as many as 8 leaves per shoot from vines. Removing fewer leaves did not have this potentially negative impact on vine capacity.

Berry temperature: Berry temperature is an important consequence of canopy management because increased berry temperature can be desirable up to an extent (~30C), but above which undesirable responses, such as reductions in color density can occur. Berry temperature was increased above ambient air temperature from 0800-1200, and from 3:00 to about 6:00 pm (Fig. 3 [note – figure numbers in this report are not sequential]). This bimodal trend of elevated berry temperature was paralleled by a bimodal diurnal trend of elevated sunlight ("PAR"), particularly in the PB-4 and PB-8 treatments (Fig. 3 A, B). The bi-modal, daily pattern of fruit heating was a result of the VSP training used; the overhead canopy of foliage blocked the midday, radiant heating of the fruit. East- and west-side berry temperatures were similar (-0.2 – 0.7 °C) to ambient air temperature when no leaves were removed (Figure 3C).

Crop yield components and vine fruitfulness: When compared to PB-NO in 2013, PB-8 generally reduced components of crop yield to a greater extent than did PB-4. Crop yield, cluster weight, and berry number per cluster were reduced by PB-4 (30, 43, and 44%, respectively) when compared to PB-NO (Table 2). Crop yield, cluster weight, berry number per cluster, and berry weight were all reduced by the most severe leaf removal treatment, PB-8 (62, 62, 57, and 9%, respectively) when compared to PB-NO, and crop yield and cluster weight were reduced by PB-8 (46 and 33%, respectively) when compared to PB-4. Crop load, which is the ratio of crop to pruning weight for a given vine, was reduced by both PB-4 and PB-8 when compared to PB-NO. Re-implementing the pre-bloom leaf removal treatments on the same vines in 2014 and 2015 tended to result in a similar reduction in crop yield components as described for the initial year (2013) of treatment, whereas post-fruit set leaf removal had minor or no impact of crop yield components.

Seasonal berry weight development: Berry weight tended to be reduced by PB-8 but not by PB-4 or PFS-6 (Fig. 8). In 2015, PB-8 reduced berry weight on all dates by an average of 15% when compared to the average berry weight of PB-NO and PB-4 (Fig. 8 C). Berry weight was reduced by PB-4 by 8% when compared to PB-NO only on 8 September. Re-implementation of pre-bloom leaf removal treatments in the third consecutive season (PB-4/8‘13re) did not further reduce berry weight. Berry weight increase from 2 Aug to 8 Sep was 0.15 g greater in PB-NO, and 0.7 g greater in PB-4, when compared PB-8.

Components of cluster compactness: Pre-bloom leaf removal of either 4 or 8 leaves per shoot reduced cluster compactness in each of the 3 years evaluated (Table 3). This was mainly due to a reduction in berries per cluster; however, the more severe leaf removal treatment also slightly reduced rachis length in 2013 and 2015. The rachis is the stem of the cluster. There was a direct, positive relationship between reduction in berry number per cluster and cluster compactness, but concomitant reductions in rachis length confounded this relationship.

Grape carotenoids: Our interest in the secondary metabolites, carotenoids, stems from their role as precursors to certain aroma compounds in grapes and resultant wines. For example, the norisoprenoid compound β-ionone, an important component of Cabernet franc and Petit Verdot aroma, is derived from both lutein and β-carotene. If the synthesis of carotenoids can be enhanced, or their conversion to aroma metabolites increased, improvements in wine quality might be realized. Increased grape exposure through leaf removal could be one means of achieving that outcome. The data of table 4 are one set of many samples that were analyzed in 2015 for carotenoid concentration. These data are for Cabernet franc treatments from the 2013 season. Of the 4 carotenoids analyzed, zeaxanthin and lutein tended to be increased by the more severe leaf pulling in Cabernet franc (Table 4). Similar trends were seen in other years and also with Petit Verdot; however, β-carotene was also significant increased by leaf removal in Petit Verdot.

Wine sensory analyses: Consumer preference tests of Cabernet franc and Petit Verdot wines were conducted on the 2013 vintage in May 2015. Cabernet franc treatments included MED, HIGH, and P-B, and Petit Verdot treatments included NONE, MED, HIGH, and P-B. Panelists were weekly red wine consumers. A balanced complete block design was implemented, such that each participant evaluated wine samples from all treatments. Samples were labeled with random, three-digit codes, and served monadically. Approx. 25-28 mL of each wine was served at room temperature in clear ISO wine glasses. Consumers cleansed their palates with unsalted crackers and filtered water. Participants answered questions on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Peryam and Pilgrim 1957) for appearance, red color, aroma, overall flavor, astringency, mouthfeel, length of finish, and overall impression. Additionally, fruity aroma and flavor, vegetative aroma and flavor, and intensity of red color, astringency, mouthfeel, and length of finish were evaluated on a 5-point Just About Right (JAR) scale (1 = not nearly enough 3 = just about right; 5 = much too much). Panelists performed a side-by-side ranking of treatment wines at the end of the hedonic tests; the higher the average ranking, the more preferred the wine. Data were collected with SIMS software (Berkeley Heights, NJ).

