Refining Fruit-Zone Leaf Removal for Red-Fruited Bordeaux Varieties Grown in a Humid Environment.

Cain Charles Hickey

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Tony K. Wolf, Committee Chair
Misha T. Kwasniewski, Co-chair
Molly K. Kelly
Gregory M. Peck
Amanda C. Stewart

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Current fruit-zone management recommendation in the eastern US aims for 1-2 basal shoot leaf layers after fruit set to limit fungal disease and sunburn incidence, and prevent extreme heating of grapes. The goal of this work was to assess if fruit-zone leaf removal to an uncommonly greater extent, and/or at an earlier phenological stage, would favorably alter yield components or fruit composition in three popularly grown, red-fruited, Bordeaux varieties – Cabernet franc, Petit Verdot, and Cabernet Sauvignon. Pre-bloom leaf removal to various extents reduced crop yield by 41-78% when compared to no leaf removal across seasons and varieties. Pre-bloom leaf removal implementation in consecutive seasons tended to further reduce crop yield components compared to implementation in the first year. Pre-bloom leaf removal tended to reduce cluster compactness and bunch rot incidence when compared to post-fruit set and no leaf removal. Basal leaf removal to the greatest extents inconsistently reduced soluble solids and titratable acidity across varieties and seasons. Pre-bloom and post-fruit set leaf removal to the greatest extent consistently increased total grape phenolics and anthocyanins compared to no leaf removal in Cabernet Sauvignon, but inconsistently increased total grape phenolics compared to no leaf removal in Cabernet franc and Petit Verdot. Basal leaf removal to the greatest extents tended to increase the synthesis and degradation of carotenoids more consistently than no leaf removal, and this was particularly true for zeaxanthin. Petit Verdot and Cabernet franc wine color and aroma were inconsistently distinguishable between leaf removal treatments, and color intensity was rated higher in wines made with fruit from pre-bloom leaf removal compared to modest post fruit-set leaf removal plots. Waiting until after fruit set to remove fruit-zone leaves maintained crop yield and offered comparable improvements in fruit composition to pre-bloom leaf removal. Pre-bloom leaf removal of no more than four leaves is recommended to limit crop yield reduction, and modestly improve fruit composition. This work showed that fruit-zone leaf removal does not need to be conservative in the eastern US, particularly because the climate does not appear to be detrimental to fruit composition, and open fruit-zones reduce grape fungal disease incidence.

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Dedication

I dedicate this work to Lisa, my ever-loving wife. She has dedicated her time and patience into this process as much as I have. In fact, I would not have gone down this career path had it not been for her constant encouragement and support. Thank you for always letting me disclose my weighted mind, and for responding with logical advice and guidance. Thanks for believing in me, and for always knowing what is better for me than I do myself.

I cannot wait to be parents together!

I love you.

Table of contents

Section **Page**

List of tables

Table **Page**

Chapter 1

Table **Page**

Table **Page**

Chapter 2

Table **Page**

List of figures

Figure **Page**

Chapter 1

Figure **Page**

Chapter 2

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

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

Abstract

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-veraison 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.

Introduction

The relationship of the canopy to fruit quality, whether with respect to sink-source relations (Bravdo et al. 1985; Dokoozlian and Kliewer 1995) or indirect/direct sunlight influence (Jacksoon and Lombard 1993; Smart and Robinson 1991, Meyers and Vanden-Heuvel 2008), has been extensively researched. The impact of sunlight and temperature on grape composition has

been of high interest, particularly regarding flavonoids (Bergqvist et al. 2001, Bonada and Sadras 2014, Downey et al. 2006, Spayd et al. 2002, Tarara et al. 2008). In general, high temperature is associated with lower grape anthocyanins, although some light is important for their synthesis (Mori et al. 2007, Spayd et al. 2002, Tarara et al. 2008). Consequently, fruit-zone leaf removal has become more conservative, aiming for 1 to 2 fruit-zone leaf layers (Wolf 2008), even though: (1) exposing fruit reduces fungal disease incidence (English et al. 1989, Wolf et al. 1986); (2) anthocyanins are not always reduced in well-exposed grapes (Bogicevic et al. 2015, Chorti et al. 2010, Kotseridis et al. 2012); and (3) an overwhelming number of studies reported fruit exposure to be beneficial, and shade detrimental, to grape and wine quality (Bledsoe et al. 1988, Carbonneau 1985, Lee et al. 2007, Hunter et al. 1991, Jackson and Lombard 1993, Reynolds et al. 1996, Ryona et al. 2008, Smart et al. 1985, Smith et al. 1988, Smart and Robinson 1991).

Several studies reported that pre-bloom leaf removal increased grape anthocyanins and phenolics. Mechanisms of metabolite improvements were: an increased skin: pulp ratio (Poni et al. 2006), thicker berry skins (Palliotti et al. 2011, Pastore et al. 2013, Poni et al. 2008, Tardaguila et al. 2010) or improved sink-source dynamics, as by bringing yields into balance with the vegetative growth (Diago et al. 2012, Intrieri et al. 2008, Gatti et al. 2012, Poni et al. 2006). However, the general positive effect of sun exposure on grape phenolics could not be ruled out (Gatti et al. 2012, Pastore et al. 2013, Tardaguila et al. 2012). Early leaf removal may, thus, offset the detrimental temperature effects associated with fruit exposure via the induction of other mechanisms that are beneficial to grape anthocyanins, such as those mentioned above.

A recent keyword search of “grape flavonoid” in Web of Science (Thomson Reuters) registered 1447 records whereas searching for “grape norisoprenoid” and “grape carotenoid”

registered 65 and 176, respectively. This is a strong case that relatively more research has been conducted on grape flavonoids relative to norisoprenoids and carotenoids. Thus, given the sensory impact of flavonoids relatively more studies have been conducted on changing wine color, mouthfeel, and astringency potential compared to aroma potential. Further investigation is required to improve our understanding of how canopy management practices impact precursors to wine aroma compounds, such as carotenoids (Lee et al. 2007).

Compared to other fruits, carotenoids are found in small concentrations in grapes (Goodwin 1980), but, similar to other fruits, they are present in skins at levels two to three times greater than found in the pulp (Razungles et al. 1988). Carotenoid concentration is high early in grape development and declines thereafter, exhibiting a sharp decline at veraison (Razungles et al. 1998). Since carotenoids are specifically located in the plastids, their degradation as grape skins change from green to red corresponds to the disintegration of chloroplasts that are not transformed into chromoplasts (Okombi et al. 1975). The proposed mechanism of carotenoid degradation (and norisoprenoid formation) in plants has three steps, involving oxidative cleavage, enzymatic transformations, and acid-catalyzed conversions. More recently, carotenoid cleavage dioxygenases (CCD) were found to be responsible for the production of apocarotenoids (i.e. C_{13}-norisoprenoids) in plants (Auldridge et al. 2006). Regardless of the mechanism, carotenoid degradation results in C_{13}-norisoprenoids, which have powerful aroma properties (Baumes et al. 2002, Mendes-Pinto 2009, Winterhalter and Rousefff 2002). C_{13}-norisoprenoids have various sensory descriptors such as "violet, woody, and raspberry" (β-ionone) and "cooked apple, floral, and quince" (β-damascenone) (Winterhalter and Rouseff 2002), and can increase fruity aromas while simultaneously muting herbaceous tones (Escudero et al. 2007, Pineau et al. 2007). C_{13}-norisoprenoids have low olfactory perception thresholds (Mendes-Pinto 2009), and

thus are perceived in young, mono-varietal red wines (Ferreira et al. 2000, Pineau et al. 2007), and are important odorants of Cabernet Sauvignon, Cabernet franc, and Merlot (Fan et al. 2010).

Since light is primarily responsible for carotenoid biosynthesis and regulation (Razungles et al. 1998, Winterhalter and Rouseff 2002), carotenoid contribution to wine aroma can be affected by viticulture practices that change the fruit-zone light environment, thus altering the grape carotenoid profile (Bureau et al. 1998a and 1998b, Mendes-Pinto 2009, Oliveira et al. 2004, Razungles et al. 1988 and 1998). Carotenoids can quantitatively and qualitatively influence wine aroma due to the synthesis and degradation patterns of carotenoid subclasses. *Quantitatively*: Fruit exposure enhances both pre-veraison accumulation and post-veraison breakdown of carotenoids (Farina et al. 2010, Razungles et al. 1998), resulting in greater norisoprenoid concentrations at harvest (Bureau et al. 2000a and 2000b). *Qualitatively*: Xanthophylls exhibit different patterns than carotenes throughout berry maturation (Razungles et al. 1996, Oliviera et al. 2004, Crupi et al. 2010). Xanthophylls are subject to inter-conversion to/from epoxified, or de-epoxified, forms as a consequence of light intensity (Yamamato 1979). De-epoxified xanthophylls, such as zeaxanthin, are characteristic of high light intensity (Baumes et al. 2002, Bureau et al. 1998a). Thus, grapes differentially exposed to light can have different proportions of xanthophylls (Bureau et al. 1998a and 1998b) and norisoprenoids, the latter having specific carotenoid precursors (Crupi et al. 2010).

The combined effects of light and temperature, which could not be separated in a field setting, were responsible for increasing pre-veraison carotenoids in sun-exposed compared to shaded berries (Bureau et al. 1998a). Carotenoid concentrations were greater in grapes from a vineyard with greater radiation and from a warmer season compared to from a vineyard with lower radiation and a cooler season, respectively (Farina et al. 2010). Though berry temperature

may be an important determinant of grape carotenoids, temperature data was not elaborated upon when a 5-6 °C difference in grape temperature was reported (Bureau et al. 1998a; Razungles et al. 1998). Only one (Kwasniewski et al. 2010) grape carotenoid study has characterized the fruit-zone environment using advanced techniques, such as enhanced point quadrat analysis (EPQA) (Myers and Vanden-Heuvel 2008), even though fruit-zone leaf layer number was positively related to C_{13}-norisoprenoids because it was a good proxy for the combined effects of radiation and temperature, both of which were positively related to several C_{13}-norisoprenoids (Lee et al. 2007). Thus, a more thorough characterization of fruit-zone radiation and berry temperature is required to better understand their effects on carotenoids, similar to what has been done for anthocyanins (Bergqvist et al. 2001; Spayd et al. 2002; Tarara et al. 2008). Most studies evaluating the effects of fruit exposure on carotenoids have involved leaf pulling or shading of clusters after fruit set (Bureau et al. 1998a and 1998b, Kwasniewski et al. 2010, Razungles et al. 1998). It is, therefore, unknown if removing leaves before bloom could further increase carotenoid synthesis before veraison, thus adding improvement in wine aroma potential to the long list of other benefits of pre-bloom leaf removal (Palliotti et al. 2011, Poni et al. 2008, Taradaguila et al. 2010).

Little is known about the effects of unconventionally early leaf removal on yield and fruit composition in Cabernet franc and Petit Verdot, two varieties widely planted in the eastern U.S. Thus, it was sought to evaluate if the timing and/or magnitude of fruit-zone leaf removal would impact crop yield, fruit composition at harvest, and carotenoid synthesis and degradation patterns. It was hypothesized that pre-bloom leaf removal would reduce crop yield, and that the removal of relatively more basal leaves at the pre-bloom and post-fruit set stages would

favorably alter fruit composition and wine quality by increasing flavonoids and the synthesis and degradation of carotenoids.

Materials and Methods

Treatments and experimental design: Two separate, completely randomized designs, comprising of five-vine-panel experimental units, each replicated six times, were set up in adjacent blocks in a commercial vineyard in Shenandoah County, Virginia. The vineyard soil was an Endav silt loam. Cabernet franc (clone 214) and Petit Verdot (clone 1) vines, both grafted onto 3309 rootstocks, were planted in 2001 at a 3.0-m (row) x 1.8-m (vine) spacing. Vines were trained to bi-lateral cordons ~0.9 m above ground, and were planted in rows running generally northeast/southwest. Creeping red fescue (*Festuca rubra* L.) was established under-trellis in the Cabernet franc vines in 2012. Three post-fruit set (modified EL stage 31, Dry and Coombe 2004) leaf/lateral shoot removal treatments were evaluated in 2012: 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). An additional leaf/lateral shoot removal treatment was implemented in 2013 and repeated in 2014: removal from the six primary basal leaves at the modified EL stage 18/19 (P-B). Frequent re-visits to experimental units were made to maintain canopy porosity in the leaf removal treatments through harvest. Main plot treatments were re-randomized each year. However, in 2014, the P-B and NO plots that were used in 2013 were maintained separately from the main plots. Treatments in these separate plots were re-implemented to evaluate any carryover effects on responses noted below.

Data were collected over the 2012, 2013, and 2014 growing seasons. Missing observations in individual vines or whole experimental units were uncommon, but occurred in

some cases, such as significant wildlife depredation in 2013. As elaborated upon below, measurements were taken in each experimental unit on multiple days throughout the growing season (enhanced point quadrat analysis, berry weight, and berry temperature), at harvest (cluster compactness, crop yield components, primary chemistry), during the dormant season (dormant cane pruning weights), and during the pre-bloom stage (vine fruitfulness).

General vine management: Cordons were spur-pruned each winter. Shoot density was not adjusted in 2012, however it was adjusted to ~18-20 shoots per m of cordon in 2013, and adjusted to ~15-16 shoots per m of cordon in 2014. Shoots were trained vertically upright with the aid of catch wires. Canopies were shoot-hedged before shoots extended more than ~0.9 m above the top catch wire. Fertilizer was not applied during the study, and no nutritional deficiency symptoms were evident. Disease management was standard for the region.

Meteorology: Temperature, rainfall, and photosynthetically active radiation (PAR) were logged on 1-min intervals in 2013 and 2014 using a HOBO data logger (model H21-002, Onset Computer Corp., Bourne, MA) located ~23 m from the experimental site. Growing degree days were generated using a base temperature of 10 °C. Seasonal data are presented from 1 Jun through 28 Oct. A severe hail storm in late Jun 2014 caused severe leaf and berry scarring, berry desiccation and abscission, and depressed crop yield in 2014 relative to other years.

Enhanced point quadrat analysis and dormant cane pruning weights: Point quadrat analysis (PQA) data were collected at EL stages 31, 33, and 35 in 2012, and at EL stages 27, 33, and 35 in 2013 and 2014. A thin metal rod was inserted into the fruiting zone along the transverse axis of the canopy, using a tape measure to guide insertions, as described in Smart and Robinson (1991); this was repeated ~25 times (~ every 0.35 m) in each experimental unit. Fruit-zone photosynthetic photon flux density (PPFD) was measured between 1030-1300 hrs in one cordon

of each vine using an AccuPAR ceptometer (Model PAR-80, Decagon Devices, Inc., Pullman, WA). Measurements were taken by inserting the ceptometer inside fruit-zones, parallel to, and directly above, the cordon, and orienting the light interception side of the ceptometer in three different directions (45˚ east, vertical, 45˚ west); those readings were then averaged. Between each fruit-zone insertion of the ceptometer, the maximum ambient PPFD was recorded outside of the canopy. This permitted PPFD to be expressed as a percentage of ambient radiation for use with enhanced point quadrat analyses (EPQA version 1.6.2) (Meyers and Vanden-Heuvel 2008). EPQA software was used to generate leaf layer number (LLN) and cluster exposure flux availability (CEFA). Cane pruning weights were collected by vine using a field scale during the dormant periods of 2012, 2013, and 2014. Cane pruning weights were additionally collected in re-implemented P-B and NO plots in 2014 and 2015 to evaluate if pre-bloom leaf removal had carryover effects on vine capacity.

Berry temperature: Berry temperature was measured on six dates between Jul and Sep in 2013 and on eight dates between Jul and Oct in 2014. Berry temperature was measured in every experimental unit at three different times of day on each collection date: morning (~ 0900-1030 hrs), around solar noon (~ 1245-1415 hrs), and late afternoon (~ 1545-1715 hrs). Berry temperature was measured by inserting a mini hypodermic thermocouple (model HYP1/2, Omega Eng., Stamford, CT) beneath the skins of berries and recorded using a handheld digital thermometer (model HH 25, Omega Eng., Stamford, CT). In every experimental unit, at each time of day, on each collection date, and on both east and west canopy sides, the temperature of three berries on clusters' exterior face, positioned at the top, center, and bottom of clusters, were measured on two clusters borne on opposite cordons of one vine for a total of six temperature measurements. In 2013, the time span was recorded over temperature measurement at each time

of day (AM, NOON, PM), and in 2014, over temperature measurement in each experimental unit; the goal was to investigate the relationship of berry temperature with ambient temperature and radiation (recorded with the HOBO weather station). Time spent ≥ 30 and 35 °C was estimated using the minimum estimated ambient air temperature necessary to reach these berry temperatures in the post-veraison period in 2013 and 2014. This ambient air temperature was derived from the simple linear relationship of ambient air temperature and berry temperature over all dates of manual berry temperature measurement, which was developed for each specific leaf removal treatment.

Crop yield components, cluster compactness, and vine fruitfulness: Crop yield weight per vine was measured with a field scale at harvest on 27 Sep 2012, 3 Oct 2013, and 14 Oct 2014 for Cabernet franc, and 4 Oct 2012, 9 Oct 2013 24 Oct 2014 for Petit Verdot. Cluster weight was determined from the quotient of crop yield weight and cluster number. Berry weight at harvest was determined from 120-berry samples in 2012, 2013 and 150-berry samples in 2014. Berry number per cluster was determined from the quotient of average cluster weight and average berry weight. Crop load was obtained by dividing crop yield by pruning weight on a per vine basis. Clusters not representative of treatments were not used for calculating crop yield or average cluster weight, but were counted when summing cluster number per vine. Pre-harvest berry weight was determined from 120- or 240- composite berry samples randomly and equally collected from east and west canopy sides in each experimental unit on several pre-harvest dates in 2013 and 2014. Petit Verdot and Cabernet franc berry tissue analysis was performed on 30 randomly selected, thawed berries from frozen 120- composite berry samples collected from each experimental unit in 2013. Seeds were counted and seed and skin weights were measured to the thousandth of a gram. To evaluate pre-bloom leaf removal impact over time, crop yield

components and berry weight at harvest in 2014 were collected in P-B and NO plots originally implemented in 2013, and re-implemented in 2014. These re-implemented plots were not berry-sampled with equal frequency as current-season plots, explaining why components of yield were greater in the re-implemented plots. For this reason, re-implemented plots were not statistically compared to current-season plots in 2014.

Cluster compactness was indexed by determining the ratio of total berry number to main rachis length in 2013 and 2014. Ten clusters from each experimental unit that were +/- 25% of the treatment's average harvested cluster weight were randomly selected from harvest bins to determine cluster compactness. Vine fruitfulness was assessed at the modified EL stage 15-16 by dividing the number of inflorescences by the number of shoots on both basal (cordon-originating) and count (one-year old spur-originating) shoots. In 2014, vine fruitfulness was measured on all vines in each NO and P-B experimental unit from 2013. In 2015, vine fruitfulness was assessed on three vines in each NO and P-B experimental unit that had been implemented in both 2013 and 2014. In spring 2015, the weak and sparse shoot growth was suspected to be due to cold injury; this may have confounded accurate vine fruitfulness assessment.

Primary fruit chemistry: Juice from 60-berry samples collected immediately prior to harvest from each experimental unit was analyzed for soluble solids concentration (˚Brix), pH, and titratable acidity (TA). Juice was obtained by hand-pressing fresh berries in a plastic bag and then centrifuging for five min at ~ 3500 rpm. Soluble solids were measured with a digital refractometer (Pocket PAL-1, ATAGO USA, Inc., Bellevue, WA). Juice pH was measured, and TA was determined by titration to an endpoint of pH 8.2, using an 848 Titrino Plus auto-titrator (Metrohm USA, Riverview, FL) and 0.1 N NaOH base. Primary fruit chemistry was evaluated

on all current-season treatments, as well as in the NO and P-B plots originally implemented in 2013, and re-implemented in 2014.

Estimated total grape phenolics and anthocyanins: Absorption spectroscopy was used to estimate total grape phenolics and anthocyanins from randomly collected berry samples. Composite berry samples of 120-150 berries were collected equally from both canopy sides in every experimental unit prior to harvest and frozen at -20 to -80˚C until tests commenced. Composite berry samples from east- and west- canopy sides were collected into separate bags on 11 Aug, 10 Sep, and 22 Oct in 2014. Berry samples were thawed, homogenized with a Magic Bullet (Homeland Housewares LLC, Los Angeles, CA), and had 30 mL of 0.025 M KCl buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) added to separate 1.0 g aliquots of the berry homogenate. The homogenate-buffer samples were shaken for 10 min and then centrifuged for 5 min at ~ 3500 rpm. The supernatant was pipetted into a 10 mm path length Hellma quartz cuvette (Thermo Fisher Scientific Inc., Pittsburgh, PA), and the absorbance at 520 and 700 nm was measured in duplicate with a Genesys 8 ThermoSpectronic spectrophotometer (Cambridge, UK). The sample containing the berry homogenate and 0.025 M KCl buffer (pH 1.0) was further diluted (2 parts sample: 1 part 0.025 M KCl buffer ratio) and its absorbance was read in duplicate at 280 nm.

Carotenoid analysis: Extraction of carotenoids was done using methods described in Wrolstad et al. (2005) and Kwasniewski et al (2010), using the same randomly collected berry samples used for total phenolics and anthocyanins analysis over the 2012-2014 period. In brief, thawed berry samples were homogenized for 2 min with a Magic Bullet. Ten mL of a 50/50 methanol (MeOH)/tetrahydrofuran (THF) with 0.1% BHT solution was added to a 10 g aliquot of the berry homogenate contained in a Nalgene Teflon fluorinated ethylene propylene Oak Ridge Centrifuge

Tube (ThermoScientific, Rochester, NY), agitated by hand, centrifuged at 5,000 rpm for 5 min (Allegra 25R centrifuge, Beckman Coulter, Indianapolis, IN) then the supernatant collected; this step was repeated by adding another 10 mL of 50/50 MeOH/THF with 0.1% butylated hydroxyltoluene (BHT) solution to the precipitate. The supernatants were combined in a 100ml separatory funnel with 20 mL of petroleum ether with 0.2% BHT and 10 mL of 20% aqueous NaCl, and agitated for ~1 min. After phase separation for ~ 5 min, the organic phase was collected and dried under vacuum for 45 min at 60 °C and 3,500 rpm using a Centrivap Concentrator (model 7810014, Labconko, Kansas City, MO), fitted with a cold trap (model 7811020, Labconko, Kansas City, MO Labconko) and diaphragm pump (model 2018B-01, Dry Fast, Skokie, IL), re-dissolved in 2 mL ethanol, and syringe filtered through a 0.45 µm polyvinylidene fluoride (PVDF) membrane Millex filter (Merck Millipore Ltd., Tullagreen, Carrigtwohill Co., Cork, IRL) into a 2 mL, amber glass HPLC vial with a PTFE lid and silicone septum (Supelco Analytical, Bellefonte, PA). Saponification was not used during carotenoid extraction due to the conditions of this process resulting in astaxanthin oxidation and stereomutation of lutein and zeaxatnhin (Goodwin 1976).

UPLC- MS analysis of carotenoids was conducted using a Waters UPLC BEH C-18 column (100 x 2.1 mm, 1.7 µm particle size) (Waters, Milford, MA) held at 40°C on a Waters H-Class UPLC equipped with a Diode array detector (DAD) and Xevo Q-Tof mass spectrometer. Mobile phase A consisted of 0.1% formic acid, 5% acetonitrile and 94.9% water, mobile phase B consisted of 0.1% formic acid in water. Samples were held at 10 °C with an injection volume of 2.5 µl. Initial gradient condition was 50:50 (v/v) A and B for 0 to 1 minutes with a 2 min pre-run 1 to 3 min 20:80, 3 to 4.5 min 10:90, 4.5 to 7.5 min hold at 10:90, 7.5 to 8 min 0:100 and held at 0:100 until 15 min. Flow was set to 0.5ml/min. The DAD was set to collect 320-490nm, with a 2

nm resolution. The maximum wavelength observed for each carotenoid used for quantification. The MS was set to collect in ESCi+ (switching between ESI+ and APcI+ modes) as poor ionization of late eluting compounds occurred with ESI+ only. ESI+ parameters were a cone gas flow of 100 L/hour, desolvation 800 L/hour, desolvation temp 450 °C, source 125 °C, and kV. The corona voltage for APCi was set to 20 μA. Leucine Enkephalin was used as a lockmass compound.

β-carotene, lutein, and zeaxanthin were identified and quantified with respect to authentic standards and other xanthophylls were identified and quantified by comparison of DAD and MS spectra as well as elution order to previous reports (Crupi et al. 2010).

Winemaking: Cabernet franc and Petit Verdot wine lots were made from equal weights of fruit between all treatments. Wine lots were made in duplicate from a composite of grape clusters from all (2012), or several (2013) experimental units of the same leaf removal treatment. Fruit was processed using a de-stemmer/crusher (Wottle Type 2; Wottle Maschinen & WeinPressenbau, Austria) in 2012 and a Laguna 1R de-stemmer and 650 model crusher (Prospero, Pleasantville, NY) in 2013. Crusher wheels were set to break berry skins as uniformly as possible without breaking seeds. Treatment musts remained in the same 55L, high density polyethylene cylindrical tanks with covers (Nalgene 11100-0015, Nalgene Nunc, Rochester, NY) from processing through fermentation. Potassium metabisulfite (PMBS) was added at a rate of 40 ppm to all lots before a post-crush cold soak period that lasted ~ four days at 4.0 °C. Tartaric acid was added at a rate of 1.5 g/L to all lots after cold soak.

Musts were inoculated with Lalvin BM 4x4 yeast (Lallemand, Inc. Montreal, Canada) at a rate of ~ 0.24 g/L using standard protocol for yeast re-hydration (Scott Labs) and adding the yeast re-hydration supplement GoFerm (Lallemand, Inc. Montreal, Canada) at a rate of ~0.3 g/L.

Musts were amended with equal parts Fermaid K at ~1/3 sugar depletion (35 g/lot in 2012; 28 g/lot in 2013). Grape skin caps of all lots were punched down two to three times daily, and fermentation temperatures were monitored daily with a bulb thermometer. Soluble solids were measured once daily with a hydrometer. When soluble solids ≤ 1.0 ˚Brix, Clini-Test Reagent Tablets (Bayer AG, Leverkusen, Germany) were used to determine dryness. Wines were siphoned off the primary lees and analyzed for percent alcohol and residual sugar by the Virginia Tech Enology Analytical Services Lab. Wines were stored in glass carboys with equal headspace at 4.0 °C. After confirming the absence of sulfur-like odors and differences between duplicate lots, same-treatment wines were combined into one glass carboy and subsequently bottled in 750 mL glass bottles with synthetic cork closures (Nomacorc, Zebulon, NC). Bottled wines were held at 4.0 °C until sensory analysis.

Wine sensory analysis: Triangle difference tests were conducted on the 2012 Cabernet franc and Petit Verdot wines on 16 and 17 April 2013 at Virginia Tech Food Science Department's sensory analysis lab using a randomized complete block design. Panelists were enrolled in the "Wines and Vines" class at Virginia Tech, where they learned about sensory analysis, but had not previously received formal sensory training. Approx. 25-28 mL of wine was served at room temperature in clear international standards organization (ISO) wine glasses. Each panelist was given a set of three clear wine glasses; two of the three glasses contained wine made from a particular treatment and one glass contained wine made from a different treatment. Sample presentation order was randomized but rotated with equal frequency. In each session, panelists were asked to identify the one wine that had different aroma, color, and flavor. Panelists cleansed their palates with unsalted crackers and filtered water. There were eight total sessions, each with 26-40 panelists. Data were collected with SIMS software (Berkeley Heights, NJ).

Consumer preference tests of Cabernet franc and Petit Verdot wines were conducted on the 2013 vintage on 5 and 6 May 2015, at Virginia Tech Food Science Department's sensory analysis lab using a randomized complete block design. 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).

Statistics: One-way ANOVA was used to evaluate the fixed effects of leaf removal treatment ($\alpha \leq 0.05$) using JMP Pro 11 (SAS, Cary NC). Significant difference of least square means ($\alpha \leq 0.05$) were determined with Tukey's HSD for most responses. Student's T-test was used to determine separation of least square means ($\alpha \leq 0.05$) for the following: first round of EPQA measurements taken in 2013 and 2014, pre-bloom carry-over effects from 2013 to 2014, vine fruitfulness in 2015, and canopy side effect on total phenolics and anthocyanins in 2014. Manual

berry temperature measurements were analyzed separately by time of day and canopy side on each date. Total estimated grape phenolics and anthocyanins at harvest in 2014 were analyzed using a model containing leaf removal treatment and canopy side as fixed effects; their interaction was also evaluated. Within each date, treatment effect on grape carotenoids was analyzed using one-way ANOVA, and, in 2014, canopy sides were analyzed separately; mean separation was determined with Tukey's HSD ($\alpha \leq 0.05$). Within each year, a model was used to determine the fixed effects of, and interaction between, treatment and date on grape carotenoids; mean separation was determined with Tukey's HSD ($\alpha \leq 0.05$). Across years, a model was used to determine the fixed effect of treatment, sample date, and year, as well as their interactions, on grape carotenoids; mean separation was determined with Tukey's HSD ($\alpha \leq 0.05$). One-way ANOVA was used to analyze the difference in zeaxanthin:lutein ratios, and degradation in carotenoids from pre-veraison to harvest; mean separation between leaf removal treatments was determined with Tukey's HSD ($\alpha \leq 0.05$). Discrimination significance in the triangle difference test was determined using an alpha one-tailed analysis ($\alpha \leq 0.05$) using JMP Pro 11 (SAS, Cary, NC). Mean separation of sensory attributes and ranking in the consumer preference test was determined using Duncan's Multiple Range Test ($\alpha \leq 0.05$) using SAS 9.3 (SAS, Cary, NC).

Results

Seasonal meteorology: In general, it was warmer and drier from 1 Jun through 28 Oct in 2013 compared to the same period in 2014 (Table 1). There was 5% greater total GDD accumulation in 2013 (1604) when compared to 2014 (1530), mainly due to greater GDD accumulation in Jul and Aug 2013 compared to these same months in 2014. Mean temperature in Jul 2013 was 1.5 °C greater than Jul 2014 and maximum temperature in Aug and Oct 2013 was 2.8 and 7.2°C greater than in Aug and Oct 2014, respectively; mean and maximum temperatures in Jun and Sep were similar between years. There was 67% less total rainfall in 2013 (153 mm) when compared

to 2014 (465 mm), mainly due to greater rainfall in Jun, Jul, and Oct 2014 compared to these same months in 2013. Ambient photosynthetically active radiation (PAR) and air temperature patterns by hour and month are further elaborated upon in the following sub-sections.

Table 1. Monthly growing degree day (GDD) and rainfall sum, and monthly mean and maximum temperatures for Jun, Jul, Aug, Sep, and Oct in 2013 and 2014.

Year	Metric	Jun	Jul	Aug	Sep	Oct[b]
2013	T mean (°C)	21.3	23.3	21.2	17.9	13.4
	T max (°C)	34.3	35.2	34.7	35.4	33.3
	GDD sum[a]	369	449	383	269	134
	Rainfall sum (mm)	17.58	66.19	52.53	15.04	2.03
2014	T mean (°C)	21.7	21.8	20.8	18.1	13.4
	T max (°C)	34.8	34.9	31.9	34.8	26.1
	GDD[a]	378	394	313	251	112
	Rainfall sum (mm)	160.91	124.28	55.68	12.67	111.28

[a]Growing degree days calculated using base 10 °C.
[b]Cabernet franc harvested on 3-Oct-2013 and 14-Oct-2014 and Petit Verdot harvested on 9-Oct-2013 and 24-Oct-2014.

Hourly PAR and temperature: Considering solar noon over the course of the season (1 Jun through 28 Oct) to be ~1330, average ambient PAR was always greater during the morning compared to afternoon (Fig. 1 A, B). In 2013, ambient PAR ranged 598-1239 µmol $m^{-2}s^{-1}$ from 900-1200, and 272-1050 µmol $m^{-2}s^{-1}$ from 1500-1800 (Fig. 1 A). In 2014, average PAR ambient PAR ranged 679-1227 µmol $m^{-2}s^{-1}$ from 900-1200, and 277-1060 µmol $m^{-2}s^{-1}$ from 1500-1800 (Fig. 1 B). In both seasons, the standard deviation of PAR was greater from 900-1200 (513-522 µmol $m^{-2}s^{-1}$) compared to 1500-1800 (434-457 µmol $m^{-2}s^{-1}$) (Fig. 1 A, B). This suggested greater variability in sky conditions, or more drastic changes in solar angle, before solar noon compared to after solar noon. However, when expressed as a percentage of ambient PAR, standard deviation was lower from 900-1200 (52-55%) compared to 1500-1800 (72-75% and 75%) (Fig. 1 A, B).

Hourly ambient air temperature was generally greater in 2013 than in 2014, and was greatest at 1400-1500 (24.2-24.7 °C) (Fig. 1 A, B). Air temperature was greater when averaged

over 1500-1800 (23.3-23.8 °C) compared to 900-1200 (21.8-22.0 °C). Standard deviation in air temperature was virtually the same before (900-1200) and after (1500-1800) solar noon in both seasons, but was greater over the course of 2013 (5.2) compared to 2014 (4.5).

Monthly PAR and temperature from 800-1330 and 1330-1800: Monthly PAR was greater in the morning (800-1330) compared to afternoon (1330-1800) in both seasons (Fig. 1 C, D). Ambient PAR was 43-157 μmol $m^{-2}s^{-1}$ greater in the morning compared to the afternoon over Jun- Oct in 2013 (Fig. 1 C). Ambient PAR was 60-177 μmol $m^{-2}s^{-1}$ greater than in the morning compared to the afternoon over Jun-Oct in 2014 (Fig. 1 D). Excepting Oct, monthly standard deviation of ambient PAR was lower in the morning compared to the afternoon. Monthly air temperature was greater in the afternoon compared to the morning in both seasons (Fig. 1 C, D). Afternoon air temperature was 1.7-3.3 °C greater in the afternoon than in the morning over Jun-Oct in 2013 (Fig. 1 C). Afternoon air temperature was 1.8-2.8 °C greater in the afternoon than in the morning over Jun-Oct in 2014 (Fig. 1 D). Though morning air temperature was virtually the same from Aug-Oct 2013 and 2014, afternoon air temperature from Aug-Oct 2013 was greater than in 2014. Excepting Oct, monthly standard deviation of air temperature was typically greater in the morning compared to the afternoon.

Time spent at each 25% ambient PAR range from 800-1330 and 1330-1800: The difference in time spent at the 0-50% ambient PAR range between morning and afternoon was greater during the whole season and post-veraison periods of 2014 compared to 2013 (Fig. 1 E, F). More time was spent under cloudy conditions (≤ 25% PAR) in the afternoon compared to morning, and more time was spent under cloudy conditions during the 2014 compared to 2013 post-veraison period. By contrast, more time was spent under sunnier conditions (25-75% PAR ranges) in the morning compared to the afternoon in both seasons. More time was spent under

sunnier conditions in the 2013 compared to 2014 post-veraison period, and more time was spent under sunny conditions in the morning compared to the afternoon during post-veraison in 2014. Compared to other PAR ranges, there was little difference in time spent at the 75-100% PAR range between morning and afternoon.

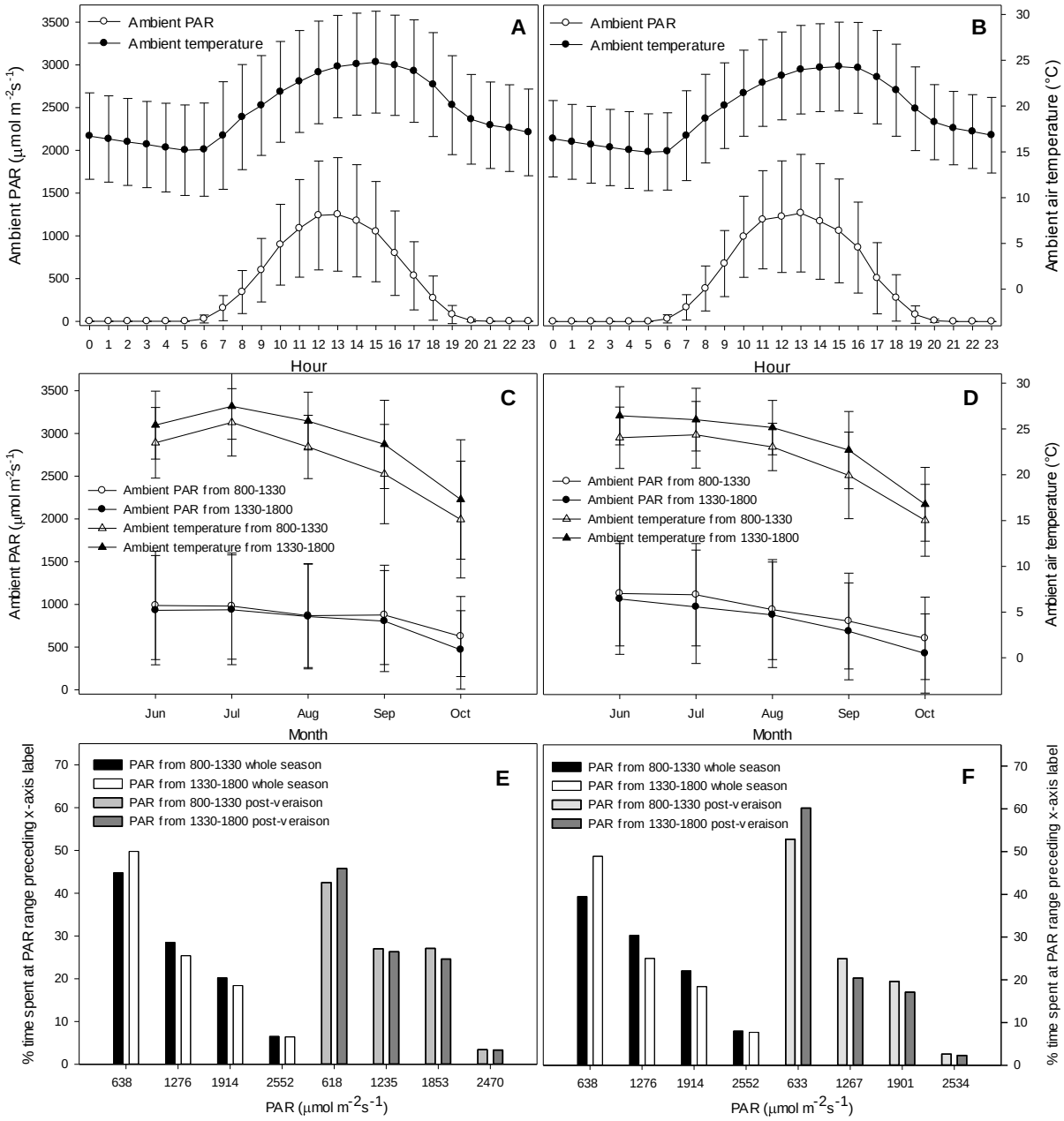

Fig. 1 Hourly average ambient photosynthetically active radiation (PAR) and air temperature (A, B), monthly average ambient PAR and air temperature during 0800-1330 and 1330-1800 periods (C, D), and percent time spent at 25% increments of the seasonal (1 Jun - 28 Oct) and post-verasion (15 Aug - 9/19 Oct 2013/2014) ambient PAR ranges during 0800-1330 and 1330-1800 periods (E, F) in 2013 (A, C, E) and 2014 (B, D, F). Data logged every minute from 1 Jun - 28 Oct.

Enhanced point quadrat analysis and dormant cane pruning weights: Cabernet franc and Petit Verdot. There was no difference in fruit-zone leaf layer number (LLN) or cluster exposure flux availability (CEFA) before leaves were removed (EL 31) from Cabernet franc vines in 2012 (Table 2). After leaves were removed (EL 33), CEFA followed a logical increase as a function of the degree of leaf removal: HIGH > MED > NO; while LLN showed a corresponding decrease. This relative fruit-zone porosity was maintained through veraison (EL 35) in 2012. Similarly, when LLN and CEFA were measured at EL 27, results showed that P-B resulted in more open fruit-zones early in the 2013 and 2014 seasons. Though LLN and CEFA readings were not taken on MED and HIGH panels at EL 27, it is assumed that their values were similar to NO, given that no leaves had been removed from those plots at that stage. As phenology progressed in 2013 and 2014, fruit-zone porosity was affected by leaf removal treatment in a logical fashion: NO > MED > HIGH = P-B (LLN), and P-B = HIGH > MED > NO (CEFA). Besides having comparatively lower CEFA at EL 27 and EL 35 in 2013, LLN and CEFA values in Petit Verdot paralleled those of Cabernet franc (Table 3). This fruit-zone data validated that the forthcoming response data was a function of both the extent and timing of leaf removal. Dormant cane pruning weight was not affected by any leaf removal treatment in either variety in any year (Tables 2 and 3). In 2014, the re-implementation of pre-bloom leaf removal in Cabernet franc plots originally imposed in 2013 did not affect cane pruning weight compared to no leaf removal plots originally implemented in 2013 (data not shown). Similarly, pruning weight was not affected by re-implementation of these treatments in Petit Verdot in 2014 (data not shown).

Table 2. Leaf removal effect on fruit-zone leaf layer number (LLN) and cluster exposure flux availability (CEFA) collected at three periods (EL 31, 33, and 35), and cane pruning weights collected during vine dormancy, in Cabernet franc, 2012-2014.

Treatment[a]	2012						
	EL 31		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	1.88	0.24	1.97 a	0.30 c	1.99 a	0.26 c	0.32
MED	1.88	0.30	1.36 b	0.50 b	1.26 b	0.48 b	0.31
HIGH	1.94	0.26	0.44 c	0.77 a	0.57 c	0.71 a	0.35
Significance[b]	ns	ns	<0.0001	<0.0001	<0.0001	<0.0001	ns
Treatment[a]	**2013**						
	EL 27		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	2.03 a	0.29 b	2.29 a	0.18 c	2.49 a	0.13 c	0.38
MED	n/a	n/a	1.82 b	0.37 b	1.54 b	0.45 b	0.33
HIGH	n/a	n/a	0.09 c	0.69 a	0.11 c	0.73 a	0.36
P-B	0.10 b	0.85 a	0.24 c	0.68 a	0.16 c	0.80 a	0.32
Significance[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns
Treatment[a]	**2014**						
	EL 27		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	2.42 a	0.25 b	2.34 a	0.24 c	2.03 a	0.24 c	0.32
MED	n/a	n/a	1.22 b	0.47 b	1.17 b	0.46 b	0.31
HIGH	n/a	n/a	0.03 c	0.73 a	0.00 c	0.70 a	0.28
P-B	0.00 b	0.79	0.36 c	0.69 a	0.00 c	0.73 a	0.24
Significance[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	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.
[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 Student's T-test (only for EL 27 in 2013 and 2014) or Tukey's HSD.

Table 3. Leaf removal effect on fruit-zone leaf layer number (LLN) and cluster exposure flux availability (CEFA) collected at three periods (EL 31, 33, and 35), and cane pruning weights collected during vine dormancy, in Petit Verdot, 2012-2014.

Treatment[a]	2012						
	EL 31		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	2.3	0.15	2.54 a	0.09 c	2.71 a	0.09 c	0.33
MED	2.41	0.16	1.40 b	0.41 b	1.58 b	0.34 b	0.32
HIGH	2.53	0.13	0.54 c	0.67 a	0.89 c	0.59 a	0.32
Significance[b]	ns	ns	<0.0001	<0.0001	<0.0001	<0.0001	ns
Treatment[a]	**2013**						
	EL 27		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	2.13 a	0.21 b	2.35 a	0.14 c	2.35 a	0.14 c	0.35
MED	n/a	n/a	1.25 b	0.38 b	1.21 b	0.39 b	0.35
HIGH	n/a	n/a	0.07 c	0.65 a	0.12 c	0.61 a	0.35
P-B	0.13 b	0.65 a	0.21 c	0.69 a	0.37 c	0.57 a	0.39
Significance[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns
Treatment[a]	**2014**						
	EL 27		EL 33		EL 35		Pruning weight (kg / m row)
	LLN	CEFA	LLN	CEFA	LLN	CEFA	
NO	2.36 a	0.22 b	2.43 a	0.15 c	1.99 a	0.21 c	0.29
MED	n/a	n/a	1.16 b	0.45 b	1.17 b	0.47 b	0.31
HIGH	n/a	n/a	0.01 c	0.72 a	0.02 c	0.69 a	0.32
P-B	0.00 b	0.78 a	0.11 c	0.69 a	0.00 c	0.75 a	0.26
Significance[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	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.
[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 Student's T-test (only for EL 27 in 2013 and 2014) or Tukey's HSD.

Berry temperature, and ambient temperature and radiation on berry temperature measurement dates: Cabernet franc and Petit Verdot. Berry temperature was largely driven by ambient air temperature, but was confounded by fruit-zone leaf removal and canopy side (Table 4). The linear air-berry temperature relationship was similar between leaf removal treatments at times of day when the sun was not directed on the fruit-zone. Due to the general north-south orientation of the vineyard rows, this was in the afternoon (PM) for the east canopy side and in the morning (AM) for the west canopy side. When the sun was cast on the east canopy side in the AM, leaf removal tended to reduce the ability to predict berry temperature with air temperature when compared to no leaf removal; this was particularly true for HIGH in 2014. The air-berry temperature relationship was most similar between leaf removal treatments around solar noon (NOON), regardless of canopy side. There was a greater difference in the ability to predict berry temperature with air temperature (R^2) between NO and HIGH/P-B when the sun was cast on the west canopy side in the PM in 2013 compared to 2014. It was likely that berry temperature was measured on sunnier afternoons in 2013 compared to 2014, which follows the general sunnier/warmer weather patterns of 2013 compared to 2014 (see above meteorology).

Table 4. Simple linear relationship (R^2) between ambient air and berry temperature as a function of fruit-zone leaf removal, canopy side, and time of day in Cabernet franc and Petit Verdot in 2013 and 2014.

2013[b]						
Cabernet franc						
	EAST			**WEST**		
Treatment[a]	**AM**	**NOON**	**PM**	**AM**	**NOON**	**PM**
NO	0.899	0.964	0.964	0.942	0.964	0.866
MED	0.899	0.964	0.956	0.944	0.963	0.790
HIGH	0.877	0.968	0.925	0.947	0.949	0.825
P-B	0.876	0.961	0.926	0.948	0.951	0.809
Petit Verdot						
	EAST			**WEST**		
Treatment[a]	**AM**	**NOON**	**PM**	**AM**	**NOON**	**PM**
NO	0.920	0.965	0.935	0.915	0.950	0.806
MED	0.883	0.964	0.939	0.940	0.949	0.791
HIGH	0.882	0.952	0.927	0.943	0.916	0.717
P-B	0.907	0.967	0.933	0.955	0.926	0.774
2014[c]						
Cabernet franc						
	EAST			**WEST**		
Treatment[a]	**AM**	**NOON**	**PM**	**AM**	**NOON**	**PM**
NO	0.852	0.845	0.783	0.871	0.782	0.758
MED	0.833	0.853	0.792	0.874	0.787	0.717
HIGH	0.789	0.852	0.805	0.877	0.777	0.752
P-B	0.835	0.831	0.801	0.879	0.772	0.763
Petit Verdot						
	EAST			**WEST**		
Treatment[a]	**AM**	**NOON**	**PM**	**AM**	**NOON**	**PM**
NO	0.796	0.953	0.970	0.933	0.931	0.889
MED	0.779	0.946	0.940	0.926	0.923	0.867
HIGH	0.670	0.939	0.960	0.945	0.945	0.831
P-B	0.722	0.924	0.968	0.931	0.952	0.870

[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.
[b]Data collected on 15 Jul, 29 Jul, 12 Aug, 26 Aug, 10 Sep, and 23 Sep in 2013; berry temperature averaged by each experimental unit and ambient temperature averaged by each time of day; AM = ~900-1030, NOON = 1245-1415; PM = 1545-1715.
[c]Data collected on 8 Jul, 21 Jul, 5 Aug, 19 Aug, 27 Aug, 8 Sep, 23 Sep, and 7 Oct in 2014; berry and ambient temperature averaged by each experimental unit; AM = ~900-1030, NOON = 1245-1415; PM = 1545-1715.

The reduced air-east berry temperature relationship in leaf removal treatment plots appeared to be due to greater and more frequent radiant heating of exposed compared to shaded grapes in the AM of 2014. The frequency of radiant heating of exposed grapes was demonstrated by the relative greater amount of MED, P-B, and (particularly) HIGH data points above the air-berry temperature trend line (Fig. 2 A, B). The magnitude of radiant heating of grapes was demonstrated by the distance of these data points from the air-berry temperature trend line; again particularly in HIGH. The lack of treatment effect on the air-berry temperature relationship around NOON was attributed to the sun being blocked by above-head canopies in the vertically-shoot positioned vineyard. As a result, data points from both canopy sides and all leaf removal treatments were close to the air-berry temperature trend line (Fig. 2 C, D).

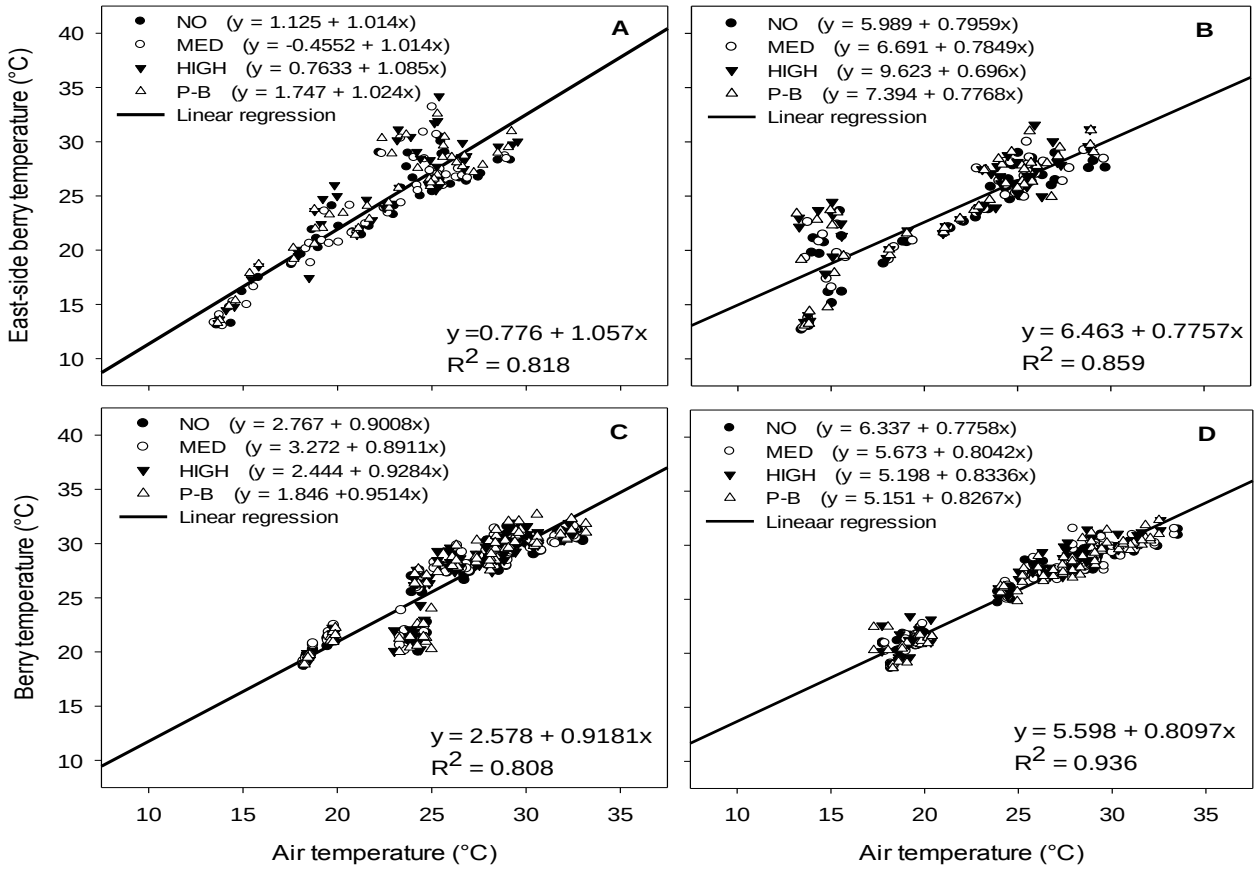

Fig. 2 The linear air-berry temperature relationship for east-exposed berries in the AM (A,B), and for east- and west-exposed berries at NOON (C,D) in Cabernet franc (A,C) and Petit Verdot (B,D) in 2014. NO, MED, and HIGH = no leaf removal, and leaf removal to medium and high extents, respectively; P-B = pre-bloom leaf removal. Each data point represents six berry temperature measurements; n = 45 in A and B; n = 264 for C and D.

The similarity in the air-west berry temperature relationship between leaf removal and no leaf removal plots appeared to be due to more variable cloud coverage in the PM periods on manual berry temperature measurement dates in 2014. There was greater across-date (in 2014) and within-date (in 2013 and 2014) standard deviation of PAR in the PM compared to AM, suggestive of greater variability in sky conditions in the PM (Fig. 3 A, B). Cloudy skies would attenuate radiant heating of grapes and thus limit differences in the air-berry temperature relationship between leaf removal treatments. Average ambient PAR was in fact greater in the AM period than the PM period in 2014 (Fig. 3 B), the year that air-berry temperature trends were generated (Table 4). There was not as great of a difference in ambient PAR between AM and

PM periods in 2013, however (Fig. 3 A). Ambient temperature and PAR were greater during all time periods of berry temperature measurement in 2013 compared to 2014, and the within-date standard deviation of air temperature tended to be greater in the AM compared to NOON or PM.

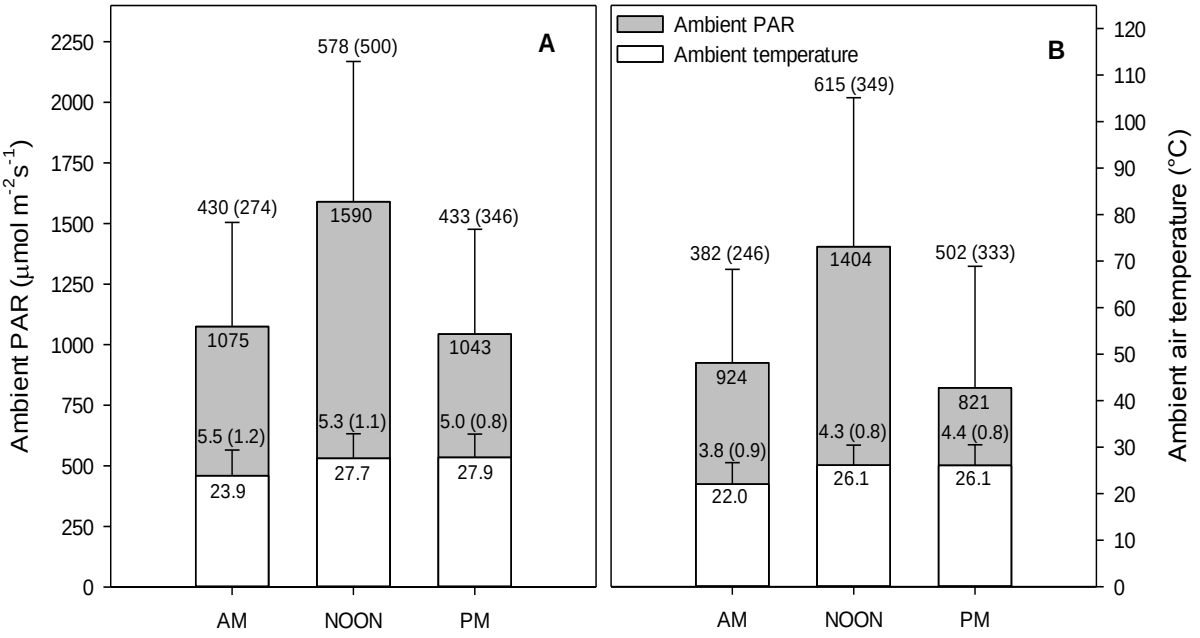

Fig. 3 Average ambient photosynthetically active radiation (PAR) and temperature on berry temperature measurement dates in 2013 (A) and 2014 (B). Category means (under bars) generated across berry temperature measurement dates. The across-date standard deviation (outside parentheses) was averaged across dates; the within-date standard deviation (in parentheses) was averaged within date, then averaged. Standard deviation generated 1 Jun - 28 Oct.

Manual berry temperature measurement in Cabernet franc. When averaged across all treatments, times of day, and measurement dates, Cabernet franc berry temperature was similar between canopy sides (Figs. 4 and 5). In 2013, east side berry temperature was 27.1 °C (Fig. 4 A, B, C) and west side berry temperature was 27.1 °C (Fig. 4 D, E, F). In 2014, east side berry temperature was 25.6 °C (Fig. 5 A, B, C) and west side berry temperature was 25.5 °C (Fig. 5 D, E, F). East and west side berry temperature ranged from 26.6-27.4 °C across all treatments in 2013 (Fig. 4 A-F), and ranged from 25.2-25.9 °C across all treatment in 2014 (Fig. 5 D, E, F). West side berry temperature was greater in the PM compared to east side berry temperature in the AM (Figs. 4 and 5 A, F). Greater differences in berry temperature were observed between

leaf removal treatments when the sun was angled on the fruit-zone. In the AM, east side berry temperature ranged 24.5-26.1 °C across treatments in 2013, and ranged 23.3-24.5 °C across treatments in 2014 (Fig. 4 and 5 A). In the PM, west side berry temperature ranged 30.1-32.0 °C across treatments in 2013, and ranged 28.1-28.7 °C across treatments in 2014 (Fig. 4 and 5 F),

Though ambient PAR levels were greater at NOON compared to AM or PM, leaf removal treatment never affected berry temperature at NOON. Ambient PAR levels were at least one of the top two recorded in each "time of day quadrant" when leaf removal *statistically* increased berry temperature. In the AM on 29 Jul 2013, all leaf removal treatments increased east side berry temperature ($p > F = 0.0046$) when compared to NO (Fig. 4 A). In the PM on 15 Jul 2013, only P-B increased west-side berry temperature ($p > F = 0.0204$) when compared to NO (Fig. 4 F). In 2014, HIGH increased east-side berry temperature when compared to NO in the AM on 8 Jul ($p > F = 0.0171$), 27 Aug ($p > F = 0.0678$), and 23 Sep ($p > F = 0.0058$) (Fig. 4 A), and in PM of 5 Aug ($p > F = 0.0101$) (Fig. 5 C). Additionally, HIGH increased west-side berry temperature ($p > F = 0.0278$) when compared to NO in PM of 5 Aug 2014 (Fig. 5 F).

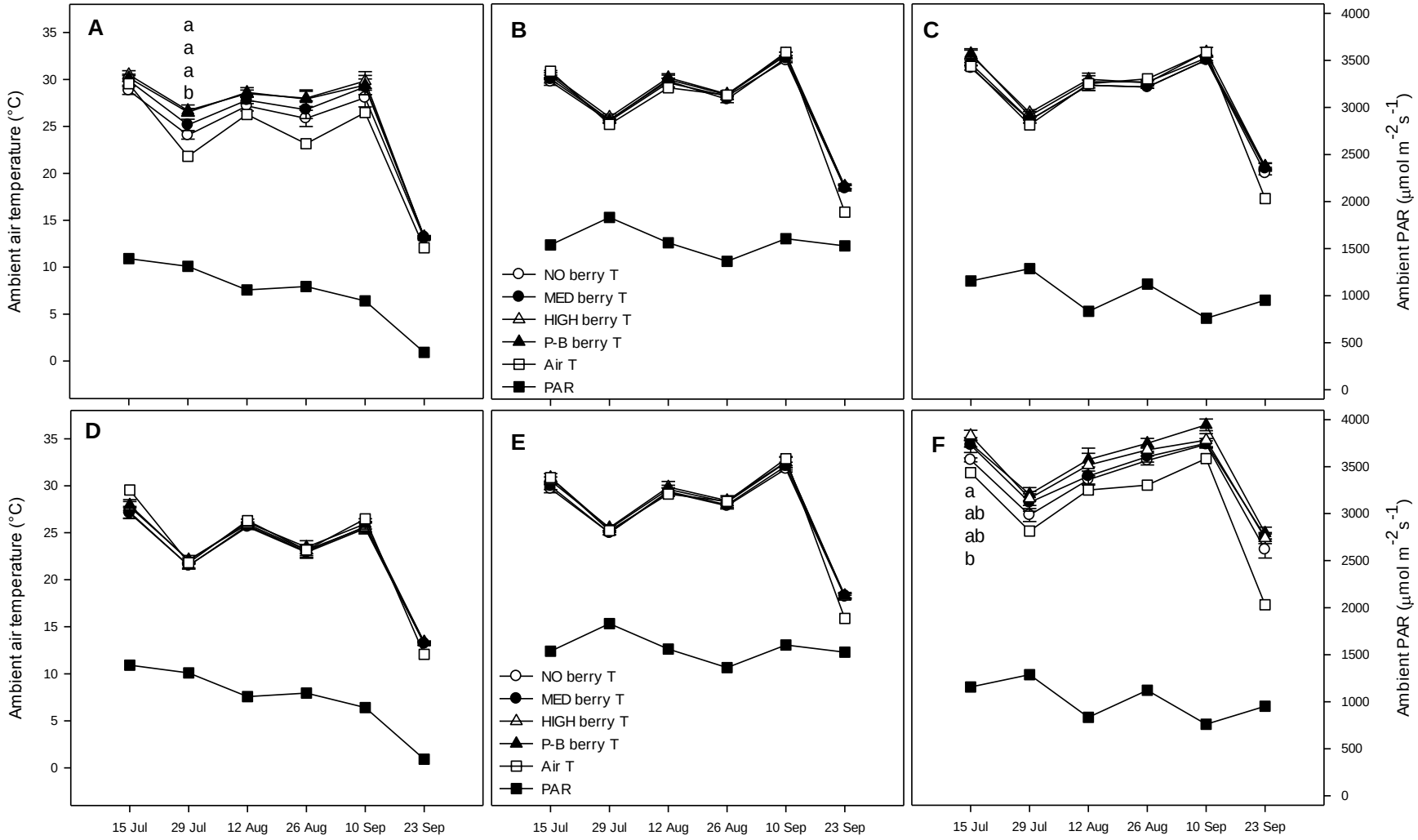

Fig. 4 2013 Cabernet franc berry temperature on EAST (A, B, and C) and WEST (D, E, and F) canopy sides in AM (A and D), NOON (B and E), and PM (C and F) as affected by leaf removal (NO = no removal; MED and HIGH = post-fruit set removal to medium and high extents; P-B = pre-bloom removal). Data points are an average of six measurements in each experimental unit; n = 6. Treatment means within a date not sharing a letter are different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

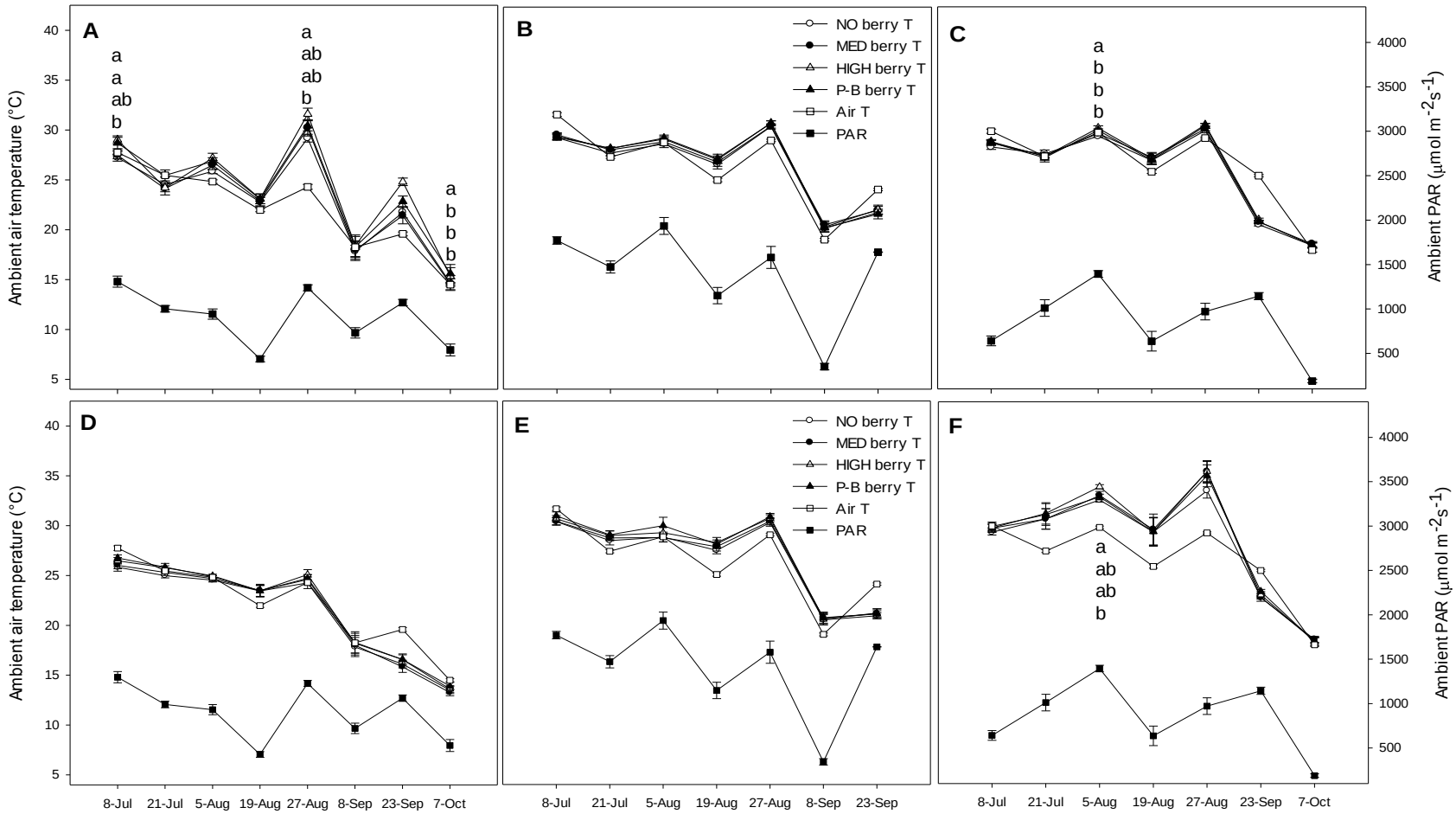

Fig. 5 2014 Cabernet franc berry temperature on EAST (A, B, and C) and WEST (D, E, and F) canopy sides in AM (A and D), NOON (B and E), and PM (C and F) as affected by leaf removal (NO = no removal; MED and HIGH = post-fruit set removal to medium and high extents; P-B = pre-bloom leaf removal). Data points are an average of six measurements in each experimental; unit; n = 6. Treatment means within a date not sharing a letter are different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

Commonly cited critical grape temperature thresholds for anthocyanin accumulation (30 or 35 °C) were observed in Cabernet franc in 2013 and 2014, when 108 and 132 berry temperature measurements (n = 6) were taken on each canopy side of each experimental unit, respectively. In 2013, berry temperature ≥ 30 °C was observed 37 and 39 times in HIGH, 33 and 34 times in MED, 26 and 33 times in NONE, and 40 and 37 times in P-B on the east and west canopy sides, respectively. Berry temperature ≥ 35 °C was observed one time in HIGH on the east canopy side in the PM, and 11 times in HIGH, six times in MED, one time in NONE, and four times in P-B on the west canopy side in the PM in 2013. In 2014, berry temperature ≥ 30 °C was observed 32 and 35 times in HIGH, 19 and 28 times in MED, 13 and 28 times in NONE, and 27 and 35 times in P-B on the east and west canopy sides, respectively. Berry temperature ≥ 35 °C was observed two times in HIGH and NONE, and three times in MED and P-B only on the west canopy side in the PM in 2014. (NOTE: data from above paragraph not shown).

Cabernet franc berries spent more *estimated* time ≥ 30 °C in 2013 compared to 2014, and on the west compared to east canopy side (Table 5). Typically, more aggressive leaf removal required lower ambient air temperature for berries to reach ≥ 30 and 35 °C. Consequently, berries spent more time above these temperatures in leaf removal plots compared to no leaf removal plots. In 2013, berries on both canopy sides spent more time ≥ 30 °C in HIGH plots compared to all other treatment plots, including P-B. In 2014, east-exposed berries attained greatest duration ≥ 30 °C in HIGH plots, and west-exposed berries attained greatest duration ≥ 30 °C in both HIGH and P-B plots. Berry temperature ≥ 35 °C was estimated to occur only in west-exposed berries, and for no more than a total of 1.5 hours in any leaf removal treatment.

Table 5. Leaf removal treatment effect on the simple linear relationship (R^2) between ambient air temperature and Cabernet franc berry temperature over all times of day, and the *estimated* minimum ambient air temperature (T) required for berries to reach, and amount of time spent at, ≥ 30 and 35 °C on east can west canopy sides, in the post-veraison periods of 2013 and 2014.

		EAST-2013[b]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.932	30.1	37.4	36.2	0.0
MED	0.911	29.7	45.6	35.8	0.0
HIGH	0.887	28.9	60.7	34.9	0.0
P-B	0.886	29.1	56.8	35.2	0.0
		WEST-2013[b]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.866	30.0	40.8	35.7	0.0
MED	0.821	29.5	49.9	35.3	0.0
HIGH	0.805	28.7	65.4	34.2	1.5
P-B	0.827	28.9	61.9	34.4	0.9
		EAST-2014[c]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.814	30.2	14.5	35.8	0.0
MED	0.798	29.8	17.9	35.3	0.0
HIGH	0.757	29.4	22.5	34.9	0.0
P-B	0.788	29.6	20.3	35.2	0.0
		WEST-2014[c]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.825	29.2	24.8	33.8	0.5
MED	0.811	28.9	30.0	33.3	0.9
HIGH	0.813	28.7	32.7	33.0	1.2
P-B	0.822	28.7	32.7	33.1	1.1

[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.
[b]Data collected on 15 Jul, 29 Jul, 12 Aug, 26 Aug, 10 Sep, and 23 Sep in 2013; berry temperature averaged by each experimental unit and ambient temperature averaged by each time of day; AM = ~900-1030, NOON = 1245-1415; PM = 1545-1715.
[c]Data collected on 8 Jul, 21 Jul, 5 Aug, 19 Aug, 27 Aug, 8 Sep, 23 Sep, and 7 Oct in 2014; berry and ambient temperature averaged by each experimental unit.

Relatively lower ambient PAR was required to heat berry temperatures ≥ 30 °C in leaf removal plots compared to no leaf removal plots during direct-sun time periods, especially on the east side in the AM (Table 6). This trend was not evident during NOON measurement periods (data not shown). Relatively lower ambient temperature and higher PAR resulted in berry temperatures ≥ 30 °C on the east-side in the AM, while relatively higher ambient temperatures and lower PAR resulted in berry temperatures ≥ 30 °C on the west-side in the PM. Relatively lower ambient PAR was required to heat west-side berries ≥ 35°C in HIGH and P-B plots compared to NONE and MED plots during PM periods.

Table 6. Average ambient temperature (T) and photosynthetically active radiation (PAR) during direct-sun time periods when east and west-side Cabernet franc berry temperature was manually measured ≥ 30 and 35 °C in individual experimental units in 2014.

	≥ 30 °C[b]			
	EAST side in AM		**WEST side in PM**	
Treatment[a]	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**
NO	25.5	1416	29.0	1174
MED	24.5	1299	29.1	1168
HIGH	25.1	1295	29.1	1151
P-B	25.2	1323	29.2	1144
	≥ 35 °C[c]			
	EAST side in AM		**WEST side in PM**	
Treatment[a]	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$))**	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**
NO	n/a	n/a	28.6	1507
MED	n/a	n/a	28.9	1550
HIGH	n/a	n/a	29.4	1406
P-B	n/a	n/a	28.7	1230

[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.
[b]Ambient conditions an average of 2 and 18 (NONE). 4 and 20 (MED), 7 and 21 (HIGH), and 5 and 20 (P-B) measurements on east and west canopy sides respectively
[c]No temperatures ≥ 35 °C were measured on the east side in the AM; ambient conditions an average of 2 (NONE). 3 (MED). 2 (HIGH), and 3 (P-B) measurements on the west canopy side.

Manual berry temperature measurement in Petit Verdot. When averaged across all treatments, times of day, and measurement dates, Petit Verdot berry temperature was similar between canopy sides (Figs. 6 and 7). In 2013, east side berry temperature was 26.9 °C (Fig. 6 A, B, C) and west side berry temperature was 27.0 °C (Fig. 6 D, E, F). In 2014, east and west side berry temperature was 25.1 °C (Fig. 7 A-F). East and west side berry temperature ranged from 26.3-27.5 °C across all treatments in 2013 (Fig. 6 A-F). East and west side berry temperature ranged from 24.8-25.4 °C across all treatments in 2014 (Fig. 7 A-F). West side berry temperature was greater in the PM compared to east-side berry temperature in the AM. Greater differences in berry temperature were observed between leaf removal treatments when the sun was angled on the fruit-zone. In the AM, east side berry temperature ranged 24.4-26.0 °C across treatments in 2013, and ranged 22.9-23.9 °C across treatments in 2014 (Fig. 6 and 7 A). In the PM, west side berry temperature ranged 29.6-31.9 °C across treatments in 2013, and ranged 27.1-28.0 °C across treatments in 2014 (Fig. 6 and 7 F).

Though ambient PAR levels were greater at NOON compared to AM or PM, leaf removal treatment never affected berry temperature at NOON. Leaf removal *statistically* increased Petit Verdot berry temperature when PAR levels were relatively high, as on the following dates. In the PM of 15 Jul 2013, HIGH and P-B increased east side berry temperature ($p > F = 0.0002$), and all leaf removal treatments increased west side berry temperature ($p > F = 0.0027$), when compared to NO (Fig. 6 C, F). In the PM of 26 Aug 2013, HIGH increased east side berry temperature compared to NO ($p> F = 0.0174$), and both HIGH and P-B increased west side berry temperature ($p > F = 0.0002$), when compared to NO and MED (Fig. 6 C, F). In the PM of 23 Sep 2013, HIGH increased west side berry temperature ($p > F = 0.0025$) compared to NO and MED (Fig. 6 F). In the AM on 23 Sep 2014, both HIGH and P-B had greater east side

berry temperature ($p > F = 0.0027$) when compared to NO and MED (Fig. 7 A), and, in the PM on 5 Aug 2014, HIGH had greater east side berry temperature ($p > F = 0.0082$) when compared to NO and MED (Fig. 7 C).

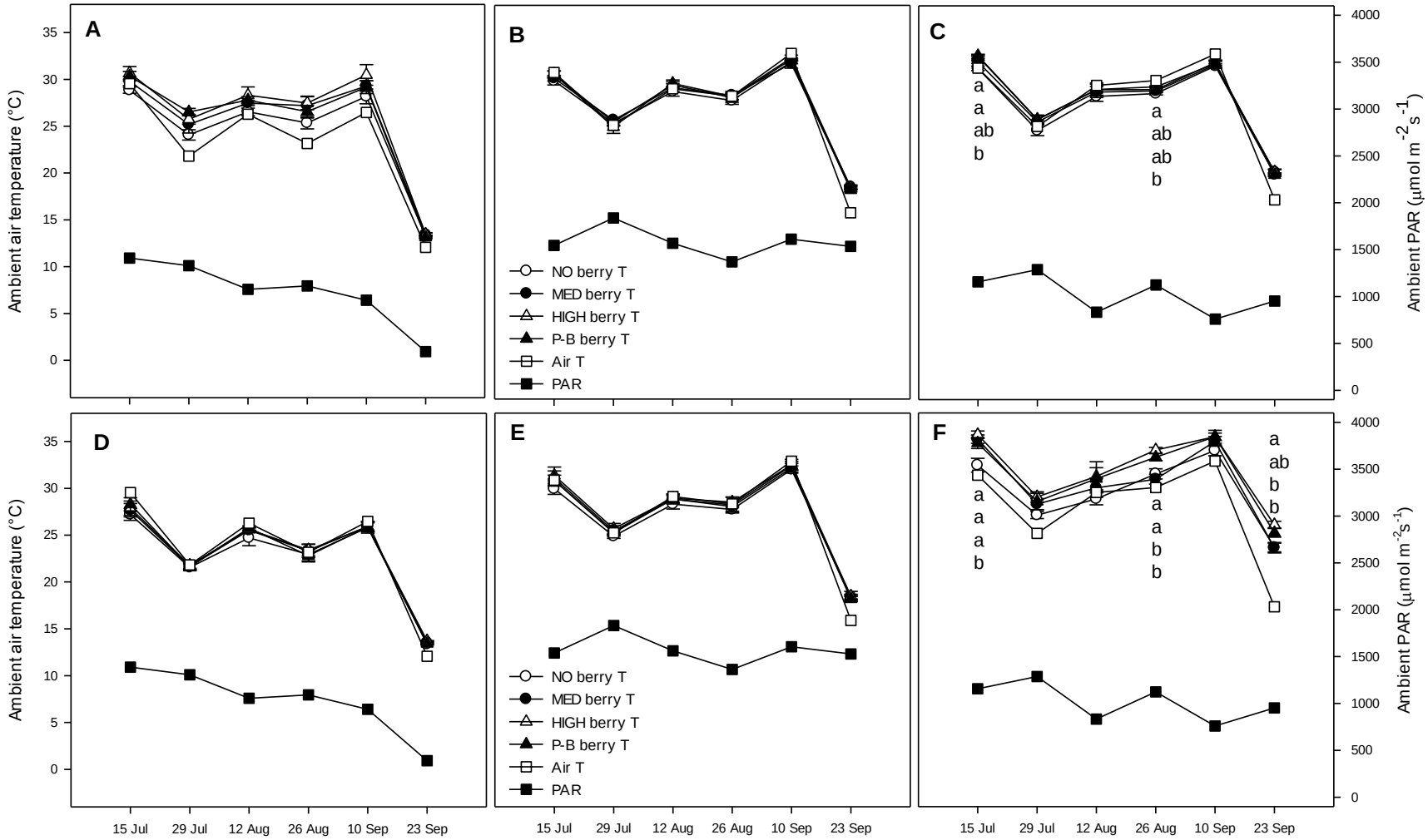

Fig. 6 2013 Petit Verdot berry temperature on EAST (A, B, and C) and WEST (D, E, and F) canopy sides in AM (A and D), NOON (B and E), and PM (C and F) as affected by leaf removal (NO = no removal; MED and HIGH = post-fruit set removal to medium and high extents; P-B = pre-bloom leaf removal). Data points are an average of six measurements in each experimental unit; n = 6. Treatment means within a date not sharing a letter are different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

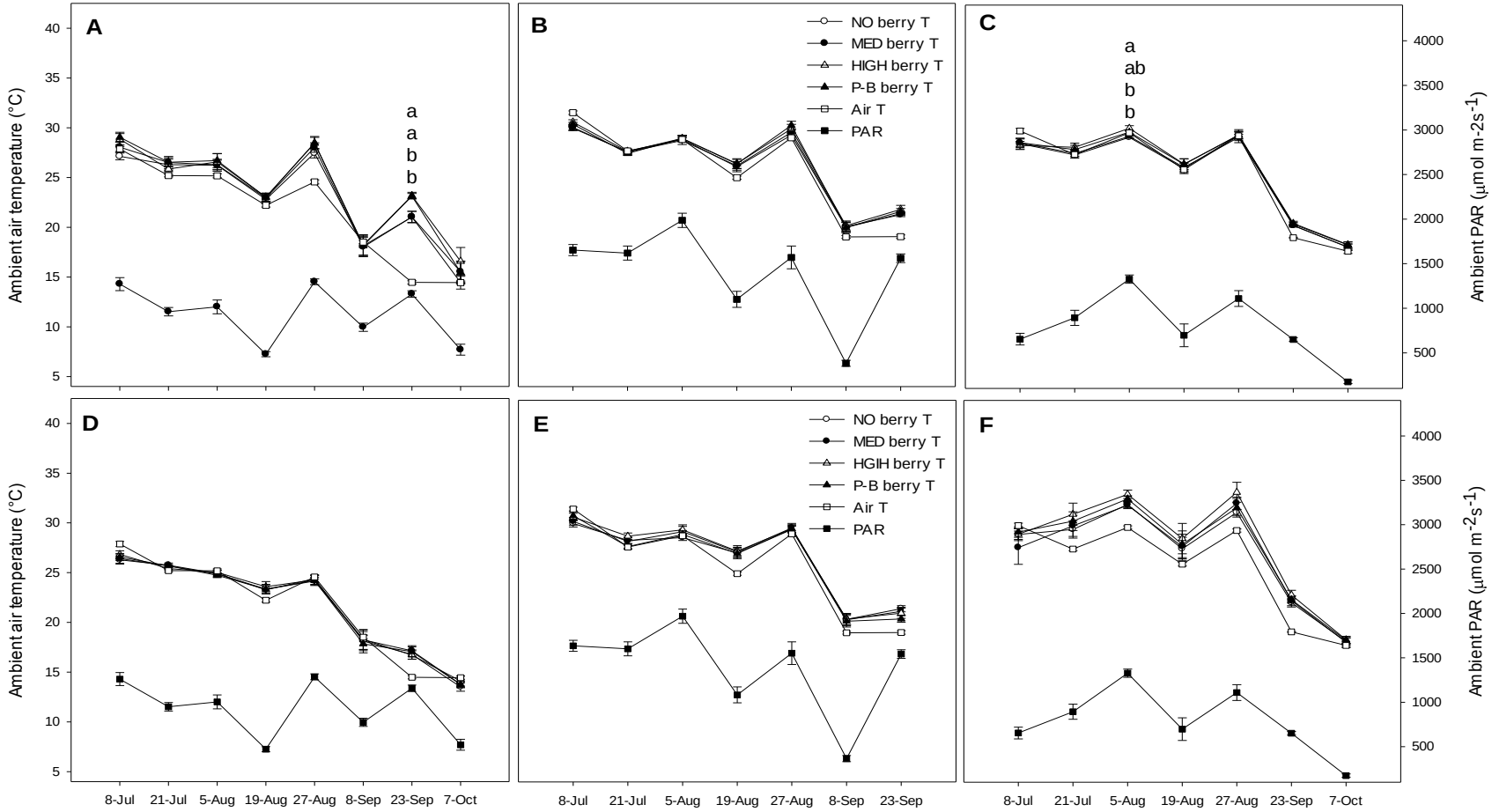

Fig. 7 2014 Petit Verdot berry temperature on EAST (A, B, and C) and WEST (D, E, and F) canopy sides in AM (A and D), NOON (B and E), and PM (C and F) as affected by leaf removal (NO = no removal; MED and HIGH = post-fruit set removal to medium and high extents; P-B = pre-bloom removal). Data points are an average of six measurements in each experimental unit; n = 6. Treatment means within a date not sharing a letter are different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

Commonly cited critical grape temperature thresholds for anthocyanin accumulation (30 or 35 °C) were observed in Petit Verdot in 2013 and 2014, when 108 and 132 berry temperature measurements (n = 6) were taken on each canopy side of each experimental unit, respectively. In 2013, berry temperature ≥ 30 °C was observed 33 and 34 times in HIGH, 30 and 29 times in MED, 24 and 27 times in NONE, and 33 and 33 times in P-B on the east and west canopy sides, respectively. Berry temperature ≥ 35 °C was observed one time in HIGH on the west canopy side at NOON, and 12 times in HIGH, seven times in MED, three times in NONE, and eight times in P-B on the west canopy side in the PM in 2013. In 2014, berry temperature ≥ 30 °C was observed 17 and 28 times in HIGH, 9 and 24 times in MED, 6 and 22 times in NONE, and 14 and 26 times in P-B on the east and west canopy sides, respectively. Berry temperature ≥ 35 °C was observed two times in HIGH only on the west canopy side in the PM in 2014 (NOTE: data from above paragraph not shown).

Petit Verdot berries spent more *estimated* time ≥ 30 °C in 2013 compared to 2014, and on the west compared to east canopy side (Table 7). As leaves were removed more aggressively, lower ambient air temperature was required for berries to reach ≥ 30 and 35 °C. Consequently, berries spent more time above these temperatures in leaf removal plots compared to no leaf removal plots. Besides east-exposed berries in 2014, in which case there was little difference in time that berries spent ≥ 30 °C between treatments, the time that berries spent ≥ 30 °C was in the following order of hierarchy: HIGH > P-B > MED > NO. Berry temperature ≥ 35 °C was estimated to rarely occurred in Petit Verdot.

Table 7. Leaf removal treatment effect on the simple linear relationship (R^2) between ambient air temperature and Petit Verdot berry temperature over all dates and times of day, and the *estimated* minimum ambient air temperature (T) required for berries to reach, and amount of time spent at, ≥ 30 and 35 °C on east can west canopy sides, during the post-veraison periods in 2013 and 2014.

		EAST-2013[b]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.933	30.6	30.6	36.7	0.0
MED	0.899	29.9	42.9	36.1	0.0
HIGH	0.875	29.3	52.9	35.4	0.0
P-B	0.908	29.5	49.6	35.5	0.0
		WEST-2013[b]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.850	30.4	32.8	36.5	0.0
MED	0.845	29.5	49.6	35.3	0.0
HIGH	0.774	28.8	61.7	34.5	0.7
P-B	0.811	29.1	57.5	34.7	0.4
		EAST-2014[c]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.897	30.6	0.9	36.9	0.0
MED	0.875	30.3	1.0	36.6	0.0
HIGH	0.827	30.2	1.0	36.9	0.0
P-B	0.841	30.1	1.0	36.5	0.0
		WEST-2014[c]			
		≥ 30 °C		**≥ 35 °C**	
Treatment[a]	**R^2**	**Minimum air T (°C)**	**Time (hrs.)**	**Minimum air T (°C)**	**Time (hrs.)**
NO	0.906	29.7	1.4	34.5	0.0
MED	0.897	29.4	1.7	34.7	0.0
HIGH	0.887	28.9	3.5	34.0	0.0
P-B	0.905	29.3	2.0	34.6	0.0

[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.
[b]Data collected on 15 Jul, 29 Jul, 12 Aug, 26 Aug, 10 Sep, and 23 Sep in 2013; berry temperature averaged by each experimental unit and ambient temperature averaged by each time of day; AM = ~900-1030, NOON = 1245-1415; PM = 1545-1715.
[c]Data collected on 8 Jul, 21 Jul, 5 Aug, 19 Aug, 27 Aug, 8 Sep, 23 Sep, and 7 Oct in 2014; berry and ambient temperature averaged by each experimental unit.

The only time that Petit Verdot berry temperature was measured ≥ 30 °C in NO plots was on the west canopy side in the PM (Table 8). The only time that berry temperature was measured ≥ 35 °C was when it was measured in HIGH plots on the west canopy side in the PM. Relatively lower ambient temperature and higher PAR resulted in berry temperatures ≥ 30 °C on the east-side in the AM, while relatively higher ambient temperatures and lower PAR resulted in berry temperatures ≥ 30 °C on the west-side in the PM.

Table 8. Average ambient temperature (T) and photosynthetically active radiation (PAR) during direct-sun time periods when east and west-side Petit Verdot berry temperature was manually measured ≥ 30 and 35 °C in individual experimental units in 2014.

≥ 30 °C[b]				
	EAST side in AM		**WEST side in PM**	
Treatment[a]	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**
NO	n/a	n/a	29.2	1187
MED	25.5	1494	29.3	1233
HIGH	27.2	1539	29.1	1281
P-B	27.3	1536	29.2	1243
≥ 35 °C[c]				
	EAST side in AM		**WEST side in PM**	
Treatment[a]	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**	**Ambient T (°C)**	**Ambient PAR (µmol $m^{-2}s^{-1}$)**
NO	n/a	n/a	n/a	n/a
MED	n/a	n/a	n/a	n/a
HIGH	n/a	n/a	29.6	1401
P-B	n/a	n/a	n/a	n/a

[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.
[b]No temperatures ≥ 30 °C were measured on the east side in the AM in NO plots; ambient conditions an average of 0 and 18 (NONE). 1 and 16 (MED), 3 and 19 (HIGH), and 2 and 17 (P-B) measurements on east and west canopy sides respectively
[c]No temperatures ≥ 35 °C were measured on the east side in the AM nor on the west-side in the PM in NO, MED, and P-B plots; ambient conditions in HIGH an average of 2 measurements on the west canopy side.

Crop yield components and vine fruitfulness: Cabernet franc. Post-fruit set leaf removal had no effect on components of yield in Cabernet franc in 2012 (Table 9). When averaged over 2013-2014 and compared to NO, P-B reduced cluster number per vine by 10% (significant only in 2014), berry weight by 6%, berry number per cluster by 33%, cluster weight by 39%, and crop yield by 50%. Due to lower crop yield without a concomitant decrease in dormant cane pruning weight (see Table 3), P-B reduced crop load by an average (2013-2014) of 37% when compared to NO. The only time that a post-fruit set leaf removal treatment affected crop yield components over 2013-2104 was when MED reduced berry number per cluster by 20%, cluster weight by 23%, and crop yield by 26% when compared to NO in 2013. When pre-bloom leaf removal treatments originally implemented in 2013 were re-implemented in the same plots in 2014 (P-B '13) and compared to no leaf removal (NO '13), P-B '13 reduced berry weight by 13%, berry number per cluster by 42%, cluster weight by 50%, cluster number per vine by 21%, and crop yield by 61%. Count shoot fruitfulness was reduced by 0.18 cluster/shoot in 2015 due to repeated pre-bloom leaf removal implementation over 2013-2014 (P-B '13), and basal shoot fruitfulness was reduced by 0.09 cluster/shoot due to pre-bloom leaf removal implementation in the previous season (P-B '14).

Table 9. Pre-bloom and post-fruit set leaf removal effect on crop yield components, cluster compactness, crop load, and count and basal shoot fruitfulness in Cabernet franc, 2012-2014.

Treatment[a]	Crop yield (kg / vine)	Cluster # / vine	Cluster weight (g)	Berry # /cluster	Berry weight (g)	Crop load	Fruitfulness[b] (count / basal)
2012							
NO	4.11	31	131.9	85	1.55	7	n/a
MED	3.44	28	123.1	78	1.58	6.1	n/a
HIGH	3.49	29	121.9	81	1.51	5.6	n/a
Significance[c]	ns	ns	ns	ns	ns	ns	n/a
2013							
NO	2.90 a	36	83.5 a	60 a	1.38	4.2 a	n/a
MED	2.14 bc	36	64.3 b	48 bc	1.36	3.7 ab	n/a
HIGH	2.38 ab	37	76.6 a	56 ab	1.38	3.9 ab	n/a
P-B	1.61 c	34	55.6 b	42 c	1.35	2.9 ab	n/a
Significance[c]	<0.0001	ns	<0.0001	<0.0001	ns	0.0599	n/a
2014							
NO	2.83 a	51 a	54.2 a	38 a	1.43 a	5.0 a	1.63 / 0.44
MED	2.93 a	48 ab	60.4 a	41 a	1.47 a	5.4 a	n/a
HIGH	2.40 a	47 ab	50.5 a	35 a	1.43 a	4.8 a	n/a
P-B	1.24 b	44 b	28.5 b	22 b	1.29 b	2.9 b	1.38 / 0.36
Significance[c]	<0.0001	0.0391	<0.0001	<0.0001	0.0008	0.0006	ns
Carryover effects on 2014 crop yield / 2015 vine fruitfulness							
NO '13	3.98 a	48 a	81.8 a	50 a	1.63 a	6.7 a	1.75 / 0.26
PB- '13	1.57 b	38 b	41.3 b	29 b	1.42 b	3.3 b	1.57 / 0.26
Significance[c]	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	0.0383 / ns
NO '14	n/a	n/a	n/a	n/a	n/a	n/a	1.67 / 0.30 a
P-B '14	n/a	n/a	n/a	n/a	n/a	n/a	1.50 / 0.21 b
Significance[c]	n/a	n/a	n/a	n/a	n/a	n/a	ns / 0.0396

[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; NO '13, P-B '13 = no and pre-bloom leaf removal originally implemented in 2013 re-implemented in 2014, respectively; NO '14 and P-B '14 = no and pre-bloom leaf removal originally implemented in 2014.

[b]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.

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

*Means in same treatment group not sharing a letter are significantly different at 0.05 level based on adjusted p-values using Student's T-test (only 2014 carryover effects and 2015 vine fruitfulness) or Tukey's HSD.

Petit Verdot. Post-fruit set leaf removal to the medium extent (MED) reduced crop yield compared to no leaf removal (NO) in 2012 (Table 10). When averaged over 2013-2014 and compared to the average of all other treatments, P-B reduced cluster number per vine by 29% (significant only in 2014), berry weight by 25%, berry number per cluster by 18% (significant only in 2013), cluster weight by 37%, and crop yield by 53%. Post-fruit set leaf removal did not affect any component of yield in 2013, but in 2014, HIGH reduced cluster number per vine by 25% when compared NO. Pre-bloom leaf removal (P-B) reduced crop load only when compared to MED (66%) and NO (72%) in 2014. When pre-bloom leaf removal treatments originally implemented in 2013 were re-implemented in the same plots in 2014 (P-B '13) and compared to no leaf removal (NO '13), P-B '13 reduced berry weight by 24%, berry number per cluster by 36%, cluster weight by 50%, cluster number per vine by 35%, and crop yield by 66%. Count shoot fruitfulness in 2014 was increased by P-B when compared to NO, albeit only by 0.13 clusters/shoot. Count shoot fruitfulness was reduced by 0.18 clusters/shoot in 2015 due to repeated pre-bloom leaf removal implementation over 2013-2014 (P-B '13), and by 0.25 cluster/shoot due to pre-bloom leaf removal implementation in the previous season (P-B '14).

Table 10. Pre-bloom and post-fruit set leaf removal effect on crop yield components, cluster compactness, crop load, and count and basal shoot fruitfulness in Petit Verdot, 2012-2014.

Treatment[a]	Crop yield (kg / vine)	Cluster # / vine	Cluster weight (g)	Berry # /cluster	Berry weight (g)	Crop load	Fruitfulness[b] (count / basal)
			2012				
NO	3.69 a	54	68.3	59	1.16	6.2	n/a
MED	2.96 b	48	61.5	55	1.12	5.2	n/a
HIGH	3.41 ab	50	67.6	59	1.15	6.3	n/a
Significance[c]	0.0213	ns	ns	ns	ns	ns	n/a
			2013				
NO	6.30 a	68	93.4 a	75 a	1.25 a	10.0 a	n/a
MED	5.91 a	64	92.4 a	75 a	1.23 a	8.9 a	n/a
HIGH	5.58 a	59	93.8 a	76 a	1.23 a	9.6 a	n/a
P-B	3.68 b	57	64.4 b	63 b	1.02 b	5.3 b	n/a
Significance[c]	<0.0001	ns	<0.0001	0.003	<0.0001	0.0002	n/a
			2014				
NO	1.95 a	64 a	30.1 a	30	1.00 a	3.9 a	2.38 b / 1.24
MED	1.67 a	60 a	27.2 a	29	0.93 a	3.2 ab	n/a
HIGH	1.31 a	48 b	26.1 a	29	0.91 a	2.3 bc	n/a
P-B	0.54 b	33 c	16.1 b	24	0.66 b	1.1 c	2.51 a / 1.24
Significance[c]	0.0002	<0.0001	0.0004	ns	<0.0001	0.0005	0.0177 / ns
			Carryover effects on 2014 crop yield / 2015 vine fruitfulness				
NO '13	2.98 a	69 a	43.3 a	42 a	1.05 a	4.9 a	2.29 a/ 0.95
PB- '13	1.00 b	45 b	21.5 b	27 b	0.80 b	1.5 b	2.11 b/ 0.93
Significance[c]	<0.0001	<0.0001	<0.0001	0.0017	0.0003	<0.0001	0.0350 / ns
NO '14	n/a	n/a	n/a	n/a	n/a	n/a	2.26 a / 0.98
P-B '14	n/a	n/a	n/a	n/a	n/a	n/a	2.01 b / 0.87
Significance[c]	n/a	n/a	n/a	n/a	n/a	n/a	0.0185 / 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; NO '13, P-B '13 = no and pre-bloom leaf removal originally implemented in 2013 re-implemented in 2014, respectively; NO '14 and P-B '14 = no and pre-bloom leaf removal originally implemented in 2014.

[b]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.

[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 Student's T-test (only 2014 carryover effects and 2015 vine fruitfulness) or Tukey's HSD.

Pre-bloom reduction in crop yield components over time: Cabernet franc and Petit Verdot. When pre-bloom leaf removal yield components were expressed as a percent reduction compared to no leaf removal, cluster number per vine, berry weight, berry number per cluster, cluster weight, and crop yield tended to be *further* reduced in the second year of a two-year consecutive treatment implementation (Fig. 8). Berry weight was the yield component that was least affected by the second consecutive year of pre-bloom leaf removal implementation. In Cabernet franc (Fig. 8 A), the following yield components were *further* reduced in the second consecutive year of pre-bloom leaf removal implementation: cluster number per vine (16%), berry weight (7%), berry number per cluster (13%), cluster weight (16%), and crop yield (16%). In Petit Verdot (Fig. 8 B), the following yield components were *further* reduced in the second consecutive year of pre-bloom leaf removal implementation: cluster number per vine (18%), berry weight (5%), berry number per cluster (19%), cluster weight (19%), and crop yield (25%).

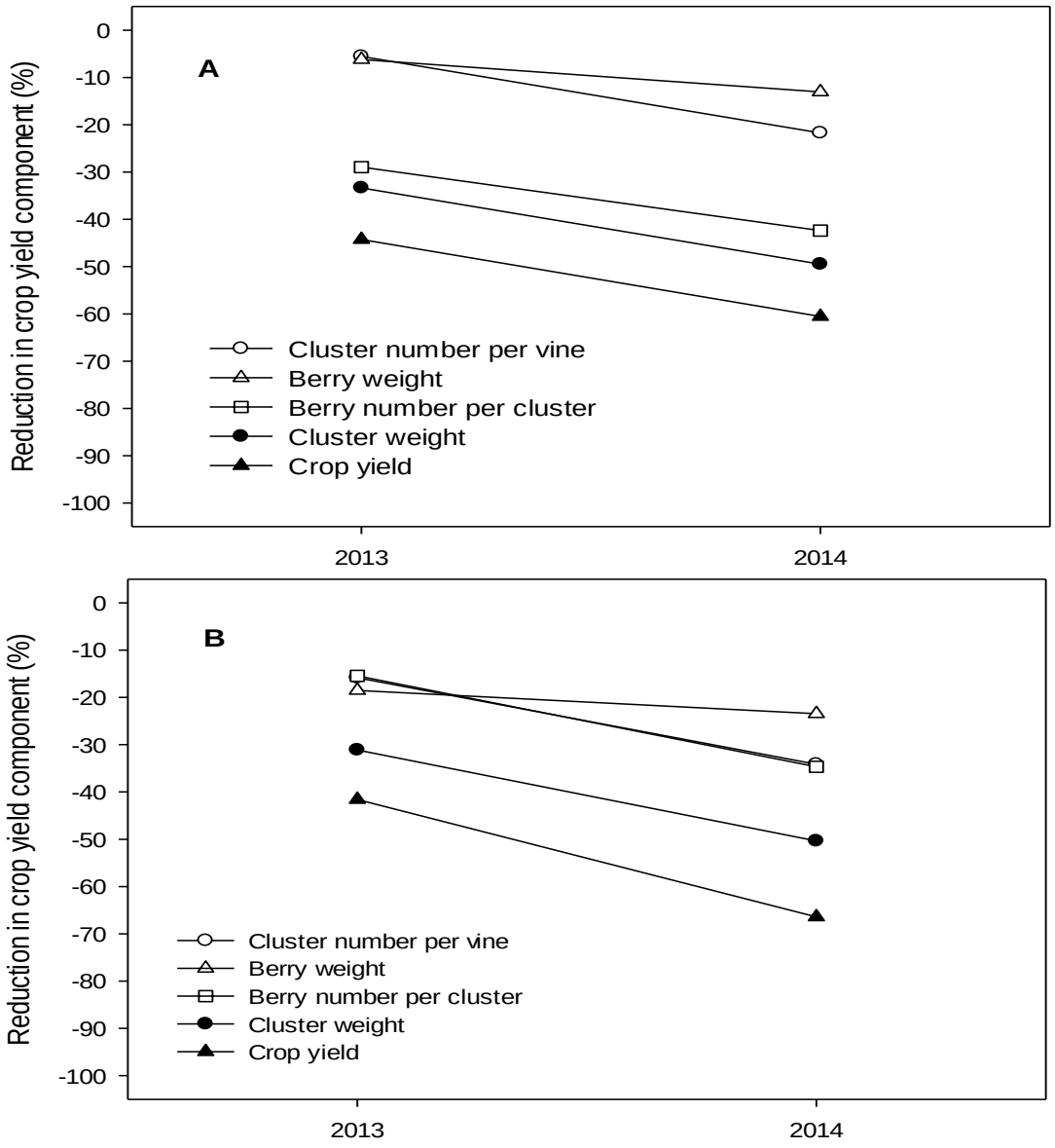

Fig. 8. The effect of pre-bloom leaf removal implementation in two consecutive years on the percent reduction of crop yield components when compared to no leaf removal in Cabernet franc (A) and Petit Verdot (B). Each data point is an average of 30 vines, except berry weight, which is an average over each five-vine experimental unit; n = 6. NOTE: data not collected in these plots in 2012.

Pre-bloom leaf removal effect on the proportional reduction in crop yield components: Cabernet franc and Petit Verdot. The proportional reduction in crop yield components, presented as *ratios* of crop yield components: crop yield, were studied to investigate which component of yield most explained the general crop yield depression due to pre-bloom leaf removal (Table 11). The proportional reduction in cluster weight was similar between varieties, and greater than the proportional reduction in other yield components. Thus, reduction in cluster weight was most responsible for crop yield reduction in both varieties. However, the proportional reduction in berry number per cluster was reduced by an average of 52% more so in Cabernet franc compared to Petit Verdot. By contrast, the proportional reduction in cluster number per vine and berry weight were reduced by an average of 88% and 122% more so, respectively, in Petit Verdot compared to Cabernet franc. Thus, berry number per cluster reduction was relatively more responsible for yield reduction in Cabernet franc, whereas the reduction in cluster number per vine and berry weight were more responsible for yield reduction in Petit Verdot.

Table 11. The effect of pre-bloom leaf removal on the *ratio* of % reduction* in yield components: % reduction* in crop yield in Cabernet franc and Petit Verdot in 2013 and 2014.

Year	Cluster # /vine: crop yield	Cluster weight: crop yield	Berry # / cluster: crop yield	Berry weight: crop yield
		Cabernet franc		
2013	0.12	0.75	0.65	0.14
2014	0.36	0.82	0.70	0.22
		Petit Verdot		
2013	0.38	0.75	0.37	0.45
2014	0.51	0.76	0.52	0.35

*% reduction calculated in comparison to no leaf removal (NO).

The means that pre-bloom leaf removal reduced cluster weight was variety-dependent (Table 12). The following % reductions in ratios are all averaged over 2013-2014. The ratio of berry weight: berry number per cluster reduction was 252% greater in Petit Verdot compared to Cabernet franc; the ratio opposite this (berry number per cluster: berry weight) was 237% greater

in Cabernet franc compared to Petit Verdot. Similarly, the ratio of berry weight: cluster weight reduction was 130% greater in Petit Verdot compared to Cabernet franc, whereas the ratio of berry number per cluster: cluster weight reduction was 46% greater in Cabernet franc compared to Petit Verdot. This, again, illustrated that pre-bloom leaf removal had a greater impact on berry number per cluster in Cabernet franc while and berry weight was more substantially affected in Petit Verdot.

Table 12. The effect of pre-bloom leaf removal on the *ratio* of % reduction* in components of cluster weight in Cabernet franc and Petit Verdot in 2013 and 2014.

Year	Berry weight: berry # / cluster	Berry # / cluster: berry weight	Berry weight: cluster weight	Berry # / cluster: cluster weight
		Cabernet franc		
2013	0.22	4.64	0.19	0.87
2014	0.31	3.18	0.27	0.85
		Petit Verdot		
2013	1.22	0.82	0.60	0.49
2014	0.67	1.49	0.46	0.68

*% reduction calculated in comparison to no leaf removal (NO)

Components of cluster compactness: Cabernet franc and Petit Verdot. In 2013, cluster compactness was not affected by any leaf removal treatment in Cabernet franc (Table 13). Pre-bloom leaf removal (P-B) reduced berry number per cluster to the greatest extent (32%) when compared to NO. However, pre-bloom leaf removal resulted in numerically shorter rachis lengths. The result was a numerical reduction in cluster compactness (18%) compared to all other treatments. In 2014, P-B significantly reduced Cabernet franc cluster compactness when compared to NO (39%) and MED (48%) due primarily to a similar reduction in berry number per cluster compared to NO (38%) and MED (44%). In 2013, P-B reduced Petit Verdot cluster compactness compared to HIGH (20%) due to a 15% reduction in berry number per cluster and minimal reduction in rachis length (7%). In 2014, all leaf removal treatments reduced cluster

compactness compared to NO (average of 22%) due to numerically longer rachis lengths (10%) and similar berry numbers per cluster.

Table 13. Post-fruit set and pre-bloom leaf removal effect on components of cluster compactness in 2013 and 2014.

Treatment[a]	Cabernet franc			Petit Verdot		
	Berry # per cluster	Rachis length (cm)	Cluster compactness[c]	Berry # per cluster	Rachis length (cm)	Cluster compactness[b]
	2013			2013		
NO	56 a	9.62	6.08	37 b	9.55	4.05 a
MED	45 c	8.45	5.54	41 a	10.11	4.69 a
HIGH	49 b	8.71	5.87	35 b	10.33	3.47 ab
P-B	38 d	7.95	5.01	23 c	9.79	2.46 b
Significance[c]	<0.0001	ns	ns	<0.0001	ns	0.0061
	2014			2014		
NO	75 a	8.34	9.21 ab	30	7.61	4.24 a
MED	75 a	8.47	9.12 ab	28	8.37	3.42 b
HIGH	76 a	8.19	9.68 a	29	8.83	3.40 b
P-B	61 b	7.59	8.23 b	24	8.01	3.13 b
Significance[c]	<0.0001	ns	0.0092	ns	ns	0.0066

[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.
[b]Berry number per length of main rachis; cluster shoulders not counted or measured.
[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's HSD.

Pre-harvest berry weight: Cabernet franc. In 2013, leaf removal inconsistently affected Cabernet franc berry weight (Fig. 9 A). When compared to NO, P-B reduced berry weight by 0.05-0.08 g on 14 Jul and 19 Sep, and both P-B and HIGH reduced berry weight on 26 Jul. Berry weight was more consistently reduced by P-B in 2014 (Fig 9 B). In 2014, pre-bloom leaf removal (P-B) reduced berry weight by 10-12% during the 11 Aug through 14 Oct period when compared to all other treatments. Additionally, P-B reduced berry weight compared to MED on 17 Jul, and compared to NO and MED on 29 Jul. Berry weight increase was 0.04 g less in P-B compared to other leaf removal treatments over 29 Jul to 11 Aug. Berry weight was lower on the west (Fig. 9

D) compared to east (Fig. 9 C) canopy side in the post-veraison of 2014. It was of interest to evaluate if there were canopy side differences in berry weight due to hail damage on the west canopy side in 2014. Canopy side differences in berry weight were greatest in HIGH (0.11-0.21 g), moderate in MED, and least in NO. On the east canopy side, P-B reduced berry weight compared to all treatments on 11 Aug (0.11 g) and 2 Sep (0.18 g), and only when compared to MED and HIGH on 22 Sep (0.17 g), and MED on 14-Oct (0.18 g) (Fig. 9 C). On the west canopy side, P-B reduced berry weight compared to all treatments on 11 Aug (0.10 g) and 14-Oct (0.16 g), and compared to NO and MED on 22-Sep (0.16 g).

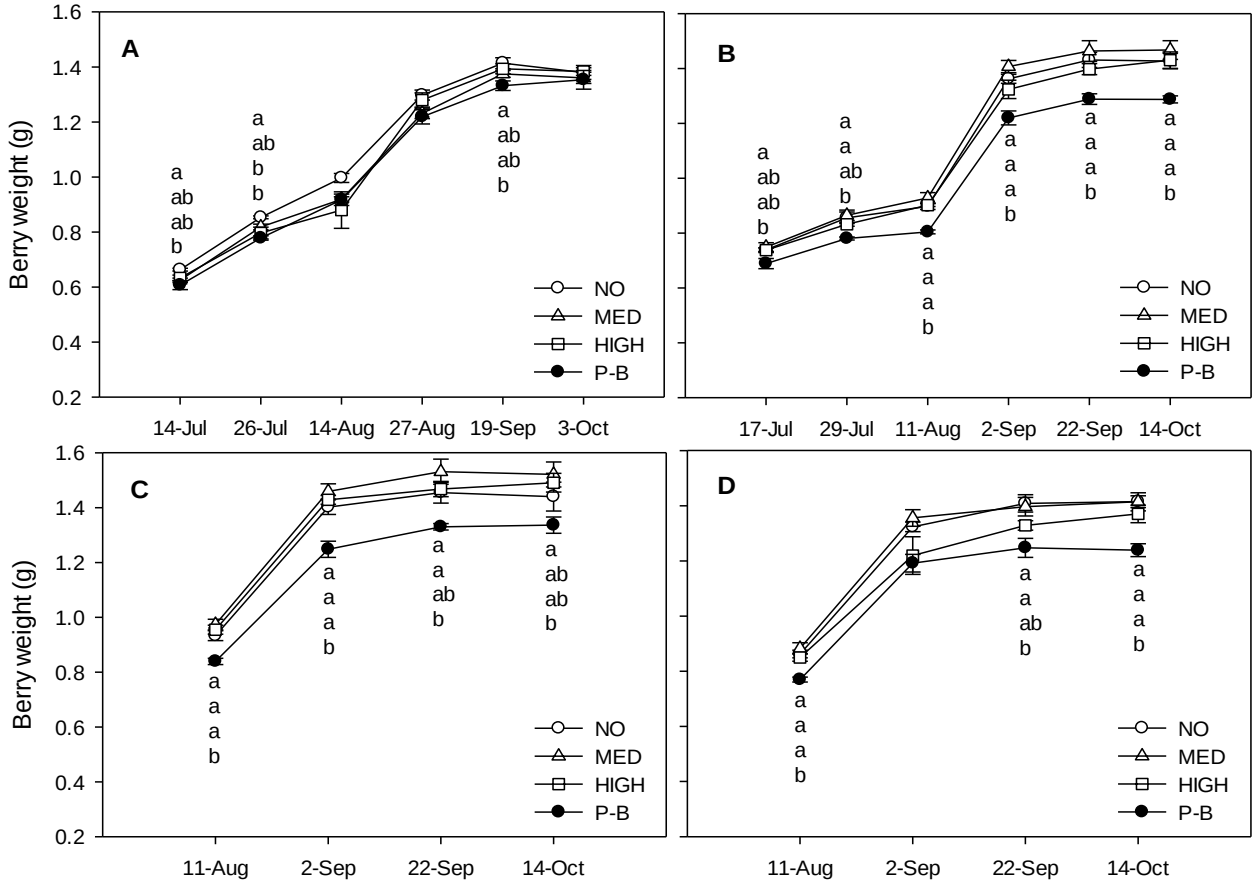

Fig. 9. Pre-bloom (P-B), post-fruit set (MED = medium extent, HIGH = high extent), and no (NO) leaf removal effect on Cabernet franc berry weight over the course of 2013 (A) and 2014 (B = average, C = east canopy side, D = west canopy side). Each data point is an average of 120 (pre-harvest) or 150 (harvest) berries evenly collected from all vines in each experimental unit; n = 6. Error bars are +/- standard error.