The intensity of red color and astringency of P-B wines ranked higher on the “just about right” scale compared to MED wines in Cabernet franc (Table 5). The intensity of red color of P-B and HIGH wines ranked higher on the “just about right” scale compared to MED wines in Petit Verdot.

Discussion: Removing leaves before bloom consistently reduced crop yield whereas removing leaves after fruit set did not. Cluster weight was the primary yield component reduced by pre-bloom leaf removal (likely due to reduced fruit set), and it was differentially reduced by leaf removal extent and between varieties. For example, pre-bloom leaf removal of eight leaves reduced berry number per cluster and cluster weight to a greater extent than pre-bloom removal of four leaves. Berry number per cluster was reduced to a greater extent in Cabernet franc, and berry weight and cluster number per vine were reduced to a greater extent in Petit Verdot. We suggest that these differences were due to the direct relationship between leaf area and fruit set. Aggressive leaf removal tended to reduce soluble solids and TA of harvested fruit, but these reductions were not consistent. Reduction in leaf area was likely responsible for reduction in soluble solids, and the sparser canopy in Cabernet franc likely resulted in greater incidence of temperature-driven malic acid respiration compared to in Petit Verdot. Aggressive leaf removal before bloom increased total grape phenolics more consistently than anthocyanins across all three varieties. This appeared to be partially, but not exclusively, due to the concentrating effect of smaller berries with this treatment. While leaf removal never increased total grape anthocyanins in Petit Verdot or Cabernet franc, anthocyanins were consistently increased in Cabernet Sauvignon, suggestive that temperature/radiation-induced increases in grape anthocyanins is variety-dependent. Bunch rot incidence was reduced to a greater extent in pre-bloom compared to post-fruit set leaf removal plots, perhaps due to looser clusters, better early-season fruit-zone spray coverage, or both. Fungal disease management and total grape phenolics and anthocyanins can be improved with aggressive leaf removal in humid regions. While reduced TA can result in less tart red wine, reduced soluble solids is an unwelcome response in a region that often experiences adverse ripening period weather. The labor and crop yield debts incurred with pre-bloom leaf removal may not be offset or even recovered by an increased bottle price. Furthermore, repeated (over years) pre-bloom leaf removal has the potential to reduce vine capacity, making the recovery from this practice even longer. Thus, the potential benefits need to be weighed against these potential and real negatives.

Impact statement:
The research has quantified the impact of grape cluster exposure on expression of aroma precursors in three red Bordeaux varieties under the variable climate growing conditions of Virginia. Findings have reduced our concern for sun-burning of fruit (so long as vines are well hydrated), potentially realizing greater benefits of exposure to disease management, and wine quality potential enhancements. We have not yet assessed the impact of revised canopy management recommendations.

Presentations in this period:
HICKEY C.*, T. Wolf. Magnitude and timing of fruit zone leaf removal changes yield and fruit composition of Cabernet Sauvignon in a humid region. Oral presentation at 66th annual meeting of the American Society for Enology and Viticulture, Portland, OR., 15-18 June 2015.

Tables, figures and abstracts:
Detailed data can be found on in appendices on pages 6-15 of this report.

Table 1. Pre-bloom and post-fruit set leaf/lateral removal effect on fruit-zone leaf layer number (LLN) and cluster exposure flux availability (CEFA) measured at veraison, and dormant cane pruning weights, 2013-2015.

Treatment[a]	2013			2014			2015		
	LLN	CEFA	Pruning weight (kg / m row)	LLN	CEFA	Pruning weight (kg / m row)	LLN	CEFA	Pruning weight (kg / m row)
PB-NO	2.48 a	0.15 b	0.87 a	2.52 a	0.21 b	1.02 a	2.65 a	0.18 b	0.99 a
PB-4	0.04 b	0.54 a	0.91 a	0.02 b	0.75 a	0.99 ab	0.05 b	0.82 a	0.94 a
PB-8	0.00 b	0.59 a	0.74 b	0.00 b	0.82 a	0.82 ab	0.00 b	0.82 a	0.69 ab
PB-4 ’13re	n/a	n/a	n/a	n/a	n/a	0.90 ab	n/a	n/a	0.79 ab
PB-8 ‘13re	n/a	n/a	n/a	n/a	n/a	0.66 b	n/a	n/a	0.60 b
Significance[b]	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.0446	<0.0001	<0.0001	0.0086
PFS-NO	n/a	n/a	n/a	2.73 a	0.19 b	0.99	2.66 a	0.14 b	1.31
PFS-6	n/a	n/a	n/a	0.00 b	0.77 a	1.02	0.00 b	0.77 a	1.18
Significance[b]	n/a	n/a	n/a	<0.0001	<0.0001	ns	<0.0001	<0.0001	ns

[a]2013: PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight leaves, respectively; 2014: PB-4 ‘13re, PB-8 ‘13re = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2013; 2015: PB-4, PB-8 = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2014. PFS-NO and PFS-6 = post-fruit set removal of no and six leaves, respectively.

[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level).

*Means in same treatment group (columns) not sharing a letter are significantly different at 0.05 level based on adjusted p-values using Tukey HSD (PB treatments) and Student’s T-test (PFS treatments).

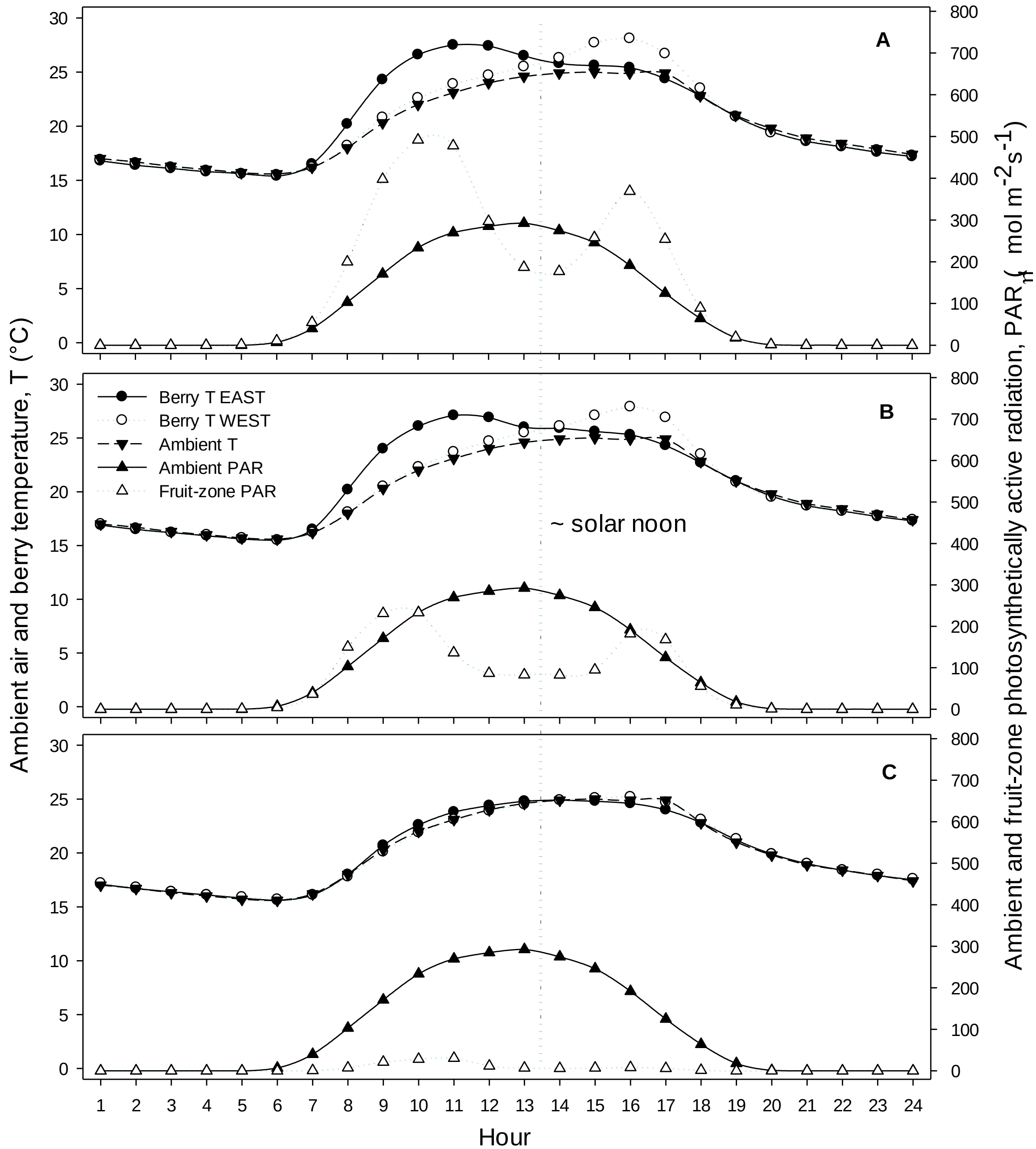

Fig. 3 Diurnal pattern of ambient air temperature, ambient and fruit-zone PAR, and berry temperature as affected by pre-bloom removal of eight (A), four (B), and no (C) fruit-zone leaves/laterals. Data logged on 15- and 1-min intervals, and averaged over 2013-2015 seasons. Ambient PAR was logged on 15- and 1-min intervals, and averaged over 2014-2015 seasons. NOTE: Ambient PAR presented as 20% of actual value to ease visualization of data plots.

Table 2. Pre-bloom and post-fruit set leaf removal effects on crop yield components, crop load, and count and basal shoot fruitfulness from 2013-2015.

Treatment[a]	Crop yield (kg/ vine)	Cluster number	Cluster weight (g)	Berry # /cluster	Berry weight (g)	Crop load	Fruitfulness[b] (count/basal)
				2013			
PB-NO	3.75 a	36 b	105.0 a	89 a	1.18 a	2.9 a	n/a
PB-4	2.63 b	44 a	59.8 b	50 b	1.20 a	1.9 b	n/a
PB-8	1.42 c	35 b	40.1 c	38 b	1.07 b	1.3 b	n/a
Significance[c]	0.0003	0.0011	<0.0001	<0.0001	0.0094	0.0013	n/a
				2014			
PB-NO	2.87 a	31	92.5 a	64 a	1.51 a	1.9	1.41 / 0.98
PB-4	1.36 b	30	45.2 b	33 b	1.42 a	0.9 b	1.61 / 1.11