Petit Verdot. In 2013, P-B reduced berry weight compared to all other leaf removal treatments on every sampled date (Fig.10 A). The difference in berry weight increased over time due to a relatively slower increase in berry weight in P-B compared to other treatments. Berry weight increase was 0.03-0.08 g less in P-B compared to other treatments, depending on sample date. In 2014, P-B reduced berry weight on every sampling date compared to all other leaf removal treatments (Fig. 10 B). Similar to 2013, berry weight increase was 0.03 – 0.09 g less in P-B compared to other treatments, depending on the time period observed. It was of interest to evaluate if there were canopy side differences in berry weight due to hail damage on the west canopy side in 2014. Petit Verdot berry weight was lower on the west (Fig. 10 D) compared to east (Fig. 10 C) canopy side, albeit to a lesser magnitude than in Cabernet franc. Pre-bloom leaf removal (P-B) reduced berry weight on both canopy sides by a range of 0.13-0.32 g, compared to other treatments; however, that reduction was less on the west compared to east side canopy side.

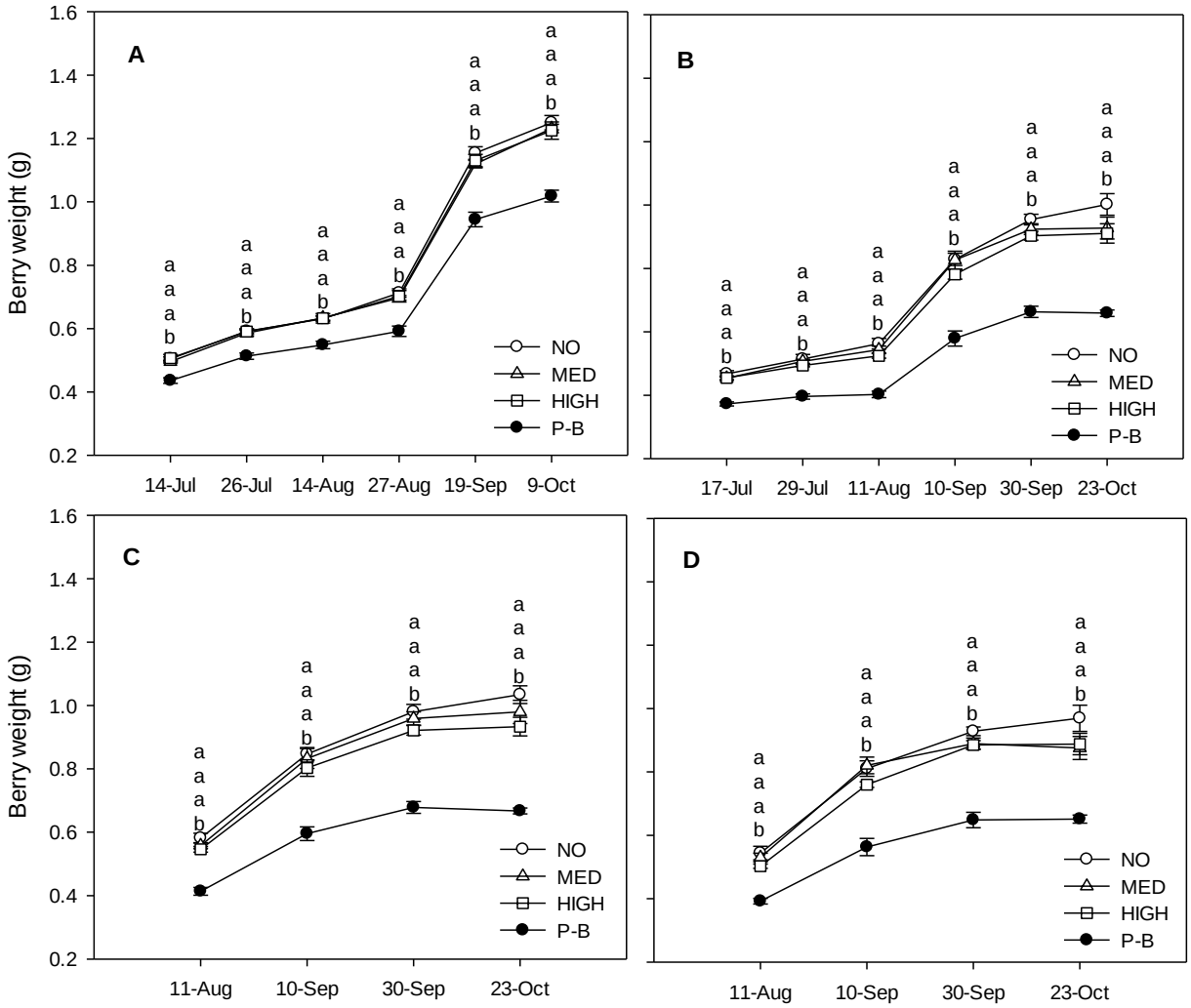

Fig. 10. Pre-bloom (P-B), post-fruit set (MED = medium extent, HIGH = high extent), and no (NO) leaf removal effect on Petit Verdot berry weight over the course of 2013 (A) and 2014 (B = average, C = east canopy side, D = west canopy side). Each data point is an average of 120 (pre-harvest) or 150 (harvest) berries evenly collected from all vines in each experimental unit; n = 6. Error bars are +/- standard error.

Components of berry weight: Cabernet franc and Petit Verdot. Pre-bloom leaf removal reduced estimated Cabernet franc pulp weight by 8% when compared to HIGH (Table 14). Pre-bloom leaf removal reduced Petit Verdot berry weight by 11% when compared to the average of all other treatments. While berries weighed less, the weight of berry skins was not concomitantly reduced by P-B in Petit Verdot. Pre-bloom leaf removal reduced estimated Petit Verdot pulp weight by 16% when compared to the average of MED and HIGH.

Table 14. Pre-bloom and post-fruit set leaf removal effect on components of Cabernet franc berry weight on 9 Sep 2013, and components of Petit Verdot berry weight on 19 Sep 2013.

Treatment[a]	Berry weight (g)	Seed # / berry	Total seed weight (g)	Individual seed weight (g)	Skin weight (g)	Estimated pulp weight[b] (g)	Total seed: berry weight ratio	Skin: berry weight ratio	Estimated skin: pulp weight ratio
Cabernet franc									
NO	1.439	1.856	0.087	0.047	0.424	0.928 ab	0.060	0.259	0.465
MED	1.391	1.928	0.097	0.050	0.412	0.882 ab	0.070	0.297	0.476
HIGH	1.440	1.844	0.084	0.046	0.374	0.982 a	0.058	0.295	0.382
P-B	1.357	1.783	0.084	0.047	0.419	0.854 b	0.061	0.309	0.496
Significance[c]	ns	ns	ns	ns	ns	0.0464	ns	ns	ns
Petit Verdot									
NO	0.993 a	2.26	0.093	0.042	0.237	0.664 ab	0.092	0.242	0.36
MED	0.994 a	2.37	0.094	0.039	0.229	0.671 a	0.091	0.236	o.343
HIGH	1.035 a	2.38	0.087	0.037	0.253	0.695 a	0.084	0.249	0.365
P-B	0.890 b	2.35	0.082	0.035	0.229	0.579 b	0.091	0.263	0.398
Significance[c]	0.0045	ns	ns	ns	ns	0.0070	ns	ns	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.
[b]Calculated by subtracting the sum of skin and total seed weight from berry weight.
[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's HSD.

Primary fruit chemistry: Cabernet franc and Petit Verdot. Leaf removal treatment did not affect juice soluble solids in any year in Cabernet franc (Table 15). In 2013 only, HIGH and P-B *increased* juice pH compared to NO. Juice titratable acidity (TA) was reduced by HIGH (17%) in 2012, by HIGH (15%) and P-B (17%) in 2013, and only by P-B (11%) in 2014, when compared to NO. Re-implementation of pre-bloom leaf removal in 2014 in plots originally implemented in 2013 reduced juice TA by 0.36 g/L when compared to re-implementation of no leaf removal plots in 2014 (data not shown). In Petit Verdot, juice soluble solids was reduced by HIGH by 2% in 2013, and by HIGH (5%) and P-B (7%) in 2014, when compared to NO (Table 12). Juice pH was increased by HIGH by 3% when compared to NO in 2012, and reduced by HIGH (3%) and P-B (6%) compared to MED in 2014. Juice TA was reduced by HIGH by an average of 15% when compared to NO and MED in 2012, and by 10% when compared to NO in 2013. Juice TA was increased by P-B by 20% when compared to the average of all other treatments in 2014. Re-implementation of pre-bloom leaf removal in 2014 in the same plots reduced soluble solids by 0.6 °Brix and pH by 0.07 units, and increased TA by 1.53 g/L when compared to no leaf removal plots re-implemented in 2014 (data not shown).

Table 15. Post-fruit set and pre-bloom leaf removal effect on primary fruit chemistry at harvest in Cabernet franc and Petit Verdot, 2012-2014.

Treatment[a]	Cabernet franc			Petit Verdot		
	Soluble solids (°Brix)	pH	Titratable acidity (g/L)	Soluble solids (°Brix)	pH	Titratable acidity (g/L)
	2012			**2012**		
NO	23.3	3.49	2.92 a	24.7	3.13 b	6.82 a
MED	22.7	3.51	2.92 a	24.5	3.16 b	6.67 a
HIGH	23.0	3.53	2.43 b	24.4	3.21 a	5.73 b
Significance[b]	ns	ns	0.0015	ns	<0.0001	0.0121
	2013			**2013**		
NO	23.6	3.47 c	8.31 a	23.0 a	3.55	7.02 a
MED	23.6	3.49 bc	7.98 a	22.8 ab	3.57	6.71 ab
HIGH	23.4	3.55 a	7.08 b	22.5 b	3.55	6.31 b
P-B	23.8	3.54 ab	6.92 b	22.9 ab	3.56	6.76 ab
Significance[b]	ns	0.0026	<0.0001	0.0385	ns	0.0117
	2014			**2014**		
NO	22.9	3.68	5.34 a	24.3 a	3.41 ab	9.71 b
MED	23.0	3.71	4.93 ab	24.5 a	3.48 a	8.95 b
HIGH	22.9	3.73	4.79 ab	23.0 b	3.38 b	9.22 b
P-B	23.1	3.71	4.73 b	22.6 b	3.28 c	11.11 a
Significance[b]	ns	ns	0.0346	<0.0001	<0.0001	<0.0001

[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.
[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's HSD.

Estimated total grape phenolics and anthocyanins: Cabernet franc and Petit Verdot. Leaf removal treatment did not affect total berry anthocyanins in Cabernet franc (Table 16). In 2014, P-B increased total berry phenolics by 20% compared to NO and 21% compared to MED; HIGH increased total berry phenolics by 12% compared to MED. In 2014, Cabernet franc berry anthocyanins were greater on the east compared to west canopy side, and vice-versa for berry phenolics. Total berry phenolics were increased by P-B by an average of 18% in 2013 and 28% in 2014 when compared to the average of all other treatments.

Table 16. Pre-bloom and post-fruit set leaf removal effect on total berry anthocyanins (TBA) and total berry phenolics (TBP) in Cabernet franc and Petit Verdot from 2012-2014.

Treatment and Canopy side	Cabernet franc					
	2012		2013		2014	
	TBA (mg/g berry)	TBP[b] (au/g berry)	TBA (mg/g berry)	TBP[b] (au/g berry)	TBA (mg/g berry)	TBP[b] (au/g berry)
NO	0.69	71.69	0.88	79.39	0.73	87.35 bc
MED	0.63	68.88	0.88	81.02	0.71	86.56 c
HIGH	0.69	74.06	0.77	77.63	0.74	96.65 ab
P-B	n/a	n/a	0.86	85.46	0.76	104.75 a
EAST	n/a	n/a	n/a	n/a	0.77 a	90.46 b
WEST	n/a	n/a	n/a	n/a	0.70 b	97.20 a
Significance[c]						
Leaf removal (LR)	ns	ns	ns	ns	ns	<0.0001
Canopy side (CS)	n/a	n/a	n/a	n/a	0.0104	0.0120
LR*CS	n/a	n/a	n/a	n/a	ns	ns
	Petit Verdot					
Treatment and Canopy side	2012		2013		2014	
	TBA (mg/g berry)	TBP[b] (au/g berry)	TBA (mg/g berry)	TBP[b] (au/g berry)	TBA (mg/g berry)	TBP[b] (au/g berry)
NO	1.23	98.28	1.18	91.61 b	1.10	115.07 b
MED	1.30	104.36	1.12	91.38 b	1.14	121.71 b
HIGH	1.29	103.58	1.12	91.54 b	1.18	127.17 b
P-B	n/a	n/a	1.22	108.09 a	1.13	155.27 a
EAST	n/a	n/a	n/a	n/a	1.14	132.18
WEST	n/a	n/a	n/a	n/a	1.13	127.44
Significance[c]						
Leaf removal (LR)	ns	ns	ns	0.0007	ns	<0.0001
Canopy side (CS)	n/a	n/a	n/a	n/a	ns	ns
LR*CS	n/a	n/a	n/a	n/a	ns	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.
[b]au = absorbance units when read at 280 nm wavelength
[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's HSD.

There was a significant, negative linear relationship between harvest berry weight and total berry phenolics in two out of three years in both varieties (Table 17). There was never a significant linear relationship between harvest berry weight and total berry anthocyanins in any year or variety.

Table 17. The simple linear regression relationship between harvest berry weight and total berry phenolics (TBP) and total berry anthocyanins (TBA) in Cabernet franc and Petit Verdot from 2012-2014.

	Cabernet franc			
	TBP		TBA	
Year	R^2	Significance	R^2	Significance
2012	0.4273	0.0044	0.2064	ns
2013	0.1457	ns	0.0531	ns
2014	0.2369	0.0159	0.0681	ns
	Petit Verdot			
	TBP		TBA	
Year	R^2	Significance	R^2	Significance
2012	0.1413	ns	0.0321	ns
2013	0.4659	0.0002	0.1197	ns
2014	0.4575	0.0003	0.0073	ns

Grape carotenoids: Cabernet franc. When averaged across all dates in 2012, HIGH increased zeaxanthin compared to MED and NO (Table 18). While lutein 5,6-epoxide was greater at post-fruit set (12 Jul) and pre-veraison (24 Jul) compared to veraison (7 Aug), zeaxanthin was lower at pre-verasion compared to post-fruit set and veraison. Lutein and β-carotene were relatively lower at each subsequent sample date as the season progressed. Zeaxanthin was increased by HIGH compared to NO and MED at pre-veraison, and by HIGH compared to NO at veraison (Fig. 11).

Table 18. Post-fruit set leaf removal treatment effect on Cabernet franc grape carotenoids in 2012.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.014	0.020 b	1.35	1.23
MED	0.014	0.021 b	1.20	1.06
HIGH	0.014	0.028 a	1.33	0.99
Date[b]				
12-Jul	0.017 a	0.027 a	2.02 a	1.99 a
24-Jul	0.016 a	0.018 b	1.76 b	1.35 b
7-Aug	0.010 b	0.024 a	1.22 c	0.77 c
24-Sep	nd	nd	0.18 d	0.27 d
Significance[c]				
Treatment	ns	0.0010	ns	ns
Date	<0.0001	0.0011	<0.0001	<0.0001
Treatment*Date	ns	ns	ns	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.
[b]Lutein 5,6-eopoxide and zeaxanthin below detection threshold (nd) at 24 Sep sample. When detected, zeaxanthin ranged 0.0091 to 0.0108 µg/g berry across treatments.
[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's HSD.

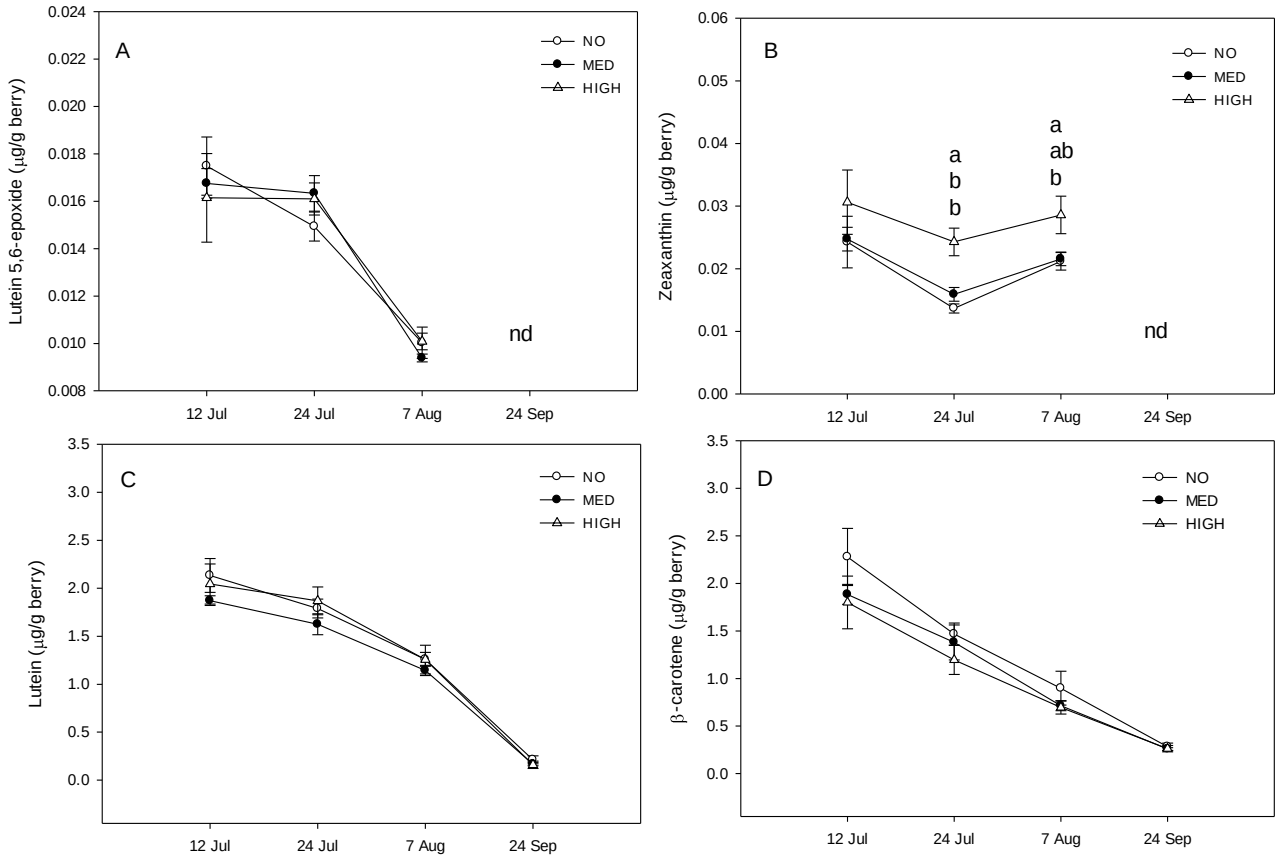

Fig. 11 Post-fruit set leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Cabernet franc grape berries over the course of 2012. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 24 Sep. Means derived from composite 120-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

When averaged across all dates in 2013, HIGH increased lutein 5,6-epoxide compared to MED and NO (Table 19). Both HIGH and P-B increased zeaxanthin compared to NO and MED, and increased lutein compared to only NO. Lutein 5,6-epoxide was lower at every subsequent sample date in 2013, while peak zeaxanthin occurred at pre-veraison (26 Jul), which was greater than at post-fruit set (14 Jul) and veraison (14 Aug). Both lutein and β-carotene were greater at all three pre-harvest time periods compared to at harvest. Lutein 5,6-epoxide was increased by HIGH when compared to MED at post-fruit set (Fig. 12). Zeaxanthin was increased by HIGH and P-B when compared to NO and MED at post-fruit set, and was increased by HIGH when compared to NO and MED, and by P-B when compared to NO, at pre-veraison. Lutein was increased by P-B and NO when compared to MED at pre-veraison and harvest, respectively.

Table 19. 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

[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.
[b]Lutein 5,6-epoxide and zeaxanthin below detection threshold (nd) in 30 Sep sample. When detected, zeaxanthin ranged 0.0091 to 0.0137 µg/g berry across treatments.
[c]Significance of treatment effects (p > F; ns = not significant at 0.05 level).

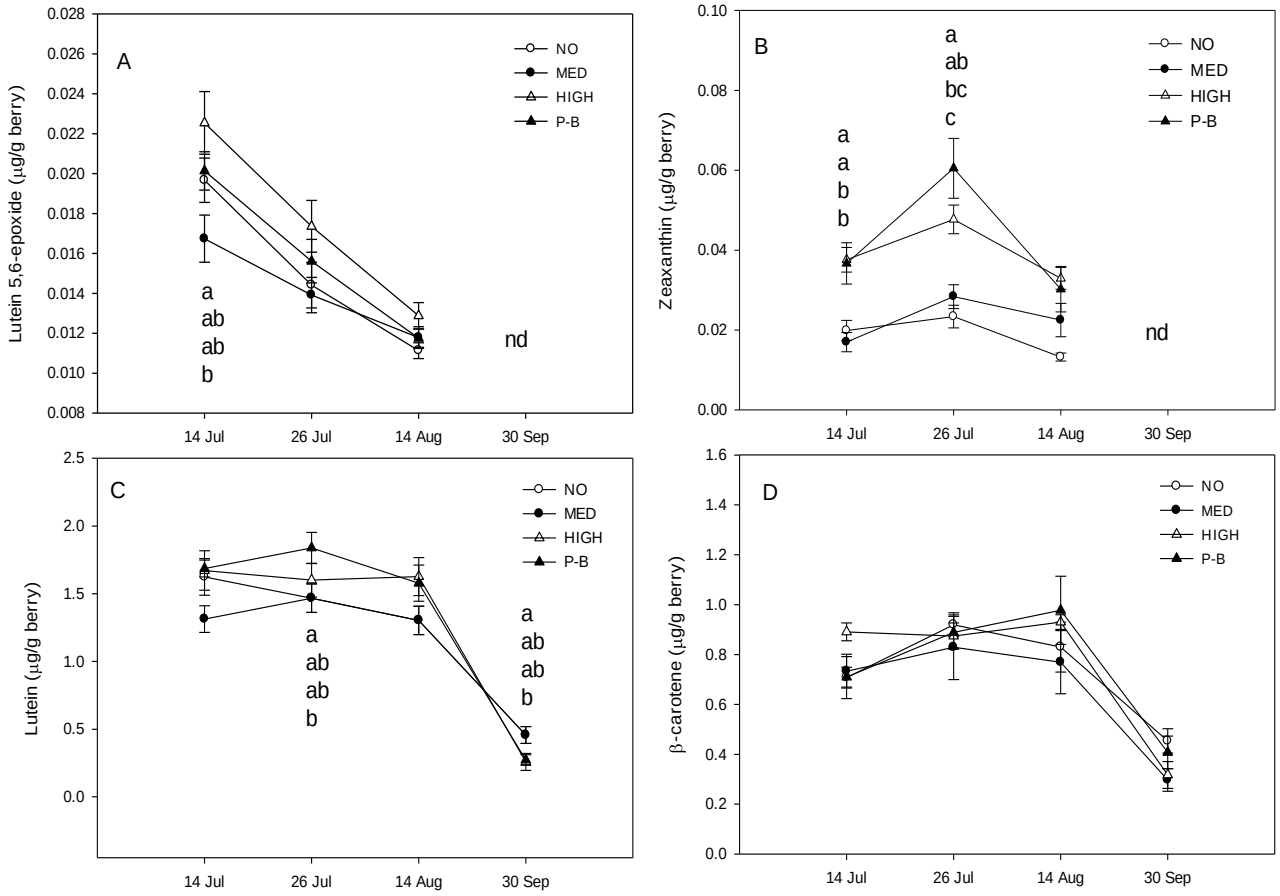

Fig. 12 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Cabernet franc grape berries over the course of 2013. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 30 Sep. Means derived from composite 120-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

When grapes were sampled from the east canopy side and averaged over all dates in 2014, HIGH increased lutein 5,6-epoxide when compared to NO, and both HIGH and P-B increased zeaxanthin when compared to NO and MED (Table 20). Whereas lutein 5,6-epoxide was greater at post-fruit set (16 Jul) compared to pre-veraison (29 Jul) and veraison (11 Aug), zeaxanthin was greater at post-fruit set and pre-veraison compared to veraison. Lutein was greater at veraison compared to pre-veraison and harvest (14 Oct), and β-carotene was greater at all pre-harvest sample dates compared to at harvest. Zeaxanthin was increased by HIGH and P-B when compared to NO and MED at pre-veraison, and by P-B when compared to all other

treatments at veraison (Fig. 13). Lutein was increased by P-B when compared to NO and MED at pre-veraison.

Table 20. Pre-bloom and post-fruit set leaf removal treatment effect on Cabernet franc grape carotenoids from the EAST canopy side in 2014.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.014 b	0.027 b	1.00	0.83
MED	0.015 ab	0.030 b	1.05	0.77
HIGH	0.017 ab	0.047 a	1.14	0.79
P-B	0.018 a	0.057 a	1.17	0.88
Date[b]				
16-Jul	0.021 a	0.045 a	1.30 ab	1.32 a
29-Jul	0.014 b	0.048 a	1.12 b	0.76 b
11-Aug	0.013 b	0.027 b	1.54 a	0.59 b
14-Oct	nd	nd	0.40 c	0.59 b
Significance[c]				
Treatment	0.0091	<0.0001	ns	ns
Date	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	ns	ns	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.
[b]Lutein 5,6-epoxide and zeaxanthin below detection threshold (nd) in 14 Oct sample. When detected, lutein 5,6-epoxide ranged 0.0091 to 0.0113 µg/g berry, and zeaxanthin ranged 0.0089 to 0.0103 µg/g berry, across treatments.
[c]Significance of treatment effects (p > F; ns = not significant at 0.05 level).

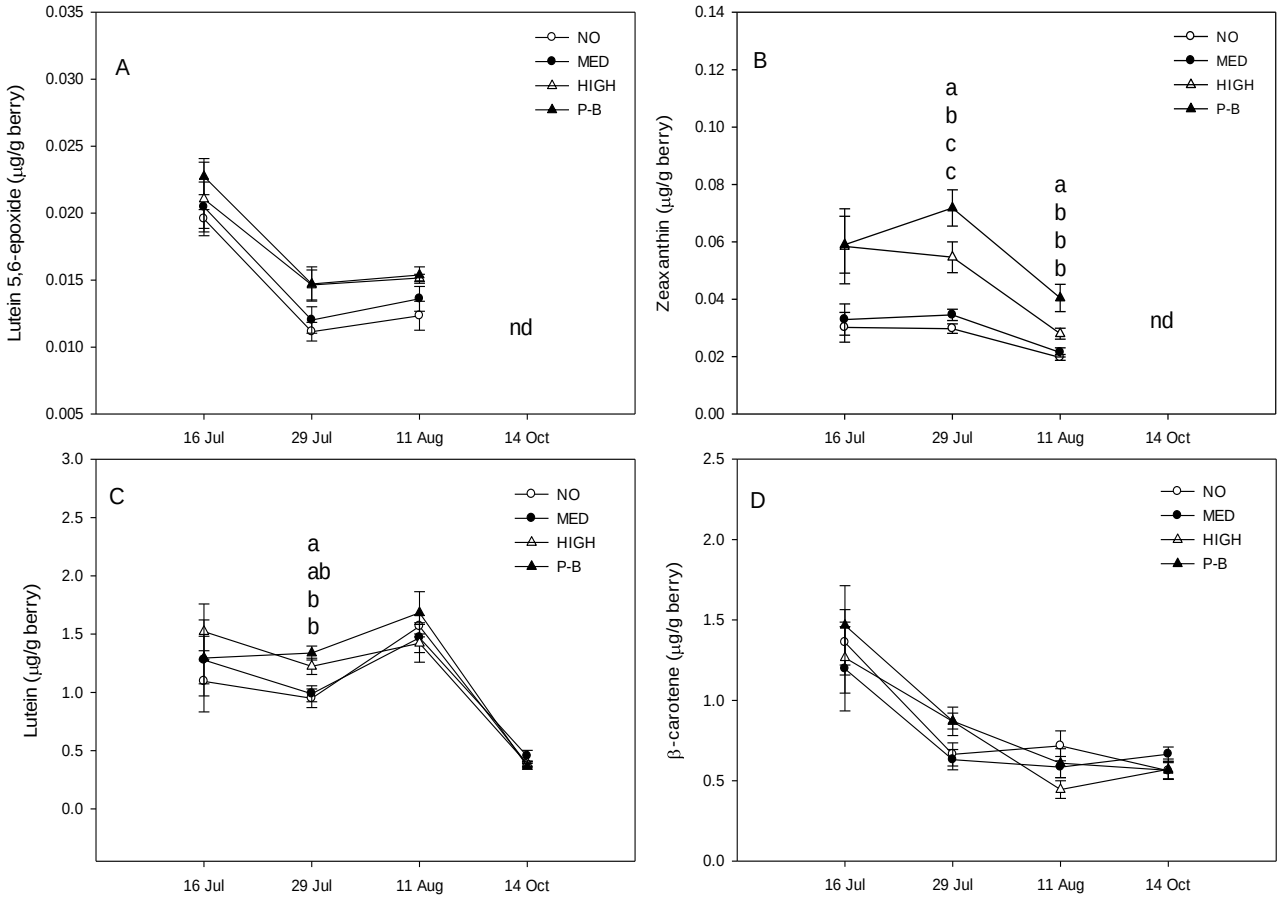

Fig. 13 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Cabernet franc grape berries collected from the EAST canopy side over the course of 2014. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 14 Oct. Means from composite 150-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error. NOTE: 16 Jul is an average of east and west canopy sides.

When grapes were sampled from the west canopy side and averaged over all dates in 2014, both HIGH and P-B increased zeaxanthin when compared to MED and NO (Table 21). Lutein 5,6-epoxide followed similar trends over the season compared to the east canopy side and was greater at post-fruit set (16 Jul) compared to the subsequent two sampled dates. Zeaxanthin and lutein followed similar trends, reaching peak levels at pre-veraison (29 Jul) compared to prior and subsequent sample dates. β-carotene was greater at post-fruit set and pre-veraison compared to veraison (11 Aug) and harvest (14 Oct). Lutein 5,6-epoxide was increased by HIGH when compared to all other treatments at pre-veraison (Fig. 14). Zeaxanthin was

increased by HIGH when compared to MED and NO at both pre-veraison and veraison, and was increased by P-B when compared to NO at pre-veraison and when compared to MED and NO at veraison.

Table 21. Pre-bloom and post-fruit set leaf removal treatment effect on Cabernet franc grape carotenoids from the WEST canopy side in 2014.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.017	0.035 b	1.22	0.98
MED	0.017	0.041 b	1.32	0.96
HIGH	0.018	0.065 a	1.47	1.03
P-B	0.018	0.071 a	1.36	1.09
Date[b]				
16-Jul	0.021 a	0.045 b	1.30 b	1.32 a
29-Jul	0.016 b	0.073 a	2.10 a	1.37 a
11-Aug	0.015 b	0.042 b	1.54 b	0.74 b
14-Oct	nd	nd	0.42 c	0.63 b
Significance[c]				
Treatment	ns	<0.0001	ns	ns
Date	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	ns	ns	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.
[b]Lutein 5,6-epoxide and zeaxanthin below detection threshold (nd) in 14 Oct sample. When detected, lutein 5,6-epoxide ranged 0.0091 to 0.0188 µg/g berry, and zeaxanthin ranged 0.0089 to 0.0130 µg/g berry, across treatments.
[c]Significance of treatment effects (p > F; ns = not significant at 0.05 level).

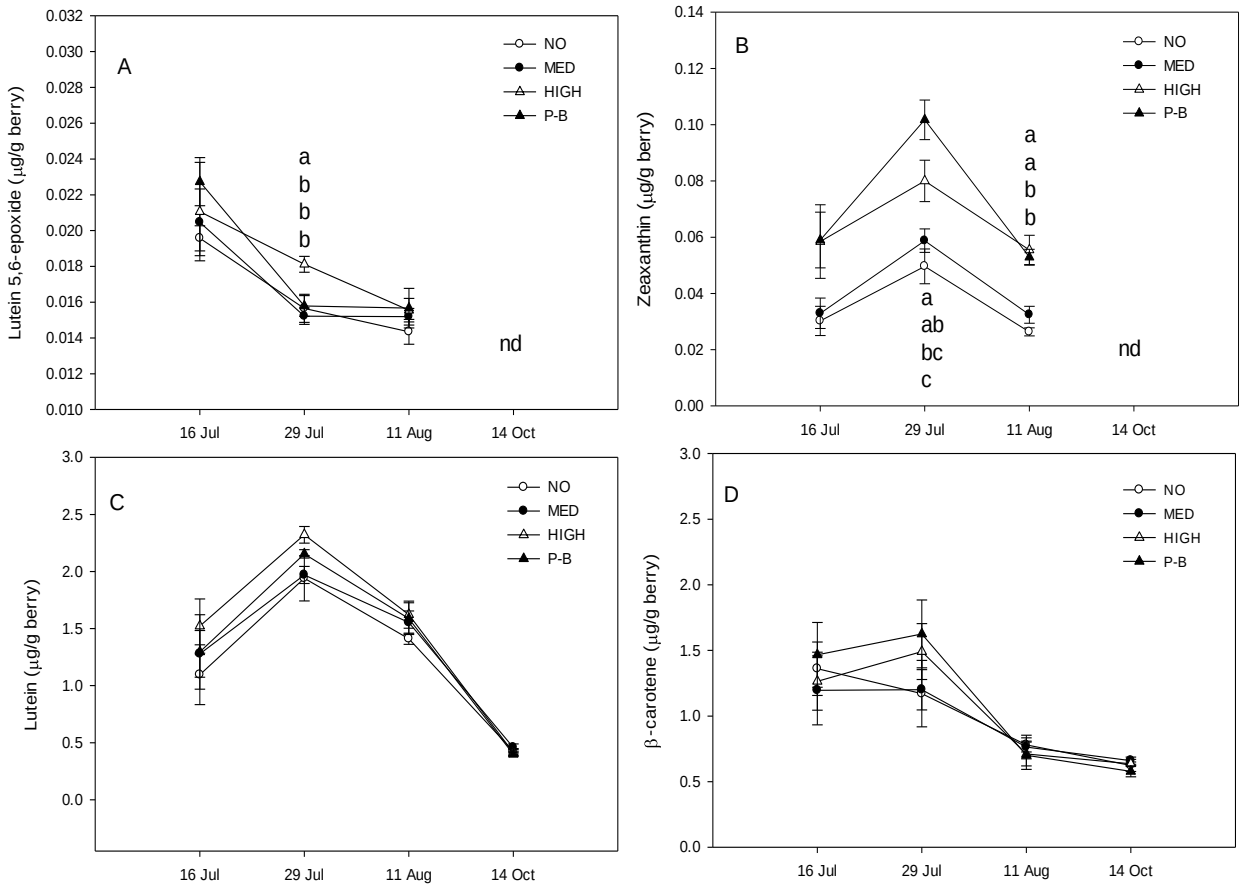

Fig. 14 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Cabernet franc grape berries collected from the WEST canopy side over the course of 2014. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 14 Oct. Means from composite 150-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error. NOTE: 16 Jul is an average of east and west canopy sides.

When averaged over the course of 2012-2014, HIGH increased lutein 5,6-epoxide and zeaxanthin compared to NO and MED, and increased lutein when compared to only MED (Table 22). Lutein 5,6-epoxide and β-carotene were relatively lower at each subsequent sample date compared to previous sample dates. Zeaxanthin and lutein followed similar patterns – levels of both carotenoids were greater at post-fruit set and pre-verasion compared to later sample dates. Lutein 5,6 epoxide was greater in 2013 and 2014 compared to 2012 while zeaxanthin was greater in 2014 compared to 2012 and 2013. When compared to 2014, β-carotene was greater in 2012 and lower in 2013.

The interaction between treatment and year for lutein 5,6-epoxide showed that it was comparatively greater in HIGH compared to other treatments in 2013, and there was relatively less difference between treatments in 2012 and 2014. The interaction between normalized sample date (NSD) and year for lutein 5,6-epoxide revealed that its relationship changed across sample dates over 2012 to 2014 such that it was similar at pre-veraison and post-fruit set in 2012, relatively different between all NSDs at 2013, but similar at pre-veraison and veraison in 2014. The interaction between treatment and year for zeaxanthin showed that the magnitude of difference in its levels changed between HIGH compared to MED and NO over the years: zeaxanthin levels in HIGH were relatively similar to NO and MED in 2012, while zeaxanthin levels in HIGH were realtively greater than NO and Med in 2013 and 2014. The interaction between NSD and year for zeaxanthin showed that it was lower at pre-veraison compared to post-fruit set and veraison in 2012, and, in 2013 and 2014, it was relatively greater at pre-veraison compared to post-fruit set and veraison. The interaction between NSD and year for lutein revealed that its relationship between sample dates changed over 2012-2104: it was greater at post-fruit set than at pre-veraison and veraison in 2012, but greater at pre-veraison and veraison compared to post-fruit set in 2014. The interaction between NSD and year for β-carotene showed that its relationship between several NSDs changed over the course of 2012-2014, but namely that it was different between all NSDs in 2012, relatively similar between post-fruit set, pre-veraison, and veraison, all which were greater than at harvest, in 2013, and relatively similar between veraison and harvest, which were lesser than at post-fruit set and pre-veraison, in 2014.

Table 22. Post-fruit set leaf removal treatment effect on Cabernet franc grape carotenoids over 2012-2014.

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	0.023 b	1.22 ab	0.96
MED	0.015 b	0.026 b	1.15 b	0.86
HIGH	0.016 a	0.041 a	1.31 a	0.88
NSD[b]				
Post-fruit set	0.019 a	0.31 a	1.62 a	1.35 a
Pre-veraison	0.015 b	0.34 a	1.60 a	1.08 b
Veraison	0.012 c	0.026 b	1.39 b	0.76 c
Harvest	nd	nd	0.30 c	0.42 d
Year[c]				
2012	0.014 b	0.023 b	1.29	1.09 a
2013	0.016 a	0.027 b	1.19	0.71 c
2014	0.016 a	0.041 a	1.2	0.89 b
Significance[d]				
Treatment	0.0015	<0.0001	0.0062	ns
NSD	<0.0001	0.0004	<0.0001	<0.0001
Year	<0.0001	<0.0001	ns	<0.0001
Treatment*NSD	ns	ns	ns	ns
Treatment*Year	0.0472	0.0057	ns	ns
NSD*Year	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*NSD*Year	ns	ns	ns	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; NOTE: P-B left out of analysis because it was not implemented in 2012.
[b]NSD = normalized sample date in order to evaluate the effect of sample date order across years. Lutein 5,6-eopoxide and zeaxanthin were below detection threshold (nd) in several harvest samples. When detected, lutein 5,6-epoxide ranged 0.0095 to 0.0131 µg/g berry across treatments in 2014, and zeaxanthin ranged 0.0093 to 0.0109 µg/g berry across treatments over 2012-2014.
[c]2014 data was averaged across canopy sides.
[d]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's HSD.

Petit Verdot. When averaged across all dates in 2012, MED increased lutein 5,6-epoxide compared to NO and HIGH, and MED and HIGH increased zeaxanthin compared to NO (Table 23). Excepting lutein 5,6-epoxide, carotenoids were greater at pre-harvest compared to at harvest (1 Oct). Peak zeaxanthin was observed at post-fruit set (12 Jul) and veraison (20 Aug) while peak β-carotene was observed at post-fruit set. The interaction was the reversed relationship between zeaxanthin in MED and HIGH from pre-veraison (24 Jul) to veraison, or

the general decrease in the magnitude of difference in zeaxanthin between treatments at harvest when compared to all previous periods (Fig. 15). Lutein 5,6-epoxide was increased by MED and HIGH when compared to NO at pre-veraison (Fig. 15). Zeaxanthin was increased by MED and HIGH when compared to NO at post-fruit-set, and increased by HIGH when compared to NO at pre-veraison, and by MED when compared to NO at veraison. Lutein was greater in NO compared to HIGH at harvest.

Table 23. Post-fruit set leaf removal treatment effect on Petit Verdot grape carotenoids in 2012.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.019 b	0.022 b	3.04	1.72
MED	0.022 a	0.034 a	3.13	1.77
HIGH	0.020 b	0.035 a	2.93	1.71
Date[b]				
12-Jul	0.022	0.042 a	3.93 a	2.49 a
24-Jul	0.020	0.028 b	3.52 a	1.67 b
20-Aug	0.020	0.037 a	3.54 a	2.00 ab
1-Oct	nd	0.014 c	1.14 b	0.76 c
Significance[c]				
Treatment	0.0296	<0.0001	ns	ns
Date	ns	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	0.0013	ns	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.
[b]Lutein 5,6-eopoxide below detection threshold (nd) in 1 Oct sample
[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's HSD.

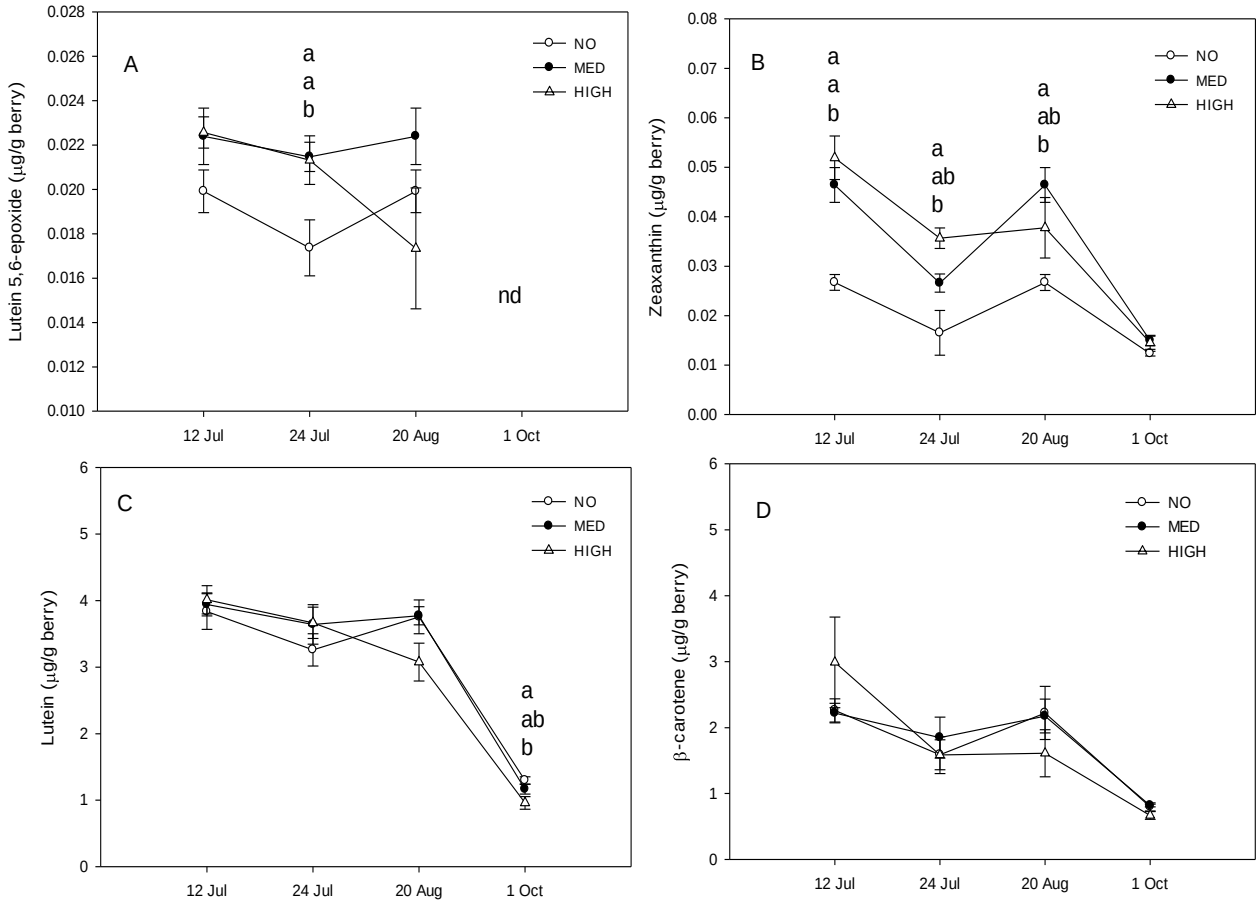

Fig. 15 Post-fruit set leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Petit Verdot grape berries over the course of 2012. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 1 Oct. Means derived from composite 120-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

When averaged across all dates in 2013, HIGH increased lutein 5,6-epoxide compared to MED, and both HIGH and P-B increased zeaxanthin and lutein compared to NO and MED (Table 24). Lutein 5,6-epoxide was greater at post-fruit set (14 Jul) and pre-veraison (26 Jul) compared to at verasion (27 Aug), and zeaxanthin was greater at all three pre-harvest dates compared to at harvest (9 Oct). Peak lutein and β-carotene was observed at post-fruit set, at which time both of these carotenoids were greater compared to all prior and subsequent sample dates. The interaction of treatments and date on lutein was either that the magnitude of difference in lutein changed between HIGH and P-B from post-fruit set to pre-veraison, or the

general decrease in the magnitude of difference in lutein between treatments over the course of the 2013 season (Fig. 16). Lutein 5,6-epoxide was increased by HIGH when compared to NO at post-fruit set, and, at pre-veraison, was greater in HIGH when compared to MED and NO and greater in P-B when compared to MED (Fig. 16). Zeaxanthin was increased by HIGH and P-B when compared to NO and MED at pre-veraison, and by only HIGH when compared to NO and MED at veraison. Lutein was increased by both HIGH and P-B when compared to NO and MED at pre-veraison. β-carotene was greater in NO compared to HIGH at harvest.

Table 24. Pre-bloom and post-fruit set leaf removal treatment effect on Petit Verdot 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	0.021 ab	0.015 b	2.26 b	1.47
MED	0.021 b	0.016 b	2.14 b	1.28
HIGH	0.025 a	0.027 a	2.90 a	1.55
P-B	0.023 ab	0.023 a	2.72 a	1.47
Date[b]				
14-Jul	0.026	0.024 a	3.37 b	1.17 b
26-Jul	0.023	0.023 a	3.92 a	3.27 a
27-Aug	0.019	0.020 a	1.84 c	0.68 c
9-Oct	nd	0.014 b	0.89 d	0.64 c
Significance[c]				
Treatment	0.0297	<0.0001	<0.0001	ns
Date	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	ns	0.0074	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.
[b]Lutein 5,6-eopoxide below detection threshold (nd) in 9 Oct sample. When detected, values ranged 0.0092 to 0.0094 µg/g berry across treatments.
[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's HSD.

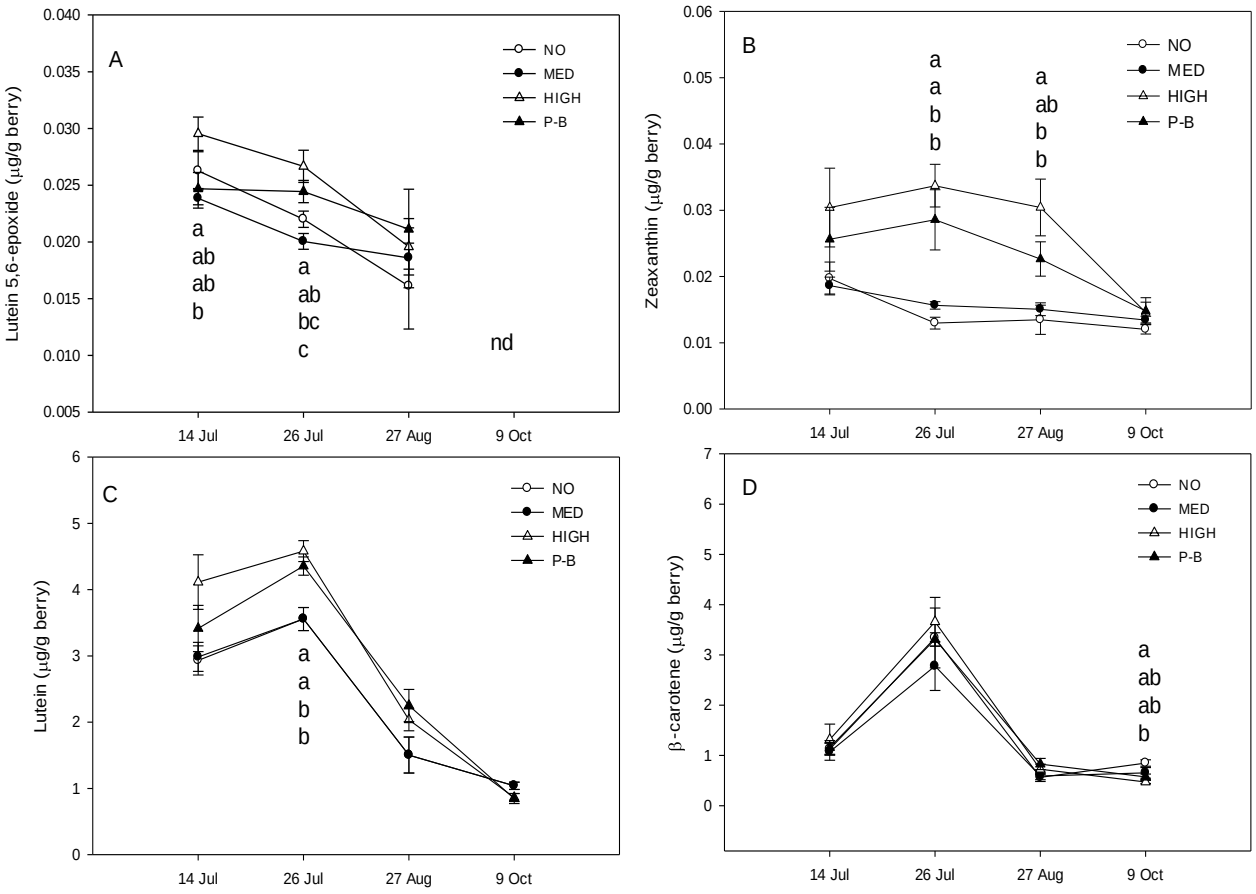

Fig. 16 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Petit Verdot grape berries over the course of 2013. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 9 Oct. Means derived from composite 120-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

When grapes were sampled from the east canopy side and averaged over all dates in 2014, HIGH increased lutein 5,6-epoxide compared to NO and MED, and P-B increase lutein 5,6-epoxide compared to NO (Table 25). While both HIGH and P-B increased zeaxanthin, only HIGH increased lutein, when compared to NO and MED. Peak lutein 5,6-epoxide and lutein was observed at pre-veraison (11 Aug), at which time these carotenoids were greater compared to all prior and subsequent sample dates. Zeaxanthin was greater at post-fruit set (16 Jul) and veraison (10 Sep) compared to harvest (22 Oct), and β-carotene was greater at pre-veraison and veraison compared to harvest.

There was an interaction between treatment and date for every carotenoid, as well as for total carotenoids and adjusted total carotenoids. The interaction for lutein 5,6-epoxide and lutein was the reversed relationship between these carotenoids in HIGH and P-B from pre-veraison to veraison (Fig. 17). Further, there was a general decrease in the magnitude of difference in lutein observed between treatments, particularly from veraison to harvest. The interaction for zeaxanthin appeared to be the change in its relationship between HIGH and P-B over the course of the season, or the decrease in the magnitude of difference observed between P-B and HIGH in comparison to MED and NO, particularly from veraison to harvest. The interaction for β-carotene was the change in the relationship between P-B and HIGH from pre-veraison to veraison, or between P-B and NO from veraison to harvest. Lutein 5,6-epoxide was greater in HIGH when compared to all treatments at post-fruit set, and when compared to MED and NO at pre-veraison (Fig. 19). Pre-bloom leaf removal (P-B) increased lutein 5,6-epoxide when compared to NO and MED at veraison. Both HIGH and P-B increased zeaxanthin compared to NO and MED at every pre-harvest sample date. While HIGH had greater lutein concentration when compared to all other treatments at pre-veraison, P-B had greater lutein concentration when compared to all other treatments at veraison. β-carotene was increased by only P-B when compared to NO at veraison.

Table 25. Pre-bloom and post-fruit set leaf removal treatment effect on Petit Verdot grape carotenoids from the EAST canopy side in 2014.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.018 bc	0.019 b	2.63 b	2.06
MED	0.018 c	0.028 b	2.64 b	2.33
HIGH	0.022 a	0.050 a	3.22 a	2.74
P-B	0.021 ab	0.054 a	2.94 ab	2.73
Date[b]				
16-Jul	0.021 b	0.052 a	3.35 b	2.25 ab
11-Aug	0.023 a	0.041 ab	3.95 a	2.94 a
10-Sep	0.015 c	0.036 b	2.54 c	2.87 a
22-Oct	nd	0.023 c	1.59 d	1.81 b
Significance[c]				
Treatment	<0.0001	<0.0001	0.0003	0.0395
Date	<0.0001	<0.0001	<0.0001	0.0001
Treatment*Date	0.0029	0.0028	0.0002	0.0026

[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.
[b]16 Jul is an average across both canopy sides, all other dates were sampled from the east canopy side only. Lutein 5,6-eopoxide below detection threshold (nd) in 22 Oct sample. When detected, values ranged 0.0091 to 0.0122 µg/g berry across treatments.
[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's HSD.