PB-8	0.90 b	27	32.3 b	26 b	1.30 b	0.7 b	1.35 / 1.01
Significance[c]	0.0028	ns	<0.0001	0.0003	0.0009	0.0124	ns / ns
PB-NO-est.	3.39 a	26	139.3 a	98 a	1.51	2.3	n/a
PB-4 ’13re	2.89 ab	36	81.4 b	50 b	1.63	2.2	n/a
PB-8 ’13re	1.71 b	31	56.6 c	40 c	1.43	1.7	n/a
Significance[c]	0.0204	ns	<0.0001	<0.0001	ns	ns	n/a
PFS-NO	3.37	26	149.7	104	1.47	2.2	n/a
PFS-6	3.35	25	138.7	94	1.45	2.2	n/a
Significance[c]	ns	ns	ns	ns	ns	ns	n/a
				2015			
PB-NO	4.76 a	39	121.2 a	83 a	1.47 a	3.2 a	1.66 / 0.33
PB-4 ‘14re	2.25 bc	39	57.3 b	42 b	1.37 a	1.7 ab	1.52 / 0.36
PB-8 ‘14re	1.00 d	37	26.5 c	22 c	1.17 b	1.0 b	1.35 / 0.19
PB-4 ’13re	2.38 b	43	55.4 b	40 b	1.39 a	2.1 ab	1.53 / 0.33
PB-8 ’13re	1.09 cd	40	27.2 c	22 c	1.21 b	1.2 b	1.46 / 0.21
Significance[c]	<0.0001	ns	<0.0001	<0.0001	<0.0001	0.0022	ns / ns
PFS-NO	4.32	31	139.3	89	1.59 a	2.2	1.57 / 0.29
PFS-6	3.99	31	129	89	1.46 b	2.3	1.57 / 0.25
Significance[c]	ns	ns	ns	ns	<0.0001	ns	ns / ns

[a]2013: PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight leaves, respectively; 2014: PB-NO (est.) = estimated yield of PB-NO vines by adding back harvest weight of sampled berries throughout season; PB-4 ‘13re, PB-8 ‘13re = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2013; 2015: PB-4 ‘14re, PB-8 ‘14re = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2014. PFS-NO, PFS-6 = post-fruit set removal of no and six leaves, respectively.

[b]Presented as cluster number per shoot; count = one-year old spur-originating shoot, basal = cordon-originating shoot. Fruitfulness assessed in year presented, but effects attributed to previous season’s leaf removal.

Table 3. Pre-bloom leaf removal effect on components of cluster compactness from 2013-2015.