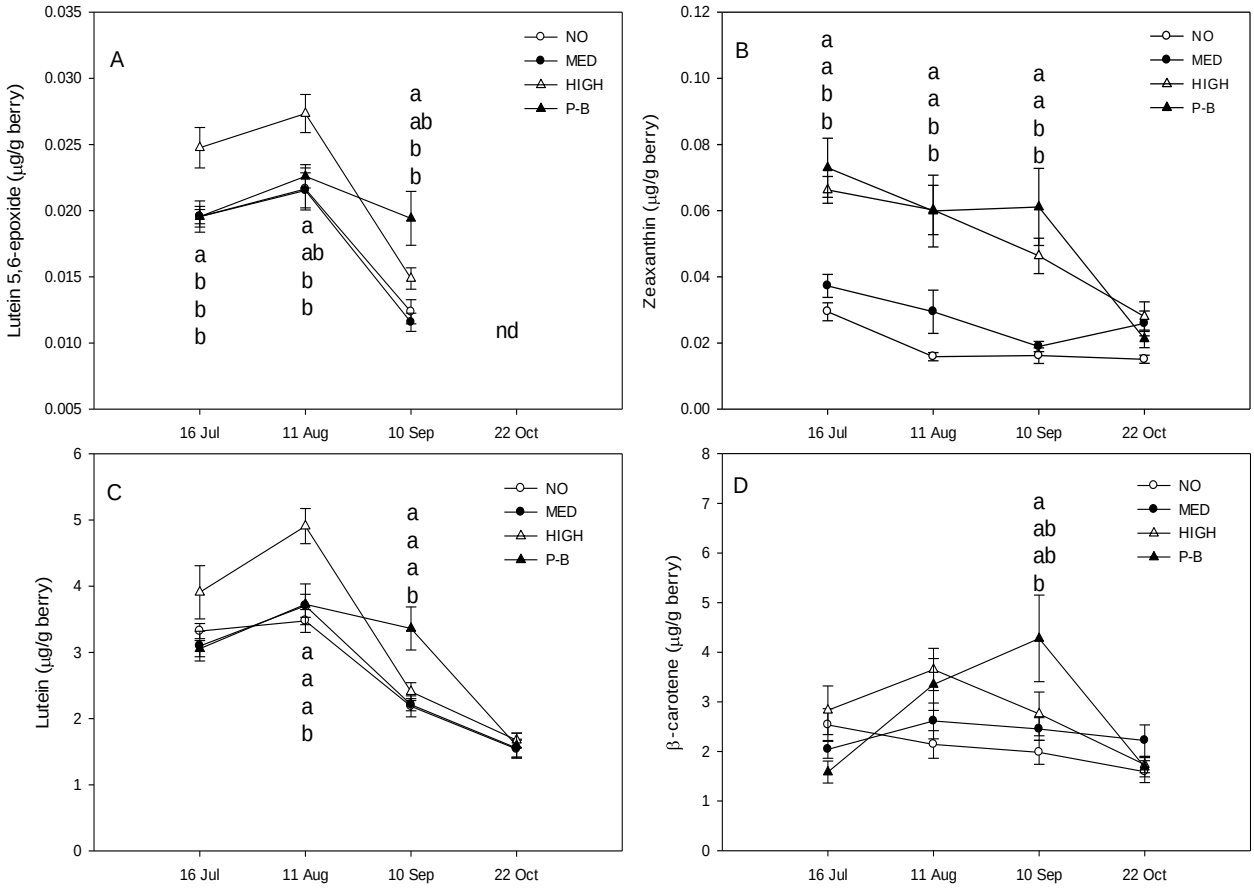

Fig. 17 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Petit Verdot grape berries collected from the EAST canopy side over the course of 2014. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 22 Oct. Means from composite 150-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error. NOTE: 16 Jul is an average of east and west canopy sides.

When grapes were sampled from the west canopy side and averaged over all dates in 2014, HIGH and P-B increased zeaxanthin when compared to NO and MED, and only HIGH increased lutein when compared to MED (Table 26). Excepting lutein 5,6-epoxide, carotenoids were greater at pre-veraison (11 Aug) when compared to post-fruit set (16 Jul) and harvest (22 Oct). The interaction for zeaxanthin appeared to be the change in its relationship between HIGH and P-B between post-fruit set and veraison, or the general decrease in its magnitude of difference between treatments, particularly from veraison to harvest (Fig. 18). The interaction for lutein was the change in its relationship between P-B and all other treatments from pre-verasion to verasion. This interaction may also have been due to the general decrease in the magnitude of difference in lutein between HIGH and MED/NO from pre-veraison to veraison, or the decrease in its magnitude of difference observed between P-B and other treatments from veraison to harvest. Lutein 5,6-epoxide was increased by HIGH when compared to all other treatments at post-fruit set, and increased by P-B when compared to NO and MED at veraison (Fig. 18). Zeaxanthin was increased by HIGH and P-B compared to NO and MED at post-fruit set, and, at pre-veraison, by HIGH when compared to NO and MED and by P-B when compared to MED. At veraison, zeaxanthin was also increased by HIGH when compared to all other treatments, and by P-B when compared to NO and MED. Lutein was increased by HIGH when compared to P-B at pre-veraison, and by P-B when compared to all other treatments at veraison. β-carotene was increased by P-B when compared to all other treatments at veraison.

Table 26. Pre-bloom and post-fruit set leaf removal treatment effect on Petit Verdot grape carotenoids from the WEST canopy side in 2014.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.018	0.023 b	2.70 ab	2.38
MED	0.016	0.028 b	2.61 b	2.31
HIGH	0.020	0.046 a	2.99 a	2.54
P-B	0.018	0.054 a	2.77 ab	2.74
Date[b]				
16-Jul	0.021 a	0.052 a	3.35 b	2.25 b
11-Aug	0.021 a	0.038 b	3.72 a	3.24 a
10-Sep	0.012 b	0.036 b	2.32 c	2.51 ab
22-Oct	nd	0.025 c	1.68 d	1.98 b
Significance[c]				
Treatment	ns	<0.0001	0.0529	ns
Date	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*Date	ns	<0.0001	<0.0001	0.0164

[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.
[b]16 Jul is an average across both canopy sides, all other dates were sampled from the east canopy side only. Lutein 5,6-eopoxide below detection threshold (nd) in 22 Oct sample. When detected, values ranged 0.0098 to 0.0115 µg/g berry across treatments.
[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's HSD.

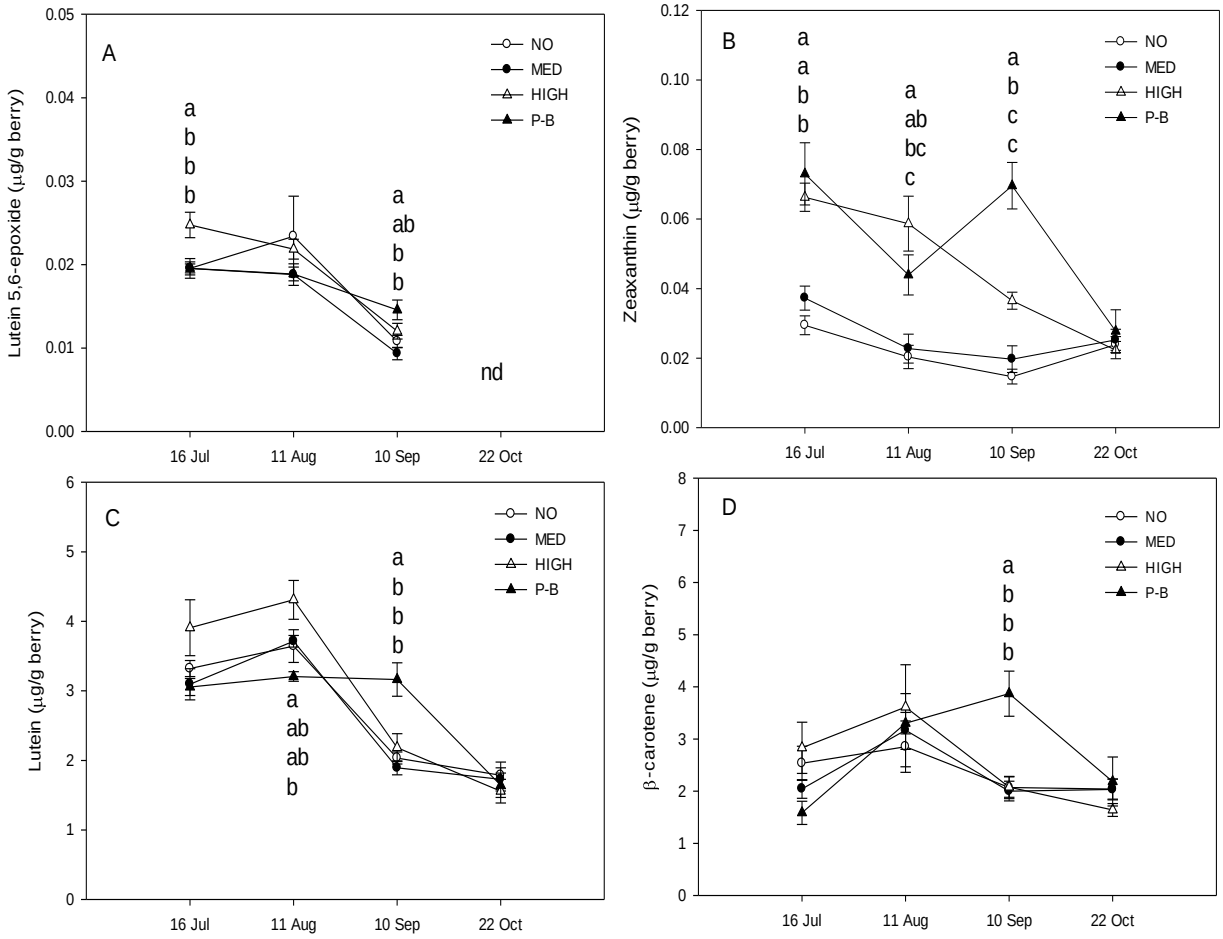

Fig. 18 Post-fruit set and pre-bloom leaf removal effect on lutein 5,6-epoxide (A), zeaxanthin (B), lutein (C), and β-carotene (D) in Petit Verdot grape berries collected from the WEST canopy side over the course of 2014. NOTE: lutein 5,6-epoxide and zeaxanthin consistently not deteced (nd) in berries at 22 Oct. Means from composite 150-berry samples; n = 6. Treatments not sharing a letter within a date are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error. NOTE: 16 Jul is an average of east and west canopy sides.

When averaged over the course of 2012-2014, HIGH increased the level of all carotenoids except β-carotene when compared to NO and MED (Table 27). Excepting zeaxanthin, carotenoids were greater at post-fruit set and pre-veraison compared to at veraison and harvest, and were greater at verasion compared to at harvest. Lutein 5,6-epoxide was greater in 2013 compared to 2012 and 2014 whereas zeaxanthin, lutein, and β-carotene were lower in 2013 compared to 2012 and 2014.

The interaction between treatments and normalized sample date (NSD) for zeaxanthin showed that there was relatively no difference in it between treatments at harvest compared to all other sample dates, particularly those at post-fruit set and pre-veraison, when trends were HIGH > MED > NO. There was a similar interaction between treatment and NSD for lutein, which revealed that there was no difference in it between treatments at both veraison and harvest, but trends were HIGH > MED = NO at both post-fruit set and pre-veraison. The interaction between treatment and year for lutein 5,6-epoxide showed that levels were greater in MED compared to HIGH and NO in 2012, but were greater in HIGH compared to MED and NO in 2013 and 2014. The interaction between treatment and year for zeaxanthin revealed that it was differentially affected by MED over 2012 and 2013: MED had similar levels to HIGH in 2012, but similar levels to NO in 2013. The interaction between treatment and year for lutein showed that levels were similar between treatments in 2012, but relatively greater in HIGH compared to MED and NO in 2013 and 2014.

The observed interaction between NSD and year for lutein 5,6-epoxide revealed that it was relatively similar between sample periods in 2012 compared to 2013, or that levels were greater at post-fruit set compared to pre-verasion in 2013, and vice-versa in in 2014. The interaction between and NSD and year for zeaxanthin revealed that there was less difference in it between sample dates in 2013 compared to 2012 and 2014, when the largest difference was between post-fruit set and harvest. The interaction between NSD and year for lutein revealed that there was less difference in it at veraison compared to post-fruit set and pre-bloom in 2012, and relatively greater difference in it between these time periods in 2013 and 2014. Another observed interaction for lutein was that it was greater at post-fruit set than pre-veraison in 2012, and vice-versa in 2013. The interaction between NSD and year for β-carotene showed that it was

relatively greater at pre-verasion than other sampled dates in 2013 compared to 2012 and 2014, and that it was greater at post-fruit set and verasion compared to pre-verasion in 2012, but greater at pre-verasion compared to post-fruit set and veraison in 2013 and 2014. The three-way interaction between treatment, NSD, and year showed that the difference in zeaxanthin between all pre-harvest and harvest samples over 2012-2014 was greatest in HIGH, followed by MED, and then NO. Further, this three-way interaction showed that there was relatively little difference in zeaxanthin between sampled dates in 2013 compared to 2012 and 2014, but this was only the case in MED and NO treatments.

Table 27. Post-fruit set leaf removal treatment effect on Petit Verdot grape carotenoids over 2012-2014.

Treatment[a]	Lutein 5,6-epoxide (µg/g berry)	Zeaxanthin (µg/g berry)	Lutein (µg/g berry)	β-carotene (µg/g berry)
NO	0.019 b	0.019 c	2.66 b	1.80
MED	0.020 a	0.026 b	2.63 b	1.79
HIGH	0.022 a	0.037 a	2.98 a	1.97
NSD[b]				
Post-fruit set	0.023 a	0.036 a	3.57 a	2.05 b
Pre-veraison	0.022 a	0.028 b	3.76 a	2.65 a
Veraison	0.017 b	0.028 b	2.46 b	1.62 c
Harvest	nd	0.017 c	1.23 c	1.10 d
Year[c]				
2012	0.021 b	0.030 a	3.03 a	1.73 b
2013	0.023 a	0.019 b	2.43 c	1.43 c
2014	0.019 c	0.032 a	2.80 b	2.40 a
Significance[d]				
Treatment	0.0006	<0.0001	<0.0001	ns
NSD	<0.0001	<0.0001	<0.0001	<0.0001
Year	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*NSD	ns	<0.0001	<0.0001	ns
Treatment*Year	0.0053	<0.0001	<0.0001	ns
NSD*Year	<0.0001	<0.0001	<0.0001	<0.0001
Treatment*NSD*Year	ns	0.007	ns	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; NOTE: P-B left out of analysis because it was not implemented in 2012.
[b]NSD = normalized sample date in order to evaluate the effect of sample date order across years. Lutein 5,6-eopoxide and zeaxanthin below detection threshold (nd) in several harvest samples. When detected, Lutein 5,6-epoxide and zeaxanthin values ranged 0.0091 to 0.0107 µg/g berry across treatments over 2013-2014.
[c]2014 data was averaged across canopy sides. NOTE: no lutein 5,6-epoxide detected at harvest in 2012.
[d]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's HSD.

The zeaxanthin: lutein ratio in Cabernet franc grapes was greater in HIGH when compared to NO at every sample date, and when compared to MED at harvest (Fig. 19). The zeaxanthin: lutein ratio in Petit Verdot grapes was greater in HIGH when compared to NO at every sample date, and when compared to MED at pre-veraison and veraison. The zeaxanthin: lutein ratio in Petit Verdot grapes was greater in MED when compared to NO at harvest.

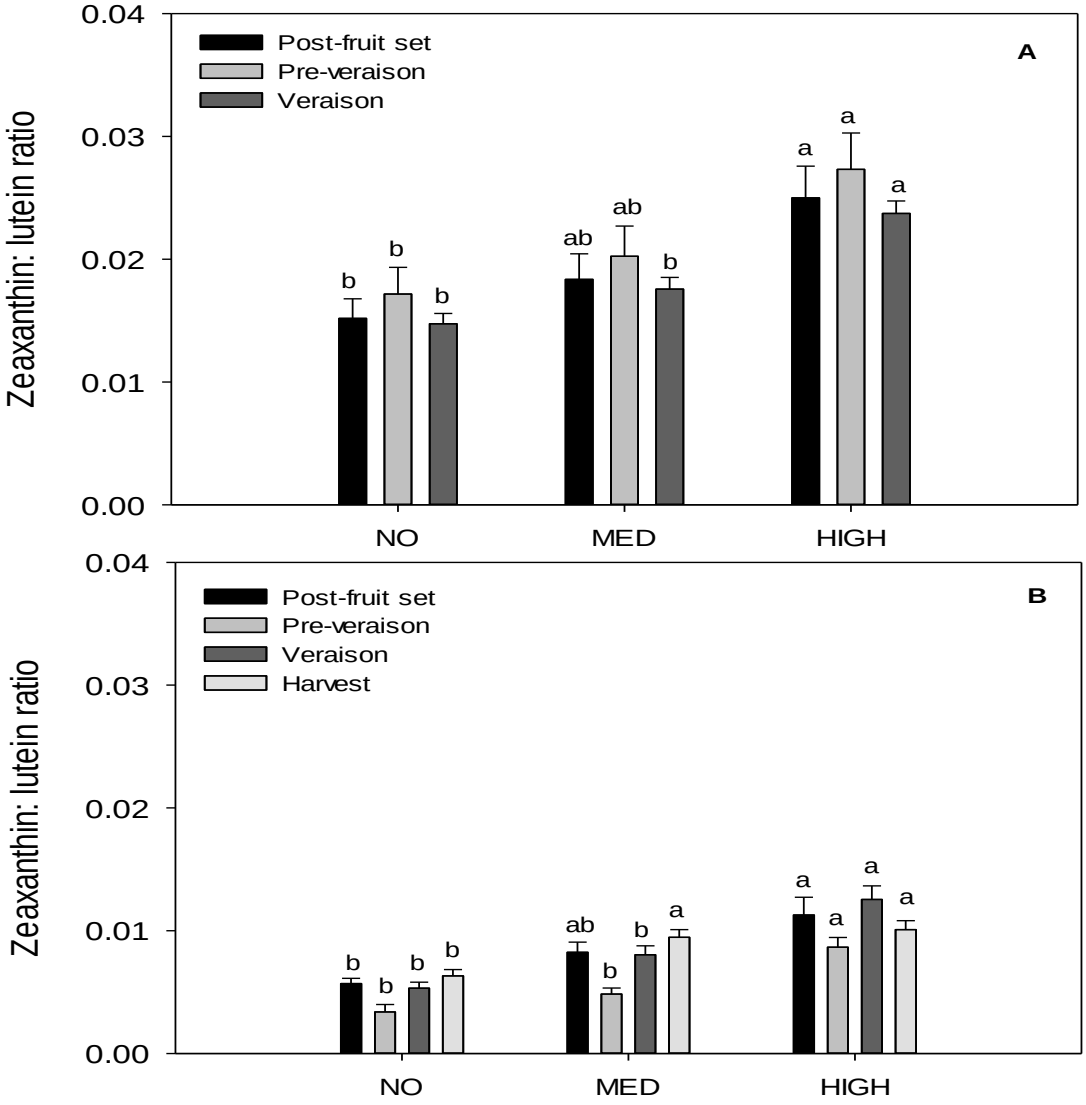

Fig. 19. Cabernet franc (A) and Petit Verdot (B) grape zeaxanthin: lutein ratios at different phenological stages as affected by no leaf removal (NO), and post-fruit set leaf removal to medium (MED) and high (HIGH) extents. Data averaged over 2012-2104; n = 18. Treatment bars not sharing a letter within a phenologiical stage are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

The change in Cabernet franc grape carotenoids from pre-veraison to harvest (ΔC) was greater in HIGH compared to MED and NO in 2014 only (Fig. 20). The Petit Verdot ΔC was greater in HIGH compared to MED in 2013, and greater in HIGH compared to NO in 2014.

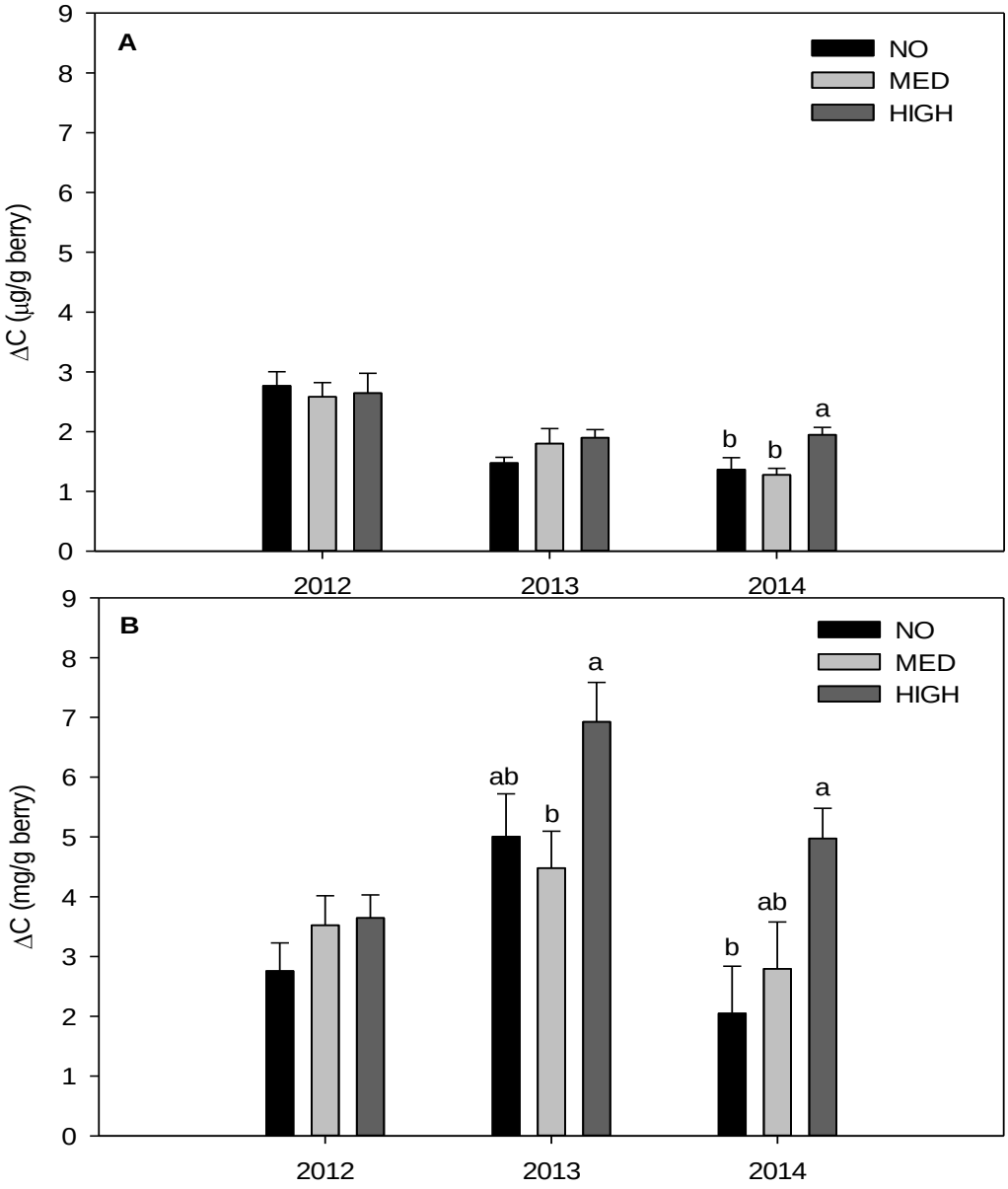

Fig. 20. The change in Cabernet franc (A) and Petit Verdot (B) grape carotenoids (ΔC) from pre-veraison to harvest as affected by no leaf removal (NO), and post-fruit set removal to medium (MED) and high (HIGH) extents from 2012-2014. Treatment bars not sharing a letter within a year are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error, n = 6. NOTE: Lutein and β-carotene totaled in Cabernet franc; lutein, β-carotene, and zeaxanthin totaled in Petit Verdot.

Triangle difference test: Cabernet franc and Petit Verdot. Panelists were able to determine the difference in Cabernet franc wine color between HIGH and NONE, and the difference in Cabernet franc wine aroma between MED and NO (Table 28). Panelists were able to determine the difference in Petit Verdot wine aroma between MED and NO, and MED and HIGH.

Table 28. Triangle difference test of Cabernet franc and Petit Verdot wines from the 2012 vintage.

Cabernet franc				
Treatment comparison[a]	**Attribute**	**Panelist #**	**Successes**	**Significance[b]**
NO vs. HIGH	Color	26	14	0.0248
NO vs. HIGH	Aroma	26	9	ns
NO vs. HIGH	Taste	26	7	ns
NO vs. MED	Color	23	11	ns
NO vs. MED	Aroma	23	12	0.0481
NO vs. MED	Taste	23	7	ns
MED vs. HIGH	Color	39	16	ns
MED vs. HIGH	Aroma	39	14	ns
MED vs. HIGH	Taste	39	12	ns
Petit Verdot				
Comparison	**Attribute**	**Panelist #**	**Successes**	**Significance[b]**
NO vs. HIGH	Color	21	10	ns
NO vs. HIGH	Aroma	21	3	ns
NO vs. HIGH	Taste	21	8	ns
NO vs. MED	Color	17	9	ns
NO vs. MED	Aroma	17	10	0.0273
NO vs. MED	Taste	17	8	ns
MED vs. HIGH	Color	22	9	ns
MED vs. HIGH	Aroma	22	14	0.0035
MED vs. HIGH	Taste	22	6	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.
[b]Significance of treatment effects (p > F; ns = not significant at 0.05 level) determined using an Alpha one-tailed analysis.

Consumer preference test: Cabernet franc and Petit Verdot. 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 29). 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.

Table 29. 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.
[b]84 panelists participated in the Cabernet franc consumer preference test.
[c]61 panelists participated in the Petit Verdot consumer preference test.
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 based on adjusted p-values using Duncan;s Multiple Range test.

Discussion

Berry temperature, ambient radiation, and ambient air temperature: Grape berry temperature was primarily driven by ambient air temperature, as shown before (Bergvist et al. 2001, Cola et al. 2009). The greater estimated time that berries spent ≥ 30 or 35 °C in 2013 compared to 2014 was likely due to the relatively warmer air temperatures experienced in 2013, as higher grape temperatures are characteristic of warmer regions and seasons (Bergqvist et al. 2001, Farina et al. 2010). Radiation can confound berry temperature (Cola et al. 2009). In the current study, it was shown to increase berry temperature, as others have demonstrated (Bergvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). As long as radiation was not blocked by a cloud or the grapevine canopy, higher radiation typically increased grape temperature above ambient air temperature, supportive that direct sunlight radiantly heats fruits more efficiently than diffuse light (Smart and Sinclair 1976). Sunlight and temperature are two primary sources of variation in grape flavonoids across seasons (Downey et al. 2006), and temperature and sunlight alter the synthesis and degradation patterns of grape carotenoids and norisoprenoids (Farina et al. 2010, Lee et al. 2007). Thus, it was anticipated that grapes sampled from different seasons and/or leaf removal treatments would have different concentrations of these compounds. However, the variable cloudiness and moderate climate of the current study limited temperature differences experienced between shaded and exposed berries, as elaborated upon below.

The current study was conducted in a north-south oriented, vertically-shoot positioned vineyard in a humid growing region. Consequently, variable cloudiness was common, and when solar radiation reached its diurnal apex, it was blocked by the above-head canopy. Further, while ambient PAR was relatively greater in the AM, ambient air temperature was relatively greater in the PM. Thus, the diurnal period of greatest radiant heating potential of grapes was avoided, and

concomitant high ambient temperature and radiation conditions were infrequently experienced when the sun was angled on the fruit-zone of either canopy side. These weather patterns were not conducive to extreme heating of berries, nor to remarkable differences in berry temperature between canopy sides. These patterns were anticipated to contrast those of warmer/arid regions (Bergvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). Those studies reported that exposed berry temperature greatly differed between canopy sides, and that exposed berry temperature was frequently greater than that of shaded berries, even at midday (Bergvist et al. 2001), even when leaf removal was not practiced (Tarara et al. 2008), and often to ≥ 30-35 °C (Bergvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008).

Bergqvist et al. (2001) noted that differences in berry temperature between shaded and exposed fruit-zones may be less extreme in cooler regions. The results of the current study are in agreement, but would add that temperature difference between well-exposed and shaded grapes may be less pronounced even in even warm, humid growing regions due to variable cloudiness. Variable sky conditions make berry temperature difficult to predict, particularly in humid regions (Cola et al. 2009). In agreement with previous work (Bergvist et al. 2001) the results of the current study confirm that radiant heating of grapes is the primary determinant of berry temperature difference between exposed and shaded grapes and, when often changing, would confound the ability to forecast berry temperature. Thus, it cannot be assumed that exposed grapes will necessarily experience greatly different radiation and temperatures compared to shaded berries, but this will be highly dependent on the temporal difference in sky conditions in a humid region. The climatic differences between the current study and other studies that well-characterized berry temperature (Bergqvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008)

support that modelling is an undeveloped approach when trying to compare regional difference in berry temperature and its impact on grape composition (Bonada and Sadrtas 2014).

Berry temperature was measured periodically over the seasons. It is unlikely, however, that single berry temperature events impact fruit composition, especially in a field setting where temperature and radiation cannot be manipulated without strategic experimental design (Spayd et al. 2002, Tarara et al. 2008). Thus, statistical difference in berry temperature was determined on occasion, but this was of questionable biological significance. Compared to shaded grapes, the temperature of exposed grapes was measured more frequently, and estimated to spend more time, ≥ 30 or 35 °C. However, grape temperatures in the current study were cooler than others (Bergqvist et al. 2001), and it was estimated that grapes did not spend nearly as much time ≥ 30 or 35 °C compared to other studies (Pastore et al. 2013, Tarara et al. 2008), regardless of canopy side or leaf removal magnitude. In the current study, there was likely less impact on fruit composition, particularly anthocyanins, between exposed/shaded fruit-zones and canopy sides when compared to previous studies (Bergvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). These studies were conducted in regions where afternoon temperature and radiation are likely concomitantly high, thus favoring radiant grape heating to temperatures that are detrimental to grape anthocyanin accumulation, particularly on the west or south side canopy side. In humid regions, it may be erroneous to characterize a canopy side by the relative term "hot," especially in north-south oriented rows and particularly under cloudy conditions. Due to modest berry temperature increases and decreased disease incidence experienced in open fruit-zones (English et al. 1989, Wolf et al. 1986), canopy management practices that provide direct light into the fruit-zone may be better suited for temperate humid regions, contrasting the canopy management recommendations in relatively warmer/arid regions (Bergqvist et al. 2001, Spayd et al. 2002).

Berries measured in Petit Verdot and pre-bloom leaf removal plots had relatively lower temperature and spent less estimated time ≥ 30 °C when compared to Cabernet franc and aggressive post-fruit set leaf removal, respectively. Both Petit Verdot and P-B had smaller berries and looser clusters compared to Cabernet franc and HIGH, respectively, which can result in comparatively lower berry temperatures compared to when berries touch each other in a tighter cluster (Keller 2010). Though anectodal, the greater grape surface area exposed to air flow, the greater the evaporative cooling. As such, pre-bloom leaf removal may not only be a prudent practice to reduce sunburn incidence (Pastore et al. 2013), but also alleviate heat loads on fruit when ambient weather conditions are conducive to extreme heating. When compared to Cabernet franc, Petit Verdot was more vigorous and had larger canopies, and had a post-veraison period that was relatively shorter, and later-shifted. These observations make a case for growing Petit Verdot, and other later-maturing red varieties, if temperature-induced anthocyanin degradation is of concern. Yet varieties may differ in their susceptibility to temperature-induced changes in grape anthocyanins (Kotseridis et al. 2012, Mori et al. 2007).

Components of crop yield: As shown by others, crop yield can be reduced by leaf removal timing and extent (Intrieri et al. 2008, Kliewer 1970, Pastore et al. 2013, Tardaguila et al. 2010). This reduction in crop is primarily due to a reduction in cluster weight observed in pre-bloom leaf removal plots. Removing leaves before bloom reduced crop yield more consistently, and to a greater extent, than did any post-fruit set leaf removal treatment. This was anticipated, as leaf removal had lesser impact on crop yield reduction when implemented well after compared to immediately after bloom (Candolfi-Vasconcelos and Koblet 1990). Pre-bloom leaf removal reduced crop yield by an average of 50-53% when compared to no leaf removal over 2013-2014 of the current study. Crop yield reduction ranged from 30-71% across several different

magnitudes of pre-bloom leaf removal, varieties, and climates (Diago et al. 2012, Palliotti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2008, and 2010, Gatti et al. 2012, Intrieri et al. 2008, Tardaguila et al. 2010 and 2012). Crop yield reduction was primarily a function of pre-bloom leaf removal's impact on cluster weight, which was reduced by an average of 37-39% in Cabernet franc and Petit Verdot in the current study. While pre-bloom leaf removal did not differentially impact cluster weight between these varieties, berry number per cluster and berry weight were differentially affected (discussed below). Others have reported a 20-69% reduction in cluster weight and 14-64% reduction in berry number per cluster (Diago et al. 2012, Palliotti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2008, and 2010, Gatti et al. 2012, Intrieri et al. 2008, Tardaguila et al. 2010 and 2012) as a function of pre-bloom leaf removal. These studies also reported a reduction in fruit set by 8-37%, which appears to be the primary mechanism by which pre-bloom leaf removal reduced crop yield.

Carbohydrate supply to developing flowers is necessary from inflorescence initiation in the previous summer through fruit set in the current season for successful berry formation (Candolfi-Vasconcelos and Koblet 1990, Lebon et al. 2008). Source tissue limitation before bloom can result in flower abortion, particularly by disturbing meiosis, as anthers and ovules are particularly sensitive to carbohydrate deprivation (Saini 1997, Jean and Lapointe 2001, Lebon et al. 2008). Since carbohydrate supply is important for fruit set, removal of current season source tissues can result in flower necrosis and abscission (Candolfi-Vasconcelos and Koblet 1990 and 1991, Caspari and Lang 1996, Caspari et al. 1998, Coombe 1962). It is frequently cool, cloudy, and rainy in the region that the current study was conducted in, and these conditions are unfavorable for pollen viability and germination rates (Koblet 1966). Though bloom-time weather was not recorded at the experimental vineyard, the average temperature in the week

corresponding to bloom was 20.5 °C in 2013 and 18.8 C in 2014, as logged with a weather station located ~ 29 km away from the Cabernet franc and Petit Verdot experimental vineyard. These temperatures are below 25.0 °C, the lower threshold of the optimal temperature range for pollen germination (Keller 2010). Though the entire reduction in crop yield cannot be attributed to lower bloom-time temperature, there were drastically lower berry numbers per cluster and other crop yield components in 2014 compared to 2013. Fruit set, flower abscission, and vine or inflorescence carbohydrate status were not measured in the current study. However, the reduction in berry number per cluster suggested that fruit set was reduced due to the tandem reduction in carbohydrate supply and sub-optimal temperatures surrounding bloom.

Pre-bloom leaf removal reduced berry number per cluster by 14-64% in several studies (Diago et al. 2012, Palliotti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2008, and 2010, Gatti et al. 2012, Intrieri et al. 2008, Tardiguila et al. 2010 and 2012). While carbohydrate status was not measured, many of the above studies cited the reduction in source tissue amount/carbohydrate supply before bloom to be the primary cause of reduced berry number per cluster (Candolfi-Vasconcelos and Koblet 1990, Caspari and Lang 1996, Coombe 1962). The removal of more leaves in Trebbiano resulted in a greater reduction in fruit set and berry number per cluster compared to removing fewer leaves in Sangiovese (Poni et al. 2006). While this comparison was confounded by experimental setting (Trebbiano in a field; Sangiovese in a pot), it did support that carbohydrate supply is important

Pre-bloom, or early-season, leaf removal differentially impacted berry number per cluster between varieties in the current study as well as other studies (Poni et al. 2006 and 2008, Sabbatini et al. 2010, Tardaguila et al. 2010). While Taradaguila et al. (2010) did not elaborate on findings, vigorous shoots in Vignoles were suggested to serve as more competitive sinks

compared to *V. vinifera* varieties, resulting in comparatively fewer Vignoles berries per cluster (Sabbatini and Howell 2010). In the current study, Petit Verdot (anecdotally) had relatively greater vigor than Cabernet franc, but pre-bloom leaf removal reduced Cabernet franc berry number per cluster to a comparatively greater extent. Further relatively less leaf area re-growth occurred, interpreted as lower vine vigor, in varieties in which berry number per cluster was reduced to a relatively greater extent (Poni et al. 2006 and 2008). Thus, the inherent vigor of a variety may not necessarily be a good proxy for pre-bloom leaf removal's differential impact on berry number per cluster between varieties, as suggested in Sabbatini and Howell (2010).

The relatively greater vine vigor and leaf size of Petit Verdot was perhaps advantageous in maintaining enough remaining leaf area to limit carbohydrate deficit and subsequent fruit set reduction compared to Cabernet franc. Cabernet franc was grown with under-trellis cover crops, which have been shown to reduce vine nitrogen status (Hickey et al. 2016). Though plant nutrient analyses were not conducted in the current study, it has been shown that vines grown with companion cover crops that did not receive supplemental nitrogen experienced reduced fruit set compared to those that received supplemental nitrogen (Keller et al. 2001). Flower abscission sensitivity to abiotic stressors and fruit set percentage can be variety-dependent, with greater inherent fruit set observed with greater starch and sucrose concentrations in inflorescences, and abiotic stress tolerance increased with starch presence in ovules and anthers (Lebon et al. 2004). Accounting for the stressors of pre-bloom leaf removal and sub-optimal bloom weather, it was possible that Cabernet franc had higher starch and sucrose concentrations while Petit Verdot had more starch reserves in the ovules and anthers during meiosis. Genetic predisposition for vine vigor, inherent berry number per cluster, or cultivar-specific distribution of carbohydrates in the reproductive tissues of the flower are all variety-dependent factors that

may confound pre-bloom leaf removal impact on berry number per cluster. Because both roots and leaves are carbohydrate source tissues during flowering and fruit set, variety-specific predisposition for root or rootstock for mass, as well as leaf area at bloom, may play a role in fruit set percent, and, consequently, berry number per cluster.

Reduced cluster compactness due to pre-bloom leaf removal was anticipated given previous reports to this effect (Palliotti et al. 2011, Poni et al. 2006 and 2008, Sabbatini and Howell 2010, Tardaguila et al. 2010 and 2012). Though berry number was reduced to the greatest extent by pre-bloom leaf removal, rachis length also tended to be reduced, perhaps due to distal portions of clusters being aborted. However, as a consequence, pre-bloom leaf removal often only inconsistently reduced cluster compactness. Looser clusters experience reduced bunch rot incidence (Hed et al. 2009, Palliotti et al. 2011, Poni et al. 2006, Sabbatini and Howell 2010, Tardaguila et al. 2010 and 2012), which is a desirable trait particularly in humid regions.

Pre-bloom leaf removal reduces berry weight (Gatti et al. 2012, Palliotti et al. 2011), but inconsistently (Bogicevic et al. 2015, Intrieri et al. 2008, Pastore et al. 2013, Poni et al. 2008, Tardaguila et al. 2012), and often in a variety-specific manner (Poni et al. 2006 and 2008, Sabbatini and Howell et al. 2010, Tardaguila et al. 2010). Authors did not elaborate on berry weight reduction (Gatti et al. 2012, Palliotti et al. 2011) or explained that berry size was not reduced by pre-bloom leaf removal because photosynthetic rate was increased in remaining leaves, or reduced berry number per cluster resulted in compensatory berry growth (Intrieri et al. 2008). Lateral shoots were maintained in Poni et al. (2008), and final leaf area equaled the control due to lateral growth compensation in Sangiovese (Poni et al. 2006); in these cases, the sustained leaf area deficit required to limit berry growth compensation may not have occurred. The results of the current study contrast those of Poni et al. (2006) that waiting until after

flowering is a better approach than removing leaves before bloom if the goal is to limit berry growth compensation due to reduced berry number per cluster. Interestingly, trends of the current study and others (Poni et al. 2006, Tardaguila et al. 2010) suggest that pre-bloom leaf removal impact on berry number per cluster may be indirectly related to its berry size. In these cases, it was typical for pre-bloom leaf removal to have a relatively greater effect on berry number per cluster or berry weight, but not both, when comparing varieties. Berry growth compensation occurs when berry number per cluster is reduced (Poni et al. 2008). However, the results of the current study and others (Gatti et al. 2012, Palliotti et al. 2011, Poni et al. 2006, Tardaguila et al. 2010) suggest that berry growth may not always be compensatory when berry number per cluster is reduced via early source tissues limitation.

The attenuated berry weight gain throughout berry growth stages I-III, particularly in the Petit Verdot pre-bloom leaf removal plots, paralleled the low source tissue treatment implemented shorty after fruit set in Ollat and Gaudillere (1998). Berry growth reduction may have been due to a lower solute content during berry growth stage I, as well as a *sustained* sink strength deficit (Ollat and Gaudillere 1998). Since pericarp cell division occurs during the first two weeks post-flowering (phase I) (Jona and Botta 1988), pre-bloom leaf removal may have reduced cell division. This appeared to be the case particularly in Petit Verdot, given the immediate and significant difference in berry weight at the initial sampling. Pre-bloom leaf removal reduced estimated pulp weight in Petit Verdot and Cabernet franc. If pulp weight is an estimation of cell number, then pre-bloom leaf removal may have reduced berry cell number.

Pre-bloom leaf removal can differentially affect the growth and presence of berry tissues. Pre-bloom leaf removal reduced seed number per berry in Sangiovese (Poni et al. 2006), but either increased or did not affect seed number per berry in Barbera and Lambrusco salamino

(Poni et al. 2008). While pre-bloom leaf removal did not reduce seed number, total and individual seed weight was numerically lower in Petit Verdot in the current study; these trends were similar to what Poni et al. (2006) reported, but contrasted what Poni et al. (2008) reported. Pre-bloom leaf removal can increase the berry skin: pulp ratio (Poni et al. 2006), and differentially promote berry skin growth (Pastore et al. 2013, Poni et al. 2008 and 2010) regardless of berry size (Poni et al. 2008). The suggested mechanism of the promotion of grape skin growth was due to radiation and temperature exposure for extended periods of time (Pastore et al. 2013, Pallioti et al. 2011, Poni et al. 2008 and 2010). Estimated pulp weight was the only berry tissue component that was consistently reduced by pre-bloom leaf removal in the current study. However, because lighter berries likely have a smaller surface area and heavier skins are likely thicker, pre-bloom leaf removal may have increased berry skin thickness, particularly in Petit Verdot; this was only speculation, and was not statistically proven.

Pre-bloom leaf removal reduced current-season fruitfulness by 6%, and subsequent-season fruitfulness by 21-30% (Sabbatini and Howell 2010, Tardaguila et al. 2012). However, pre-bloom leaf removal did not always impact fruitfulness Intrieri et al. 2008, Gatti et al. 2012, Palliotti et al. 2011, Pastore et al. 2013). In the current study, pre-bloom leaf removal reduced cluster number per vine in one of two years. Vine fruitfulness, (inflorescence count per shoot number) was inconsistently impacted by pre-bloom leaf removal. The disconnect between vine fruitfulness and cluster number per vine was due to the fact that fruitfulness data were collected the year *after* yield components were collected, and experimental plots were rearranged to limit carry-over effects of pre-bloom leaf removal. There were times during pre-bloom leaf removal implementation when whole shoots or parts of shoots were damaged. The result was fewer cluster number per vine, particularly in Petit Verdot, which has shoots that are more tender than

those of Cabernet franc. Aggressive fruit-zone leaf removal increases radiation penetration to basal buds. The increased radiation to the basal buds can increase fruitfulness in the following season (Perez and Kliewer 1990, Sanchez and Dokoozlian 2005). This may offset the negative impact of source tissue limitation on bud fertility, particularly in spur-pruned vines. Since vine carbohydrate status during inflorescence initiation (i.e. summer before flowering) impacts vine fruitfulness (Candolfi-Vasconcelos and Koblet 1990), aggressive leaf removal apparently did not reduce vine carbohydrate status enough to limit fruitfulness in the current study.

Pre-bloom leaf removal *further* reduced crop yield when implemented in two consecutive seasons in both Cabernet franc and Petit Verdot. Since inflorescence primordia are initiated one year before flowering, early-season leaf removal can reduce fruitfulness and, thus, crop yield in both the current and following season (Candolfi-Vasconcelos and Koblet 1990, Lebon et al. 2008). One of the yield components that was further reduced the most due to re-implementation of pre-bloom leaf removal was cluster number per vine. However, pre-bloom leaf removal did not reduce vine fruitfulness in the experimental plots used for carry-over analysis. Thus, cluster number reduction was either due to physical damage incurred when removing leaves on young, tender shoots, or due to whole-cluster abortion. Berry number per cluster and cluster weight were the other yield components that were further reduced, suggesting that repeated leaf pre-bloom leaf removal can continue to reduce berries per cluster and cluster weights (Gatti et al. 2012, Palliotti et al. 2011) as a function of *further* reduced fruit set (Sabbatini and Howell 2010). Carbohydrate reserves can be depressed due to source tissue reduction during the previous-season's fruit ripening period (Candolfi-Vasconcelos and Koblet 1990), but also due to excessive crop loads, as well as poor photosynthesis conditions prior to current-season flowering. Thus, pruning weight in the current-season plots may have been reduced due to source tissue limitation

before bloom, as well as the continual depletion of storage reserves in permanent vine parts (Palliotti et al. 2011). Since reduced carbohydrate availability is partially responsible for fruit set reduction (Candolfi-Vasconcelos and Koblet 1990, Caspari and Lang 1996, Coombe 1962), and part of the bloom and fruit set period is supported by mobilized carbohydrates (Lebon et al. 2008), recurring pre-bloom leaf removal may hasten the depletion of storage reserves, and, consequently, reduce vine size and crop yield potential at an atypically early vine age.

Primary fruit chemistry: Previous studies have frequently shown that pre-bloom leaf removal increased soluble solids concentration (Diago et al. 2012, Gatti et al. 2012, Intrieri et al. 2008, Palliotti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2008, and 2010, Sabbatini and Howell 2010, Tardaguila et al. 2012). Soluble solids were increased by greater leaf area: fruit weight ratios (Gatti et al. 2012, Pallioti et al. 2011, Tardaguila et al. 2010 and 2012), and restored and more photosynthetically efficient leaf area in the post-veraison period, resulting hastened berry sugaring (Intrieri et al. 2008, Pallioti et al. 2011, Pastore et al. 2013, Poni et al. 2006). Less absolute soluble solids are required to reach a given soluble solids concentration in relatively smaller berries (Ollat and Gaudillere 1998). This may have been why there was a more consistent reduction in soluble solids due to post-fruit set removal of leaves to the greatest extent in Petit Verdot in the current study. While neither leaf photosynthetic rates nor leaf area was measured in the current study, a similar leaf area amount was removed between pre-bloom leaf removal and post fruit set leaf removal to greatest extent. Though anecdotal, Cabernet franc canopies were sparser and shoots appeared to grow at a slower rate compared to Petit Verdot. Thus, it was possible that leaf removal did not reduce soluble solids in Cabernet franc due to relatively limited vegetative sink competition and/or increased exposed leaf area. Even though remaining leaves from partially defoliated vines are often more efficient carbon assimilators

(Candolfi Vasconcelos and Koblet 1990, Buttrose 1966, Kliewer et al. 1970, Poni et al. 2006), it was possible that leaf area in Petit Verdot was maintained at a deficit that was great enough to limit soluble solids; this may have been the case particularly in 2013, the year that crop loads were relatively greatest. Early leaf removal can delay the onset of ripening compared to later defoliation due to a leaf area deficit and a reduction in sink strength and assimilate translocation to fruit (Ollat and Gaudillere 1998, Pastore et al. 2013). A low sink strength may have been especially responsible for low soluble solids concentrations in Petit Verdot in 2014, when crop loads were about one third less than crop loads were in 2013. Most pre-bloom leaf removal studies were conducted in drier regions. Thus, it was assumed that post-veraison weather was more conducive to fruit ripening in those regions compared to the frequently cool/cloudy weather in Virginia. Growing degree day sum, and average monthly PAR, were lower in the post- compared to pre-veraison periods of 2013 and 2014. It was cooler, and more rain fell, in 2014 compared to 2013, and these patterns were maintained in the post-veraison period. Additionally, a June 2014 hail storm resulted in some leaf damage. These factors, combined with relatively lower leaf area, may have resulted in relatively greater attenuation in soluble solids accumulation in the aggressive leaf removal treatments in the 2014 season compared to in the 2013 season.

Fruit-zone leaf removal often reduces titratable acidity (TA) which can be attributed to temperature-driven malic acid respiration (Jackson and Lombard 1993, Kliewer and Schultz 1964). Aggressive leaf removal tended to reduce titratable acidity in Cabernet franc and Petit Verdot, albeit inconsistently. The relatively greater reduction in titratable acidity experienced with pre-bloom and post-fruit set removal of leaves to the greatest extent in 2013 may have been due to the general warmer temperature in 2013 when compared to 2014. Previous studies also

found that pre-bloom leaf removal inconsistently affected (Gatti et al. 2012, Intrieri et al. 2008, Palliotti et al. 2011, Poni et al. 2006, Tardaguila et al. 2010) TA. Two studies in which pre-bloom leaf removal had no effect on titratable acidity also noted the re-growth of laterals from basal nodes (Intrieri et al. 2008, Palliotti et al. 2011); in these cases, fruit-zone shading may have limited fruit temperature. Similarly, in the current study, the higher vigor Petit Verdot canopies may have blocked more incident radiation, resulting in less malic acid respiration. Pre-bloom leaf removal's variable impact on titratable acidity was suggested (Poni et al. 2006) to be due to greater carbon accumulation into tartaric acid in sun versus shade berries (Kliewer and Schultz 1964). The increase in titratable acidity in Petit Verdot pre-bloom leaf removal plots was an unexpected response in 2014. Tartaric acid reduction during ripening is a dilution effect – tartrate levels don't change as berries gain mass (Johnson and Carroll 1973). Pre-bloom leaf removal considerably reduced Petit Verdot berry weight in both 2013 and 2014. Thus, TA levels may not have been different (2013), and even increased (2014) because acids in grapes from the pre-bloom leaf removal treatment were not diluted as much as they were in the grapes from other treatments.

Total phenolics and anthocyanins: Pre-bloom leaf removal increased total grape phenolics in Petit Verdot, and less consistently in Cabernet franc in this study. Open fruit zones have been shown to increase grape phenolics (Jackson and Lombard 1993, Price et al. 1995, Smart and Robinson 1991). However, total phenolics were only increased by pre-bloom leaf removal in the current study, which suggested that leaf removal timing was important for this response. There are several subclasses of grape phenolics that accumulate at different periods of grape development, and are differentially affected by temperature and radiation (Downey et al. 2006). When these subclasses are combined into total phenolics, it is difficult to determine if specific

phenolic compounds were increased from longer exposure to increased radiation and berry temperatures. The physical changes incurred by berries, namely smaller size and lower estimated pulp weight, was the most likely mechanism behind the increase in total phenolics observed in grapes from the pre-bloom leaf-removal plots. Pre-bloom leaf removal only increased total phenolics in Cabernet franc in the same year that berry weight was also reduced. Further, the negative relationship that existed between berry weight and total phenolics was (1) significant in every instance that pre-bloom leaf removal increased total phenolics in both varieties, and (2) always greater in the Petit Verdot, the variety that had lower berry weight. Differential berry tissue growth could not be ruled as another factor that increased total phenolics, as trends suggested that there may have been relatively thicker skins in the berries from pre-bloom leaf removal plots compared to other treatments.

In the current study, aggressive fruit-zone leaf removal did not affect total grape anthocyanins in either Cabernet franc or Petit Verdot, regardless if implemented before bloom or after fruit set. Thus, total anthocyanins were not increased with the lower crop loads, smaller berries, and open fruit-zone microclimates associated with pre-bloom leaf removal plots. Since anthocyanins are one compound class of the broad subclass of phenolics called flavonoids, it was possible that they were not in high enough concentrations in skins to be *further* concentrated by smaller berry weight and, thus, differences were not detected with the quantification methods used in the current study. Anthocyanins tend to be reduced at grape temperatures of 30-35 °C (Downey et al. 2006, Spayd et al. 2002, Tarara et al. 2008, Yamane et al. 2006). However, grape anthocyanins were increased in pre-bloom compared to veraison-time leaf removal plots, even when berries spent ~300 hrs. ≥ 30 °C in both plots during the post-verasion period (Pastore et al. 2013). Pre-bloom leaf removal can thus increase anthocyanins by additional mechanisms than

the microclimate created by leaf removal, such as the promotion of thicker berry skins (Pallioti et al. 2011, Poni et al. 2008), or greater leaf area: fruit weight ratios (Gatti et al. 2012, Intrieri et al. 2008). While anthocyanins were decreased when *shaded* berries were artificially heated to detrimental temperatures for relatively shorter periods than *exposed* berries (142 hrs. ≥ 30 °C and 15 hrs. ≥ 35 °C) (Tarara et al. 2008), shaded fruit is cooler than exposed fruit, and is typically near ambient air temperature in a field setting (Spayd et al. 2002, and results presented herein). Thus, unless post-veraison weather is characterized by consistent ambient air temperatures ≥ 30-35 °C, shaded fruit may not be heated to temperatures that are detrimental to anthocyanin accumulation. In the experimental vineyard in which the current study was conducted, ambient air temperature was never ≥ 35 °C, and ≥ 30 °C for only 39 and 16 hrs. during the entire post veraison periods of 2013 and 2014, respectively. Given the grape temperature trends that were presented earlier in the discussion, it was unlikely that grape anthocyanins were limited by temperatures in the current study, even in the most exposed fruit-zones.

The general leaf removal practice in Virginia is to aim for one to two fruit-zone leaf layers (Wolf 2008). This is likely because of the documented cases that anthocyanins were reduced with aggressive leaf removal. These popular studies were conducted in regions that are warmer, drier, and sunnier compared to the humid growing regions in the eastern US (Bergqvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). That grape anthocyanins were not reduced fruit-zones with ~ 0.1 – 0.6 leaf layers was considered a positive response, as fungal disease incidence is generally improved with more open fruit-zones (English et al. 1989, Wolf et al. 1986). In the current study, aggressive leaf removal resulted in fruit-zones characterized by moderate east- and west-side berry temperatures and increased radiation penetration, a beneficial or at least not detrimental, combination for grape anthocyanins (Tarara et al. 2008). Therefore,

basal leaf removal in red-fruited varieties need not be as conservative as currently practiced in many eastern US growing regions, and need not be focused on the east canopy side, particularly if leaf removal is executed before or relatively soon after fruit set in order to reduce sunburn incidence (Pastore et al. 2013).

Grape carotenoids: Carotenoids tended to be greater in 2014, the relatively cooler year, compared to 2013, the relatively warmer year, and this was more consistent in Petit Verdot than in Cabernet franc. This result contrasts that increased carotenoid synthesis occurs in relatively warmer compared to cooler growing seasons (Farina et al. 2010). It is possible that carotenoid degradation was slower in the cooler post-veraison period of 2014 compared to 2013, as increased temperature increased post-veraison carotenoid degradation (Bureau et al. 1998b). Though temperature can apparently influence carotenoid synthesis and degradation patterns (Bureau et al. 1998b, Farina et al. 2010), separating the effect of radiation and temperature is difficult in a field setting and, thus, it was unknown if temperature plays a greater role in carotenoid synthesis or degradation, or if there are temperature thresholds that are particularly conducive or detrimental to carotenoid accumulation. Separating the effects of light and temperature was beyond the scope of this field study, but it was generally warmer, and berries spent more time ≥ 30 °C in the post-veraison period of 2013 compared to 2014. Thus, other factors equal, it is possible that carotenoid degradation increases at temperatures ≥ 30 °C. Nonetheless, Petit Verdot berry weight was lower in 2014 compared to 2013. Thus, the consistently greater carotenoid concentrations in Petit Verdot in 2014 may have simply been a function of increased concentration in smaller berries.

When averaged across all dates within a season, carotenoid differences were more consistently different between dates compared to between leaf removal treatments, and were

typically greater at all pre-harvest sample dates compared to at harvest. The trends of the current study are consistent with others' reports that carotenoids are highest early in berry formation and then progressively decrease as berries develop, with a sharp decrease seen at or after veraison (Razungles et al. 1988). The within-date increase in carotenoid concentrations in leaf removal compared to no leaf removal plots revealed that seasonal increases observed in grape carotenoids from leaf removal plots were due to increases in carotenoids during the pre-veraison period. Other studies also reported that carotenoid increases in well-exposed grapes occurs before veraison (Bureau et al. 1998 a and b) and that differences are attenuated thereafter, or that carotenoids were even greater when in shaded conditions during post-veraison period (Bureau et al. 1998 a and b, Razungles et al. 1998). In the current study, lutein and β-carotene were inconsistently greater in relatively shaded fruit-zones at harvest, consistent with other reports that these two major grape carotenoids were greater at maturity when grapes were shaded (Bureau et al. 1998 a and b). Thus, it is possible that these two major carotenoids are particularly sensitive to shade in the post-veraison period when compared to minor carotenoid classes.

When there were differences in carotenoids between leaf removal treatments at veraison, they were *typically* observed as increased zeaxanthin levels in aggressive post-fruit set and pre-bloom leaf removal plots, or as increased carotenoids in pre-bloom leaf removal plots. Zeaxanthin may have been the carotenoid that was most consistently increased by leaf removal at veraison because its patterns differ than those of the main grape carotenoids, lutein and β-carotene (Razungles et al. 1988, 1998). De-epoxidized xanthophylls, such as zeaxanthin, are favored by high light intensity whereas lutein and β-carotene are degraded by high light intensity (Bureau et al. 1998a and b, Razungles et al. 1998). Increased carotenoid concentrations at veraison was mainly observed in pre-bloom leaf removal plots; this was anecdotally observed as

a delay in grape coloration in these plots, particularly in 2014. As mentioned above regarding the lower soluble solids concentrations observed in Petit Verdot plots that had the most basal leaves removed, the attenuated grape coloration may have been due to a concomitant decrease in source tissues and sink strength during ripening in pre-bloom leaf removal plots. As such, carbohydrate supply to ripening fruit may have been a limited in these plots. A decrease in carotenoids as grapes mature may be due to competition between terpenoids (i.e. carotenoids) and phenolics (i.e. flavonoids) for common precursors, acetate and mevalonate (Razungles et al. 1988). If so, then there may not have been as much competition for these precursors in the pre-bloom leaf removal plots during veraison because coloration was delayed. Therefore, the synthesis of phenolic compounds such as anthocyanins were not in competition for common precursor compounds.

Basal leaf removal effect on grape carotenoids in the current study are consistent with findings of others that fruit exposure can enhance the pre-veraison accumulation, and post-veraison breakdown, of carotenoids (Bureau et al. 1998a and b, Razungles et al. 1998). These responses were anticipated, as light is necessary for carotenoid biosynthesis, and the activity of phytoene synthase, an enzyme gene responsible for the first committed step in the carotenoid biosynthetic pathway, is stimulated by light (Hirschberg et al. 2001 and those cited within). Further, fruit exposure enhances pre-veraison accumulation and post-veraison breakdown of grape carotenoids (Razungles et al. 1998). The difference in peak and harvest carotenoids were correlated with C_{13}-norisoprenoids in wines – the greater the magnitude of degradation in grape carotenoids, the greater the amount of C_{13}-norisoprenoids in wine (Crupi et al. 2010). Thus, in the current study, fruit-zones associated with greater fruit-zone radiation penetration potentially

had greater C_{13}-norisoprenoid concentrations in wines, as there tended to be greater degradation in carotenoids from pre-veraison to harvest when grapes were most exposed to light.

The four carotenoids analyzed in the current study were, however, differentially influenced by basal leaf removal – zeaxanthin was most consistently increased and degraded by fruit exposure, while lutein 5,6-epoxide and lutein were affected by leaf removal practice more consistently than β-carotene was. In a Syrah study, shade tended to increase epoxified xanthophyll forms (Bureau et al. 1998a), which supported that high light intensity causes de-epoxidation of violaxanthin to zeaxanthin while shade causes epoxidation of zeaxanthin to violaxanthin via antheraxathin (Baumes et al. 2002). While results of the current study cannot disprove that zeaxanthin and lutein experience similar patterns during ripening (Crupi et al. 2010), trends showed that zeaxanthin is much more influenced by fruit exposure than lutein. This may be a function of the different physiological roles these two carotenoids play: β-carotene and lutein are mainly involved in light harvesting, while xanthophylls are involved in photoprotection, and aid in plant tissue adaptation to different light conditions (Hirschberg 2001). Nonetheless, xanthophylls increase under strong light and the ratio between lutein and the xanthophylls decreases, and the mRNA levels of enzyme genes responsible for the flux of carotenoid synthesis down the "xanthophyll side" of the biosynthetic pathway were increased to a greater extent than the "lutein side" when plants were shifted from low light to strong light (Hirschberg et al. 2001 and those cited within). The results of the current study support these findings, as the zeaxanthin: lutein ratios were consistently greater in well-exposed compared to shaded Petit Verdot and Cabernet franc grapes at every sample date when averaged across all three years.

Studies conducted in more Mediterranean-type climates observed that lutein and β-carotene synthesis and degradation were increased in sun-exposed relative to shaded grapes (Bureau et al. 1998a and b, Razungles et al. 1998). The only study known to look at fruit exposure effects on carotenoids in a region analogous to the current study found that zeaxanthin was increased with basal leaf removal compared to a shaded control, while lutein and β-carotene were less consistently affected (Kwasniewski et al. 2010). Thus, in humid regions, it is possible that the required radiation and temperature thresholds needed to differentially impact lutein and β-carotene are not consistently experienced. Given the physiological role of zeaxanthin as an important photoprotector of photosystem II, where it quenches triplet state chlorophyll and reduces the likelihood of reactive singlet oxygen (Dall'Osto et al. 2012), it is likely that zeaxannthin is the most responsive carotenoid to changes in light conditions. Similar to flavonols being a good phenolic proxy for grape exposure to light (Downey et al. 2006), zeaxanthin may be the best carotenoid indicator for grape exposure to light across a wide range of climates.

It has been shown that carotenoid concentrations can greatly vary across varieties (Crupi et al. 2010), and that fruit exposure can differentially influence carotenoid degradation patterns between a white (Muscat of Frontignan) compared to a red (Syrah) variety (Bureau et al. 1998a and b). In the current study, increased grape carotenoid concentrations, particularly lutein and β-carotene, were observed in Petit Verdot when compared to Cabernet franc, and, in 2014, on the west compared to east side in Cabernet franc. Since carotenoids are found in grape skins at levels two to three times greater than in the pulp (Razungles et al. 1988), the increase in carotenoid concentrations in both of these cases was speculated to be a function of the smaller berries. The inherent smaller berry size in Petit Verdot may have been why there were relatively

more treatment*date interactions observed in Petit Verdot relative to Cabernet franc across all seasons. The relatively small difference in carotenoid concentration that was needed in order to determine treatment effect over time may have been attenuated by the dilution effect of relatively greater pulp amounts in Cabernet franc berries. Though concentrations of carotenoids changed in Cabernet franc berry skins over the season, they changed less consistently compared to Petit Verdot in 2013, the year that berry weight was unaffected by leaf removal treatment in Cabernet franc. Further, while lutein 5,6-epoxide was inconsistently detected in both Petit Verdot and Cabernet franc berry samples at harvest, zeaxanthin was also inconsistently detected in Cabernet franc berry samples at harvest. Again, this was putatively due to the dilution effect of the relatively larger berry size and relative pulp amount in Cabernet franc.

Since lutein and β-carotene were in greater concentrations particularly in Petit Verdot, and β-carotene and lutein constitute almost 85% of the total carotenoids in mature grapes (Mendes-Pinto 2002), the C_{13}-norisoprenoids derived from β-carotene and lutein may be more directly involved in wine aroma than others (Winterhalter and Rouseff 2002). From a practical winemaking standpoint, the relatively small berry size in Petit Verdot may have resulted in an increased concentration of β-ionone in wine musts, as this norisoprenoid has been identified as a product of both lutein and β-carotene (Isoe et al. 1969; Kanasawud and Crouzet 1990; Eugster and Marki-Fischer 1991; Marais 1992). Since β-ionone has sensory descriptors of "violet, woody, and raspberry" (Winterhalter and Rouseff 2002), an increase in its concentration may result in detectable increases in fruity and floral aromas in wine. These features may be the reason that regional winemaking trends have been towards decreasing the amount of Petit Verdot used in Bordeaux blends, possibly to achieve a wine characterized less by fruity and floral notes, and more aligned with classic Bordeaux characteristics such as earthy, leathery, and tannic.

Wine sensory: Cabernet franc wine color was distinguished between the most aggressive post-fruit set leaf removal treatment and the no leaf removal treatment. Further, wines made from aggressive leaf removal treatments received higher intensity ratings for red color in both varieties, and for astringency in Cabernet franc. Wine phenolics, anthocyanins, and color density tend to be increased with sun exposure (Di Profio et al. 2011, Ristic et al. 2007, Smart and Robinson 1991, Staff et al. 1997, Verzera et al. 2016). Accordingly, basal leaf removal increased Cabernet franc wine color and improved palatability (Staff et al. 2007) and Cabernet franc and Merlot wine viscosity and length (Di Profio et al. 2011). While tannins were not measured in the current study, they are the least susceptible flavonoid to light exposure (Downey et al. 2006). Shading reduced tannins in grapes and wine, which also had a lower astringency rating (Ristic et al. 2007). Thus, exposed Cabernet franc grapes may have had greater tannin concentration and, consequently, greater wine astringency, as this was a sensory attribute that has been positively correlated with wine tannins (Cassasa et al. 2013). The contribution of anthocyanins to red wine color is complex, and is a function of both monomeric forms and copigmentation (He et al. 2012). Monomeric anthocyanins are largely responsible for the color of young red wines (He et al. 2012). While monomeric anthocyanin absorbance at 520 nm is reduced as pH increases from one to five (Cabrita et al. 2000), the pH range was low, never exceeding more than a 0.09-unit difference between treatment wines of the current study, regardless of year or variety. Thus, it was unlikely that color intensity was affected by the relationship of wine pH and monomeric anthocyanins. Total grape anthocyanins were not affected by leaf removal treatment, but pre-bloom leaf removal increased grape phenolics and reduced berry size in Petit Verdot in 2013. The result may have been a net increase in phenolic and anthocyanin concentration in pre-bloom leaf removal wine musts, as smaller berries can

result in greater color saturation in wines, likely due to the concentration of skin anthocynains (Cassasa et al. 2015). Thus, red wine color intensity may have been a function of anthocyanin copigmentationn with other phenolic compounds, as copigmentation can be responsible for 30-50% of the color of young red wines, and the principal cofactors in young red wines are flavonoid and non-falvonoid phenolics (He et al. 2012).

Basal leaf removal improved Cabernet franc wine aroma (Staff et al. 2007), and increased black pepper and black fruit aroma and decreased green characteristic in Cabernet franc, possibly due to increased norisoprenoids and a concomitant reduction in methoxypyrazines, as shown by Di Profio et al. (2011). In the current study, consumers did not prefer the sensory attributes of any Cabernet franc or Petit Verdot treatment wines over another. However, Cabernet franc and Petit Verdot wine aroma was distinguished between leaf removal treatments of different magnitude. While aroma compounds were not quantified in grapes or wines, aroma compounds can be increased in well-exposed fruit-zones, or decreased in shaded fruit-zones (Lee et al. 2007, Marais et al. 1992, Razungles et al. 1998, Ristic et al. 2007, Verzera et al. 2016). Grape carotenoids are precursors to norisoprenoids, a class of aroma compounds that are important odorants in young, mono-varietal red wines (Ferreira et al. 2000, Pineau et al. 2007), and particularly for Cabernet franc (Fan et al. 2010). Post-fruit set leaf removal to the medium extent had greater zeaxanthin levels than no leaf removal in the same season that wine aroma was significantly distinguished between these two treatments. Zeaxanthin-derived norisoprenoids include 3-hydroxy-7,8-dihydro-β-ionol and 3-hydroxy-β-ionone (Mathieu et al. 2005, Crupi et al. 2010), and zeaxanthin is a potential *in vivo* precursor of TDN (Kwasniewski et al. 2010). These norisoprenoids may have resulted in the ability of panelists to detect aroma difference between these two treatment wines. Though carotenoid patterns suggested that there was greater aromatic

potential in wines made with fruit from aggressive leaf removal plots in 2013, there were no significant difference in consumer preference of aroma from wines made from well-exposed and well-shaded treatments. Thus, the concentration of measured (i.e. carotenoids) and/or unmeasured (methoxypyrazines, norisoprenoids) aroma impact compounds were not affected enough by leaf removal practice to result in difference in wine aroma preference. It was speculated to have been difficult for panelists to determine wine preference without having a point of reference for comparison, as wines were served wines monadically, yet there was little difference in treatment preferences when wines were ranked side by side.

Conclusion: Best fruit-zone management practices were historically aggressive to improve fruit composition and disease control, but then became more conservative to limit sunburn and anthocyanin reduction. Comparable to a pest control program, or other cultural practices, this report confirms that fruit-zone leaf removal should be a region-specific practice. Aggressive leaf removal modestly improved fruit composition and wine color intensity, and has potential to reduce fungal disease incidence and, if implemented relatively close to fruit set, reduce sunburn. While very aggressive leaf removal before bloom can result in uneconomical reduction in crop yield, it is probable that similar fruit composition is attainable without drastic crop yield reduction if either fewer leaves are removed before bloom, or just as many leaves are removed immediately after fruit set. Carotenoids increased and decreased to a greater extent when leaves were removed from fruit-zones, and zeaxanthin appeared to be the carotenoid that was most susceptible to changes in radiation and temperature. Future work should focus on removing fewer leaves before bloom or immediately after fruit set in red and white varieties in order to evaluate if fruit composition can be maintained or improved without drastic crop yield reduction across varieties used for different enological goals.

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

Abstract

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 of the humid eastern US 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.

Introduction

Fruit-zone leaf removal reduces disease incidence and severity (English et al. 1989, Wolf et al. 1986). Accordingly, fruit-zone leaf removal is almost a ubiquitous recommendation, especially in humid climates where fungal diseases are prevalent. Early works on fruit exposure and diseases, in addition to general canopy management and environmental impact studies (Jackson and Lombard 1993, Smart and Robinson 1991), collectively recommended aggressive leaf removal to improve grape disease management and wine quality potential. More recent studies implied that the "more open the fruit-zone, the better" recommendation may not be true, as excessive fruit exposure can radiantly heat fruit to temperatures that are particularly detrimental to anthocyanins (Bergqvist et al. 2001, Mori et al. 2007, Spayd et al. 2002, Tarara et al. 2008). Though these field studies were conducted in relatively warm/dry growing regions of southeastern WA (Spayd et al. 2002, Tarara et al. 2008) and the San Joaquin Valley of CA (Bergqvist et al. 2001), fruit-zone management recommendations became, or remained, conservative - even in humid/temperate grape growing regions (1-2 leaf layers, Wolf 2008), even though anthocyanins are but one class of the several classes of sensory impact compounds in grapes, and even when many studies reported grape and wine quality to be increased with fruit exposure and reduced with shade (Lee et al. 2007; Ryona et al. 2008, Hunter, 1991, Jackson and Lombard 1993, Smith et al. 1988, Smart et al. 1985, Reynolds et al. 1996, Bledsoe et al. 1988, Carbonneau 1985, Di Profio et al. 2011, Staff et al. 1997, Smart and Robinson 1991).

Excessive fruit exposure can be deleterious to anthocyanins (Bergqvist et al. 2001, Mori et al. 2007, Spayd et al. 2002, Tarara et al. 2008). However, aggressive leaf removal increased anthocyanins (Di Profio et al. 2011, Staff et al. 2007), even when conducted in regions typically drier and warmer than the eastern US (Diago et al. 2012, Kotseridis et al. 2012). In Greece, post-

flowering leaf removal increased anthocyanins in Cabernet Sauvignon and Merlot, but not in Sangiovese, putatively a function of cultivar-specific tolerance of anthocyanins to detrimental temperatures, or a greater source: sink ratio dominated by more photosynthetically active apical leaves (Kotseridis et al. 2012). Post-fruit set leaf removal improved anthocyanins in Tempranillo grapes in Rioja, Spain, which was attributed to increased berry skin mass and/or a greater leaf area: crop weight ratio (Diago et al. 2012). Similar factors were at least partially responsible for improving anthocyanins and total phenols in pre-bloom leaf removal studies conducted on several varieties (Gatti et al. 2012, Pallioti et al. 2011, Poni et al. 2006 and 2008, Tardaguila et al. 2010). While the benefits of a more open fruit-zone microclimate could not be ruled out (Kotseridis et al. 2012, Taradaguila et al. 2012), pre-bloom leaf removal improved grape anthocyanins by other mechanisms, such as reduced berry size and increased skin: pulp ratio (Bogicevic et al. 2015, Poni et al. 2006) thicker berry skins (Pallioti et al. 2011, Poni et al. 2008, Tardaguila et al. 2010) and greater leaf area: fruit weight ratios (Diago et al. 2012, Intrieri et al. 2008, Gatti et al. 2012, Poni et al. 2006).

There are other documented benefits of pre-bloom leaf removal. Pre-bloom leaf removal improved canopy efficiency and hastened soluble solids accumulation (Pallioti et al. 2011, Poni et al. 2006), reduced cluster compactness (Pallioti et al. 2011) and bunch rot (Tardaguila et al. 2010 and 2012), and did not cause sunburn (Bogicevic et al. 2015) or reduced sunburn incidence compared to leaf removal at veraison (Pastore et al. 2013). Thus, aggressive pre-bloom removal warrants consideration for use in more humid, temperate growing regions, where detrimental grape temperatures were speculated to occur less frequently, diseases are more prevalent, and fruit ripening is occasionally delayed by inclement weather.

Light is necessary for anthocyanin synthesis, particularly if uncoupled from temperature extremes (Tarara et al. 2008). However, the eastern US tends to be cloudier and more temperate than regions where the above cited studies were conducted. Thus, it was hypothesized that aggressive fruit-zone leaf removal in a humid region would increase radiation penetration to grapes and only modestly increase berry temperatures, conditions that favor grape anthocyanins relative to berry temperature extremes in either sun or shade (Tarara et al. (2008). Removing leaves at the pre-bloom stage reduced cluster compactness and, consequently, bunch rot incidence (Hed et al. 2009, Pallioti et al. 2011, Poni et al. 2006, Sabbatini and Howell 2010, Tardaguila et al. 2010 and 2012). It was hypothesized that fruit-zone leaf removal in a humid region such as Virginia could be more aggressive than currently recommended (1-2 leaf layers, Reynolds and Wolf 2008) in order to improve disease management without compromising anthocyanins or other aspects of fruit chemistry.

The goal of this experiment was to evaluate aggressive pre-bloom or post-fruit set leaf/lateral shoot removal as a tool to alter crop yield components, or grape anthocyanins and phenolics, in Cabernet Sauvignon. An additional goal was to quantify the temperatures typical of shaded and exposed berries in a humid region. It was anticipated that the results of this study would characterize how fruit exposure can impact berry temperature, anthocyanins, and phenolics in the macroclimate of the humid eastern US.

Materials and Methods

Treatments and experimental design: Two experiments were conducted, both of which used Cabernet Sauvignon ENTAV-INRA® clone 337 vines, grafted onto 420-A rootstock, and grown at Virginia Tech's AHS, Jr. Agricultural Research and Extension Center (AHS, Jr. AREC) near Winchester, VA (39°11'N; 78°28'W). In both experiments, vines were planted in May 2006 in

rows running generally northeast/southwest at a 3.0-m (row) x 1.5-m (vine) spacing and were trained to bilateral cordons with vertically-positioned shoots. The soil was a Poplimento Hagerstown sandy loam (A. Blackburn, personal communication, 2013). The inter-row groundcover, established in 2001, initially comprised a mixture of orchard grass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*); cv. 'Shenandoah', with the fescue dominating after ~ six years. The intra-row groundcover in the pre-bloom leaf/lateral shoot removal experiment consisted of perennial creeping red fescue (*F. rubra*), established in Sep 2008, and an 85-cm wide herbicide-treated strip in the post-fruit set leaf/lateral shoot removal experiment. Leaf/lateral shoot removal treatments in the two separate experiments were as bulleted below (NOTE: from this point forward, treatments will be referred to as *leaf* removal treatments):

Expt. I: Pre-bloom leaf/lateral shoot removal experiment (2013-2015):

PB-NO - no leaves/lateral shoots removed; lateral shoots maintained at ~3-4 nodes.

PB-4 - removal of leaves/lateral shoots from primary shoot nodes 1-4 before bloom, [modified Eichorn and Lorenz (EL) stage 18-19 (Dry and Coombe 2004)]; distal lateral shoots maintained at ~3-4 nodes.

PB-8 - removal of leaves/lateral shoots from primary shoot nodes 1-8 before bloom, [modified Eichorn and Lorenz (EL) stage 18-19 (Dry and Coombe 2004)]; distal lateral shoots maintained at ~3-4 nodes.

Expt. II: Post-fruit set leaf/lateral shoot removal experiment (2014-2015):

PFS-NO - no leaves/lateral shoots removed; lateral shoots maintained at ~3-4 nodes.

PFS-6 - removal of leaves/lateral shoots from primary shoot nodes 1-6 at pea-berry size / bunch closure (modified EL stage 31/32); distal lateral shoots maintained at ~3-4 nodes.

A complete block design was used in each experiment, partitioning the experimental area into six blocks, each separated by five-vine border plots within the row and by bordering buffer rows. Within each block, leaf removal treatments were randomly assigned to either one-vine experimental units (pre-bloom experiment) or two-vine experimental units (post-fruit set experiment). Pre-bloom and post-fruit set leaf removal experimental units were no more than ~ 45 m away from each other in each vineyard block. Fruit-zone porosity was monitored weekly, and maintained from treatment initiation through harvest by removing any green tissue re-growth into the fruit-zone. Pre-bloom leaf removal in 2013 was re-implemented on the same vines in 2014 and 2015; these vines are henceforth referred to as "PB-4 '13re, or PB-8 '13re." Both pre-bloom and post-fruit set leaf removal were re-implemented in 2015 on vines initially used in 2014; only the pre-bloom vines are henceforth referred to as "PB-4 '14re, or PB-8 '14re." Figure 1 depicts the chronology of current-season and re-implemented pre-bloom leaf removal spatially and temporally, using the vines in experimental block 1 as an example.

2013	ROW POST	PB-NO X	PB-4 X	X	PB-8 X	X	ROW POST
2014	ROW POST	PB-NO X	PB-4 '13re X	PB-8 X	PB-8 '13re X	PB-4 X	ROW POST
2015	ROW POST	PB-NO X	PB-4 '13re X	PB-8 '14re X	PB-8 '13re X	PB-4 '14re X	ROW POST

Fig. 1 Chronology of pre-bloom leaf removal implementation and re-implementation in experimental block 1 over the course of the entire study, 2013-2015. "X" = vine; "PB-4/8'13re" = re-implementation of pre-bloom leaf removal that initially occurred in 2013; "PB-4/8'14re" = re-implementation of pre-bloom leaf removal that initially occurred in 2014.

Meteorology: Rainfall and ambient air temperature were recorded hourly from 1-Apr through 24-Jun 2013 using an ET106 weather station (Campbell Scientific, Logan, UT) located ~150 m from the experimental vineyard. Rainfall and ambient air temperature were also recorded hourly from 25-Jun 2013 through 31-Oct 2013, and 1-Apr through 31-Oct 2014 and 2015 using a CS215 temperature/relative humidity probe (Campbell Scientific, Logan, UT) and a TE525WS-L rain gauge (Texas Electronics, Dallas, TX) located ~25 and ~350 m away from the experimental vineyard, respectively. Growing degree days (GDD) were summed from 1 Apr using a base temperature of 10 °C.

Canopy characterization: To characterize fruit-zone architecture, point quadrat analysis (PQA) data were collected between EL stages 33 and 35 (veraison), as described in Smart and Robinson (1991). A thin metal rod was inserted into the fruiting zone along the transverse axis of the canopy, using a tape measure to guide insertions. This process was repeated 10 times in each experimental unit (vine) to quantify fruit-zone leaf layer number (LLN). Fruit zone sunlight

penetration was evaluated within several days of collecting PQA data using an AccuPAR ceptometer (Model PAR-80, Decagon Devices, Inc., Pullman, WA). Photosynthetic photon flux density (PPFD) was assessed by inserting the ceptometer inside canopy fruit zones parallel to, and directly above, the cordon and orienting the light interception side of the ceptometer in three different directions (45˚ east, vertical, 45˚ west) and then averaging those readings. Two PPFD readings were taken in every experimental unit between 1100-1400 hrs under clear skies. An ambient PPFD reading was taken with each experimental unit reading in order to express fruit-zone PPFD as a percentage of the ambient radiation in order to generate cluster exposure flux availability (CEFA), with enhanced point quadrat analysis (EPQA version 1.6.2) software (Meyers and Vanden-Heuvel 2008).

The "datalogger vine panel:" The following measurements were logged at specified intervals in each respective season.

2013: berry and ambient temperature, and fruit-zone PAR were logged on 15-min intervals from 30 Jul 2013 through 9 Oct 2013.

2014: berry and ambient temperature, and fruit-zone and ambient PAR were logged on 15-min intervals from 25 Jul 2014 through 4 Sep 2014, and on 1-min intervals from 6 Sep 2014 through16 Oct 2014, and from 22 Jun 2015 through 5 Oct 2015.

2015: berry and ambient temperature, and fruit-zone and ambient PAR were logged on 1-min intervals from 22 Jun 2015 through 5 Oct 2015.

One experimental unit (vine) of each *pre-bloom* leaf removal treatment (post-fruit set leaf removal vines were never used) in the same vineyard panel (max. 7.6 m apart) was subjected to logging of berry temperature and fruit-zone photosynthetically active radiation (PAR). This was done to measure berry temperatures typical of our region, and evaluate how light and

temperature impact berry temperature. In addition to these leaf removal treatment-specific measurements, ambient temperature and PAR were logged immediately surrounding and above the "datalogger vine panel," respectively. The temperature of two outside-facing berries on both east and west canopy sides of each leaf removal treatment were measured with a mini hypodermic thermocouple (type T, model HYP1/2, Omega Eng., Stamford, CT) inserted approx. 0.6 cm beneath berry skins and affixed with a strong, all-purpose glue. Fruit-zone PAR was measured with a SQ-316 quantum sensor (Apogee Instruments, Logan, UT) placed on top, and parallel to, the orientation of one cordon in each leaf removal treatment. Ambient PAR was measured with a SQ-316 quantum sensor, mounted approx. 3 m above the ground (approx. 0.6 m above vine canopies) at the middle of the vineyard panel where all data were logged. Ambient temperature was measured with two ST-100 thermistors (Apogee Instruments, Logan, UT). The thermistors were placed on the north and south bordering ends (~ 7.6 m apart) of the "datalogger vine panel," and mounted at fruit-zone height inside a naturally aspirated 6-panel radiation shield (model 41303-5A, R.M. Young, Traverse City, MI). Thermocouples were logged with an AM25T solid-state multiplexer (Campbell Scientific, Logan, UT) attached to a CR1000 datalogger (Campbell Scientific, Logan, UT), which also logged the PAR and ambient temperature data. All sensors, excepting the ambient temperature sensors, were taken down at the end of each field season and re-mounted in the following season.

Manual berry temperature: Berry temperature was manually measured on several dates over the course of the three seasons in order to better characterize berry temperatures across all six experimental blocks (as opposed to just using data logged in one block). Berry temperatures were manually measured on five dates between Jul and Sep in 2013 (pre-bloom leaf removal treatments), six dates between Jul and Sep in 2014, and seven dates between Jun and Sep in 2015

(both pre-bloom and post-fruit set leaf removal treatments). Measurements were taken in every experimental unit at three different times of day on each collection date: morning (~0900-1030 hrs), around solar noon (~1245-1415 hrs), and late afternoon (~1545-1715 hrs). Measurements were taken by inserting a mini hypodermic thermocouple (model HYP1/2, Omega Eng., Stamford, CT) beneath the skins of berries, which was connected to a handheld digital thermometer (model HH 25, Omega Eng., Stamford, CT). Each measurement took ~ 2 sec. At each time of day, on each collection date, and on both east and west canopy sides, three berries on the cluster's exterior face, positioned at the top, center, and bottom of clusters were measured on two clusters borne on opposite vine cordons in each experimental unit. Thus, a total of twelve total single-berry temperature measurements, or six single-berry temperature measurements on each canopy side, were taken in each experimental unit. The timeframe that encompassed manual berry temperature measurement in each experimental unit was recorded starting in Aug 2014; this was done in order to further investigate the relationship between berry temperature, and ambient air temperature and radiation.

Estimated post-veraison berry temperature in post-fruit set leaf removal plots: An attempt was made to *estimate* the cumulative length of time that berries in the post-fruit set removal plots were subjected to two benchmark temperatures, ≥ 30 and 35 °C, in the post-veraison period. The percent difference between average manually-measured berry temperature in pre-bloom removal and post-fruit set leaf removal plots during direct-sun times of day was calculated to be 4-5%, depending on canopy side and season (Table 1). This 4-5% increase was adjusted for berries in the PFS-6 over each hour period between 0800-1800, as follows: First, the average hourly percent increase of *logged* berry temperature in pre-bloom leaf removal plots compared to ambient air temperature was calculated between 0800-1800 (Table 1). The hourly percent

increase in berry temperature in pre-bloom leaf removal plots was normalized according to the peak average percent increase of logged berry temperature, making 0900-1059 and 1500-1659 100% (or, 1.00) (Table 1). The percent increase in manually measured post-fruit set berry temperature during direct-sun time periods (4-5%) was then multiplied by the normalized percent increase of berry temperature in pre-bloom leaf removal plots for each respective hour. The result was an estimation of the °C that berry temperature was greater in post-fruit set leaf removal compared to pre-bloom leaf removal plots (Table 1, furthest column to right). This number was added back to the average of every logged temperature of PB-4 and PB-8 berries in each respective hour over the entire 2014 and 2015 post-veraison periods. The total *estimated* time that berries in the post-fruit set leaf removal plots spent above 30 and 35 °C was then summed.

Table 1. Method used to estimate hourly percent increase in PFS-6 berry temperature compared to the average of PB-4/8 berry temperature in 2014 and 2015

	2014									
	% increase in average logged PB-4/8 berry vs. ambient air temperature						**% increase in PFS-6 vs. PB-4/8 berry temperature**			
	Actual % increase		**Average % over direct-sun periods**		**Adjusted based off peak % increase**		**Calculated % increase in manually-measured berry temperature during direct-sun time periods**		**Adjusted % increase in PFS-6 berry temperature based off peak % increase of logged PB-4/8 berry vs. ambient air temperature**	
Hour	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**
0800	11.30	5.40	11.30	5.40	0.63	0.63	5.00	5.00	3.17	3.14
0900	17.90	4.30	**17.85**	4.30	**1.00**	0.50	5.00	5.00	**5.00**	2.50
1000	17.80	2.20	**17.85**	2.20	**1.00**	0.26	5.00	5.00	**5.00**	1.28
1100	17.00	1.80	17.00	1.80	0.95	0.21	5.00	5.00	4.76	1.05
1200	16.00	2.60	16.00	2.60	0.90	0.30	5.00	5.00	4.48	1.51
1300	9.25	3.40	9.25	3.40	0.52	0.40	5.00	5.00	2.59	1.98
1400	4.00	3.20	4.00	3.20	0.22	0.37	5.00	5.00	1.12	1.86
1500	3.00	8.80	3.00	**8.60**	0.17	1.00	5.00	5.00	0.84	**5.00**
1600	3.00	8.40	3.00	**8.60**	0.17	1.00	5.00	5.00	0.84	**5.00**
1700	0.00	3.90	0.00	3.90	0.00	0.45	5.00	5.00	0.00	2.27
1800	0.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	0.00	0.00
	2015									
Hour	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**	**EAST**	**WEST**
0800	12.70	1.20	12.70	1.20	0.82	0.12	4.00	5.00	3.30	0.58
0900	15.90	2.50	**15.40**	2.50	**1.00**	0.24	4.00	5.00	**4.00**	1.20
1000	14.90	2.60	**15.40**	2.60	**1.00**	0.25	4.00	5.00	**4.00**	1.25
1100	13.30	3.00	13.30	3.00	0.86	0.29	4.00	5.00	3.45	1.44
1200	9.00	3.40	9.00	3.40	0.58	0.33	4.00	5.00	2.34	1.63
1300	4.80	4.00	4.80	4.00	0.31	0.38	4.00	5.00	1.25	1.92
1400	3.70	6.00	3.70	6.00	0.24	0.58	4.00	5.00	0.96	2.88
1500	3.20	9.20	3.20	**10.40**	0.21	**1.00**	4.00	5.00	0.83	**5.00**
1600	2.70	11.60	2.70	**10.40**	0.18	**1.00**	4.00	5.00	0.70	**5.00**
1700	1.50	9.20	1.50	9.20	0.10	0.88	4.00	5.00	0.39	4.42
1800	0.00	2.40	0.00	2.40	0.00	0.23	4.00	5.00	0.00	1.15

Crop yield components, vine fruitfulness, berry weight, cluster compactness, and botrytis bunch rot and bunch stem necrosis incidence: Crop was harvested at commercially acceptable composition and integrity. Crop yield per vine was measured with a field scale at harvest on 9 Oct 2013, 20 Oct 2014, and 5 Oct 2015 to obtain components of yield. Cluster weight was estimated from the quotient of crop yield weight and cluster number. Berry weight was determined from 120-berry samples, half of which were randomly collected from each of east and west canopy sides immediately prior to harvest. Berry number per cluster was derived from the quotient of average cluster weight and average berry weight. Crop load was calculated using crop yield weight and dormant pruning weight on a per-vine basis. Berry weight gain over time was determined from 120- or 200-berry samples collected from each experimental unit at various times before harvest in 2013-2015.

Cluster compactness was measured in Expt. I to evaluate if pre-bloom leaf removal impacted cluster architecture. Cluster compactness was measured at harvest on ten clusters that were +/- 25% of the average harvested cluster weight from each experimental unit. Compactness was indexed by the ratio of total berry number by the main rachis length. Berries were not counted, and rachis sections not measured, on cluster "shoulders" or "wings" in order to reduce confounding the compactness of the main body of the cluster. Vine fruitfulness was assessed in 2014 and 2015 at the modified EL stage 15/16 by dividing the number of inflorescences by the number of shoots originating from both the cordon (basal) and one-year old spurs (count).

Crop yield components were also evaluated in experimental units subjected to pre-bloom leaf removal re-implementation. In 2014, the second year of Expt. I, current-season pre-bloom leaf removal treatments were berry sampled five times; re-implemented treatments were not berry sampled until harvest. Consequently, the absolute values of components of yield and

cluster compactness were greater in the re-implemented (PB-4/8 ‘13re) compared to current-season plots. Therefore, in 2014, the yield components of PB-4/8 ‘13re were compared to *estimated* yield components of the frequently-berry sampled PB-NO. Yield components were estimated by adding the product of berry weight at harvest and sampled berry number back to the actual harvest weight for each experimental unit; components of yield were then calculated as described above. Cluster compactness of PB-NO in 2014 was estimated by adding the average berry number removed per cluster in each experimental unit to the average berry number per cluster measured during the cluster compactness assessment; this was then divided by the main rachis length. All pre-bloom leaf removal treatments were berry-sampled with equal frequency in 2015 to eliminate the need for estimation.

Botrytis bunch rot (BBR) and bunch stem necrosis (BSN) incidence was evaluated by visual inspection of every harvested cluster in 2015. A severity of ~ 5% of the entire cluster was measured as an incident, but severity of both BBR and BSN was greater in most cases. In each experimental unit, percent incidence was calculated as the number of clusters with BBR and/or BSN divided by the total number of clusters.

Primary fruit chemistry: Primary chemistry was measured on juice obtained from composite samples of 30 east- and 30 west- canopy side positioned berries that were randomly collected from each experimental unit immediately prior to harvest. Juice samples were obtained from fresh (non-frozen) berries by equally hand-pressing samples in a plastic bag for ~ two min, and then immediately centrifuging for five min at ~ 3500 rpm. Soluble solids were measured with a digital refractometer (Pocket PAL-1, ATAGO USA, Inc., Bellevue, WA). Juice pH was measured, and TA was determined by titration to an endpoint of pH 8.2, using an 848 Titrino Plus auto-titrator (Metrohm USA, Riverview, FL) and 0.1 N NaOH base.

Estimated total grape phenolics and anthocyanins: Absorption spectroscopy was used to estimate total grape phenolics and anthocyanins as affected by pre-bloom and post-fruit leaf removal. Immediately prior to harvest in 2013 (just pre-bloom experiment), 2014, and 2015 (pre-bloom and post-fruit set experiments), 60-berry samples were randomly collected from both east and west canopy sides of each experimental unit, kept separate, and frozen at -20˚C until tests commenced. Excepting 2014 post-fruit set samples, which were homogenized with an immersion blender (Waring), berry samples were homogenized for two min with a Magic Bullet (Homeland Housewares LLC, Los Angeles, CA), and had 30 mL of 0.025 M KCl buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) added to separate 1.0 g aliquots of the berry homogenate. The homogenate-buffer samples were shaken for 10 min and then centrifuged for five min at ~ 3500 rpm. The supernatant was pipetted into a 10 mm path length Hellma quartz cuvette (Thermo Fisher Scientific Inc., Pittsburgh, PA), and the absorbance at 520 and 700 nm was measured in duplicate with a Genesys 8 ThermoSpectronic spectrophotometer (Cambridge, UK). The absorbance of the sample containing the berry homogenate and 0.025 M KCl buffer (pH 1.0) was also read at 280 nm in duplicate after diluting to a 2:1 sample-buffer ratio.

Statistics: A mixed model was used in JMP Pro 11 (SAS, Cary NC) to evaluate the random effect of Block and fixed effects of pre-bloom and post-fruit set leaf removal treatment ($\alpha \leq 0.05$). Canopy side and treatment interaction with canopy side (east and west) was added to the model to evaluate estimated total berry phenolics and anthocyanins. Significant difference of least square means ($\alpha \leq 0.05$) was determined with Tukey's HSD for pre-bloom leaf removal. Significant difference of least square means ($\alpha \leq 0.05$) was determined with Student's T-test for post-fruit set leaf removal, as well as canopy side effect on estimated total phenolics and anthocyanins. Manual berry temperature measurements were analyzed separately by time of day

and canopy side on each date. Due to differential berry sampling between current-season and re-implementation treatments in Expt. I, components of yield data were analyzed separately in 2014. Treatment effect on crop yield at harvest was reversed in block 5 in 2013, and block 4 in 2014, when compared to all other experimental blocks in Expt. I. In 2013, this was due to no leaf removal having at least 22% fewer clusters per vine compared to pre-bloom leaf removal treatments. In 2014, this was due to no leaf removal having at least 61% fewer clusters per vine compared to the pre-bloom leaf removal treatments. As such, all components of yield data from block 5 in 2013 and block 4 in 2014, except berry weight, was removed from the analyses presented in Table 4. Simple linear regressions were run to determine the relationship between berry weight at harvest and total berry phenolics and anthocyanins – harvest berry weights from all blocks were included in these relationships.

Results

Meteorology: Seasonal growing degree day (GDD) accumulation in 2015 was ~ 8% greater than in 2013 and 2014, mainly attributed to greater GDD accumulation in the post-veraison period (Aug and Sep) (Table 2). The relatively low GDD accumulation in Oct 2015 was unlikely detrimental to fruit maturation, as harvest occurred earlier in 2015 than in 2013 or in 2014. Monthly mean temperature was greatest in Jun 2013, and Aug and Sep 2015 compared to the same months in the other two years. Monthly maximum temperatures were greatest in Jun and Oct 2013, and Aug 2015 compared to the same months in other years. Seasonal rainfall was 25% greater in 2013 compared to 2014 and 2015 seasons, partially a function of rainfall in Jul 2013, which was ~2.6 times greater than Jul 2014, and 3.7 times greater than Jul 2015. Rainfall was lowest in Aug 2015, and greatest in Sep 2015, compared to these months in 2013 and 2014.

Table 2. Seasonal and monthly growing degree day (GDD) and rainfall accumulation, and mean and maximum monthly temperatures for Jul, Aug, Sep, and Oct at the Alson H. Smith, Jr. AREC in Winchester, VA, 2013-2015.

Year	Metric	Season total (1 Apr - 31 Oct)	Jul	Aug	Sep	Oct[b]
2013	GDD[a] sum	1878	441	368	258	143
	Rainfall sum (mm)	667	201	77	18	113
	T mean (°C)	n/a	22	19.5	15.6	12.2
	T max (°C)	n/a	34.4	30.3	33	31.1
2014	GDD sum	1858	397	356	286	124
	Rainfall sum (mm)	519	78	74	10	91
	T mean (°C)	n/a	20.4	19.4	17.2	12.8
	T max (°C)	n/a	33.4	31.1	32.9	27.4
2015	GDD sum	2014	424	409	316	103
	Rainfall sum (mm)	551	54	31	96	68
	T mean (°C)	n/a	21.3	20.7	18.4	12.5
	T max (°C)	n/a	33.5	33.5	32.2	25.4

[a]Growing degree days calculated using base 10 °C.
[b]Fruit harvested on 9-Oct-2013, 20-Oct-2014, and 5-Oct-2015.

The range of seasonal ambient PAR was divided into arbitrary 25% increments, and the time spent in each quartile was summed to determine how ambient radiation influenced diurnal east- and west-side berry temperature. In general, less time was spent at lower ambient PAR ranges, and more time at higher ambient PAR ranges, when sun was cast on the east-canopy side during the morning (Fig. 2 A, C), compared to when the sun was cast on the west-canopy side in the afternoon (Fig. 2 B, D). In both 2014 and 2015, more time was spent ≤ 25 to 50% ambient PAR ranges during 1500-1800 compared to 0900-1200 (Fig. 2 A-D). Over the course of the entire diurnal period that berries were heated above ambient air temperature (0800-1800), ambient PAR trends were similar between 2014 and 2015, although more time was spent at the 25-75% ambient PAR ranges in 2015 (Fig. 2 E, F).

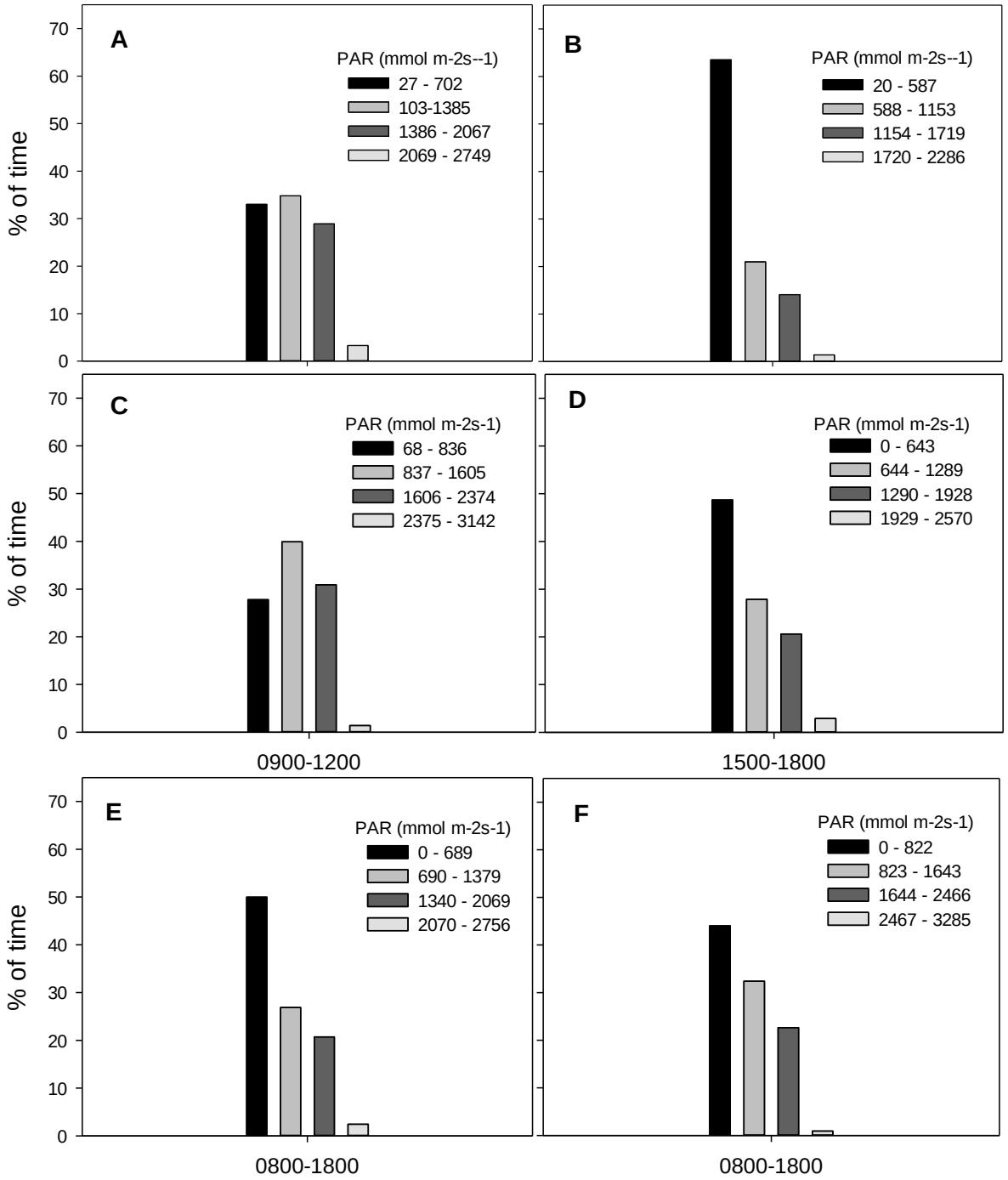

Figure 2. Percent of time from veraison to harvest spent at 25% photosynthetic active radiation (PAR) increments from 0900-1200 in 2014 (A) and 2015 (B), from 1500-1800 in 2014 (C) and 2015 (D), and from 0800-1800 in 2014 (E) and 2015 (F).

Canopy characterization and dormant cane pruning weight: Leaf removal resulted in a fruit-zone leaf layer number (LLN) of zero in all years (Table 3). 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. Pruning weight was reduced by PB-8 in 2013. Pruning weight tended to be 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.

Table 3. 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).

Berry temperature: Berry temperature was driven by ambient air temperature, but was confounded by leaf removal treatment (Table 4). The air-berry temperature relationship was stronger when leaves were maintained in the fruit-zone compared to when leaves were removed from the fruit-zone, regardless if berry temperatures were logged or manually measured. The air-berry temperature relationship was stronger on the west compared to the east canopy side, especially when fruit-zone leaves were removed. Accordingly, this relationship was weaker in the morning (AM), when the sun was cast on the east canopy side, compared to the afternoon (PM), when the sun was cast on the west canopy side. Berry temperature was more closely related to air temperature at solar noon (NOON) compared to morning or afternoon hours.

Table 4. Logged and manual-measured coefficients of determination for linear relationships between ambient air and berry temperature as a function of fruit-zone leaf removal, canopy side, and time of day.

Treatment[a]	Logged[b] (R^2)					Manual[c] (R^2)				
	EAST	WEST	AM	NOON	PM	EAST	WEST	AM	NOON	PM
PB-NO	0.987	0.989	0.957	0.979	0.976	0.862	0.937	0.796	0.944	0.899
PB-4	0.913	0.967	0.771	0.936	0.887	0.647	0.832	0.607	0.923	0.663
PB-8	0.898	0.965	0.749	0.896	0.887	0.562	0.814	0.608	0.881	0.613
PFS-NO	n/a	n/a	n/a	n/a	n/a	0.853	0.902	0.752	0.938	0.852
PFS-6	n/a	n/a	n/a	n/a	n/a	0.372	0.746	0.464	0.878	0.456

[a]PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight leaves, respectively; PFS-NO, PFS-6 = post-fruit set removal of no and six leaves, respectively.

[b]Data logged and averaged over 30-Jul-2013 – 9-Oct-2013, 25-Jul-2014 – 16-Oct-2014, and 22-Jun-2015 - 5-Oct-2015; AM = 0900-1100, NOON = 1200-1400, PM = 1500-1700.

[c]Data collected and averaged on 20-Aug, 28-Aug, 4-Sep, and 16-Sep in 2014, and on 29-Jun, 13-Jul, 31-Jul, 13-Aug, 25-Aug, and 8-Sep in 2015; AM = 0900-1030, NOON = 1245-1415; PM = 1545-1715.

Both berry and ambient air temperature were greater in the afternoon compared to morning hours. The time of day that berry temperature was most different from ambient air temperature was when the sun was cast on the fruit-zone, from 0800-1200, or from 1500-1800 (Fig. 3). This bimodal diurnal trend of elevated berry temperature was mirrored by a bimodal diurnal trend of elevated PAR, particularly in the PB-4 and PB-8 treatments (Fig. 3 A, B). East- and west-canopy side berry temperature in leaf removal treatment plots was greater than ambient air temperature at times of day when the sun was cast on the fruit-zone, or when the overhead canopy was not blocking radiation transmission to the fruit-zone. For example, in PB-4 and PB-8 plots, east-side berry temperature was 1.4 – 4.1 °C greater than ambient air temperature from 0800-1300, and west-side berry temperature was 0.3 – 3.0 °C greater than ambient air temperature from 1400-1800 (Fig. 3 A, B). The greater berry temperature on the east- compared to west- canopy side was mirrored by greater ambient and fruit-zone PAR conditions between 800-1300 (515 – 1462, and 83 – 492 µmol $m^{-2}s^{-1}$, respectively) compared to 1400-1800 (321 – 1374, and 55 – 369 µmol $m^{-2}s^{-1}$, respectively). Though ambient PAR was at its greatest, there was less of a leaf removal impact on radiant berry heating around solar noon (~1330) compared to the preceding or following several hours. This was documented by a concomitant decrease in fruit-zone PAR, and the magnitude of difference between ambient air and berry temperature, particularly observed in Fig. 3 A, B. Abstaining from fruit-zone leaf removal resulted in blockage of fruit-zone radiation throughout the day (Fig. 3 C). Consequently, east- and west-side berry temperature was similar (-0.2 – 0.7 °C) to ambient air temperature during the same hours that berries in leaf removal plots were radiantly heated above ambient air temperature.

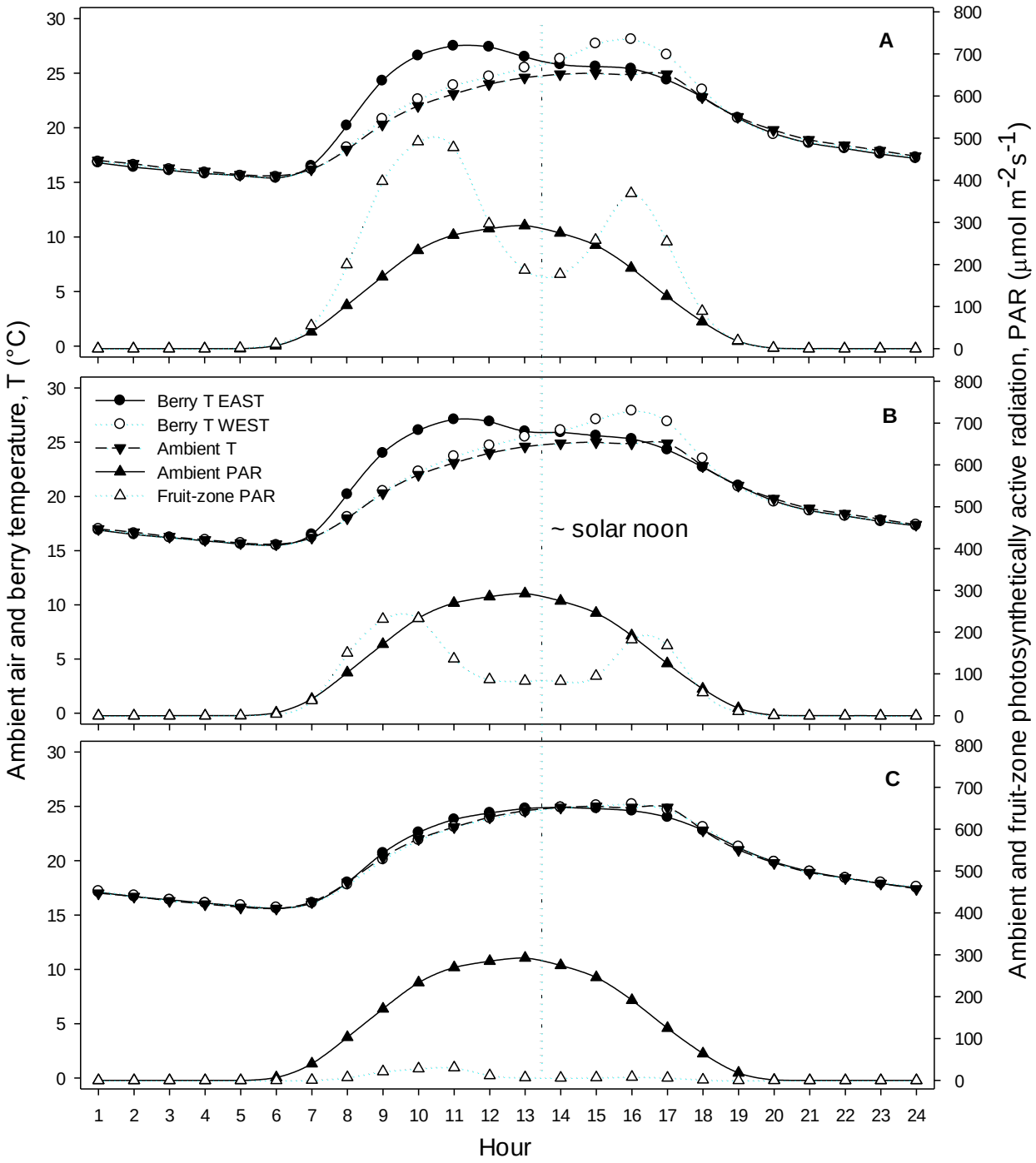

Fig. 3 Diurnal pattern of ambient air temperature, ambient and fruit-zone PAR, and berry temperature as affected by pre-bloom removal of eight (A), four (B), and no (C) fruit-zone leaves/laterals. Data logged on 15- and 1-min intervals, and averaged over 2013-2015 seasons. Ambient PAR was logged on 15- 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.

Pre-bloom and post-fruit set leaf removal had a greater effect on manually-measured east-side berry temperature during the morning (~0900-1030; Figs. 2 and 3 A), and west-side berry temperature during the late afternoon (~1545-1715; Figs. 2 and 3 C), when compared to opposite canopy sides during those periods. Berry temperature was less affected by leaf removal treatment at NOON, even though ambient PAR was frequently greater at this time compared to the AM or PM. This was a function of radiation blockage to the fruit-zone, as observed in the diurnal patterns of logged air and berry temperature, and fruit-zone and ambient PAR shown in Fig. 3, above.

When averaged across all mornings (AM) from 2013-2015, pre-bloom removal of eight (PB-8) and four (PB-4) leaves increased east-side berry temperature by an average of 2.3 °C, but west-side berry temperature by an average of only 0.6 °C, when compared to pre-bloom removal of no leaves (PB-NO) (Fig. 4 A). When averaged across all afternoons (PM) from 2013-2015, PB-8 and PB-4 increased west-side berry temperature by an average of 2.3 °C, but east-side berry temperature by an average of only 0.5 °C, when compared to PB-NO (Fig. 4 C). Leaf removal had little effect on berry temperature at NOON. When averaged across NOON time periods from 2013-2015, pre-bloom leaf removal increased both average east- and west-side berry temperature by 0.5 °C, when compared to PB-NO (Fig. 4 B).

Pre-bloom leaf removal to the extent of either four or eight leaves significantly increased manually-measured east-and west-side berry temperature during all times of day and on several different dates over 2013-2015 (Fig. 4 A-C). Pre-bloom leaf removal of eight leaves increased berry temperature compared to PB-4 on only 14 of the 102 occasions that berry temperature was manually measured during over 2013-2015 (Fig. 4 A-C). Interestingly, the most common time that PB-8 had greater berry temperature than PB-4 was when west-side berry temperature was

measured in the AM, which occurred on half of the 14 occasions that berry temperatures differed. By comparison, there were only three occasions that PB-8 had greater east-side berry temperature than PB-4 during the PM. The difference in manually-measured berry temperature between PB-8 and PB-4 was never greater than 2.0 °C, and frequently no greater than 0.7 °C.

Pre-bloom removal of eight leaves increased manually-measured berry temperature more frequently than PB-4 during time periods that the sun was cast on the *opposite* canopy side (Fig. 4 A, C). For example, when west-side berry temperature was measured in the AM, PB-8 increased berry temperature on 12 dates, and PB-4 on only two dates, over the course of 2013-2015 (Fig. 4 A). When east-side berry temperature was measured in the PM, PB-8 increased berry temperature on nine dates, and PB-4 again on only two dates, over the course of 2013-2015 (Fig. 4 C). During NOON, however, east-and west-side berry temperature was increased by PB-8 and PB-4 on virtually the same number of dates over the course of 2013-2015 (Fig. 4 B).