Treatment[a]	Berry # / cluster	Rachis length (cm)	Cluster Compactness (berry # / cm rachis length)
	2013		
PB-NO	81 a	9.98 a	8.79 a
PB-4	53 b	9.68 ab	6.55 b
PB-8	34 c	8.68 b	3.75 c
Significance[c]	<0.0001	0.0179	<0.0001
	2014[b]		
PB-NO	59 a	9.32	6.74 a
PB-4	34 cd	9.37	4.10 bc
PB-8	24 d	9.58	2.67 c
PB-4 '13re	53 ab	9.39	6.27 a
PB-8 '13re	41 bc	7.91	5.92 ab
Significance[c]	<0.0001	ns	<0.0001
	2015		
PB-NO	80 a	10.09 a	9.03 a
PB-4	41 b	8.32 b	5.59 bc
PB-8	21 c	8.35 b	2.85 cd
PB-4 '13re	41 b	8.06 b	6.36 ab
PB-8 '13re	20 c	8.80 ab	2.78 d
Significance[c]	<0.0001	0.0069	<0.0001

[a]2013: PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight leaves, respectively; 2014: PB-4 '13re, PB-8 '13re = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2013; 2015: PB-4, PB-8 = re-implementation of PB-4 and PB-8, respectively, on same vines initially used in 2014. PFS-NO and PFS-6 = post-fruit set removal of no and six leaves, respectively.
[b]NOTE: Clusters sampled non-uniformly in 2014 – six times total in PB-NO/4/8 vines and only at harvest in PB-4/8 '13revines.
[c]Significance of treatment effects (p > F; ns = not significant at 0.05 level).
*Means in same treatment group (columns) not sharing a letter are significantly different at 0.05 level based on adjusted p-values using Tukey HSD (PB treatments).

Table 4. Pre-bloom and post-fruit set leaf removal treatment effect on Cabernet franc grape carotenoids in 2013.

Treatment[a]	**Lutein 5,6-epoxide (μg/g berry)**	**Zeaxanthin (μg/g berry)**	**Lutein (μg/g berry)**	**β-carotene (μg/g berry)**
NO	0.015 b	0.019 b	1.21 ab	0.73
MED	0.014 b	0.023 b	1.07 b	0.66
HIGH	0.018 a	0.039 a	1.29 a	0.75
P-B	0.016 ab	0.042 a	1.34 a	0.75
Date[b]				
14-Jul	0.020 a	0.028 b	1.57 a	0.76 a
26-Jul	0.015 b	0.040 a	1.56 a	0.88 a
14-Aug	0.012 c	0.025 a	1.48 b	0.88 a
30-Sep	nd	nd	0.30 b	0.37 b
Significance[c]				
Treatment	0.001	<0.0001	0.004	ns
Date	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	ns	ns	ns