In 2013, PB-8 and PB-4 increased east-side berry temperature in the AM on two dates by a range of 1.1 – 3.6 °C and 0.7 – 2.9 °C, respectively, when compared to PB-NO (Fig. 4 A). When compared to PB-NO on the west-canopy side, PB-8 increased berry temperature in the PM on five dates by a range of 1.9 – 2.8 °C, and PB-4 increased berry temperature in the PM on two dates by a range of 1.3 – 2.0 °C (Fig. 4 C). In 2014, PB-8 increased east-side berry temperature in the AM on five dates by a range of 1.9 – 3.0 °C, and PB-4 increased east side berry temperature in the AM by 1.7 °C on one date, when compared to PB-NO (Fig. 4 A). When compared to PB-NO on the west canopy side, both PB-8 and PB-4 increased berry temperature in the PM on five dates by a range of 1.7 – 3.0 °C and 1.4 – 2.5 °C, respectively (Fig. 4 C). Lastly, in 2015, both PB-8 and PB-4 increased east-side berry temperature in the AM on five of the six dates by a range of 2.7 – 6.9 °C and 1.9 – 6.1 °C, respectively, when compared to PB-NO

(Fig. 4 A). When compared to PB-NO on the west canopy side, both PB-8 and PB-4 increased berry temperature in the PM on all six dates by a range of 2.8 – 4.5 °C and 2.3 – 4.5 °C (Fig. 4 C). Pre-bloom leaf removal occasionally increased berry temperature by a range of only 0.2 – 1.4 °C (2013), 0.3 °C – 1.7 °C (2014), or 0.5 – 2.1 °C (2015) when the fruit-zone did not receive direct sunlight (Fig. 4 A-C).

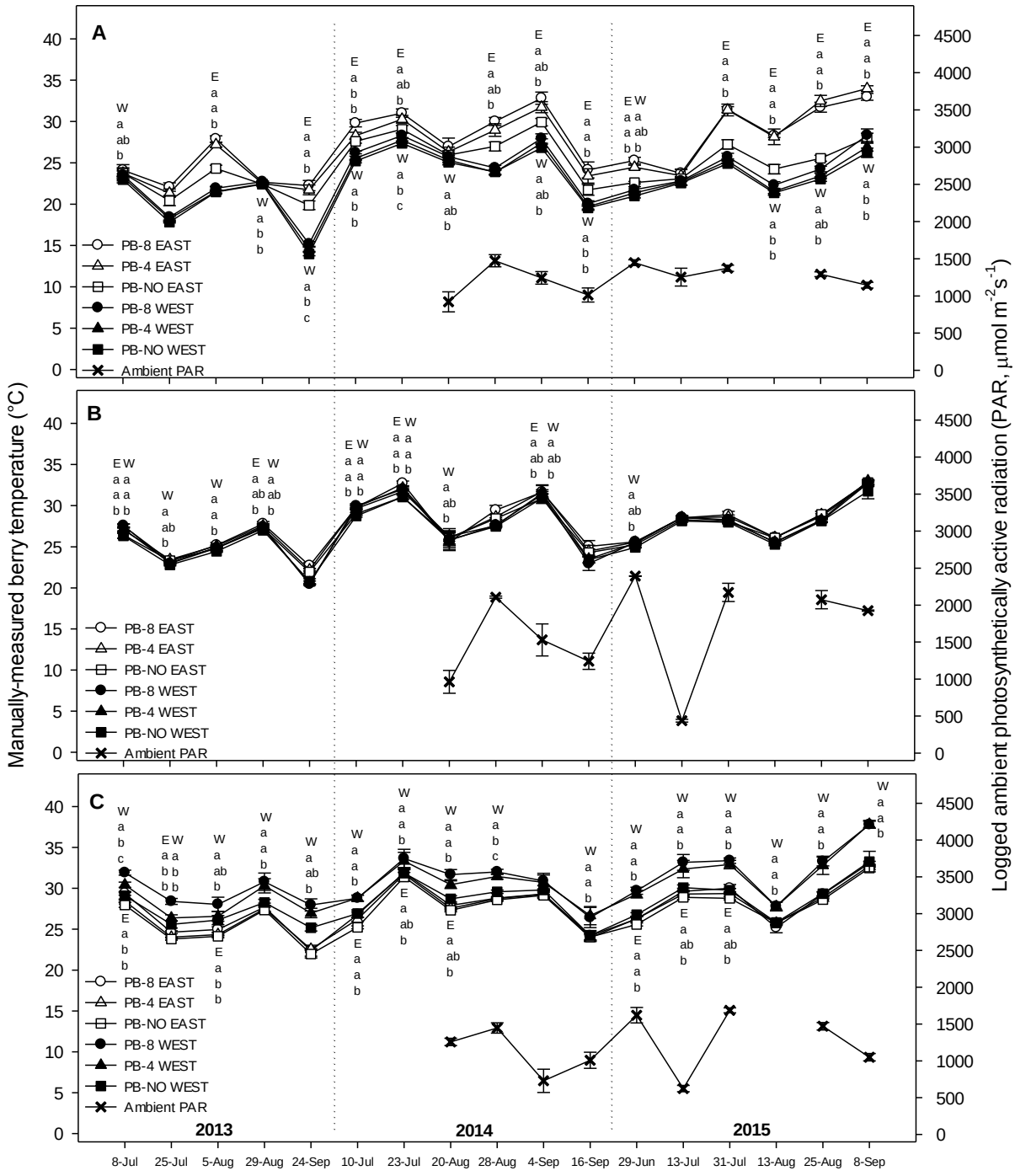

Fig. 4 Logged ambient PAR, and manually-measured berry temperature in the AM (A, 900-1030), NOON (B, 1245-1415), and PM (C, 1545-1715) on several dates over the 2013-2015 growing seasons. PB-8, PB-4, PB-NO = pre-bloom removal of eight, four, and no leaves, respectively. Data points are an average of 36 temperatures; n = 6. East (E) and west (W) berry temperature means within a date not sharing a letter are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

Post-fruit set leaf removal increased manually-measured berry temperature to a comparatively greater magnitude than pre-bloom leaf removal did (Fig. 5 A-C). When averaged across all mornings, removal of six leaves (PFS-6) increased east-side berry temperature by 4.1 °C, and west-side berry temperature by only 0.5 °C compared to removal of no leaves (PFS-NO) (Fig. 5 A). When averaged across all afternoons, PFS-6 increased west-side berry temperature by 3.7 °C and east-side berry temperature by only 0.5 °C compared to PFS-NO (Fig. 5 C). Leaf removal treatment had little effect on berry temperature at NOON. When averaged across all NOON measurements, PFS-6 increased east- and west-side berry temperature by an average of 0.5 °C when compared to PFS-NO (Fig. 5 B).

In 2014, PFS-6 increased east-side berry temperature in the AM on five of the six dates by a range of 2.8 – 5.6 °C when compared to PFS-NO (Fig. 5 A). Post-fruit set removal of six leaves increased west-side berry temperature in the PM on all six dates by a range of 1.4 – 3.8 °C when compared to PFS-NO (Fig. 5 C). Over the course of all direct-sun time periods (east in AM, west in PM) in 2014, post-fruit set leaf removal *further* increased east- and west-side berry temperature by 5% compared to pre-bloom leaf removal (data not shown). In 2015, PFS-6 increased east-side berry temperature in the AM on five of the six dates by a range of 1.0 – 8.3 °C when compared to PFS-NO (Fig. 5 A). When compared to PFS-NO on the west canopy side, PFS-6 increased berry temperature in the PM on all six dates by a range of 2.8 – 7.5 °C (Fig. 5 C). Over the course of all direct-sun time periods (east in AM, west in PM) in 2015, post-fruit set leaf removal *further* increased east-side berry temperature by 4%, and west-side berry temperature by 5% compared to pre-bloom leaf removal (data not shown). By contrast, PFS-6 occasionally increased berry temperature by a range of only 0.3 – 1.2 °C (2014) or 0.2 – 1.2 °C (2015) when berry temperature was measured during non-direct sun time periods (Fig. 5 A-C).

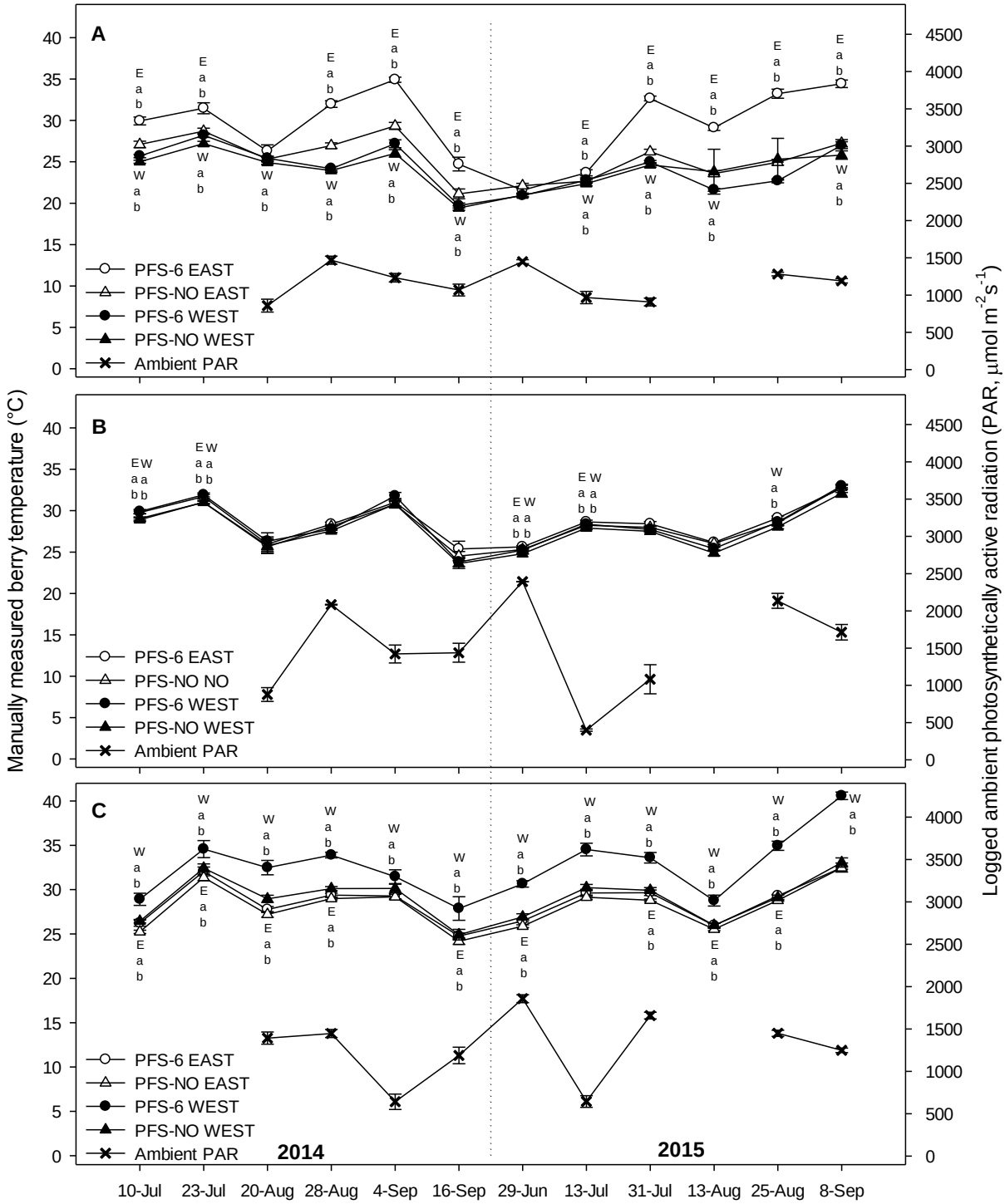

Fig. 5 Logged ambient PAR, and manually-measured berry temperature in the AM (A, 900-1030), NOON (B, 1245-1415), and PM (C, 1545-1715) on several dates over the 2014 and 2015 growing seasons. PFS-6, PFS-NO = post-fruit set removal of six and no leaves, respectively. Data points are an average of 72 temperatures; n = 6. East (E) and west (W) berry temperature means within a date not sharing a letter are significantly different ($\alpha \leq 0.05$) using Tukey's HSD. Error bars are +/- standard error.

Berries were heated ≥ 30 or35 °C for more time between 1000-1200 (east canopy side) and 1500-1700 (west canopy side) than during the middle of the day (1300-1400) during the post-veraison period (Fig. 6). Berries tended to be heated ≥ 30 and 35 °C for more time in PB-4/8 plots compared to PB-NO plots. Berries tended to be heated ≥ 30 °C for more time in 2013 and 2015 compared to 2014, and ≥ 35 °C for more time in 2015 compared to 2013 and 2014. Berry temperatures ≥ 35 °C were *never* logged in PB-NO, nor ≥ 30 °C outside the 0800-1800 diurnal period. Berries in PFS-6 plots were *estimated* to spend relatively more time ≥ 30 and 35 °C compared to the PB-4 and PB-8 plots (data not shown): 101 hrs ≥ 30 °C and 11 hrs ≥ 35 °C on the east canopy side, and 86 hrs ≥ 30 °C and 11 hrs ≥ 35 °C on the west canopy side in 2014; 117 hrs ≥ 30 °C and 27 hrs ≥ 35 °C on the east canopy side, and 110 hrs ≥ 30 °C and 33 hrs ≥ 35 °C on the west canopy side in 2015.

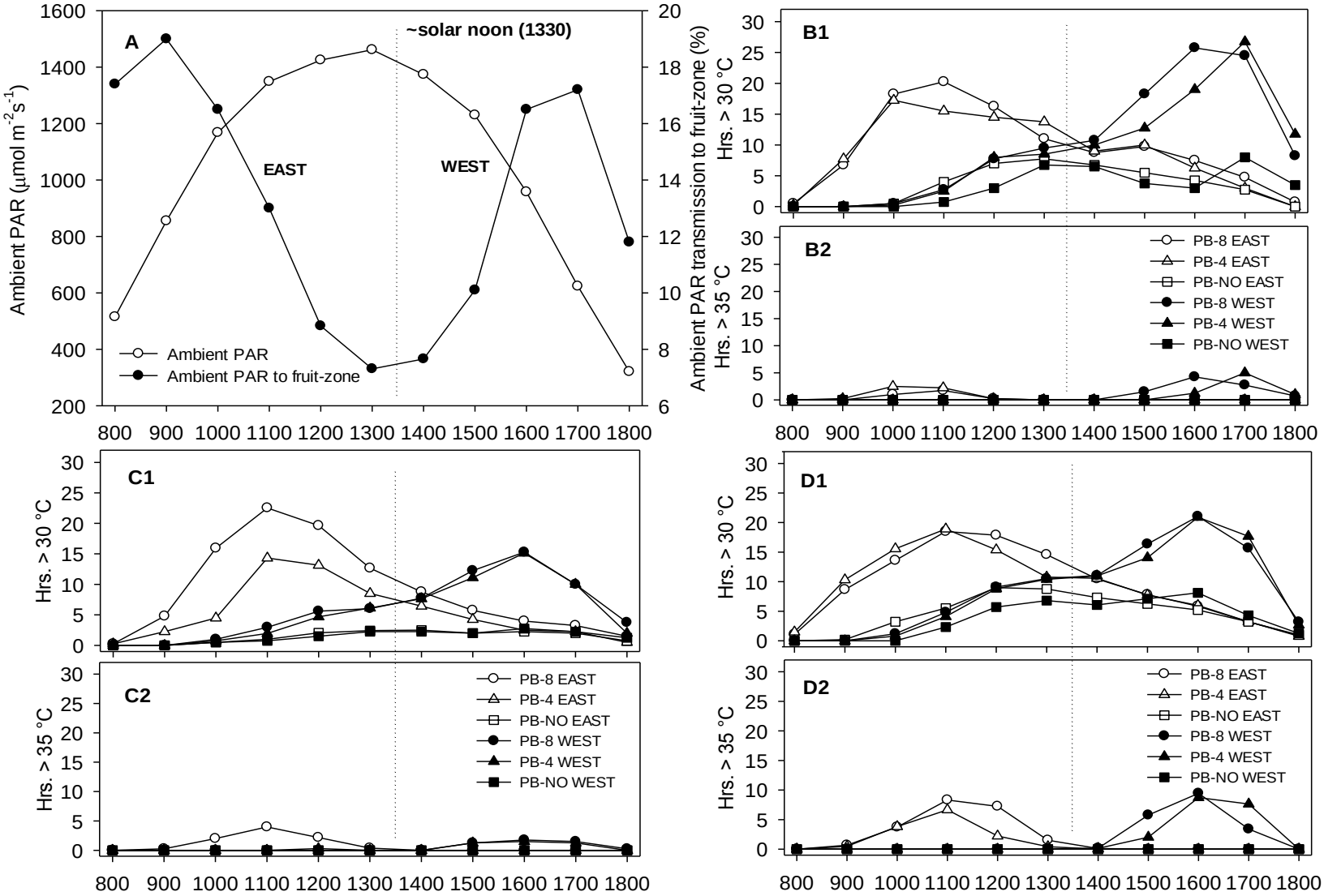

Fig. 6 Diurnal ambient PAR and percent PAR transmission to the fruit-zone logged over 2013-2015 (A), and leaf removal treatment effect on logged berry temperature hours above 30 (B1, C1, D1) and 35 (B2, C2, D2) deg. C in the post-veraison period of 2013 (B), 2014 (C), and 2015 (D).

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 5). Crop yield, cluster weight, berry number per cluster, and berry weight were reduced by 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 was reduced by PB-4 and PB-8 when compared to PB-NO. In 2014, crop yield and components were reduced in a similar fashion as in 2013. Crop yield, cluster weight, and berry number per cluster were reduced by PB-4 (53, 51, and 48%, respectively) and PB-8 (69, 65, and 59%, respectively), when compared to PB-NO. As in 2013, only PB-8 reduced berry weight (14%) compared to PB-NO. Cluster weight and berry number per cluster were reduced by PB-4 '13re (41, and 47%, respectively) and crop yield, cluster weight, and berry number per cluster were reduced by PB-8 '13re (50, 58, and 59%), when compared to PB-NO-est. As in 2013, both PB-4 and PB-8 reduced crop load when compared to PB-NO. Pre-bloom leaf removal had similar effects on crop yield components in 2015, but to a greater extent than the previous two seasons. Because no crop yield component differed between PB-4/8 '13re and PB-4/8 '14re, averages of these treatments were used for comparison to PB-NO. Crop yield, cluster weight, and berry number per cluster were reduced by PB-4 (51, 53, and 51%, respectively) and by PB-8 (78, 78, and 73%, respectively) when compared to PB-NO. As in the previous two seasons, berry weight was only reduced by PB-8 by 19% when compared to PB-NO. Only PB-8 reduced crop yield when compared to PB-NO.

Table 5. 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.

[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) and Student's T-test (PFS treatments).

Pre-bloom leaf removal tended to *further* reduce berry number per cluster, cluster weight, and crop yield over time when analyzed as a percent reduction compared to PB-NO (Fig. 7). Yield components were generally reduced to a greater absolute amount due to PB-8 compared to PB-4. In 2013, berry number per cluster, cluster weight, and crop yield was reduced by PB-4 (44, 43, and 29%, respectively) and PB-8 (57, 61, and 61%, respectively) compared to PB-NO (Fig. 7 A, B, C). The lack of linearity between these components and crop yield in PB-4 appeared to be a function of the greater cluster number (see Table 4). In 2015, berry number per cluster, cluster weight, and crop yield were all reduced by PB-4 (51, 53, and 48%, respectively) and by PB-8 (73, 77, and 76%, respectively). Thus, pre-bloom leaf removal implementation in three consecutive years resulted in berry number per cluster, cluster weight, and crop yield to be *further* reduced by PB-4 (7, 10, and 19%, respectively) and by PB-8 (16, 16, and 15%, respectively). In 2014, berry number per cluster, cluster weight, and crop yield were all reduced by PB-4 (48, 51, and 54%, respectively) and by PB-8 (58, 65, and 65%, respectively) compared to PB-NO (Fig. 7 D, E, F). In 2015, berry number per cluster, cluster weight, and crop yield were all reduced by PB-4 (50, 54, and 54%, respectively) and by PB-8 (72, 77, and 77%, respectively). Thus, pre-bloom leaf removal implementation in two consecutive years resulted in berry number per cluster, cluster weight, and crop yield to be only slightly *further* reduced in PB-4, and further reduced by PB-8 (14, 12, and 12%, respectively).

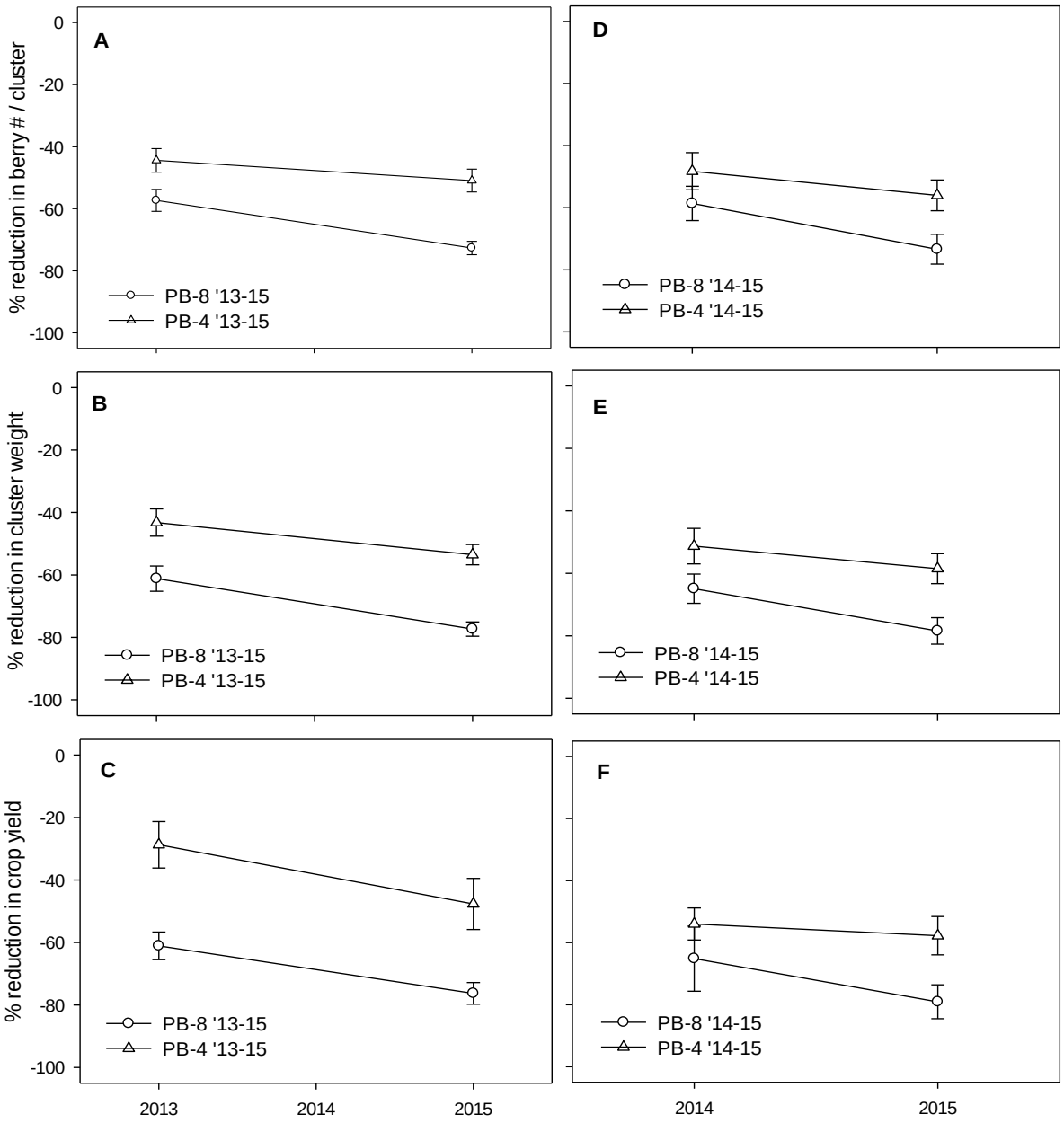

Fig. 7. The effect of re-implementation of pre-bloom removal of eight (PB-8) and four (PB-4) leaves in 2013, 2014, and 2015 (A, B, and C) and in 2014 and 2015 (D, E, and F) on percent reduction in berry number per cluster (A, D), cluster weight (B, E), and crop yield (C, F) compared to removal of no leaves; n = 6. Error bars are +/- standard error. NOTE: 2014 data is not presented in A, B, or C due to non-uniform berry sampling from PB-NO, PB-4, and PB-8.

Seasonal berry weight development: Berry weight tended to be reduced by PB-8 but not by PB-4 or PFS-6 (Fig. 8). In 2013, PB-8 reduced berry weight when compared to both PB-NO and PB-4 on 9-Jul (13%), 30-Aug (13 %), and 9-Oct (10%), and when compared to PB-4 on 24-Jul (9%) (Fig. 8 A). Average berry weight increase from 24 Jul to 9 Aug was 0.02 g and from 9 Aug to

30 Aug was 0.02 g and 0.06 g greater in PB-NO and PB-4 compared to PB-8, respectively. In 2014, PB-8 reduced berry weight when compared to both PB-NO and PB-4 on 15 Jul (8%; $p > F = 0.0029$) and 17 Oct (11%; $p > F = 0.0009$), and only when compared to PB-NO on 14 Aug (17%), 12 Sep (21%), and 30 Sep (20%). Berry weight increase from 31 Jul to 14 Aug and from 14 Aug to 12 Sep was 0.13 g and 0.14 g more in PB-NO compared to PB-8, respectively. 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 on 8 Sep only. 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.

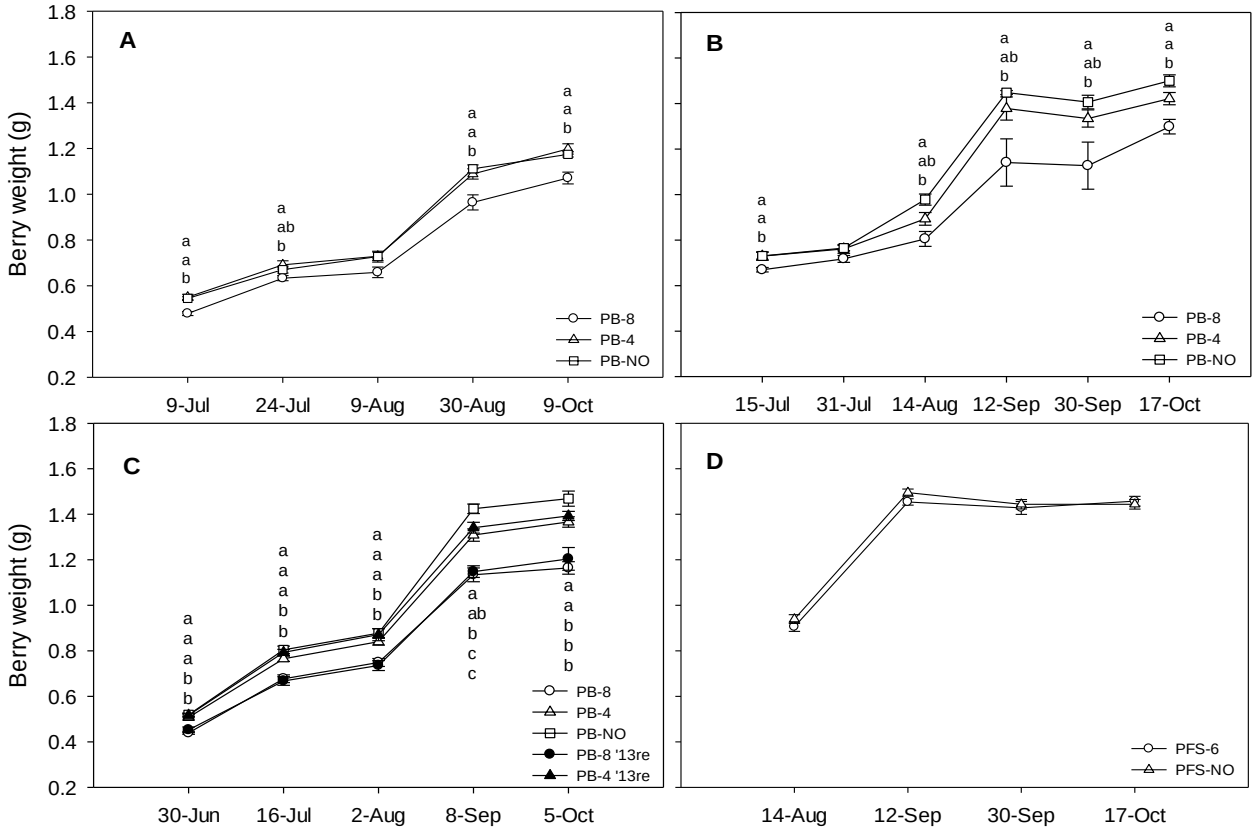

Fig. 8 Pre-bloom (A, B, C) and post-fruit set (D) leaf removal effect on berry weight over time in 2013 (A), 2014 (B, D), and 2015 (C); Each data point is an average of 120 berries; n = 6. Treatment mean berry weight within a date not sharing a letter are significantly different ($\alpha \leq 0.05$)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

Components of cluster compactness: Cluster compactness was reduced by both PB-8 (57%) and PB-4 (25%) when compared to PB-NO in 2013 (Table 6). Pre-bloom removal of eight leaves (PB-8) reduced berry number per cluster (58%) to a greater extent than did PB-4 (35%). The disproportionally lesser reduction in cluster compactness relative to berry number per cluster was due to shorter rachis lengths in PB-4 in 2013. Rachis length was not affected by leaf removal treatment in 2014, and berry number per cluster and cluster compactness were reduced similarly by PB-8 (59% and 60%, respectively) and PB-4 (42% and 39%, respectively) when compared to PB-NO in 2014. In 2014, berries were sampled on five fewer occasions in re-implemented treatments (PB-4/8 '13re) compared to current-season treatments, resulting in no difference in cluster compactness compared to PB-NO. When compared to PB-NO in 2015, PB-4/PB-4 '13re

reduced berry number per cluster by an average of 49% and PB-4 reduced cluster compactness by 38%, and PB-8/PB-8 ‘13re reduced berry number per cluster by an average of 74% and cluster compactness by an average of 69%. As in 2013, the greater reduction in berry number per cluster relative to cluster compactness in PB-4/PB-4 ‘13re plots was due to shorter rachis lengths, particularly in PB-4 ‘13re. There was a direct, positive relationship between reduction in berry number per cluster and cluster compactness, but concomitant reduction in rachis length confounded this relationship.

Table 6. Pre-bloom leaf removal effect on components of cluster compactness from 2013-2015.

Treatment[a]	2013		
	Berry # / cluster	**Rachis length (cm)**	**Cluster Compactness (berry # / cm rachis length)**
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).

Botrytis bunch rot and bunch stem necrosis incidence: Botrytis bunch rot (BBR) incidence was greater when no leaves were removed from the fruit-zones (PB-NO, PFS-NO) compared to any of the leaf removal treatments (PB-4, PB-8, PFS-6) (Table 7). Though not statistically compared, waiting until the post-fruit set period to remove leaves increased the incidence of BBR when compared to removing leaves before bloom. Bunch stem necrosis incidence was not affected by treatment.

Table 7. Botrytis bunch rot (BBR) and bunch stem necrosis (BSN) incidence as affected by pre-bloom and post-fruit set leaf removal in 2015.

Treatment[a]	BBR (%)	BSN (%)
PB-NO	18.5 a	10.9
PB-4	3.0 b	15.1
PB-8	0.0 b	13.5
PB-4 '13re	0.0 b	18.7
PB-8 '13re	0.0 b	9.3

Significance[b]	<0.0001	ns
PFS-NO	28.8 a	15.7
PFS-6	6.4 b	15.6
Significance[b]	0.0196	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).

Primary fruit chemistry: Pre-bloom removal of eight leaves (PB-8) reduced soluble solids by 5% when compared to PB-4 in 2013, and both current-season PB-8, and its re-implementation (PB-8'13re), reduced soluble solids by an average of 4% when compared to PB-NO in 2014 (Table 8). All pre-bloom leaf removal treatments significantly reduced TA by an average of 15% when compared to PB-NO in 2015. Post-fruit set removal of six leaves (PFS-6) reduced pH by 2-3% and TA by 15-19% in both years, and soluble solids by 1% in 2015, when compared to PB-NO.

Table 8. Pre-bloom and post-fruit set leaf removal effects on juice soluble solids concentration (SSC), pH, and titratable acidity (TA) in 2013-2015.

Treatment[a]	2013			2014			2015		
	SSC (°Brix)	pH	TA (g/L)	SSC (°Brix)	pH	TA (g/L)	SSC (°Brix)	pH	TA (g/L)
PB-NO	21.9 ab	3.30	8.15	21.8 a	3.50	7.91	21.7	3.27	7.81 a
PB-4	22.2 a	3.28	7.94	21.4 ab	3.48	7.39	21.9	3.37	6.92 b
PB-8	21.1 b	3.28	7.58	20.8 b	3.49	7.08	21.7	3.34	6.35 b
PB-4 '13re	n/a	n/a	n/a	21.4 ab	3.50	7.30	22.0	3.31	6.73 b
PB-8 '13re	n/a	n/a	n/a	21.1 b	3.46	7.18	21.4	3.30	6.60 b
Significance[b]	0.0287	ns	ns	0.0182	ns	ns	ns	ns	0.0003
PFS-NO	n/a	n/a	n/a	21.4	3.60 a	8.11 a	21.2 a	3.41 a	6.71 a
PFS-6	n/a	n/a	n/a	21.4	3.53 b	6.90 b	21.0 b	3.32 b	5.43 b
Significance[b]	n/a	n/a	n/a	ns	0.0194	<0.0001	0.0104	0.0013	0.0002

[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).

Total berry phenolics and anthocyanins: Pre-bloom removal of four leaves (PB-4) increased total berry anthocyanins by an average of 9% when compared to PB-NO and PB-8 in 2014 (Table 9). In 2015, PB-4 and PB-8 increased total berry anthocyanins by an average of 22% when compared to PB-NO. Pre-bloom leaf removal increased total berry phenolics by an average of 14% compared to PB-NO in both 2013 and 2014. In 2015, PB-8 increased total berry phenolics by 10% compared to PB-4 and 37% compared to PB-NO; PB-4 increased total berry phenolics by 25% compared to PB-NO. Post-fruit set removal of six leaves (PFS-6) increased total berry phenolics by 13% in 2014 and 16% in 2015 when compared to PFS-NO. In 2015, PFS-6 increased total berry anthocyanins by 16% when compared to PFS-NO.

Table 9. Pre-bloom and post-fruit set leaf removal effects on total berry anthocyanins (TBA) and phenolics (TBP) on the east and west canopy sides in 2013-2015.

Leaf removal[a] and canopy side	2013		2014		2015	
	TBA (mg/g berry)	TBP (au/g berry)	TBA (mg/g berry)	TBP (au/g berry)	TBA (mg/g berry)	TBP (au/g berry)
PB-NO	0.94	73.19 b	1.11 b	82.63 b	0.83 b	62.99 c
PB-4	1.00	83.39 a	1.22 a	94.27 a	1.00 a	78.45 b
PB-8	1.02	83.89 a	1.13 b	94.65 a	1.02 a	86.33 a
EAST	1.02	81.12	1.15	89.81	0.94	75.75
WEST	0.96	79.19	1.16	91.23	0.95	76.11
Significance[b]						
Leaf removal (LR)	ns	0.0112	0.0017	<0.0001	<0.0001	<0.0001
Canopy side (CS)	ns	ns	ns	ns	ns	ns
LR*CS	ns	ns	ns	ns	ns	ns
PFS-NO	n/a	n/a	0.61	49.38 b	0.71 b	57.61 b
PFS-6	n/a	n/a	0.62	55.71 a	0.80 a	66.55 a
EAST	n/a	n/a	0.61	51.31	0.77	62.89
WEST	n/a	n/a	0.62	53.79	0.73	61.27

Significance[b]	n/a	n/a				
Leaf removal (LR)	n/a	n/a	ns	0.0046	0.0377	0.0105
Canopy side (CS)	n/a	n/a	ns	ns	ns	ns
LR*CS	n/a	n/a	ns	ns	ns	ns

[a]PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight leaves, respectively. In 2013 and 2014, current-season leaf removal treatments were used; in 2015, re-implemented treatments from 2014 were used. 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).

There was no relationship between berry weight at harvest and total berry phenolics and anthocyanins when evaluated by combining the three years of the pre-bloom leaf removal study (Fig. 9). Rather, seasons showed a grouping of sorts, with 2014 having the greatest concentrations of these compounds in grapes across all berry weights (Fig. 9 A, E). A higher percentage of data points from 2013 and 2015 fell below the 95% confidence interval toward the lower and higher range of berry weight, respectively. There was no relationship between berry weight and total berry phenolics and anthocyanins in 2013 (Fig. 9 B, F). While there was no relationship between berry weight and total berry anthocyanins in 2014, there was a significant, (p > F = 0.0122) negative, linear relationship of berry weight and total berry phenolics in 2014 (Fig. 9 C, G). There was a significant, negative, linear relationship between berry weight and both total berry anthocyanins (p > F = 0.0179) and phenolics (p > F = 0.0003) in 2015 (Fig. 9 D,

H). A relatively high percentage of data points from PB-4 fell above the 95% confidence interval in both total berry phenolics and anthocyanins plots in 2014 and 2015 due to an increase in metabolite concentration without a concomitant decrease in berry weight. The grouping of treatment data points was well-defined only in the total berry phenolics relationship in 2014, and in both total berry phenolics and anthocyanins relationships 2015: PB-8 had lower berry weight and higher metabolite concentration, and vice-versa for PB-NO.

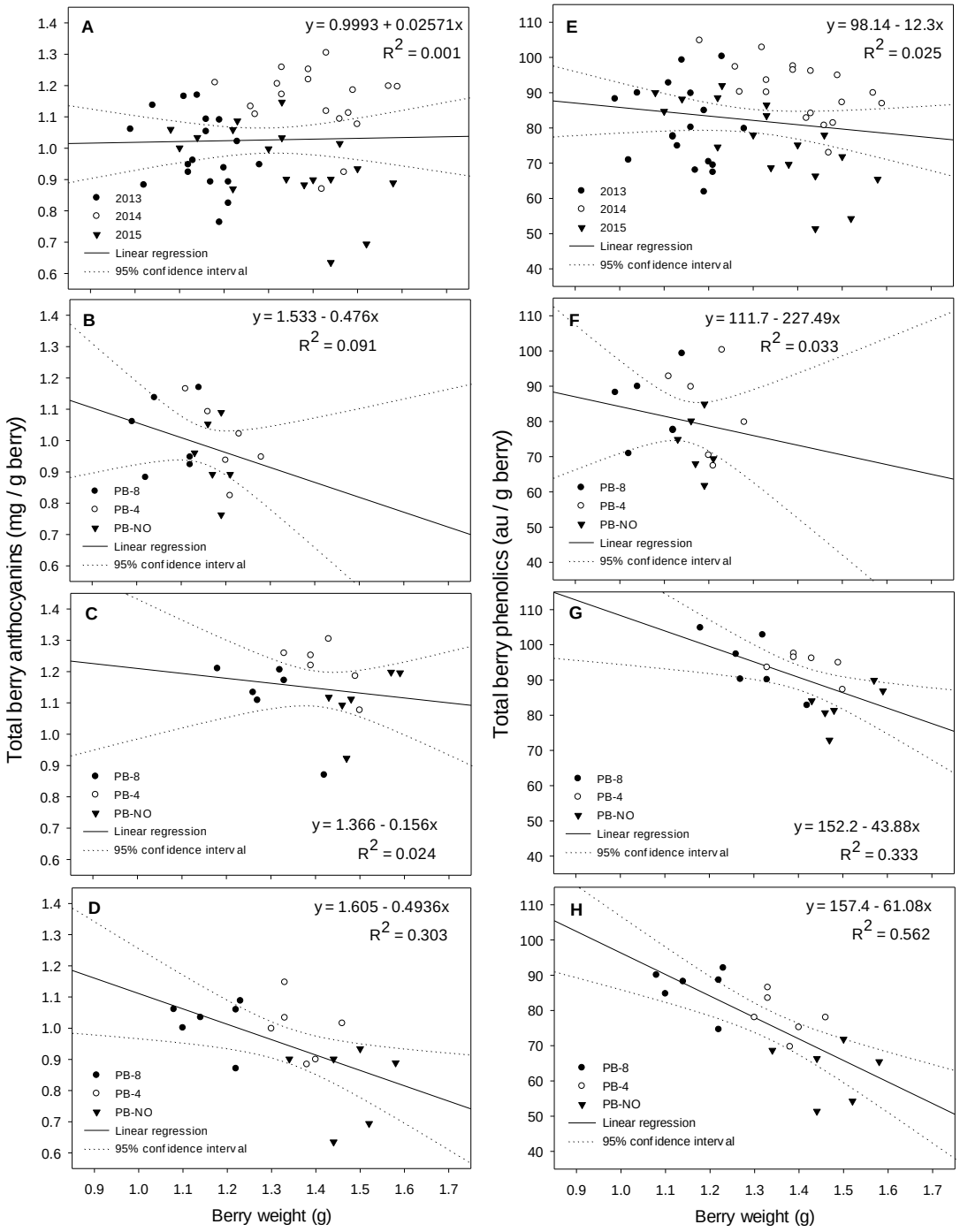

Fig. 9 The linear relationship between harvest berry weight and total berry anthocyanins (A-D) and phenolics (E-H) over 2013-2015 (A, E), and as affected by pre-bloom leaf removal treatment in 2013 (B, F), 2014 (C, G), and 2015 (D, H). PB-8, PB-4, and PB-NO = pre-bloom removal of eight, four, and no leaves, respectively. Each data point represents an average of 120 berries; n = 6.

There was no relationship between berry weight at harvest and total berry phenolics and anthocyanins when evaluated by combining the two years of the post-fruit set leaf removal study (Fig. 10). Seasons were grouped, with 2014 having lower metabolite concentrations as well as lower berry weights, whereas 2015 had comparatively higher metabolite concentrations across a broader range of berry weights (Fig. 10 A, E). There was no relationship between berry weight and total berry phenolics and anthocyanins in 2014, the year that post-fruit set leaf removal did not affect berry weight (Fig. 10 B, E). There was a significant, negative, linear relationship between berry weight and both total berry anthocyanins ($p > F = 0.0400$) and phenolics ($p > F = 0.0418$) in 2015 (Fig. 10 C, F), the year that PFS-6 reduced berry weight compared to PFS-NO. In 2015, treatment data point groups were well-defined, with PFS-6 having lower berry weight and higher metabolite concentration compared to PFS-NO.

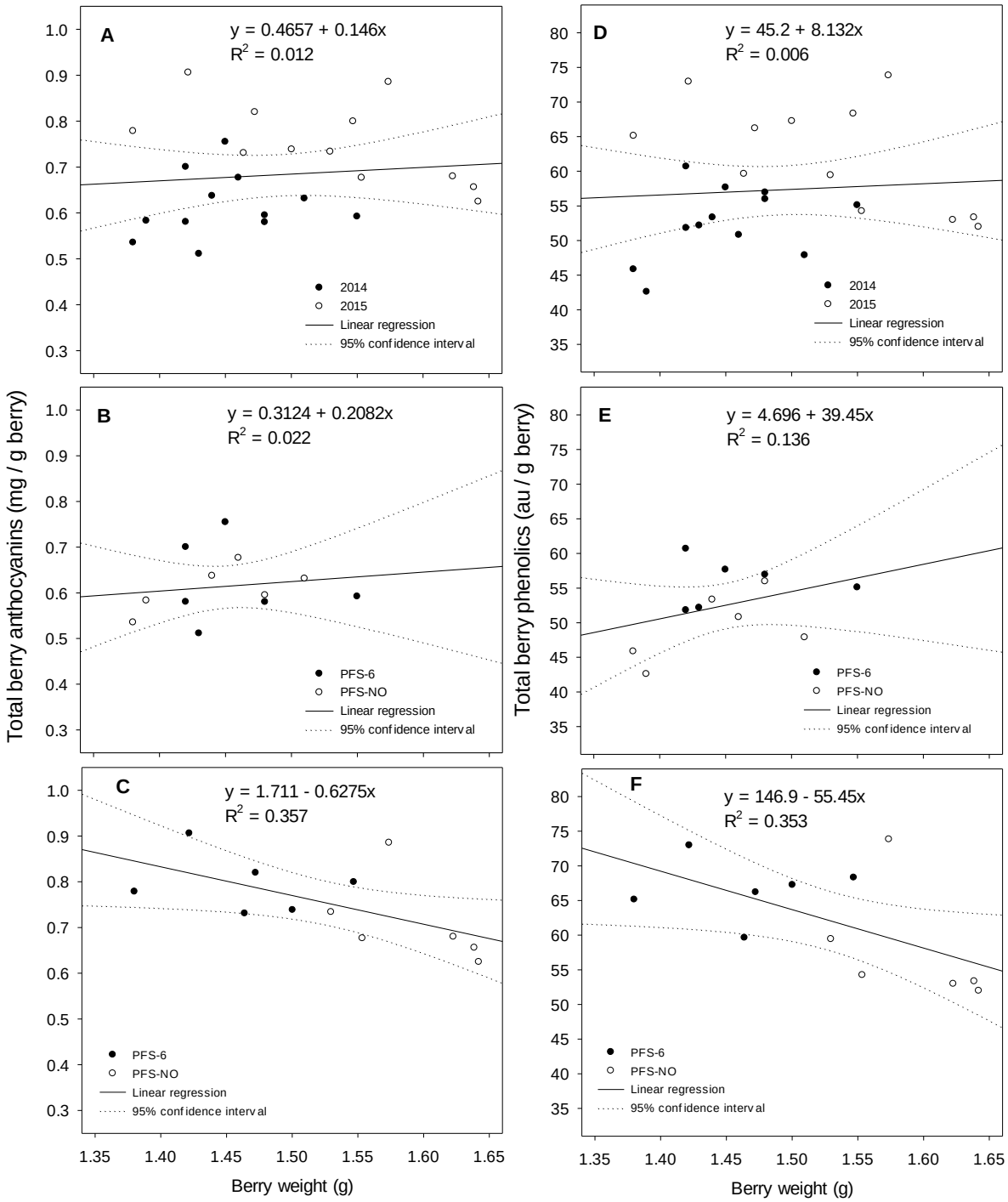

Fig. 10 The linear relationship between harvest berry weight and total berry anthocyanins (A-C) and phenolics (D-F) over 2014-2015 (A, D), and as affected by post-fruit set leaf removal of six (PFS-6) and no (PFS-NO) leaves in 2014 (B, E) and 2015 (C, F). Each data point represents an average of 120 berries; n = 6.

Discussion

The goal of these studies was to evaluate if exposing fruit to a greater extent and/or at an earlier time than currently recommended would favorably alter yield components and fruit composition of Cabernet Sauvignon. Most components of crop yield were reduced to a greater extent by pre-bloom removal of eight compared to four leaves, while post-fruit set leaf removal had minimal effect on components of yield. Fruit-zone leaf removal tended to increase total grape phenolics and anthocyanins, regardless of extent or timing of treatment execution.

Berry temperature and radiation: Comprehensive studies and reviews have documented that temperature and light are two of the most important meteorological determinants of fruit composition, wine quality, and vine growth and health (Bergqvist et al. 2001, Dokoozlian and Kliewer 1995, Downey et al. 2006, Jackson and Lombard 1993, Smart and Robinson 1991, Spayd et al. 2002). Yet, popular techniques developed to quantify canopy porosity and the amount of incident radiation reaching the fruit-zone do not quantify berry temperature (Smart and Robinson 1991, Meyers and Vanden-Heuvel 2008). It was therefore justified to characterize ambient air temperature and radiation patterns, and quantify how these related to berry temperature and, ultimately, composition of grapes from both shaded and exposed fruit-zones.

Radiation transmission to the fruit-zone was required to heat berries above ambient air temperature, as shown before (Bergqvist et al. 2001). This was a temporal phenomenon, however, given the diurnal change in solar angle and the decrease in fruit-zone radiation around solar noon. Trends of the current study showed that the percent radiation transmission to the fruit-zone typically declined as the solar angle tracked closer to noon due to the overhead canopy (Fig. 3). As such, the diurnal period of greatest *radiant* heating potential of grapes may be avoided in vertically-shoot positioned vineyards. These findings contrast Bergqvist et al. (2001),

who reported a 7-10 °C difference in berry temperature between exposed and shaded cluster at midday, and no midday depression in the difference between the temperature of ambient air and exposed berries, although that study was conducted in east-west oriented rows. It was possible that canopies were sparser, and likely that sunlight was more intense, in the relatively warmer/sunnier climate of the central San Joaquin Valley of California compared to the northern Shenandoah Valley of Virginia in which the current study was conducted. The regional contrast in climate between these two studies make ambient radiation an important consideration as a variable when modelling berry temperature, particularly under variable sky conditions (Cola et al. 2009).

Direct sunlight was shown to increase grape temperature to a greater extent than indirect sunlight (Bergqvist et al. 2001), revealing the spatiotemporal influence of solar angle on berry temperature in a given training system. The most aggressive fruit-zone leaf removal treatment frequently increased berry temperature when the sun was cast on the *opposite* canopy. However, because direct light heats plant tissues to a greater extent than diffuse light (Smart and Sinclair 1976), berry temperature was greatest when sun was cast on the east canopy side in the AM and on the west canopy side in the PM. During times of day that the sun was not blocked by the above-head canopy, a 4-5% *further* increase in berry temperature was observed in post-fruit set leaf removal plots compared to pre-bloom leaf removal plots. This was putatively a function of berries warming to a greater magnitude when touching neighboring berries in compact clusters (Keller et al. 2010), a phenomenon that might be mitigated with reduced cluster compactness afforded by pre-bloom leaf removal. As such, pre-bloom leaf removal may be a useful tool to increase the amount of surface area of berries that is exposed to air movement and, thus, ultimately reduce berry heating.

Manually measured berry temperature often showed statistical separation, but questionable biological importance, and were merely point-in-time observations. Leaf removal can biologically impact anthocyanins if berry temperatures routinely exceed 30-35 °C (Bergqvist et al. 2001, Mori et al. 2007, Spayd et al. 2002, Tarara et al. 2008). Although temperature is known to affect anthocyanin development, it was unclear whether sustained periods of elevated temperature were more or less important than shorter periods of possibly higher temperature (Tarara et al. 2008). Assuming time spent ≥ 30-35 °C impacted grape anthocyanin concentrations, the best determinant for treatment impact was logged berry temperature. Leaf removal resulted in berry temperature accumulation for 90 hrs ≥ 30 °C but only 10 hrs ≥ 35 °C, when averaged across pre-bloom removal extent and canopy side over three post-veraison periods (2013-2015). Comparatively, berry temperatures were logged for 163 hrs ≥ 30 °C and 63 hrs ≥ 35 °C when averaged across canopy side over three post-veraison periods under the more arid conditions used by Tarara et al. (2008). This comparison shows the relatively shorter time spent at these critical temperature thresholds in the current study, even when Tarara et al. (2008) did not employ fruit-zone leaf removal, which likely would have *further* increased the amount of time berry temperature was ≥ 30 and 35 °C. The comparison of berry temperature results between Tarara et al. (2008) and the current study reveals that climate largely drives berry temperature. There may be less difference between exposed and shaded grapes in warm and cool climates (Winkler et al. 1974), as cited in Bergqvist (2001), who reported point-in-time ambient air temperatures ≥ 35 °C at midday. By contrast, ambient temperature never exceeded 33.6 °C in the course of the current study.

The amount of time that berry temperature was ≥ 30 or ≥ 35 °C did not greatly differ between east and west canopy sides in this study. While the amount of time that berry

temperature was ≥ 30 °C was not different, berries spent 52 *less* hrs ≥ 35 °C on the east compared to west canopy side in Tarara et al. (2008). Ambient radiation in this study was often attenuated in the afternoon compared to the morning (Figs. 2 and 3), generally as a function of greater afternoon cloudiness. Though west-exposed berries had greater *average* berry temperature, radiation appeared to influence berry temperature on the west-side to a lesser degree than it did on the east-side. Whether this was due to greater cloud coverage in the afternoon, or that berries at a greater *current* temperature are less susceptible to radiant heating-induced temperature increase, is beyond the scope of this study. However, the net effect was less of a canopy-side difference in time spent at or above critical temperature thresholds in this study compared to others conducted in drier growing regions (Bergqvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). Variable cloudiness, particularly in the afternoon hours, is more common in Virginia than in the arid West. Thus, in humid growing regions, it may be erroneous to refer to a specific canopy side, particularly the west canopy side of north-south oriented rows, as the "hot" canopy side. Rather, the canopy side receiving more sunlight may vary from season-to-season in humid regions.

Components of yield: The close relationship between percent reduction in crop yield and percent reduction in berry number per cluster and cluster weight revealed that pre-bloom leaf removal reduced crop yield via reduction in berry number per cluster more so than reduction in berry weight or cluster number per vine. While fruit set was not measured in our study, the substantial reduction in berry number per cluster in pre-bloom plots was highly indicative of reduced set. Yield reduction was perhaps a function of source-induced carbon deficit that incurred before, and was maintained throughout, flowering (Candolfi-Vasconcelos and Koblet 1990, Caspari and Lang 1996, Coombe 1962). Further, bloom-time weather is frequently cool, cloudy, and/or rainy

in the eastern US, conditions that are unfavorable for pollen viability and germination rates (Koblet 1966). Optimum temperature for pollen germination is between 25.0 and 30.0 °C, whereas temperature below 10 or above 35 °C prevent germination (Keller 2010). Further, pollen tube growth is slowed below 25-30 °C (Keller 2010). Average temperature in the week surrounding bloom was 19.7 °C in 2013 and 2014, and 16.7 °C in 2015. Berry number per cluster was accordingly lower in 2015 compared to 2013 and 2014. Thus, source tissue reduction and sub-optimal germination temperatures at bloom may have reduced fruit set to a greater extent than would be expected to occur in regions with relatively warmer bloom periods.

Reduction in crop yield was a consistent response to pre-bloom leaf removal (Diago et al. 2012, Pallioti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2009, Gatti et al. 2012, Intrieri et al. 2008, Tardaguila et al. 2010 and 2012). In those previous studies, pre-bloom leaf removal reduced fruit set by 8-37%, berry number per cluster by 14-64%, cluster weight by 20-69%, and crop yield by 30-71%. Those broad response ranges represent different leaf removal techniques, such as manual (Gatti et al. 2012, Pallioti et al. 2011) and mechanical pre-bloom defoliation (Tardaguila et al. 2012), or both (Intrieri et al. 2008, Tardaguila et al. 2010), and were conducted in potted and field settings (Poni et al. 2006), and in several cultivars, including Tempranillo (Diago et al. 2012, Tardaguila et al. 2012), Sangiovese (Gatti et al. 2012, Intrieri et al. 2008, Pallioti et al. 2011, Poni et all. 2006), Graciano and Carignan (Tardaguila et al. 2010), Trebbiano (Poni et al. 2006), and Barbera and Lambrusco salamino (Poni et al. 2009). Only one other pre-bloom leaf removal study was known to be conducted in a region analogous to the current study - Michigan, US. (Sabbatini and Howell 2010). In the Michigan study, pre-bloom leaf removal reduced fruit set, berry number per cluster, cluster weight, and crop yield. Effects were more pronounced in Vignoles, a French-American hybrid, a response that was attributed to the

stronger shoot growth of Vignoles relative to Pinot noir and Pinot gris (Sabbatini and Howell 2012). Cabernet Sauvignon, used in the current study, is an inherently vigorous variety. As such, the strong shoot growth may have served as a strong sink competing with the flowering and fruit set process.