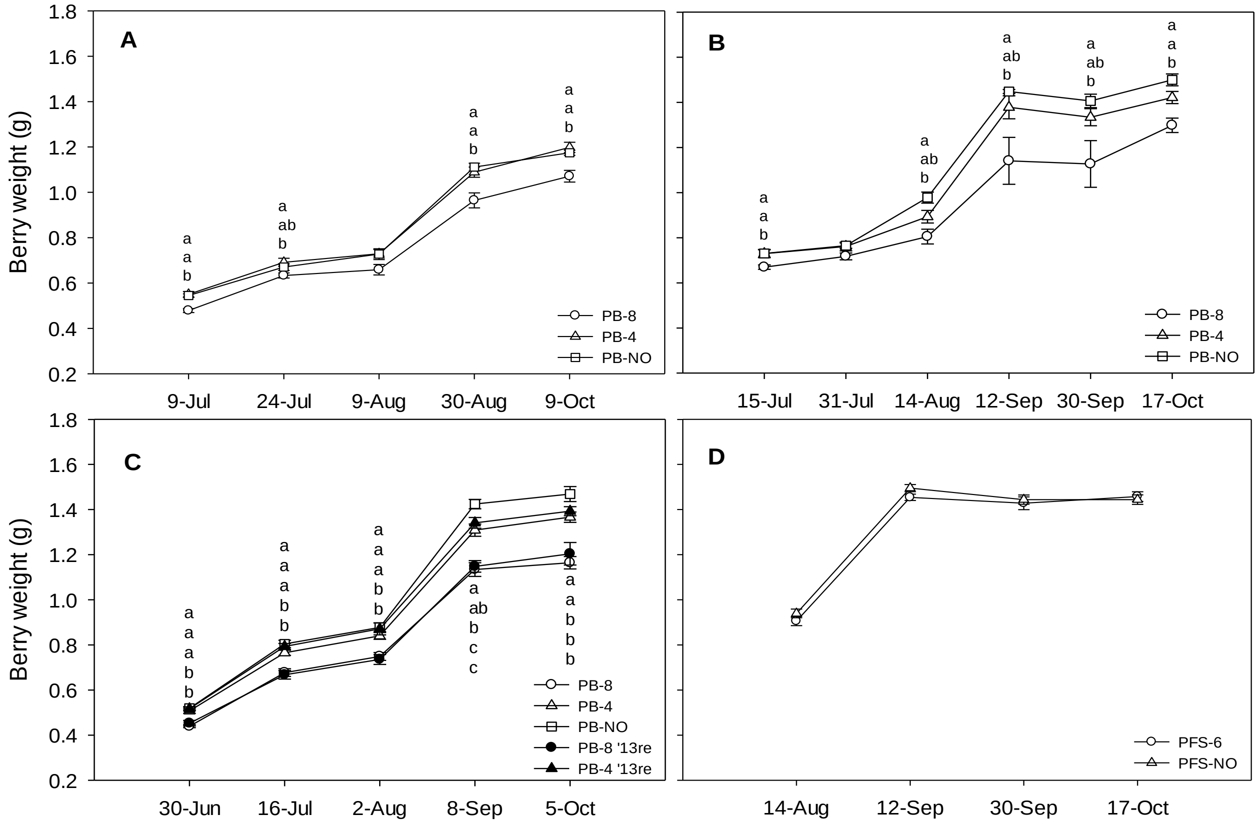

Fig. 8 Pre-bloom (A, B, C) and post-fruit set (D) leaf removal effect on berry weight over time in 2013 (A), 2014 (B, D), and 2015 (C); Each data point is an average of 120 berries; n = 6. Treatment mean berry weight within a date not sharing a letter are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

Table 5. Consumer preference of Cabernet franc and Petit Verdot wines from the 2013 vintage.

Cabernet franc[b]					
Attribute[d]	**NO[a]**	**MED[a]**	**HIGH[a]**	**P-B[a]**	**Significance[e]**
Appearance	n/a	6.58	6.32	6.74	ns
Red color	n/a	6.71	6.87	6.89	ns
Red color intensity	n/a	3.15 b	3.31 ab	3.45 a	0.0132
Aroma	n/a	6.37	6.50	6.51	ns
Fruity aroma	n/a	3.32	3.14	3.25	ns
Vegetative aroma	n/a	2.77	2.68	2.88	ns
Fruity flavor	n/a	3.36	3.27	3.29	ns
Vegetative flavor	n/a	2.81	2.93	2.99	ns
Overall flavor	n/a	6.15	6.35	6.39	ns
Astringency	n/a	6.04	6.18	6.27	ns
Astringency intensity	n/a	3.07 b	3.15 ab	3.40 a	0.0474
Mouthfeel	n/a	6.01	6.07	6.37	ns
Mouthfeel intensity	n/a	3.24	3.25	3.39	ns
Length of finish	n/a	6.06	6.26	6.19	ns
Length of finish intensity	n/a	3.20	3.20	3.30	ns
Overall impression	n/a	6.06	6.25	6.25	ns
Side-by-side ranking	n/a	2.07	2.00	1.93	ns
Petit Verdot[c]					
Attribute[d]	**NO[a]**	**MED[a]**	**HIGH[a]**	**P-B[a]**	**Significance[e]**
Appearance	7.36	7.31	7.33	7.21	ns
Red color	7.33	7.21	7.31	7.33	ns
Red color intensity	3.84 ab	3.79 b	4.00 a	4.00 a	0.0495
Aroma	6.49	6.52	6.64	6.44	ns
Fruity aroma	3.28	3.39	3.49	3.2	ns
Vegetative aroma	3.03	2.87	2.90	3.21	ns
Fruity flavor	3.57	3.49	3.30	3.33	ns
Vegetative flavor	3.16	2.97	3.18	3.13	ns
Overall flavor	6.28	6.72	6.46	6.39	ns
Astringency	6.31	6.64	6.36	6.44	ns
Astringency intensity	3.36	3.20	3.23	3.34	ns
Mouthfeel	6.28	6.30	6.31	6.25	ns
Mouthfeel intensity	3.54	3.54	3.46	3.69	ns
Length of finish	6.33	6.31	6.48	6.30	ns
Length of finish intensity	3.41	3.36	3.21	3.52	ns
Overall impression	6.23	6.44	6.33	6.26	ns
Side-by-side ranking	2.37	2.33	2.73	2.57	ns

[a]NO = no leaf removal; MED = post-fruit set removal of leaves to medium extent; HIGH = post-fruit set removal of leaves to high extent; P-B = pre-bloom leaf removal of six basal leaves and laterals.

dThe following attributes ranked on a scale of 1-9: appearance, red color, aroma, overall flavor, mouthfeel, length of finish, and overall impression; the following attributes ranked on a scale of 1-5: red color intensity, fruity and vegetative aroma, fruit and vegetative flavor, astringency intensity, mouthfeel intensity, and length of finish intensity; side-by-side ranking was an average of treatment ranking order, with "1" being the favorite.