Few other than Sabbatini and Howell (2010) studied the severity of pre-bloom leaf removal on yield components. In that study, there was a strong (0.94-0.99) positive relationship between the number of leaves removed at trace bloom and the extent of cluster weight reduction in different varieties (Sabbatini and Howell 2010). When years were combined in the current study, there was a positive linear relationship (R^2 = 0.633-0.768) between the extent of pre-bloom leaf removal and the reduction in berry number per cluster, cluster weight, and crop yield (data not shown). When evaluated by year, this relationship was stronger in 2013 and 2015 (R^2 = 0.810-0.859) than in 2014 (R^2 = 0.545-0.722) (data not shown). This was likely because there was less *absolute* reduction in crop yield components due to pre-bloom leaf removal in 2014 compared to 2013 and 2015. The relationship between pre-bloom leaf removal extent and crop yield had lower R^2 values compared to berry number per cluster and cluster weight. This was likely due to the additional influence of berry weight and cluster number per vine on this metric. Our results suggest that there may not always be a strong linear relationship between pre-bloom source tissue removal and reduction in fruit set/cluster weight, and this relationship may vary between varieties, and across growing seasons and regions. The extent of fruit set reduction may be more proportional to the percent of leaves *retained*, rather than the percent of leaves *removed*, before bloom. This relationship was not measured in the current study, and would be difficult to quantify given the rapid increase in leaf area during bloom and fruit set.

Pre-bloom leaf removal *further* reduced crop yield when re-implemented over the following two seasons. However, when re-implemented once (2015), pre-bloom removal of eight, but not four, leaves *further* reduced crop yield. Reduction in crop yield was paralleled by a reduction in berry number per cluster and cluster weight. While some studies observed no further reduction in crop yield over multiple years (Poni et al. 2006, Intrieri et al. 2008), others' observations were similar to the current study (Gatti et al. 2012, Pallioti et al. 2011, Sabbatini and Howell 2010, Tardaguila et al. 2010 and 2012). For example, fruit set was *further* reduced by 24 and 88%, and crop yield by 55 and 83% due to re-implementation of pre-bloom removal of four and six leaves, respectively (Sabbatini and Howell 2010). If aggressive pre-bloom leaf removal is annually conducted on the same vines, crop yield may continue to decline due to reduced berries per cluster and cluster weights (Gatti et al. 2012, Pallioti et al. 2011), and as a function of reduced set (Sabbatini and Howell 2010).

Reduced carbohydrate status can limit fruit set (Candolfi-Vasconcelos and Koblet 1990, Caspari and Lang 1996, Coombe 1962). Accordingly, the *further* reduction in berry number per cluster over time observed in the current study was possibly indicative of lower carbohydrate status in the pre-bloom leaf removal vines. Carbohydrate reserves in the permanent vine parts can be continually depleted due to reduced source tissues during the *previous-season's* fruit ripening period as well as before the flowering/fruit set period of the *current-season* (Candolfi-Vasconcelos and Koblet 1990). Pre-bloom leaf removal decreased pruning weight (Pallioti et al. 2011), potentially due to pre-bloom source tissue limitation as well as the continual depletion of storage reserves in permanent vine parts (Pallioti et al. 2010). In the current study, re-implementation of pre-bloom leaf removal *further* reduced pruning weights over-time, but to a

significant within-season extent due only with pre-bloom removal of eight leaves. Therefore, repeated pre-bloom leaf removal can reduce vine capacity.

Defoliation of 50% of leaf area reduced final berry size when conducted 12 days after flowering, but has a reduced effect when executed at 35 and 58 days after flowering (Kliewer 1970). The reduction in berry and pericarp cell size was likely due to reduced assimilate supply (Ollat and Gaudillere 1998). Yet, the smaller berry size may have also been due to decreased translocation of leaf-derived hormones to the grapes from early defoliated treatments (Kliewer 1970). Early leaf removal may not completely limit berry growth potential (Ollat and Gaudillere 1998), as berry growth rates were similar between defoliated and foliated vines during the lag and second growth phase (Kliewer 1970). The current study confirms that early and aggressive source tissue removal can limit berry size, but waiting until several weeks after fruit set has little impact on berry weight. The most aggressive pre-bloom leaf removal treatment in the current study reduced the rate of berry weight gain, especially during the lag and second growth phase. This delayed berry weight gain was analogous to that of the low source tissue treatment implemented immediately after fruit set in Ollat and Gaudillere (1998). Since berry cell division occurs during the first two weeks after flowering (Jona and Botta 1988) it is possible that waiting until 12 days after flowering had little effect on berry cell division, resulting in less difference in berry weight gain during the lag and second growth phase, as in Kliewer et al. (1970). It was likely that *only* the most aggressive pre-bloom leaf removal treatment in the current study concomitantly reduced berry cell size *and* cell number enough to result in an initial, and sustained, berry weight reduction throughout the season. It was speculated that that the pre-bloom leaf removal threshold for reducing berry size was between four and eight leaves.

While some pre-bloom leaf removal studies reported a reduction in berry weight (Gatti et al. 2012, Pallioti et al. 2011, Tardaguila et al. 2010), others did not (Intrieri et al. 2008, Pastore et al. 2013, Poni et al. 2009, Sabbatini and Howell 2010, Tardaguila et al. 2010 and 2012). The latter group cited that berry growth was compensatory due to a reduction in berry number per cluster (Intrieri et al. 2008, Poni et al. 2009) and the null effect that early mechanical defoliation had on berry weight was a function of source tissue limitation before cell division in berry growth stage I (Tardaguila et al. 2012).

Since inflorescence primordia are initiated one year before they flower, early leaf removal can reduce vine fruitfulness (cluster number / unit of measure) and, thus, crop yield in both the current- and following season (Candolfi-Vasconcelos and Koblet 1990). Pre-bloom leaf removal reduced current-season fruitfulness by 6%, and subsequent-season fruitfulness by 21-30% (Sabbatini and Howell 2010, Tardaguila et al. 2012). However, in the current study, as well as others' (Intrieri et al. 2008, Gatti et al. 2012, Pallioti et al. 2011, Pastore et al. 2013), pre-bloom leaf removal did not reduce fruitfulness. While limiting source tissues at/before bloom in the previous season may negatively affect bud fertility, the increase in radiation transmission to next year's fruiting buds can improve fruitfulness (Perez and Kliewer 1990, Sanchez and Dokoozlian 2005), potentially negating the impact of source tissue limitation on bud fertility. There is likely a threshold at which the leaf area reduction negatively impacts bud fertility and fruitfulness, but this will likely vary as a function of timing and physiological status of the vines at the time of leaf removal.

Besides a small reduction in berry weight in one of two years, crop yield components were not negatively affected by post-fruit set leaf removal, even to the aggressive extent of six leaves removed. This response was anticipated, however, as fruit set and crop yield were

unaffected by leaf removal at four weeks post-bloom (Candolfi-Vasconcelos and Koblet 1990), nor due to leaf removal as early as at fruit set (Bledsoe et al. 1988). While not evaluating leaf removal extent, leaf removal that occurred further *after* bloom reduced crop yield to a lesser extent than did leaf removal performed closer to bloom (Candolfi-Vasconcelos et al. 1990). Results of our pre-bloom and post-fruit set leaf removal experiments support that crop yield can be regulated by the timing and extent of leaf removal (Intrieri et al. 2008, Kliewer 1970, Pastore et al. 2013, Tardaguila et al. 2010) primarily due to the sensitive period surrounding bloom, at which time fruit set is limited by carbohydrate supply to the flowering/setting cluster (Candolfi-Vasconcelos and Koblet 1990, Caspari and Lang 1996, Coombe 1962).

Primary fruit chemistry: Several pre-bloom leaf removal studies observed increased soluble solids (Diago et al. 2012, Gatti et al. 2012, Intrieri et al. 2008, Pallioti et al. 2011, Pastore et al. 2013, Poni et al. 2006, 2009, and 2010, Sabbatini and Howell 2010, Tardaguila et al. 2012). Pre-bloom leaf removal increased soluble solids due to: greater leaf area: fruit weight ratios (Gatti et al. 2012, Tardaguila et al. 2010 and 2012), regulated vegetative vigor, improved canopy and water use efficiency, and regulated crop load (Pallioti et al. 2011), and restored and more efficient leaf area due to lateral re-growth in the post-veraison period, and hastened assimilate translocation to the fruit (Intrieri et al. 2008, Pastore et al. 2013, Poni et al. 2006 and 2009); the last found before (Koblet et al. 1993, Quinlan and Weaver 1970). Most of the above-cited studies were conducted in drier regions, such as Italy (Gatti et al. 2012, Intrieri et al. 2008, Poni et al. 2006 and 2009, Pallioti et al. 2011) and Spain (Diago et al. 2012, Tardaguila et al. 2012), all of which likely have drier and sunnier ripening periods than typically observed in humid regions.

In the current study, pre-bloom removal of eight leaves reduced soluble solids, while removal of four and six leaves did not, regardless of when they were removed. Crop load tended to be lower in pre-bloom leaf removal plots, but also relatively low in the traditional sense in all other treatments (Bravdo et al. 1985). Though leaf area was not measured in the current study, fruit-zone and lateral re-growth were eliminated to minimize fruit-zone shading, perhaps limiting the pool of carbohydrates available for fruit ripening, even though remaining leaves on defoliated shoots are more efficient assimilators (Buttrose 1966, Kliewer et al. 1970, Candolfi-Vasconcelos and Koblet 1991, Poni et al. 2006). Because of the supra-optimal water availability in humid regions, aggressive leaf removal may not have reduced vegetative vigor to a point that carbohydrate competition with ripening fruit was alleviated. Therefore, the source sink balance was unfavorable for sugar translocation to ripening grapes potentially due to growing shoot tips serving as a competitive carbohydrate sink (Winkler et al. 1974).

Early-season (pre-bloom, immediately post-fruit set) leaf removal can delay ripening onset due to an initial leaf area deficit. Ripening was delayed particularly when leaves were removed after fruit set to result in smaller berries as sinks (Ollat and Gaudillere 1998). Genetic expression analyses indicated that pre-bloom and veraison-time leaf removal can result in a delay in the transcriptional ripening program of berries (Pastore et al. 2013). However, berry soluble solids can resume accumulating when source tissues are restored (Ollat and Gaudillere 1998, Pastore et al. 2013). In the current study, crop level and berry size may have been reduced so much in the PB-8 plots that that sink strength and assimilate translocation to fruit was reduced (Ollat and Gaudillere 1998). The combined effects of (1) a *maintained* leaf area deficit from pre-bloom through harvest, (2) a small crop sink/strength, and (3) sub-optimal assimilation rates during ripening may have resulted in reduced soluble solids. Given the inconsistent reduction of

soluble solids in PB-8 plots across all years, it is possible that the source-sink mechanisms that mitigated soluble solids accumulation in this treatment may have been slightly offset by the lesser absolute sugar required to reach a given concentration in smaller berries (Ollat and Gaudillere 1998). Nonetheless, aggressive pre-bloom leaf removal may be a useful tool to delay sugar accumulation in regions prone to hastened sugar accumulation, and in varieties prone to high °Brix, such as Petit manseng or Frontenac. This tactic may result in fruit with a better enological balance of primary fruit chemistry and varietal character from secondary metabolites.

In the current study, pre-bloom leaf removal inconsistently reduced titratable acidity (TA), while post-fruit set removal of leaves reduced TA more consistently, and to a relatively greater magnitude, than pre-bloom leaf removal. Pre-bloom leaf removal reduced TA in some cases (Pastore et al. 2013, Poni et al. 2009, Tardaguila et al. 2012), but either increased (Poni et al. 2006), or did not affect it in others (Gatti et al. 2012, Intrieri et al. 2008, Pallioti et al. 2011). Though genetic analysis suggested that tartaric acid synthesis in sun-exposed grapes from pre-bloom leaf removal plots was prolonged, higher berry temperatures likely increased malic acid degradation, ultimately resulting in lower titratable acidity compared to shaded grapes (Pastore et al. 2013). Interestingly, two pre-bloom leaf removal studies that did not observe TA reduction also permitted the re-growth of laterals from basal nodes (Intrieri et al. 2008, Pallioti et al. 2010). In those studies, lateral leaf shading may have mitigated the temperature-driven malic acid respiration frequently observed in exposed fruit-zones (Jackson and Lombard 1993, Kliewer and Schultz 1964), emphasizing the importance of reporting fruit-zone architecture, as by cluster exposure flux availability and/or leaf layer number. Pre-bloom leaf removal's variable effect on TA was potentially due to increased tartaric acid synthesis (Gatti et al. 2012, Poni et al. 2006), as greater carbon accumulation into tartaric acid occurs in sun versus shade berries (Kliewer and

Schultz 1964). Further, the optimum temperature range for organic acid and malic acid accumulation is between 20° and 25°C, and malic acid levels decline above 38 °C (Kliewer 1964, Lakso and Kliewer 1975). During the pre-veraison period of organic acid accumulation in grapes (Kliewer 1965), average logged berry temperature in both pre-bloom leaf removal and no leaf removal plots was within optimal range (20.3-23.8 °C). Thus, in humid climates, the temperature of exposed and shaded berries during pre-veraison may be optimal, or at least not inhibitory, for organic acid synthesis; this may not be true in warmer climates. Unlike malic acid, tartaric acid degrades very little throughout berry ripening (Ruffner, 1982), and the apparent reduction in tartrate levels is actually a dilution due to water and sugar influx into the berry (Johnson and Carroll 1973). That pre-bloom leaf removal had less consistent effects on TA than post-fruit set leaf removal in the current study may be explained by a combination of the last few points. The relatively earlier exposure of grapes in the pre-bloom plots may have enhanced organic acid accumulation in grapes compared to the several-week delay in grape exposure in the post-fruit set leaf removal plots. Furthermore, any high temperature-induced malic acid degradation may have been offset by the lack of tartaric acid dilution in smaller berries from the pre-bloom removal of eight leaf-plots.

Total berry phenolics and anthocyanins: Total phenolics were increased every year due to leaf removal, regardless of timing or magnitude. While the mechanisms were not measured, greater fruit-zone radiation and the concentrating effect of smaller berries were likely candidates, the latter particularly in the pre-bloom removal of eight leaves treatment. There was a stronger negative relationship between berry weight and phenolics than berry weight and anthocyanins. However, if a decrease in berry weight was solely responsible for an increase in berry phenolics, then phenolics should have been increased only by PB-8. This was not the case, however, as PB-

4 also increased phenolics in all years, but did not retard berry weight increase. The 2015 results, where phenolics were greater in PB-8 and PB-4 compared to PB-NO, but also greater in PB-8 compared to PB-4, were interpreted as concentration having a partial, but not sole, role of increasing berry phenolics. In this particular case, it was speculated that relatively higher incident radiation to the fruit-zone equally increased phenolics in both PB-4 and PB-8, and the greater phenolics observed in PB-8 plots was due to the additional concentration effect.

Other studies similarly found that total berry phenolics were increased with pre-bloom leaf removal (Intrieri et al. 2008, Poni et al. 2006, Tardaguila et al. 2010 and 2012), albeit inconsistently at times (Diago et al. 2012). Mechanisms behind increased grape phenolics were increased skin: pulp ratios (Poni et al. 2006), thicker berry skins (Pallioti et al. 2011, Poni et al. 2008, Tardaguila et al. 2010), greater and more efficient leaf area: fruit weight ratios (Diago et al. 2012, Intrieri et al. 2008, Gatti et al. 2012, Poni et al. 2006), and the positive effects of sun exposure on grape phenolics (Gatti et al. 2012, Tardaguila et al. 2012) such as skin flavonols and anthocyanins (Pastore et al. 2013). Phenolics include several flavonoid and non-flavonoid subclasses, the former subclass containing compounds that accumulate at different times during berry development and are differentially affected by environmental conditions such as light and temperature (Downey et al. 2006). It was, therefore, difficult to pinpoint a specific environmental effect or mechanism behind the increased grape phenolics observed in leaf removal plots. Nonetheless, leaf removal at either the pre-bloom or at the post-fruit set stage increased radiation to the fruit-zone, and increased berry phenols, a correlative response reported in other studies as well (Jackson and Lombard 1993, Price et al. 1995, Smart and Robinson 1991). Other factors, such as berry skin thickening, greater leaf area: fruit weight ratios, more

efficient remaining-leaf assimilation, and/or hastened assimilate translocation to clusters might also have been responsible for additional increases in berry phenolics.

Removal of fruit-zone leaves before bloom (Diago et al. 2012, Gatti et al. 2012, Intrieri et al. 2008, Pallioti et al. 2011, Poni et al. 2009, Tardaguila et al. 2010 and 2012) and after bloom (Diago et al. 2012; Kotseridis et al. 2012, Poni et al. 2006) has increased anthocyanins. There were several postulated mechanisms behind increased anthocyanins in pre-bloom leaf removal plots: greater and more efficient leaf area and greater leaf area: fruit weight ratios (Gatti et al. 2012, Intrieri et al. 2008, Pallioti et al. 2011, Diago et al. 2012), lateral shoot-shading of the fruit-zone (Intrieri et al. 2008, Pallioti et al. 2011), modifications to the flavonoid biosynthetic pathway (Pastore et al. 2013), greater skin: pulp ratios (Poni et al. 2006), and greater berry skin mass (Pastore et al. 2013, Poni et al. 2008 and 2010), regardless of berry size (Poni et al. 2008). Yet, the benefit of open fruit-zones could not be ruled out, even though fruit-zone light and berry temperatures were not well-characterized (Gatti et al. 2012, Kotseridis et al. 2012, Pallioti et al. 2011, Taradaguila et al. 2012). None of these postulated mechanisms that increased grape anthocyanins were measured in the current study.

The inconsistent relationship observed between berry weight and anthocyanin concentration in the current study suggests that anthocyanins were not simply increased in concentration due to berry weight reductions. The increased anthocyanins observed here with aggressive leaf removal was a contrast to the well-documented reduction in anthocyanin synthesis (Yamane et al. 2006) or increase in degradation (Mori et al. 2007) due to excessive berry temperatures attained when grapes are exposed to the sun (Bergqvist et al. 2001, Downey et al. 2006, Spayd et al. 20002, Tarara et al. 2008). Several studies observed decreased grape anthocyanins at 30-35 °C, the commonly cited temperature thresholds that are detrimental to

anthocyanin accumulation (Bergqvist et al. 2001, Mori et al. 2007, Spayd et al. 2002, Tarara et al. 2008, Yamane et al. 2006). mRNA levels of flavonoid biosynthetic pathway enzyme genes and the *VvmybA1* transcription factor, responsible for enhanced expression of genes in the flavonoid biosynthetic pathway, were lower in grapes held at 30 compared to 20 °C (Yamane et al. 2006). However, in apple, it was shown that a similar transcription factor responsible for anthocyanin biosynthesis in red-skinned apple cultivars was induced by light exposure (Takos et al. 2006). These studies collectively showed that light is necessary, but extreme temperatures deleterious, for anthocyanin biosynthesis and accumulation. Thus, the general mechanism of increased anthocyanins in the current study appeared to be greater fruit-zone radiation coupled with sun-exposed berries that spent less time at critical temperature thresholds compared to other studies (Spayd et al. 2002, Tarara et al. 2008, Pastore et al. 2013).

Previous studies have reported the duration of time that berries spent above critical temperature thresholds for grape anthocyanin degradation (Spayd et al. 2002, Tarara et al. 2008). Working in the sunny Yakima Valley of Washington State, Tarara et al. (2008) found that exposed berries that had lower anthocyanins spent 166 hrs ≥ 30 °C and 89 hrs ≥ 35 °C during the post-veraison period (Tarara et al. 2008). However, anthocyanins were reduced when shaded grapes were artificially heated for comparatively less time (142 hrs ≥ 30 °C and 15 hrs ≥ 35 °C) during the post-veraison period (Tarara et al. 2008). In another study in the Yakima Valley, sun-exposed berries on the west canopy side that spent 150-181 hrs > 30 °C and 67-74 hrs > 35°C from bunch closure to harvest had lower total grape color and anthocyanins than east-exposed berries that spent 115-148 hrs > 30 °C and 3-8 hrs > 35°C from bunch closure to harvest. In study conducted in Bologna, Italy, berry temperatures ≥ 30 °C were logged for ~ 300 hrs in both pre-bloom and veraison leaf removal treatments, but anthocyanins were *increased* in the *pre-*

bloom leaf removal and decreased in the veraison leaf removal treatment compared to a non-defoliated control (Pastore et al. 2013). Comparatively, in the most extreme cases of the current study, exposed grapes on both canopy sides spent only 105-108 hrs ≥ 30 °C and 19-22 hrs ≥ 35 °C, or when estimated in other treatments, 105-108 hrs ≥ 30 °C and 19-22 hrs ≥ 35 °C. The two weeks following color change is the critical period of susceptibility for temperature-induced color loss in red grapes (Yamane et al. 2006). The results of the current study suggest that berry temperature must be ≥ 30 °C for more than 57 hrs and/or ≥ 35 °C for more than 12 hrs during the two weeks following color change, as these were the most extreme conditions experienced by berries that did not experience reduced anthocyanins.

Collectively, the above studies suggest that sun-exposed berries can be subjected to temperatures ≥ 30 and 35 °C for a longer period of time than shaded berries before anthocyanin accumulation is inhibited or degradation is increased. If shaded berries are simultaneously heated, they are more susceptible to anthocyanin degradation than are exposed berries (Spayd et al. 2002, Tarara et al. 2008). However, the results of the current study generally showed that shaded grapes were seldom heated above ambient temperature, at least in a humid, frequently cloudy region in the eastern US. The more relevant temperature for reducing grape anthocyanins appears to be 35 °C rather than 30 °C, as anthocyanins were not reduced when berries spent 270 hrs ≥ 30 °C in a non-defoliated treatment (Pastore et al. 2013). Further, west-exposed berries that had lower anthocyanins spent 52-70 more hrs ≥ 35 °C, and only 7-33 more hrs ≥ 30 °C, when compared to east-exposed berries (Spayd et al. 2002, Tarara et al. 2008). It is unknown, however, if a combination of these two temperatures is a better determinant of grape anthocyanins than either alone. As shown in the current study and others (i.e. Pastore et al. 2013), the timing of leaf removal – and therefore the history of berry exposure – may have a

bearing on the fate of anthocyanin accumulation and degradation. To this effect, pre-bloom leaf removal apparently promotes anthocyanins relative to removing leaves later, either due to the cooling effect of looser clusters, or other mechanisms, such as the promotion of berry skin growth (Poni et al. 2006). However, in many parts of the eastern US, even waiting until after fruit set to remove leaves will likely result in fruit experiencing a combination of moderate temperatures and increased sunlight, a beneficial combination for grape anthocyanins (Tarara et al. 2008). Because anthocyanins and phenolics tended to be increased, basal leaf removal in red-fruited varieties in humid regions (1) can only benefit fruit composition; (2) need not be as conservative as currently practiced; (3) need not be focused on the east canopy side, and (4) may not result in sunburn, particularly if leaf removal occurs before bloom (Pastore et al. 2013).

Conclusion: Post-fruit set leaf removal did not reduce crop yield and concurrently increased berry phenolics and anthocyanins and decreased late season bunch rot incidence. Aggressive, pre-bloom leaf removal is not recommended in humid regions unless a crop yield reduction is acceptable. Removal of no more than four leaves before bloom would be a recommended starting point. On the other hand, basal leaf removal need not be as conservative as once thought due to frequent cloud cover and moderate temperatures experienced in humid compared to drier regions. Thus, fruit-zones can be maintained with fewer leaf layers than currently recommended in order to concomitantly improve disease management and increase total grape phenolics and anthocyanins. This work offers refinement for a ubiquitous practice that has received little modification and attention in humid growing regions over the last two decades. Future work ought to investigate long-term, cultivar-specific responses to pre-bloom leaf removal across several humid meso-climates, such as those in south and eastern Virginia, North Carolina, Georgia, Tennessee, and Missouri.

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Preliminary assessment of an hourly ambient temperature and radiation-driven model of grape berry temperature under variable sky conditions of the eastern US

Abstract

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 using the model.

Conclusions: When fruit-zones are shaded, berry temperature is highly predictable using ambient air temperature as the independent variable. The degree to 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 adjust their future fruit-zone management practices accordingly.

Introduction

Canopy management studies have been an active area of viticulture research since the 1960's (Shaulis et al. 1966). Since, several studies demonstrated that fruit exposure impacts fruit and wine quality. In general, fruit exposure to sunlight positively impacts (Bledsoe et al. 1988; Carbonneau 1985; Di Profio et al. 2011; Reynolds et al. 1996; Staff et al. 1997) and shading negatively impacts (Hunter, 1991; Jackson and Lombard 1993; Ryona et al. 2008; Smart et al. 1985; Smart and Robinson 1991) fruit and wine quality. But, compounds can be affected differentially by fruit exposure. Highly exposed fruit can reduce methoxypyrazines (Ryona et al. 2008), anthocyanins (Spayd et al. 2002), and acidity (Jackson and Lombard 1993), but increase flavonols (Price et al. 1995; Spayd et al. 2002). Light and temperature can differentially affect grape compound synthesis. Thus, the impact of light and temperature on grape composition (Dokoozlian and Kliewer 1995), particularly anthocyanins and other flavonoids (Bonada and Sadras 2015; Downey et al. 2006), but also volatile compounds (Robinson et al. 2014), has been of high interest over the last decade.

Because exposed fruit is subjected to a concurrent increase in light and temperature, an important question was if particular grape compounds were affected more or less by temperature or light. It was determined that temperature particularly affects grape anthocyanins (Bergqvist et

al. 2001; Spayd et al. 2002; Tarara et al. 2008), being detrimental at ~ 35 °C (Downey et al. 2006; Mori et al. 2007; Tarara et al. 2008). Temperature, in addition to light, can impact the activity of glycoside-producing enzymes in fruit (Gerdes et al. 2002). Further, temperature was related to C_{13}-norisoprenoids (Lee et al. 2007) and can affect grape carotenoids (Bureau et al. 1998). Though temperature highly impacts grape compounds, some studies evaluating the effects of fruit exposure on sensory impact compounds and their precursors have not measured grape berry temperature (Kwasniweski et al. 2010) or not elaborated on its impact, even when there was a 5 °C difference in temperature between sun-exposed and shaded clusters (Razungles et al. 1998).

Environmental factors can affect approx. 18% of grape genes (Dal Santo et al. 2013). Temperature, along with sunlight, are the environmental factors that vary most across vineyard sites and seasons and, therefore, account for flavonoid differences between sites and season (Downey et al. 2006). While both temperature and sunlight can affect aromatic compounds and their precursors (Gerdes et al. 2002), temperature was only briefly mentioned in terms of its impact on aroma compounds in a recent review (Robinson et al. 2014). This suggests that relatively few studies analyzing grape composition have sought to measure grape berry temperature, potentially because it is difficult to experimentally separate light and temperature effects in a field setting (Bonada and Sadras 2015, as in Spayd et al. 2002 and Tarara et al. 2008), or because the effect of temperature on grape composition is not appreciated as much as the effects of sunlight.

Strategies to achieve optimal canopies and metrics to quantify them, called point-quadrat analyses (PQA), have been used and cited extensively over the last 25 years (Smart and Robinson 1991). Proportion of exterior (from the canopy) fruit was given a high rating for its

impact on wine quality, citing that shaded fruit is inferior for winemaking. An enhancement of PQA, EPQA, added photon flux assessment, allowing quantification of canopy biomass distribution, light environment, and the efficacy of treatment (i.e. leaf removal, shoot thinning) implementation (Meyers and Vanden-Heuvel 2008). Cluster exposure flux availability, an EPQA metric, had the highest predictive strength for glycosylated aroma compounds in Riesling when compared to other metrics (Meyers et al. 2013). Given the impact that the work of Smart and Robinson (1991) and Meyers and Vanden-Heuvel (2008) has had on shaping viticulture practice and research, it is evident that the fruit-zone microclimate can have a greater impact on grape composition compared to any other environmental feature of the vineyard. Yet PQA or EPQA metrics do not quantify berry temperature.

Indirect methods to evaluate berry temperature are often imprecise (Bonada and Sadras 2015). Furthermore, canopy management practices tailored for a particular macroclimate might not be appropriate in other macroclimates. In order to better understand how berry temperature affects grape berry composition, quantitative and direct temperature measures are critical, and should be done in the field to limit confounding effects associated with growth chambers or artificial heating or cooling of fruit (Bonada and Sadras 2015). The last sentence of a recent review on the effect of temperature on grape composition read: "Modeling remains an underdeveloped approach to investigate berry composition in response to temperature" (Bonada and Sadras 2015). Only one other berry temperature prediction model was known to be developed (Cola et al. 2009). This model was based on a technique called energy balance to simulate thermal regimes of living bodies, and only required minimum and maximum daily ambient air temperature as inputs. The authors cited that direct berry temperature measurement was costly and complicated, and that other meteorological metrics, such as solar radiation and

relative humidity, were difficult to obtain. However, again, indirect methods to evaluate berry temperature are inconclusive and subject to misinterpretation (Bonada and Sadras 2015).

Temperature highly impacts fruit composition, yet relatively few fruit exposure studies have quantified grape temperature. Thus, we sought to develop a model to predict grape berry temperature, particularly by using readily available meteorological metrics of ambient light and temperature as inputs. Permitting the prediction of grape berry temperature will allow accurate and quantitative comparisons to be made between regions that greatly differ in temperature and sunlight conditions. Consequently, fruit-zone management can become fine-tuned, and more vineyard- and region-specific. Our goal was to produce a model that was perhaps more pragmatic and data driven by taking a more "boots-on-the-ground," rather than math-driven approach of another berry temperature model study (in Cola et al. 2009). This was accomplished by logging berry temperature and meteorology at the scale of the canopy. It was hypothesized that berry temperature could be predicted using only ambient air temperature and radiation, but that berry temperature may also be affected by relative humidity, berry color, and canopy-side position. All of these metrics were thus logged over the course of the study.

Materials and Methods

Treatments and experimental design: Two separate timings of leaf removal treatments were implemented, both of which used Cabernet Sauvignon ENTAV-INRA® clone 337 vines, grafted onto 420-A rootstock, and grown at Virginia Tech's AHS, Jr. Agricultural Research and Extension Center (AHS, Jr. AREC) near Winchester, VA (39°11'N; 78°28'W). Vines were planted in May 2006 in rows running generally northeast/southwest at a 3.0-m (row) x 1.5-m (vine) spacing and were trained to bilateral cordons with vertically-positioned shoots. The soil was a Poplimento Hagerstown sandy loam (A. Blackburn, personal communication, 2013). The

inter-row groundcover, established in 2001, initially comprised a mixture of orchard grass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*); cv. 'Shenandoah', with the fescue dominating after ~ six years. The intra-row groundcover in the pre-bloom leaf/lateral shoot removal plots consisted of perennial creeping red fescue (*F. rubra*), established in Sep 2008, and an 85-cm wide herbicide-treated strip in the post-fruit set leaf/lateral shoot removal plots. Leaf/lateral shoot removal treatments were as bulleted below:

Expt. I: Pre-bloom leaf/lateral shoot removal treatments (2013-2015):

PB-NO - no leaves/lateral shoots removed; lateral shoots maintained at ~3-4 nodes.

PB-4 - removal of leaves/lateral shoots from primary shoot nodes 1-4 before bloom, [modified Eichorn and Lorenz (EL) stage 18-19 (Dry and Coombe 2004)]; distal lateral shoots maintained at ~3-4 nodes.

PB-8 - removal of leaves/lateral shoots from primary shoot nodes 1-8 before bloom, [modified Eichorn and Lorenz (EL) stage 18-19 (Dry and Coombe 2004)]; distal lateral shoots maintained at ~3-4 nodes.

Expt. II: Post-fruit set leaf/lateral shoot removal treatments (2014-2015):

PFS-NO - no leaves/lateral shoots removed; lateral shoots maintained at ~3-4 nodes.

PFS-6 - removal of leaves/lateral shoots from primary shoot nodes 1-6 at pea-berry size / bunch closure (modified EL stage 31/32); distal lateral shoots maintained at ~3-4 nodes.

A complete block design was used for each set of leaf removal timing, partitioning the experimental area into six blocks, each separated by five-vine border plots within the row and by bordering buffer rows. Within each block, leaf removal treatments were randomly assigned to either one-vine experimental units (pre-bloom experiment) or two-vine experimental units (post-

fruit set experiment). Pre-bloom and post-fruit set leaf removal experimental units were no more than ~ 45 m away from each other in each vineyard block.

General vine management: Cordons were spur-pruned each winter. Shoot density was adjusted, aiming for ~ 15-16 shoots per m of cordon. Shoots were maintained vertically upright with the aid of catch wires. Shoots were shoot-hedged before they extended more than ~0.9 m above the top catch wire. Frequent re-visits to experimental units were made to ensure that canopy porosity was maintained through harvest. No vine nutritional deficiency symptoms were evident, and disease management was standard for the region.

Canopy characterization: Point quadrat analysis (PQA) data was collected between EL stages 33 and 35 (veraison), as described in Smart and Robinson (1991), in order to characterize fruit-zone architecture. This was performed in the Cabernet Sauvignon pre-bloom study from 2013-2015, the Cabernet Sauvignon post-fruit set study from 2014-2105. A thin metal rod was inserted into the fruiting zone along the transverse axis of the canopy, using a tape measure to guide insertions. This process was repeated 10 times in each experimental unit (vine) in order to quantify fruit-zone leaf layer number (LLN). Photosynthetic photon flux density (PPFD) was assessed by inserting the ceptometer inside canopy fruit zones parallel to, and directly above, the cordon and orienting the light interception side of the ceptometer in three different directions (45˚ east, vertical, 45˚ west) and then averaging those readings. Two PPFD readings were taken in every experimental unit (one reading above each cordon) between ~ 1030-1400 hrs under consistent ambient radiation conditions. An ambient PPFD reading was taken between with each fruit-zone reading in order to express fruit-zone PPFD as a percentage of the ambient radiation. This data was used to generate the cluster exposure flux availability (CEFA) metric with

enhanced point quadrat analysis (EPQA version 1.6.2) software (Meyers and Vanden-Heuvel 2008).

The "datalogger vine panel:" The following measurements were logged at specified intervals in each respective season. **2013:** berry and ambient temperature, and fruit-zone PAR were logged on 15-min intervals from 30 Jul 2013 through 9 Oct 2013. **2014:** berry and ambient temperature, and fruit-zone and ambient PAR were logged on 15-min intervals from 25 Jul 2014 through 4 Sep 2014, and on 1-min intervals from 6 Sep 2014 through16 Oct 2014, and from 22 Jun 2015 through 5 Oct 2015. **2015:** berry and ambient temperature, and fruit-zone and ambient PAR were logged on 1-min intervals from 22 Jun 2015 through 5 Oct 2015.

One experimental unit (vine) of each *pre-bloom* leaf removal treatment (post-fruit set leaf removal vines were never logged) in the same Cabernet Sauvignon vineyard panel (max. 7.6 m apart) was subjected to logging of berry temperature and fruit-zone photosynthetically active radiation (PAR). This was done to measure berry temperatures typical of our region, and evaluate how light and temperature impact berry temperature. In addition to these leaf removal treatment-specific measurements, ambient temperature and PAR were logged immediately surrounding and above the "datalogger vine panel," respectively. The temperature of two outside-facing berries on both east and west canopy sides of each leaf removal treatment were measured with a mini hypodermic thermocouple (type T, model HYP1/2, Omega Eng., Stamford, CT) inserted approx. 0.6 cm beneath berry skins and affixed with a strong, all-purpose glue. Fruit-zone PAR was measured with a SQ-316 quantum sensor (Apogee Instruments, Logan, UT) placed on top, and parallel to, the orientation of one cordon in each leaf removal treatment. Ambient PAR was measured with a SQ-316 quantum sensor, mounted approx. 3 m above the ground (approx. 0.6 m above vine canopies) at the middle of the vineyard panel where

all data were logged. Ambient temperature was measured with two ST-100 thermistors (Apogee Instruments, Logan, UT). The thermistors were placed on the north and south bordering ends (~ 7.6 m apart) of the "datalogger vine panel," and mounted at fruit-zone height inside a naturally aspirated 6-panel radiation shield (model 41303-5A, R.M. Young, Traverse City, MI). Thermocouples were logged with an AM25T solid-state multiplexer (Campbell Scientific, Logan, UT) attached to a CR1000 datalogger (Campbell Scientific, Logan, UT), which also logged the PAR and ambient temperature data. All sensors, excepting the ambient temperature sensors, were taken down at the end of each field season and re-mounted in the following season.

Logged green and red berry temperature: One Cabernet Sauvignon (red fruit) vine panel and one Petit manseng (green fruit) vine panel were located directly across the row from the above-mentioned "datalogger vine panel" located in the Cabernet Sauvignon vineyard The basal leaves and lateral shoots were removed up to the second catch wire (i.e. ~ 10 basal leaves and laterals) from the primary shoots growing from one cordon of one vine in each variety panel to prevent berry temperature from being differentially affected by fruit exposure extent. On the leaf-pulled half of the vine, the temperature of one Cabernet Sauvignon and one Petit manseng berry positioned on the exterior face of an east- and west- exposed cluster was logged on 1 min intervals from 3 Oct to 16 Oct 2014 (a total of 12,356 total logged measurements of color/canopy side specific berry temperature), and from 22 Aug to 25 Sep 2015 (a total of 38,645 total logged measurements of color/canopy side specific berry temperature). The logged Cabernet Sauvignon and Petit manseng berries were ~0.6 m away from each other. Berry temperature was measured with a mini hypodermic thermocouple (type T, model HYP1/2, Omega Eng., Stamford, CT) inserted approx. 0.6 cm beneath berry skins and affixed with an all-purpose glue. Data was logged with the same CR1000 datalogger (Campbell Scientific, Logan, UT) mentioned above.

Manual berry temperature measurement for model validation: Berry temperature was manually measured on 29 Jun, 31 Jul, 25 Aug in 2015 in the same Cabernet Sauvignon vineyard that the datalogger was located. Measurements were taken in every experimental unit at three different times of day on each collection date: morning (~0900-1030 hrs), around solar noon (~1245-1415 hrs), and late afternoon (~1545-1715 hrs). Measurements were taken by inserting a mini hypodermic thermocouple (model HYP1/2, Omega Eng., Stamford, CT) beneath the skins of berries, which was connected to a handheld digital thermometer (model HH 25, Omega Eng., Stamford, CT). Each measurement took ~ 2 sec. At each time of day, on each collection date, and on both east and west canopy sides, three berries on the cluster's exterior face, positioned at the top, center, and bottom of the clusters, were measured on two clusters borne on opposite vine cordons in each experimental unit. Thus, a total of 12 single-berry temperature measurements, or six single-berry temperature measurements on each canopy side, were taken in each experimental unit. The timeframe that encompassed manual berry temperature measurement was recorded in order to validate the prediction of berry temperature with ambient air temperature and PAR using the herein developed model. The ambient air temperature and PAR were retrieved from the "datalogger panel," and logged on one-min intervals.

Model development: Hour angle for each logged data point was computed in Excel (Microsoft Corporation, Redmond, Washington, USA) using formulas adapted from a spreadsheet posted by the National Oceanic & Atmospheric Administration Earth System Research Laboratory (www.esrl.noaa.gov). Hour angle converts local solar time into degrees with which the sun moves across the sky. Hour angle is negative in the morning, 0° at solar noon, and positive in the afternoon. Accordingly, negative and positive hour angles were those associated with radiation directed on the east and west canopy sides, respectively. Each hour away from solar

noon corresponds to 15° angular motion of the sun in the sky. Hour angle was rounded to 15° intervals to correspond to hourly changes in local solar time as the season progressed. Berry temperature differential was calculated for each logged data point by subtracting ambient temperature from canopy side-specific berry temperature. Summary statistics, and linear correlations between ambient PAR and berry temperature differential, were calculated using MATLAB (The Mathworks, Natick, Massachusetts) for each treatment-hour angle combination in 2014 and 2015 only (NOTE: ambient PAR was not logged in 2013).

Statistics: All linear relationships were analyzed with simple one-way ANOVA using the bivariate analysis function in JMP Pro 11 (SAS, Cary, NC). Linear relationship graphs were built using the graph builder function in JMP Pro 11, and hourly relationship graphs were built using Sigma Plot 12.5 (Systat Software, Inc., San Jose, CA).

Results

Canopy characterization in plots used for manual berry temperature measurement and model validation: Leaf removal in the Cabernet Sauvignon plots resulted in zero fruit-zone leaf layers (LLN) at veraison 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) at veraison compared to removing no leaves, and there was no difference in CEFA between PB-4 and PB-8.

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 in Cabernet Sauvignon over 2013-2015.

Treatment[a]	2013		2014		2015	
	LLN	CEFA	LLN	CEFA	LLN	CEFA
PB-NO	2.48 a	0.15 b	2.52 a	0.21 b	2.65 a	0.18 b
PB-4	0.04 b	0.54 a	0.02 b	0.75 a	0.05 b	0.82 a
PB-8	0.00 b	0.59 a	0.00 b	0.82 a	0.00 b	0.82 a
Significance[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PFS-NO	n/a	n/a	2.73 a	0.19 b	2.66 a	0.14 b
PFS-6	n/a	n/a	0.00 b	0.77 a	0.00 b	0.77 a
Significance[b]	n/a	n/a	<0.0001	<0.0001	<0.0001	<0.0001

[a]PB-NO, PB-4, PB-8 = pre-bloom leaf removal of no, four, and eight 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).

Ambient air temperature vs. berry temperature: Berry temperature was highly correlated with air temperature, as shown by the highly significant (p > F = < 0.0001), positive, linear relationship between these two variables (Fig. 1); this relationship will henceforth be referred to as the "air-berry temperature relationship." NOTE: all linear relationships henceforth presented are significant (p > F = < 0.0001). The air-berry temperature relationship was maintained in each of the three years that both of these metrics were logged (Fig. 2). The air-berry temperature relationship was relatively stronger on the west compared to east-canopy side in all three years (Fig. 3).

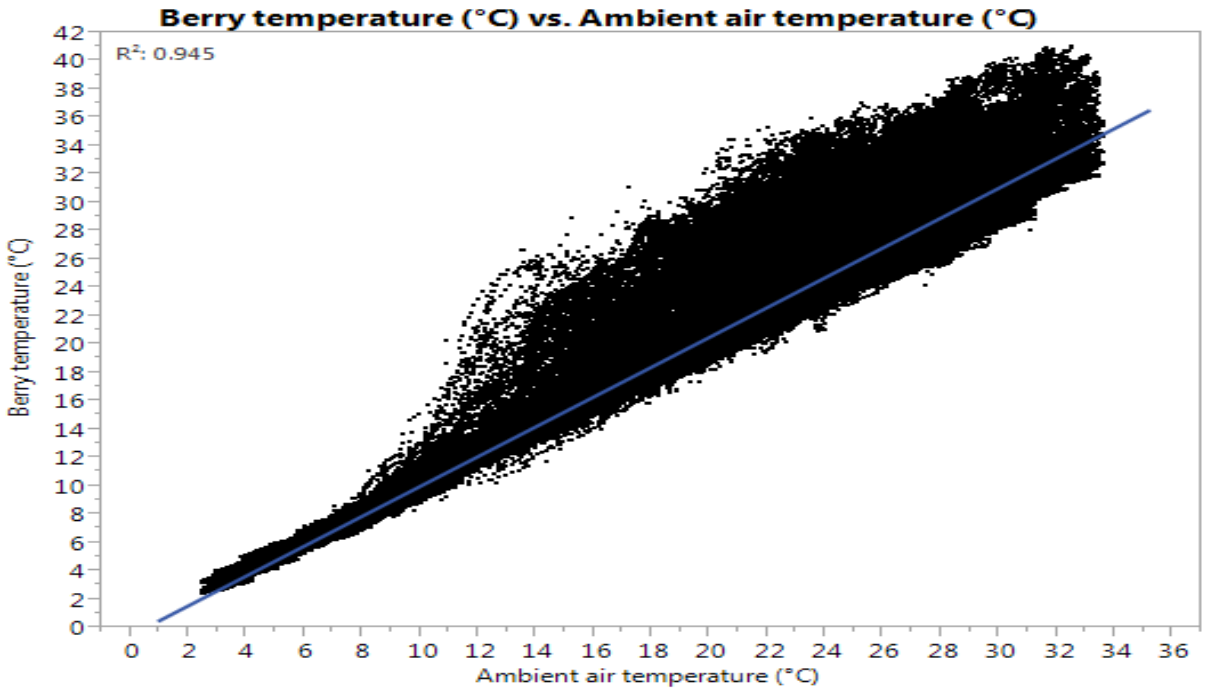

Fig. 1. The linear relationship between ambient air temperature and berry temperature over 2013-2015.

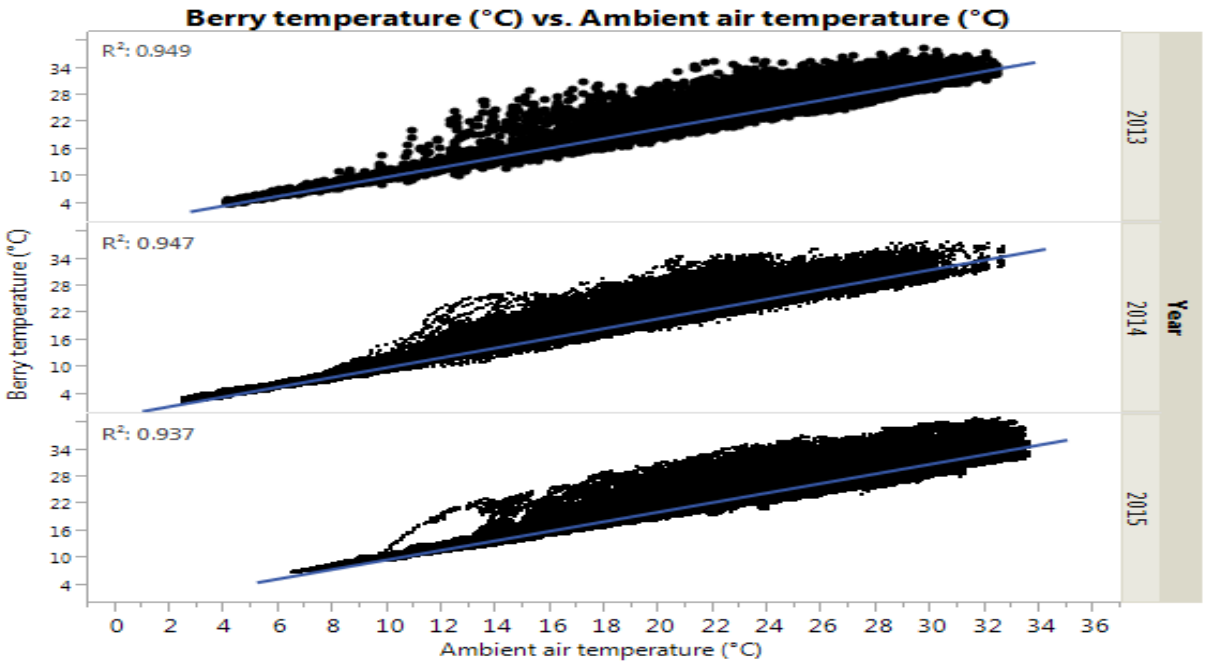

Fig. 2. The linear relationship between ambient air temperature and berry temperature in each of the three years of data collection, 2013-2015.

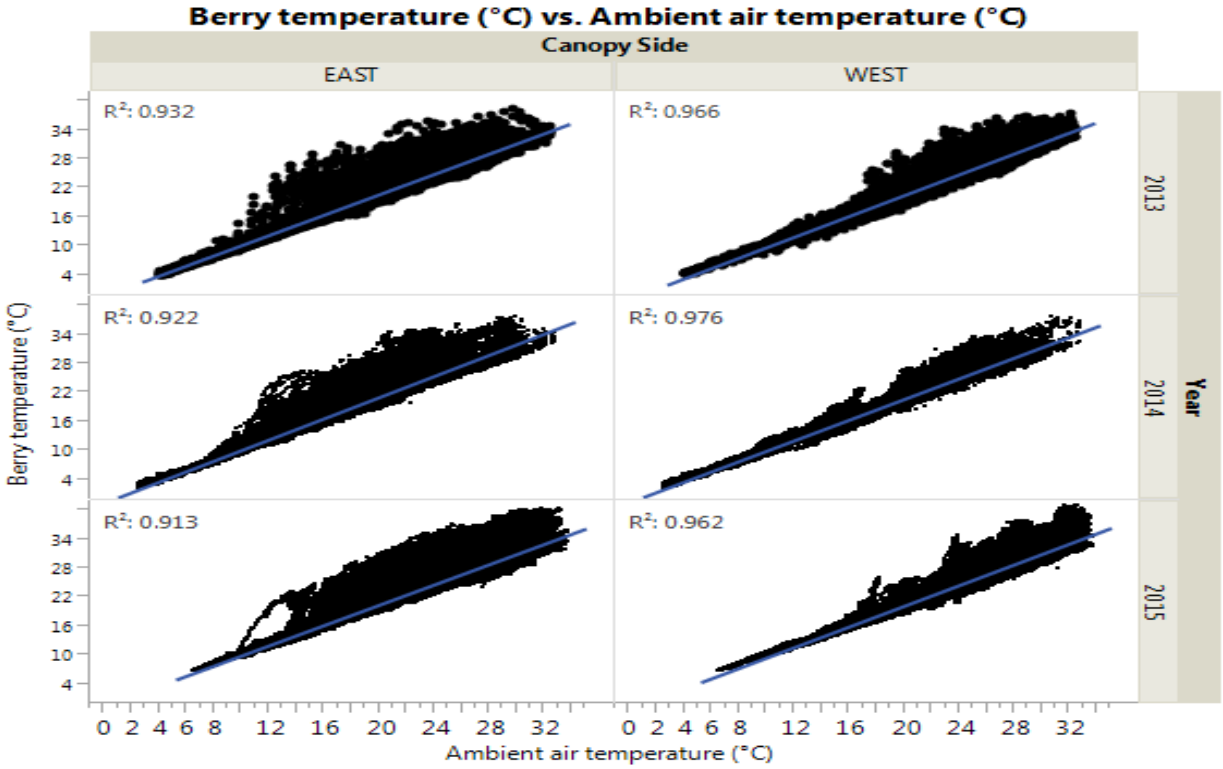

Fig. 3. The linear relationship between ambient air temperature and east- and west- canopy side berry temperature in each of the three years of data collection, 2013-2015.

A relatively greater number of data points fell above the air-berry temperature trend line compared to below the air-berry temperature trend line (Figs. 1-3). Thus, it was suspected that there was another meteorological factor that increased berry temperature without concomitantly increasing air temperature. This appeared to be a function of removing leaves and lateral shoots from the fruit-zone, as relatively more data points fell above the air-berry temperature trend line in the pre-bloom removal plots (PB-4/PB-8) compared to when no leaves were removed (PB-NO) (Fig. 4). Accordingly, the air-berry temperature relationship was stronger when no leaves were removed from the fruit-zone compared to when leaves were removed from the fruit-zone (Fig. 5). The air-berry temperature relationship in the fruit-zone of each leaf removal treatment was consistent across all three years of the study (Fig. 6).

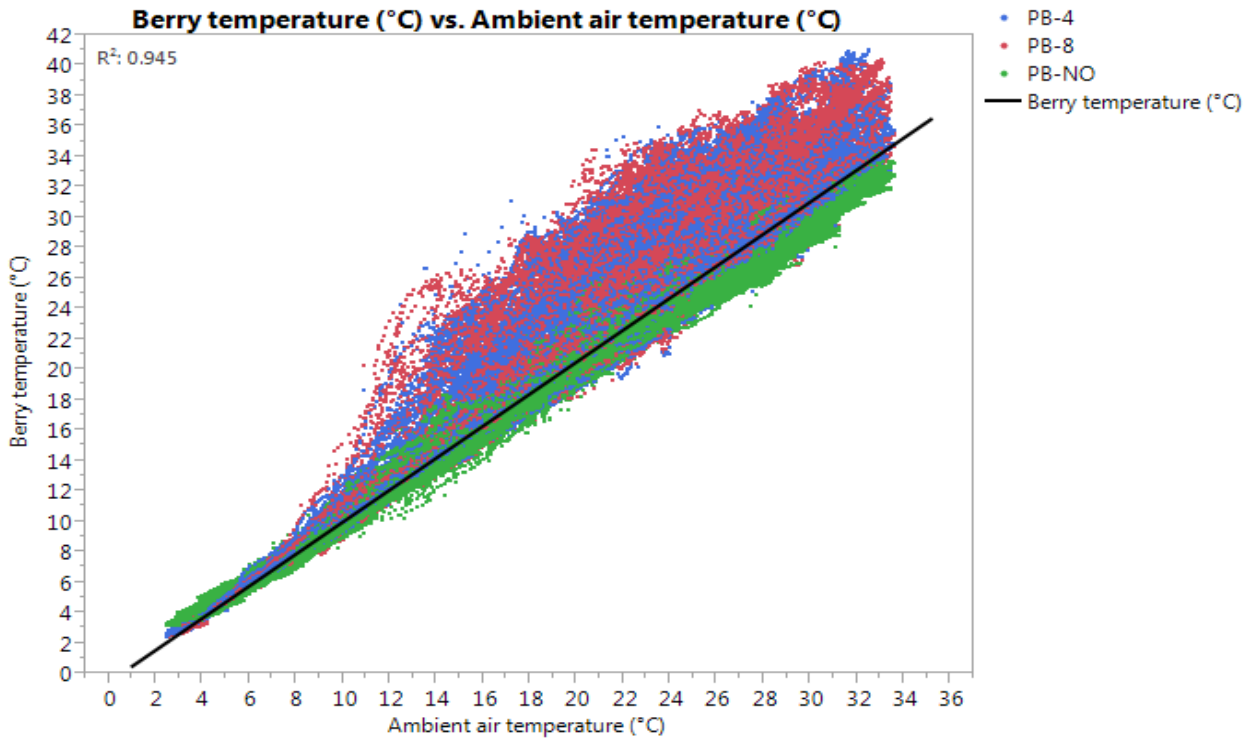

Fig. 4. The linear relationship between ambient air temperature and berry temperature, accounting for pre-bloom leaf removal treatments over 2013-2015.