[e]Significance of treatment effects (p > F; ns = not significant at 0.05 level).

*Means in same treatment group (rows) not sharing a letter are significantly different at 0.05 level.

Appendix A

Extent and timing of fruit-zone leaf and lateral shoot removal alters yield components and fruit composition in Cabernet Sauvignon grapes.

Background and aims: Aggressive fruit-zone leaf removal can improve disease management, particularly in humid regions. However, current fruit-zone leaf removal practices tend to be conservative and have seen little refinement over the last decade or more. We hypothesized that pre-bloom removal of basal leaves/lateral shoots would reduce crop yield and that aggressive basal leaf/lateral shoot removal would improve total phenolics and anthocyanins in Cabernet Sauvignon.
Methods and results: Two experiments were conducted to evaluate the effects of pre-bloom removal of no (PB-NO), four (PB-4), or eight (PB-8) basal leaves/lateral shoots (*Experiment 1*, 2013-2015) and post-fruit set removal of no (PFS-NO) and six (PFS-6) basal leaves/lateral shoots (*Experiment 2*, 2014-2015) on crop yield components, and total grape phenolics and anthocyanins in Cabernet Sauvignon. *Experiment 1:* Pre-bloom removal of eight leaves/lateral shoots reduced all yield components to a greater extent than did PB-4. When compared to PB-NO, PB-4 reduced berry number per cluster by 35-51%, cluster weight by 33-53%, and crop yield by 51-53% over 2013-2015. When compared to PB-NO, PB-8 reduced berry weight by 9-19%, berry number per cluster by 52-73%, cluster weight by 57-78%, and crop yield by 55-78% over 2013-2015. When compared to PB-NO, yield components tended to be reduced by a greater percentage due to re-implementation of both PB-4 and PB-8 in consecutive seasons. Compared to PB-NO, PB-4 reduced cluster compactness by 25-39%, and PB-8 reduced cluster compactness 58-68% over 2013-2015. Botrytis bunch rot incidence was reduced by PB-4 by 87% and by PB-8 by 100% when compared to PB-NO in 2015. Pre-bloom removal of eight leaves/lateral shoots reduced soluble solids in two of three years, and both PB-4 and PB-8 reduced titratable acidity when compared to PB-NO in 2015. Both PB-4 and PB-8 increased total grape phenolics by an average of 14-31% when compared to PB-NO over 2013-2015. While PB-4 increased total grape anthocyanins by an average of 9% when compared to PB-NO in 2014, both PB-4 and PB-8 increased total grape anthocyanins by an average of 22% when compared to PB-NO in 2015. *Experiment 2:* Botrytis bunch rot incidence was reduced by 78% by PFS-6. Post-fruit set removal of six leaves/lateral shoots reduced soluble solids in one year, and pH and titratable acidity in both years. Post-fruit set removal of six leaves/lateral shoots increased total grape phenolics in 2014 (13%) and 2015 (16%), and increased total grape anthocyanins in 2015 (13%).
Conclusions: Aggressive removal of fruit-zone leaves/lateral shoots tended to increase grape phenolics and anthocyanins and reduce botrytis bunch rot incidence, regardless of timing of removal. While pre-bloom leaf/lateral shoot removal resulted in greater concentrations of these compounds in grapes compared to post-fruit set leaf/lateral shoot removal, pre-bloom leaf/lateral shoot removal also reduced crop yield by an average of 57% compared to no leaf removal.
Significance of the study: Aggressive removal of fruit-zone leaves/lateral shoots improves the probability of getting disease-free fruit into the winery. Because the climate in humid growing regions is not as conducive to heating fruit to critical temperatures as in other climates, removing leaves/lateral shoots to an equivalent of 0 fruit-zone leaf layers is not deleterious to fruit quality. As such, disease management and fruit quality can be concomitantly improved with aggressive leaf removal. However, if removal of leaves/lateral shoots occurs before bloom, crop yield can be dramatically reduced, depending on the extent of green tissue removal at this critical stage.

Appendix B

Extent and timing of fruit-zone leaf and lateral removal alters yield components, grape phenolics, and carotenoids in Cabernet franc and Petit Verdot.

Background and aims: The rationale for fruit-zone leaf removal in a humid environment has been driven more by disease management than by documented changes in fruit composition. Though a common practice for several decades, leaf removal recommendations remain general and are not variety-, timing-, or magnitude-specific. We evaluated if the timing or magnitude of fruit-zone leaf and lateral removal would alter fruit composition and crop yield components of two regionally popular red-fruited varieties.

Methods and results: Two separate experiments in adjacent Cabernet franc and Petit Verdot vineyards evaluated the effects of three post-fruit set leaf/lateral shoot removal treatments [no removal (NO), removal from opposite the basal primary cluster and the node directly above (MED), and removal from the node directly above the distal primary cluster down to the cordon (HIGH)] and one pre-bloom (P-B) leaf/lateral shoot removal treatment (removal from the six primary basal nodes). Post-fruit set leaf removal had marginal, inconsistent effects on crop yield and components. In Cabernet franc, P-B reduced crop yield by an average of 50%, explained by reductions in cluster weight (39%), berry number per cluster (33%), cluster number (8%), and berry weight (6%) compared to NO. In Petit Verdot, P-B reduced crop yield by an average of 53%, explained by reductions in cluster weight (37%), cluster number per vine (32%), berry weight (25%), and berry number per cluster (18%) compared to NO. Re-implementation of P-B over two consecutive seasons caused *further* reduction in these yield components. Aggressive leaf removal (HIGH and P-B) tended to reduce soluble solids in Petit Verdot but not in Cabernet franc. HIGH tended to reduce titratable acidity (TA) in both varieties, whereas P-B tended to reduce TA only in Cabernet franc. P-B more consistently increased total berry phenolics in Petit Verdot than in Cabernet franc, but leaf removal did not increase total berry anthocyanins. When compared to NO and MED, HIGH and P-B tended to increase carotenoid accumulation to a greater extent in the pre-veraison period, and increase carotenoid degradation to a greater extent in the post-véraison period; this was particularly consistent for zeaxanthin. The color and aroma of wines from different leaf removal treatments were distinguished from one another, albeit infrequently. Color intensity was rated higher in wines made with fruit from P-B plots compared to wines made with fruit from MED plots.

Conclusions: Pre-bloom leaf removal reduced crop yield, and differentially affected crop yield components between varieties. Pre-bloom leaf removal did not affect grape anthocyanins and inconsistently improved total grape phenolics. Leaf removal of several basal leaves tended to increase carotenoid synthesis and degradation compared to removing fewer leaves, and this was more consistent in Petit Verdot compared to Cabernet franc. Leaf removal has potential to increase the color intensity of young red wines and change aroma and astringency, but preference of these attributes was not determined.

Significance of the study: Removing fewer leaves before bloom, or more leaves immediately after fruit set may be best fruit-zone management strategies to modestly improve fruit composition, sustain an economical crop yield, and create a fruit-zone environment associated with reduced disease incidence in a humid environment.

Appendix C

Building an hourly grape temperature-prediction model for vertically-shoot positioned vineyards in a humid climate

Background and aims: Light and temperature are important determinants of aroma and flavor compounds in grapes and, thus, wines. Metrics have been developed, and enhanced, that characterize fruit-zone radiation, quantify the physical nature and spatial distribution of the fruit-zone, and relate to fruit composition. However, few models are known to be available that can accurately predict grape temperature, even though it is affected by both fruit-zone architecture, and ambient air temperature and radiation. It was sought to develop a grape temperature-prediction model to aid in the meteorological risk assessment of over-heating well-exposed grapes to known critical temperature thresholds, such as those for anthocyanins (30-35 °C).

Methods and results: Ambient and fruit-zone photosynthetic active radiation (PAR), ambient and berry temperature, and ambient relative humidity were logged over three consecutive growing seasons. Berry temperature extremes were accounted for by logging the temperature of berries on the exterior face of grape clusters. The results were "grower friendly" models for each 15° hour angle that predict berry temperature differential from ambient air temperature from east and west canopy sides, in well-exposed and shaded fruit-zones, and using ambient radiation and hour angle. The difference between manually measured and predicted berry temperature ranged [0.17] to [2.84] °C across east and west canopy sides of differentially leaf-thinned fruit-zones, and across six different hour angles rounded to the nearest 15°.

Conclusions: When fruit-zones are shaded, berry temperature is highly predictable with ambient air temperature. The degree with which exposed berry temperature is heated above ambient air temperature is dependent on several factors, including, but not limited to, solar radiation, diurnal hour angle, and current ambient air temperature. The ability to predict berry temperature differential from ambient air temperature was complicated by fruit-zone leaf removal practice, canopy side, hour angle, and ambient radiation, particularly at diurnal periods of direct radiation penetration to the fruit-zone.

Significance of study: Having the ability to predict berry temperature will permit growers to have a better understanding of their site-specific risk of reaching critical grape temperatures (i.e. for anthocyanins). Growers can accordingly adjust their fruit-zone management practices.