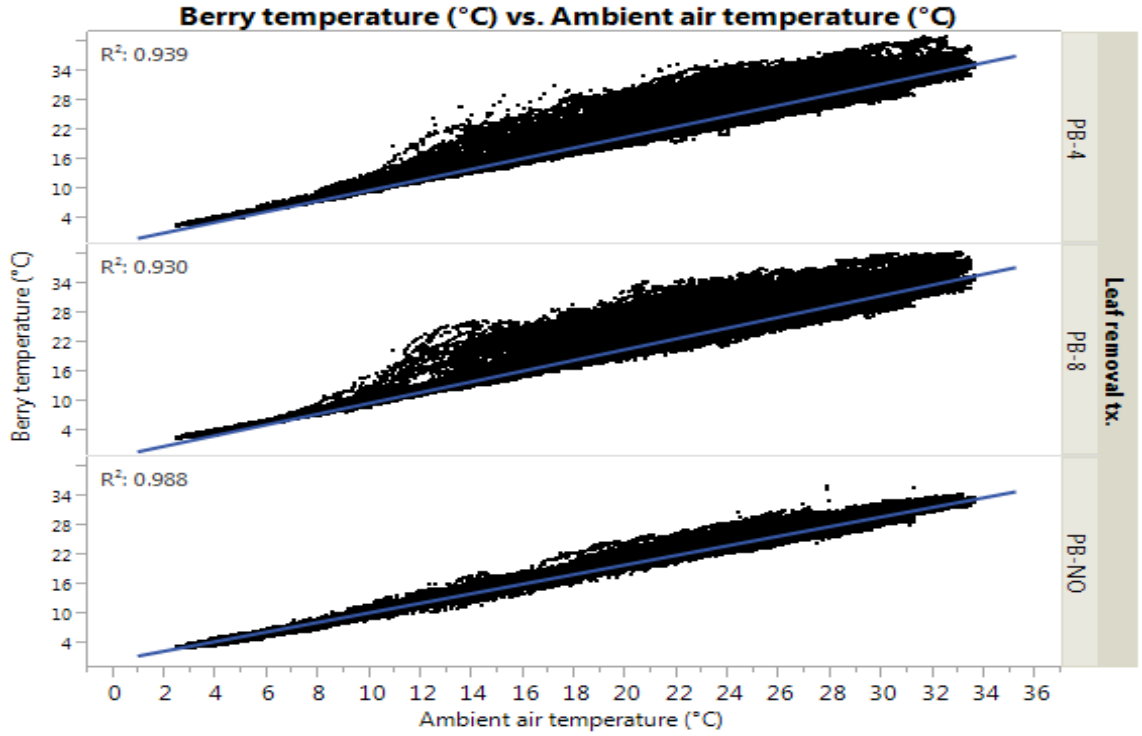

Fig. 5. The linear relationship between ambient air temperature and berry temperature in each pre-bloom leaf removal treatment over 2013-2015.

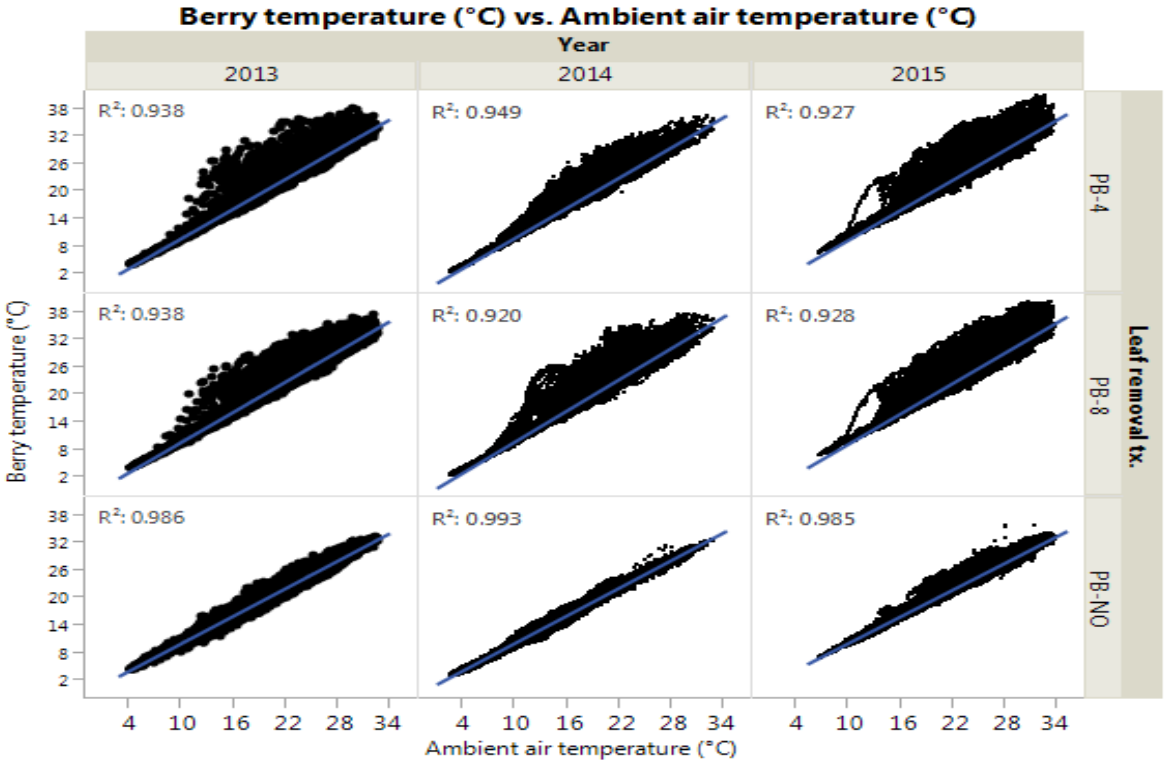

Fig. 6. The linear relationship between ambient air temperature and berry temperature in each pre-bloom leaf removal treatment in each of the three years of data collection, 2013-2015.

Canopy side had little effect on the air-berry temperature relationship in PB-NO plots, but, as observed when all treatments were combined (Fig. 3), the air-berry temperature relationship was stronger on the west compared to east canopy side in PB-4 and PB-8 plots (Fig. 7). Collectively, Figs. 1-7 showed that berry temperature could be highly predicted (at least $R^2 \geq 0.897$) by ambient air temperature alone, and without regard to time of day, or any other meteorological factor.

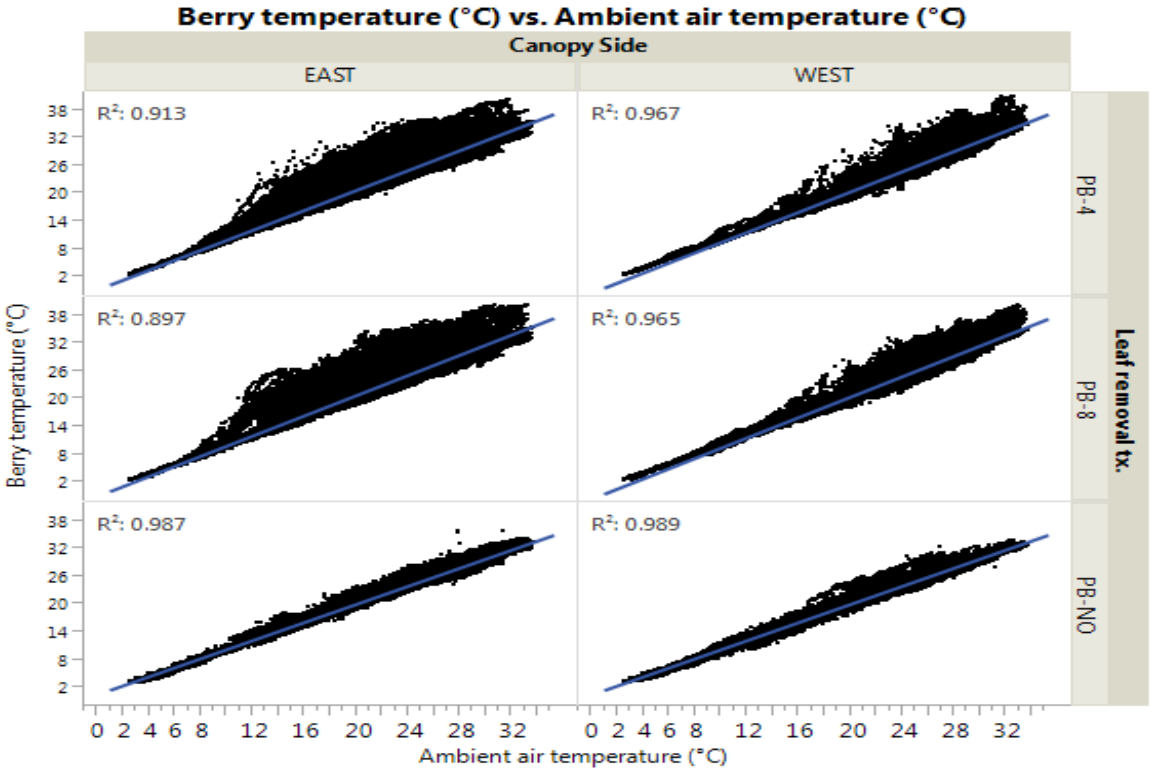

Fig. 7. The linear relationship between ambient air temperature and east- and west-canopy side berry temperature in each of the leaf removal treatment plots over 2013-2015.

Ambient photosynthetically active radiation (PAR) vs. berry temperature: The relationship between ambient PAR and berry temperature was much weaker than the air-berry temperature relationship (Fig. 8). This relationship will henceforth be referred to as the "ambient PAR-berry temperature relationship." The ambient PAR-berry temperature relationship had similar R^2 values in 2014 and 2015, the two years that both of these metrics were logged (Fig. 9). Opposite to the air-berry temperature relationship, the ambient PAR-berry temperature relationship was relatively stronger on the east- compared to west-canopy side over 2014 and 2015 (Fig. 10).

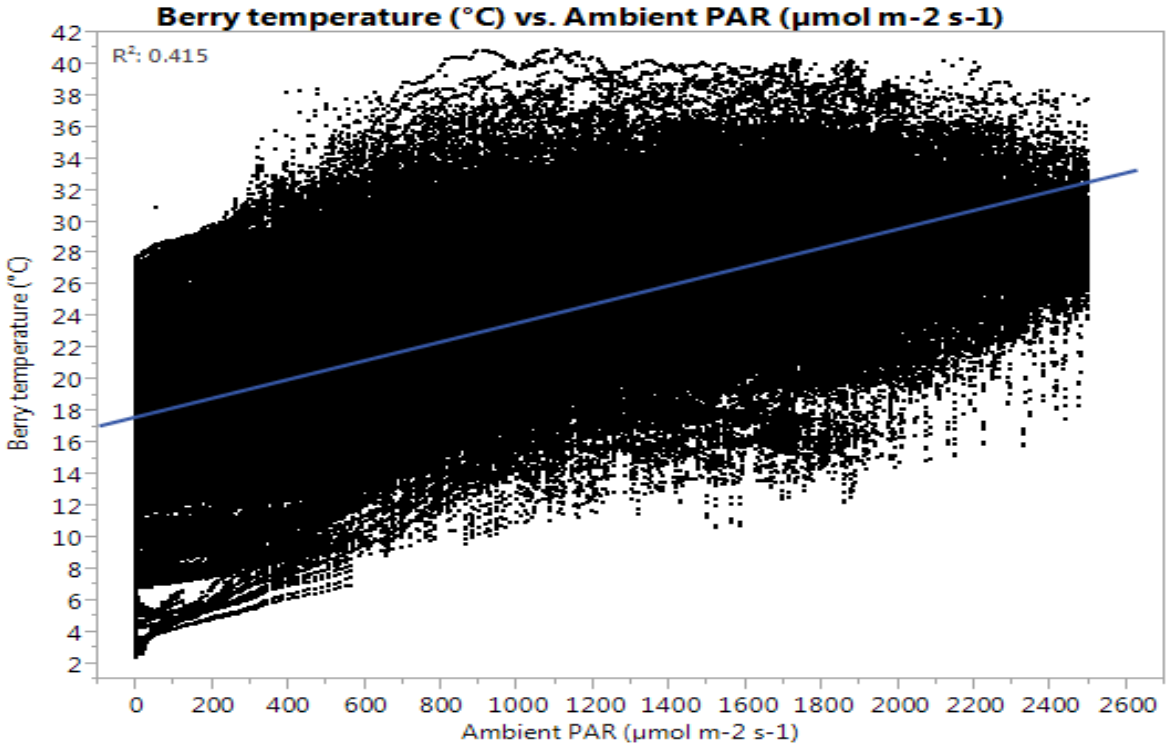

Fig. 8. The linear relationship between ambient photosynthetically active radiation (PAR) and berry temperature over 2014-2015.

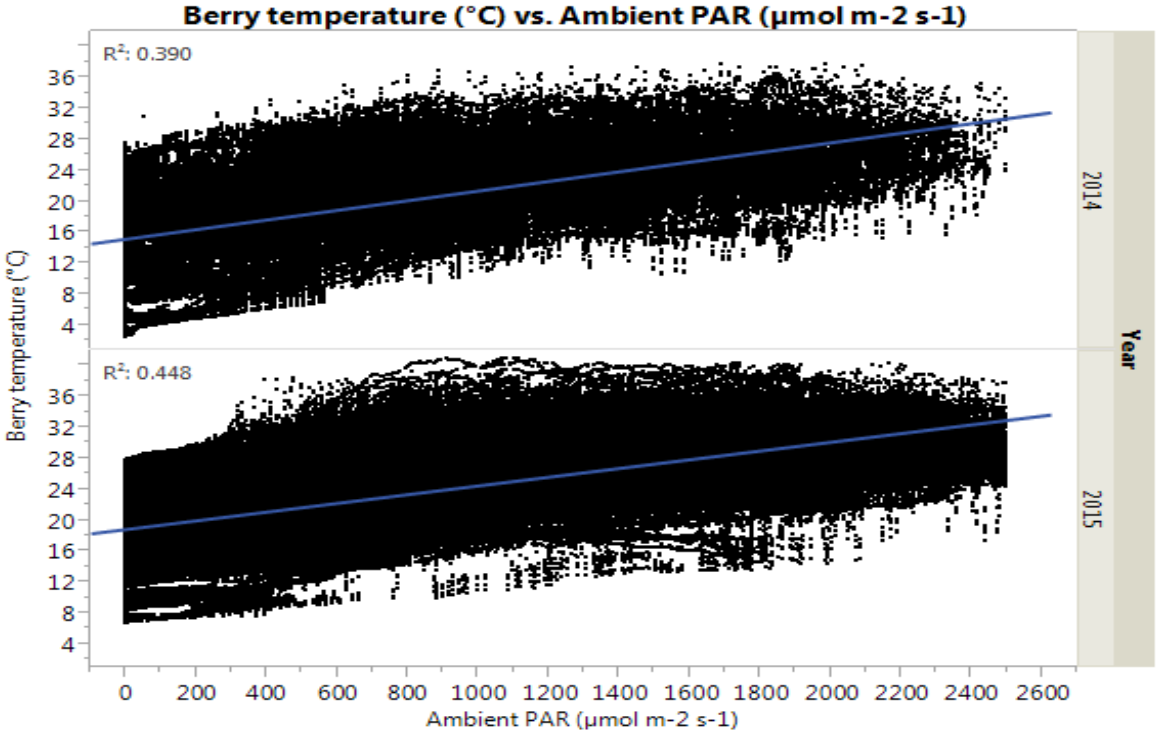

Fig. 9. The linear relationship between ambient PAR and berry temperature in each of the two years of data collection, 2014-2015.

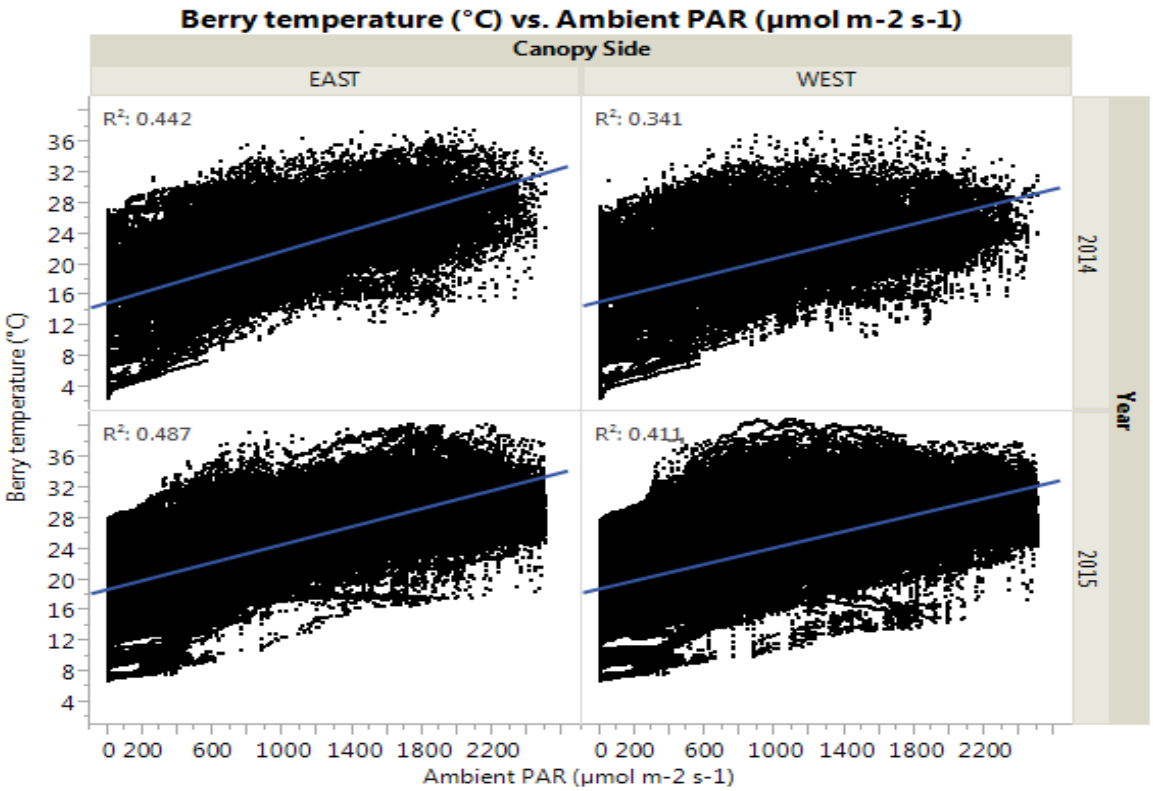

Fig. 10. The linear relationship between ambient PAR and east- and west- canopy side berry temperature in each of the two years of data collection, 2014-2015.

Unlike the air-berry temperature relationship, there was little evident separation between the number of data points that fell above or below the ambient PAR-berry temperature trend line (Figs. 8-10). Thus, it did not appear that another factor altered the relationship between ambient PAR and berry temperature. Nonetheless, there was a *less* clear separation of data points between PB-4/PB-8 and PB-NO in the ambient PAR-berry temperature relationship (Fig. 11) compared to when the air-berry temperature relationship was separated by treatment (Fig. 4). However, PB-4 and PB-8 data points were *further* above the ambient PAR-berry temperature trend line in comparison to PB-NO data points. Yet, the ambient PAR-berry temperature relationship was relatively stronger in the PB-4 and PB-8 plots compared to PB-NO plots (Fig. 12). This differed from the air-berry temperature relationship, which was stronger in the PB-NO plots compared to PB-4 and PB-8 plots (Fig. 5).

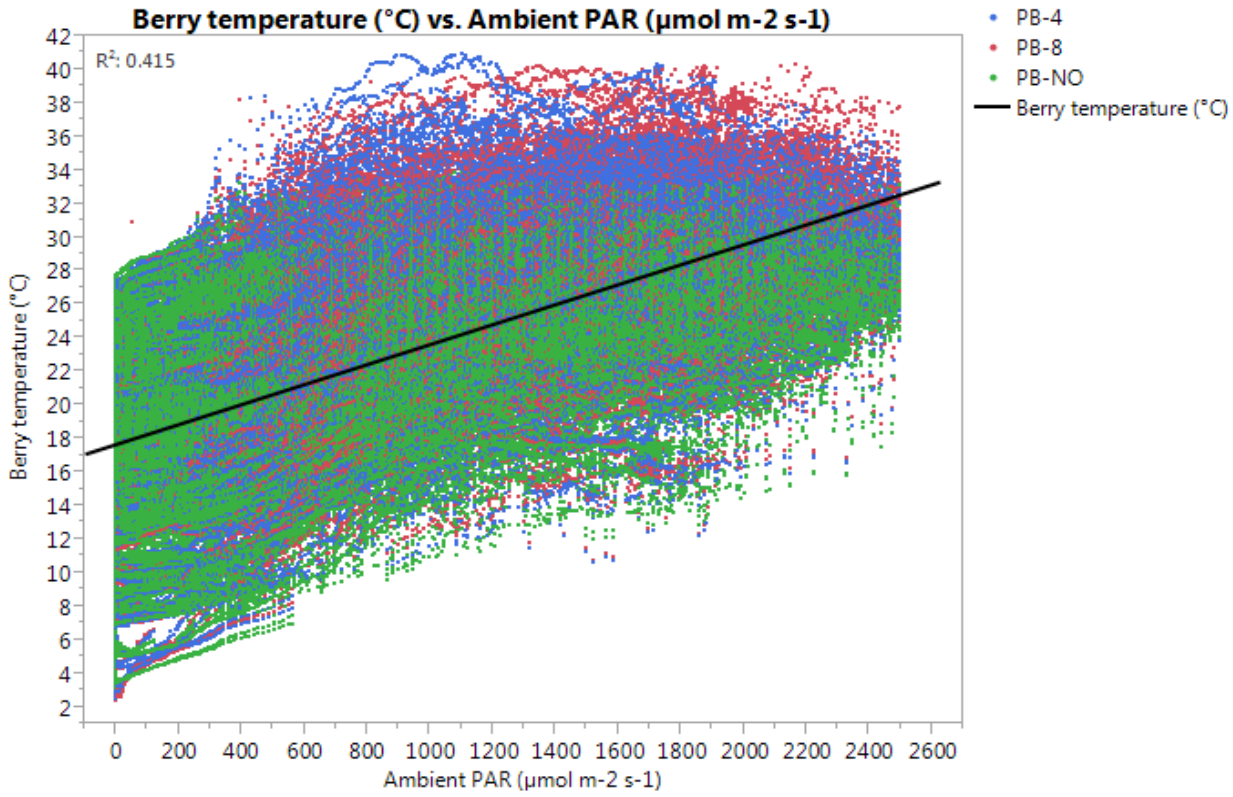

Fig. 11. The linear relationship between ambient PAR and berry temperature, accounting for pre-bloom leaf removal treatments over 2014-2015.

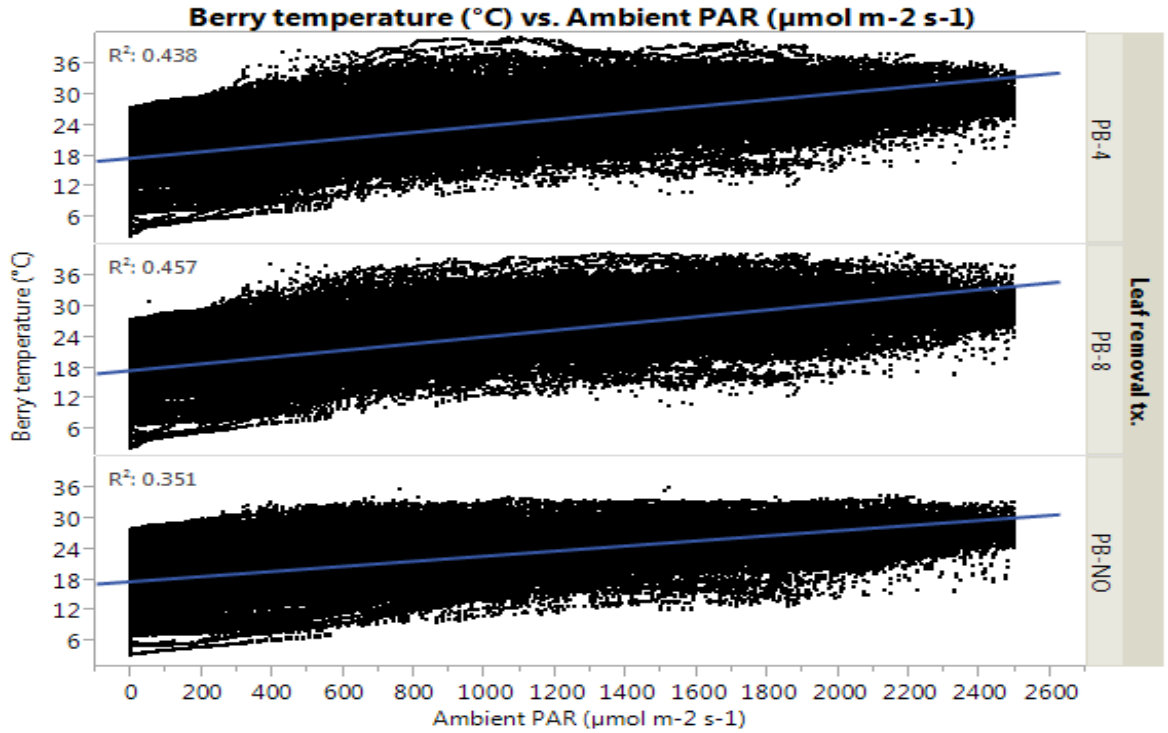

Fig. 12. The linear relationship between ambient PAR and berry temperature in each pre-bloom leaf removal treatment over 2014-2015.

The ambient PAR-berry temperature relationship in fruit-zone leaf removal treatments was consistent across the two years of the study in which both of these metrics were logged together (Fig. 13). Canopy side had little effect on the ambient PAR-berry temperature relationship in PB-NO plots, but, as observed when all treatments were combined (Fig. 10), the ambient PAR-berry temperature relationship was stronger on the east compared to west canopy side in PB-4 and PB-8 plots (Fig. 14). Collectively, Figs. 8-14 showed that berry temperature was not very predictable ($0.338 \leq R^2 \leq 0.512$) with ambient PAR alone, and without regard to time of day, or any other meteorological factor.

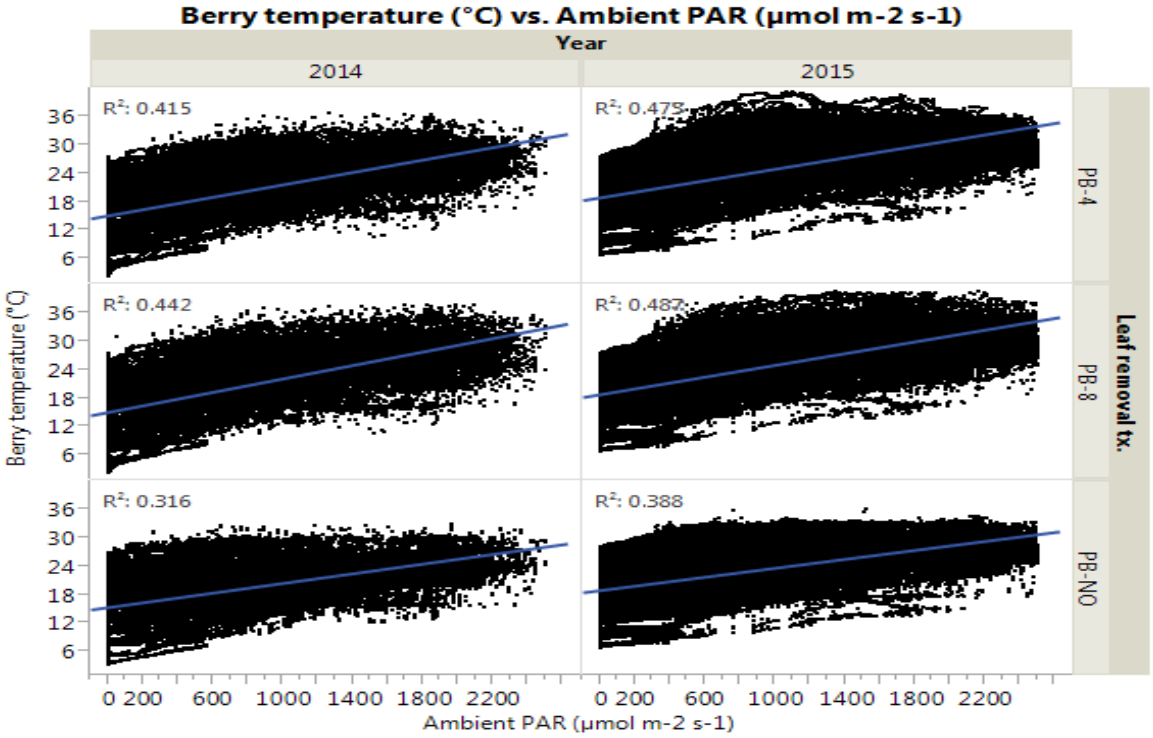

Fig. 13. The linear relationship between ambient PAR and berry temperature in each pre-bloom leaf removal treatment in each of the two years of data collection, 2014-2015.

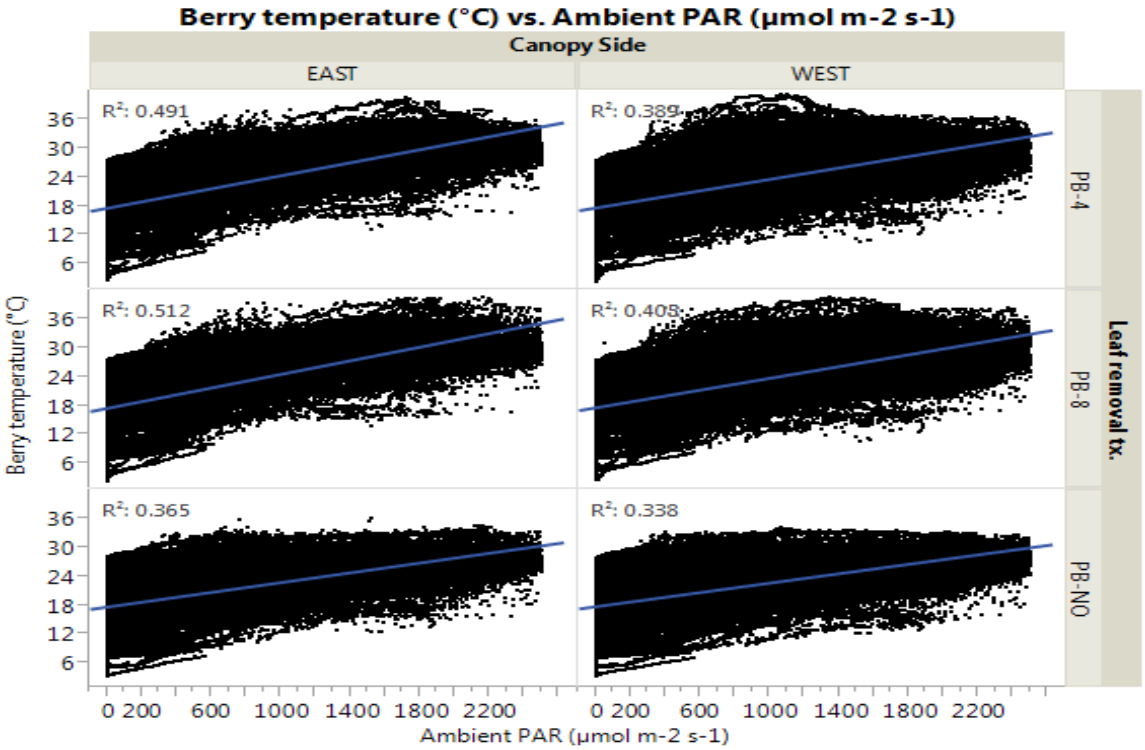

Fig. 14. The linear relationship between ambient PAR and east- and west-canopy berry temperature in each of the leaf removal treatment plots over 2014-2015.

Fruit-zone photosynthetically active radiation (PAR) vs. berry temperature: The relationship between fruit-zone PAR and berry temperature was much weaker than the air-berry temperature relationship (Fig. 15). This relationship will henceforth be referred to as the "fruit-zone PAR-berry temperature relationship." The fruit-zone PAR-berry temperature relationship had similar R^2 values over 2013-2015 (Fig. 16). The fruit-zone PAR-berry temperature relationship was relatively stronger on the east- compared to west-canopy side in all years that data was logged, similar to the ambient PAR-berry temperature relationship (Fig. 17).

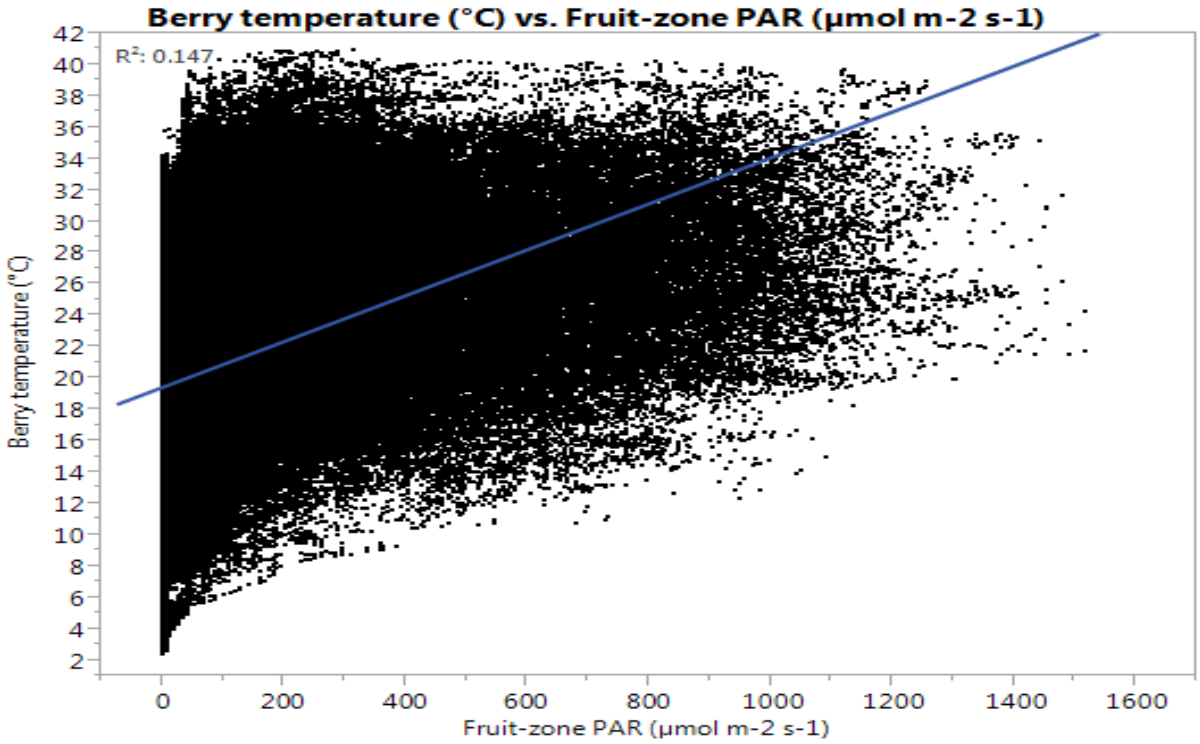

Fig. 15. The linear relationship between fruit-zone photosynthetically active radiation (PAR) and berry temperature over 2013-2015.

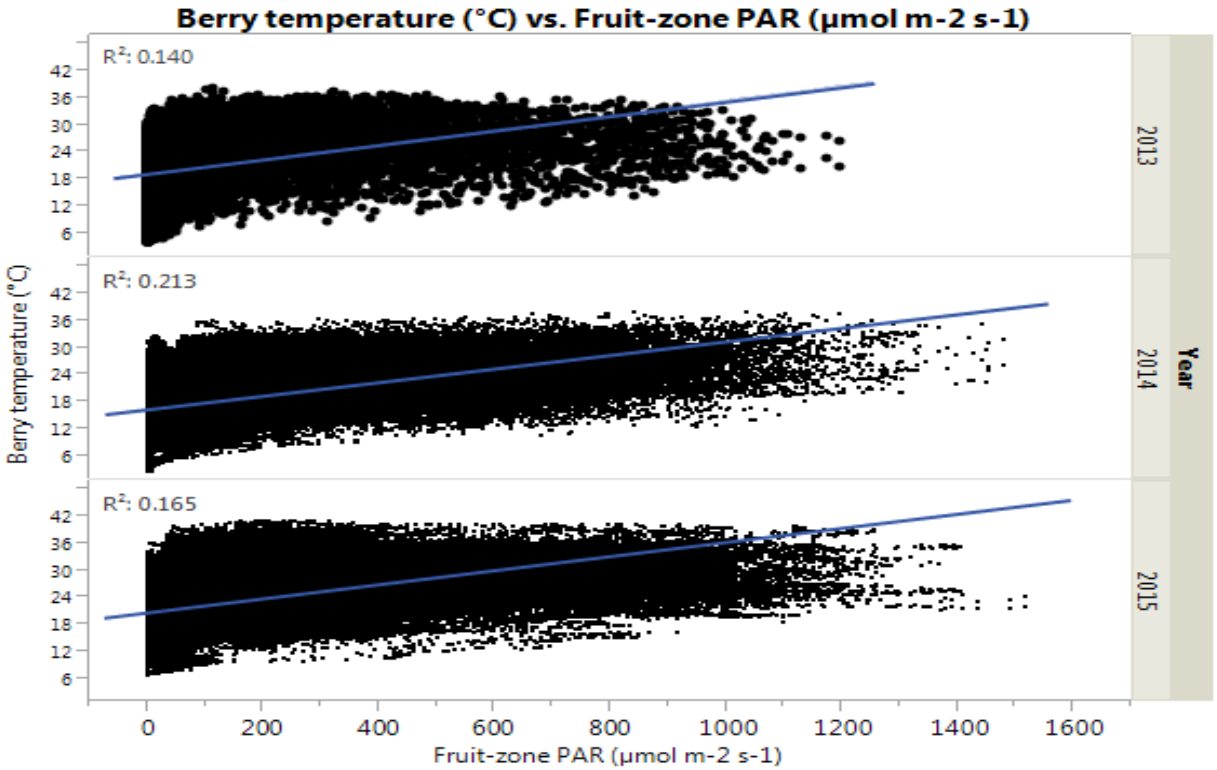

Fig. 16. The linear relationship between fruit-zone PAR and berry temperature in each of the three years of data collection, 2013-2015.

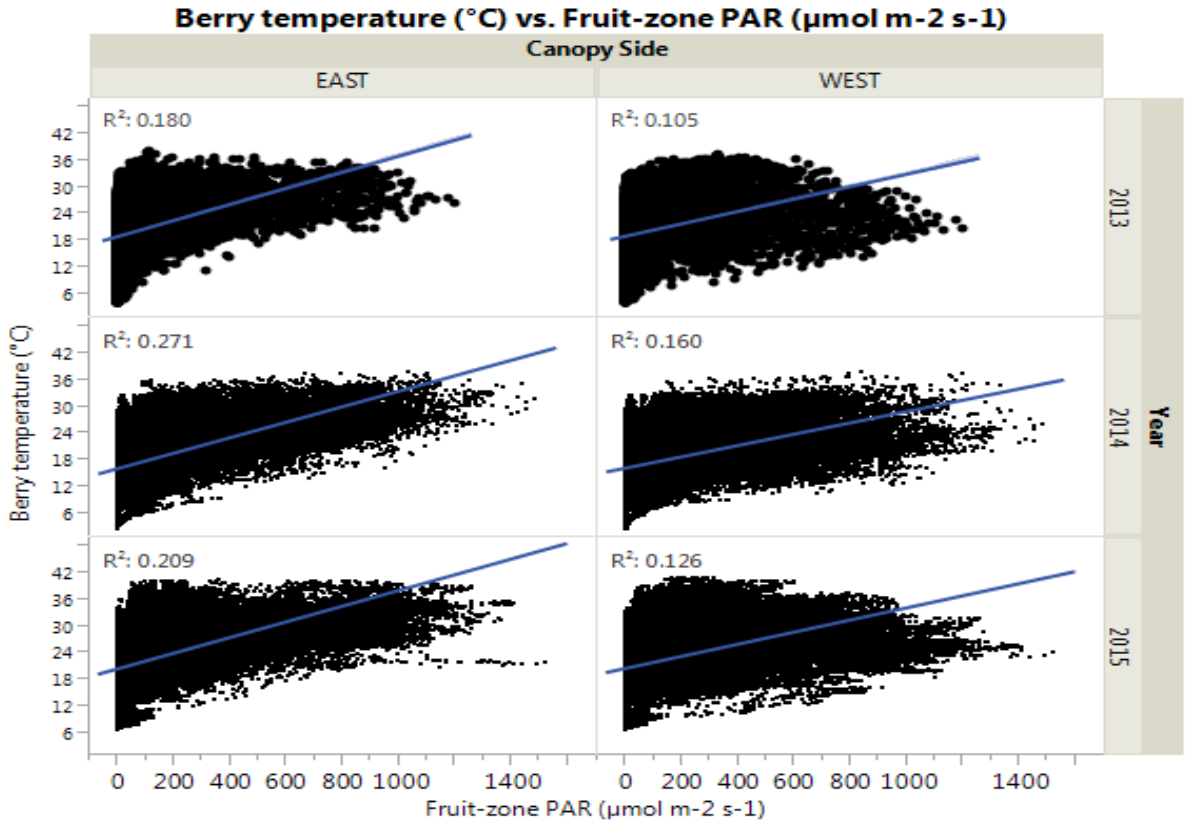

Fig. 17. The linear relationship between fruit-zone PAR and east- and west- canopy side berry temperature in each of the three years of data collection, 2013-2015.

There was a clear separation between the data points from the PB-4/PB-8 and PB-NO treatments in the fruit-zone PAR-berry temperature relationship (Fig. 18). However, the separation was not above or below the fruit-zone PAR-berry temperature trend line. Rather, there were relatively more PB-4 and PB-8 data points as fruit-zone PAR increased from left to right, while most PB-NO data points fell in the lower fruit-zone PAR range. The strength of the fruit-zone PAR-berry temperature relationship was PB-8 > PB-4 > PB-NO (Fig. 19), and this relationship was consistent over 2013-2105 (Fig. 20). The fruit-zone PAR-berry temperature relationship was relatively stronger on the east- compared to west-canopy side in PB-4 and PB-8 plots, but canopy side had lesser bearing on this relationship in PB-NO plots (Fig. 21). Collectively, Figs. 15-21 showed that fruit-zone PAR had more of an effect on berry temperature in leaf removal plots compared to no leaf removal plots. However, fruit-zone PAR alone was not

a particularly effective predictor of berry temperature. These figures also showed that fruit-zone PAR had a relatively greater effect on east- compared to west-side berry temperature. The relationship between fruit-zone PAR and berry temperature was consistent over 2013-2015.

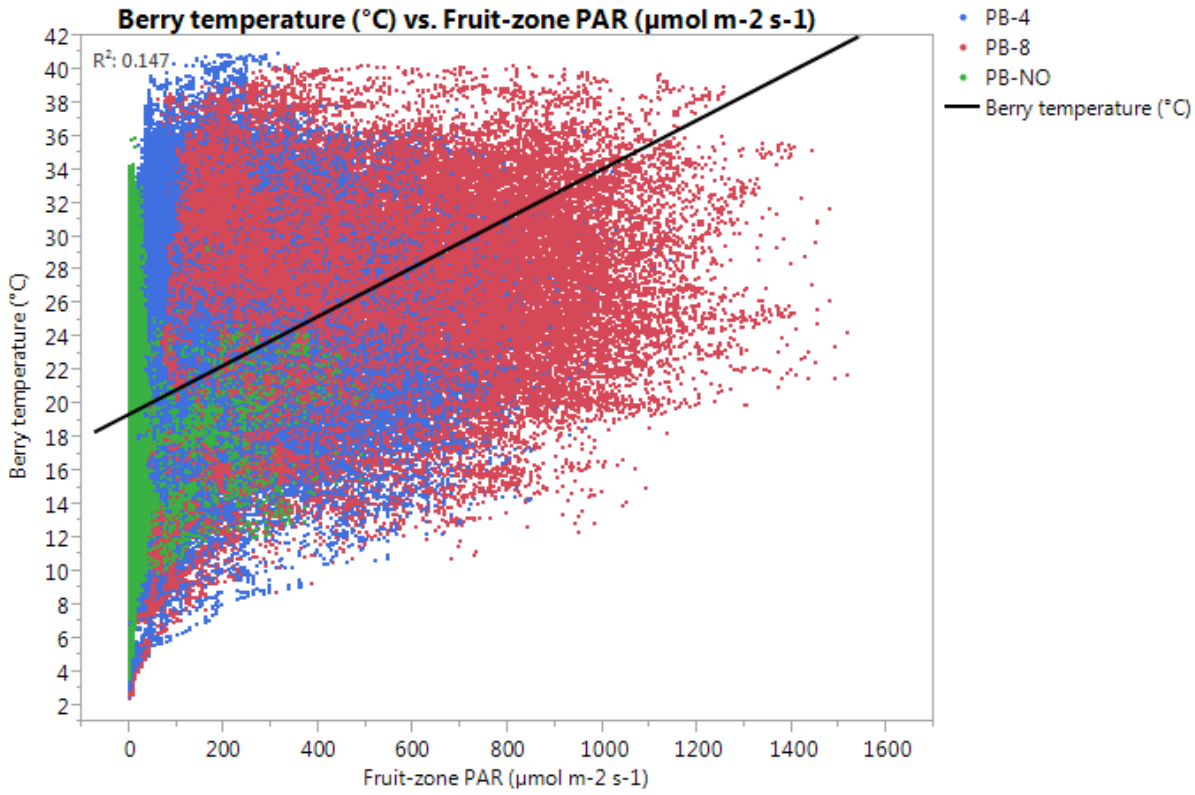

Fig. 18. The linear relationship between fruit-zone PAR and berry temperature, accounting for pre-bloom leaf removal treatments over 2013-2015.

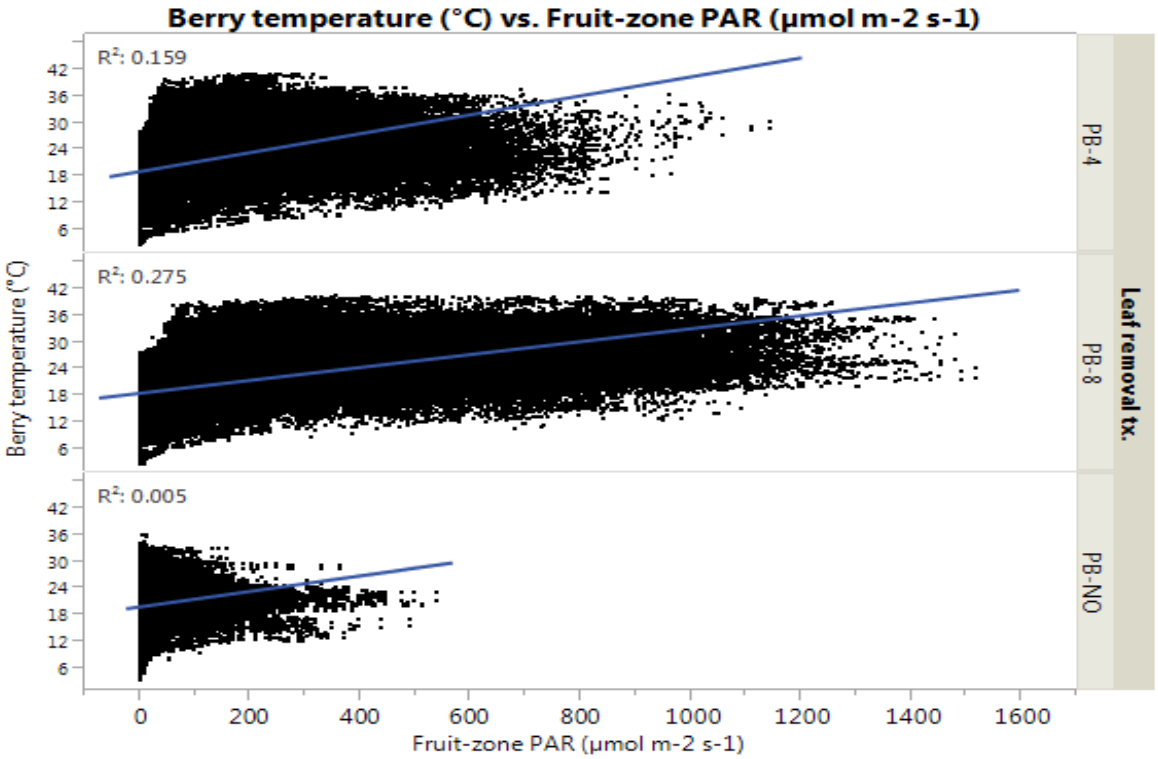

Fig. 19. The linear relationship between fruit-zone PAR and berry temperature in each pre-bloom leaf removal treatment over 2013-2015.

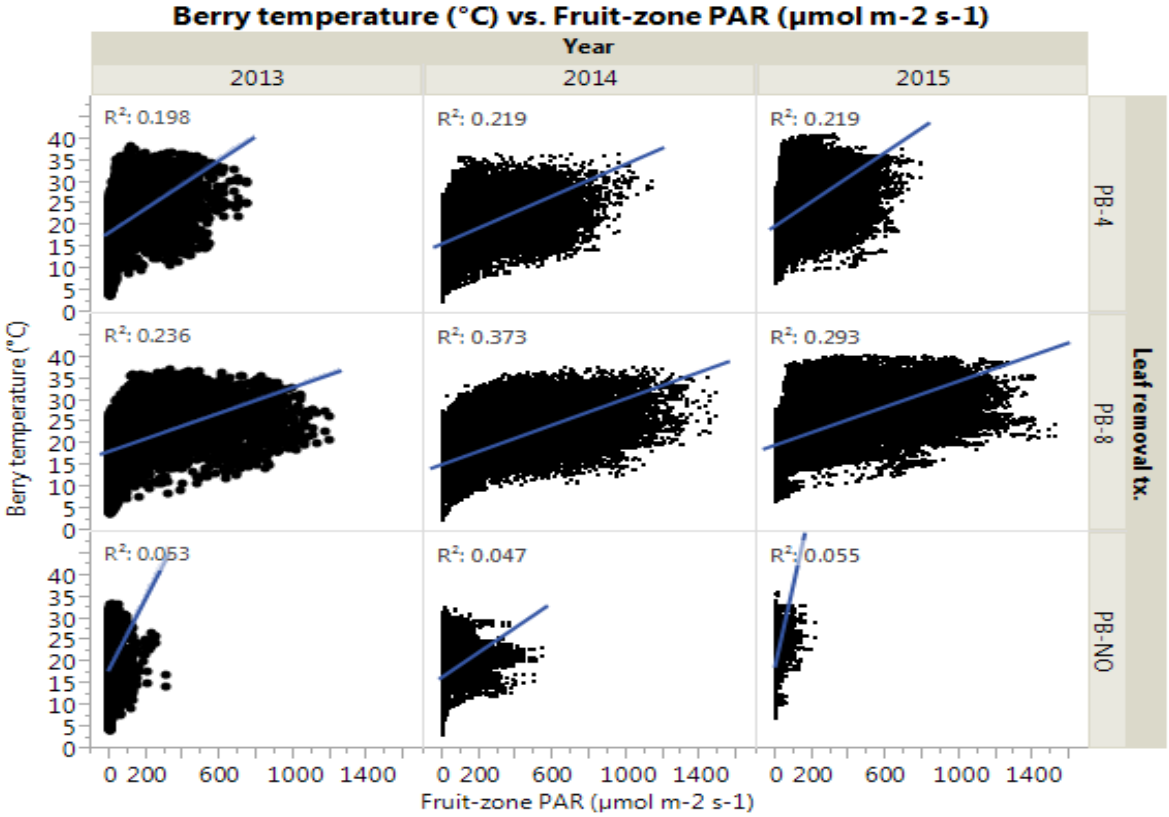

Fig. 20. The linear relationship between fruit-zone PAR and berry temperature in each pre-bloom leaf removal treatment in each of the three years of data collection, 2013-2015.

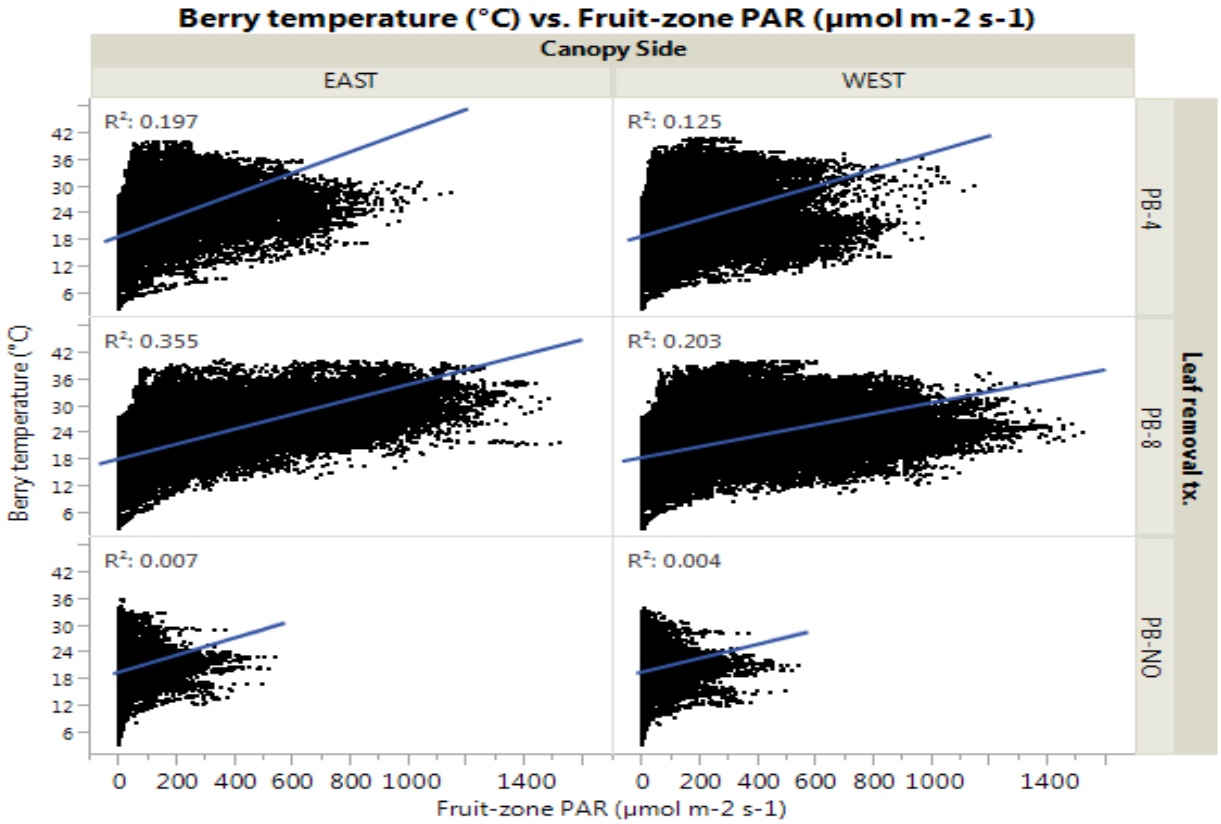

Fig. 21. The linear relationship between fruit-zone PAR and east- and west-canopy berry temperature in each of the leaf removal treatment plots over 2013-2015.

Fruit-zone relative humidity vs. berry temperature: There was a weak, *negative* relationship between fruit-zone relative humidity (RH) and berry temperature (Fig. 22). This relationship will henceforth be referred to as the "fruit-zone RH-berry temperature relationship." The fruit-zone RH-berry temperature relationship was maintained over 2014-2015, but was stronger in 2015 (R^2 = 0.415) compared to 2014 (R^2 = 0.242) (data not shown). The fruit-zone RH-berry temperature relationship was similar between canopy sides, as R^2 values ranged 0.235-0.249 across canopy sides in 2014, and ranged 0.409-0.421 across canopy sides 2015 (data not shown). There was no evident difference in the fruit-zone RH-berry temperature relationship between pre-bloom leaf removal treatment other than the relatively greater berry temperatures observed in PB-4 and PB-8 compared to PB-NO plots at the same fruit-zone RH (data not shown). The fruit-zone RH-berry temperature relationship was similar between all pre-bloom leaf removal

treatments, as there was small R^2 values range (0.277-0.306) across treatments, and this small range was maintained across treatments in 2014 (0.213-0.264) and 2015 (0.404-0.427) (data not shown). Lastly, when averaged over 2014-2015, there was little difference (R^2 = 0.275-0.320) in the fruit-zone RH-berry temperature relationship when evaluated by every canopy side and pre-bloom leaf removal treatment combination (data not shown). This data showed that there was a weak, *negative* linear relationship between fruit-zone RH and berry temperature. While there was no apparent difference in the fruit-zone RH-berry temperature relationship between treatment or canopy side, this relationship was relatively stronger in 2015 compared to 2014.

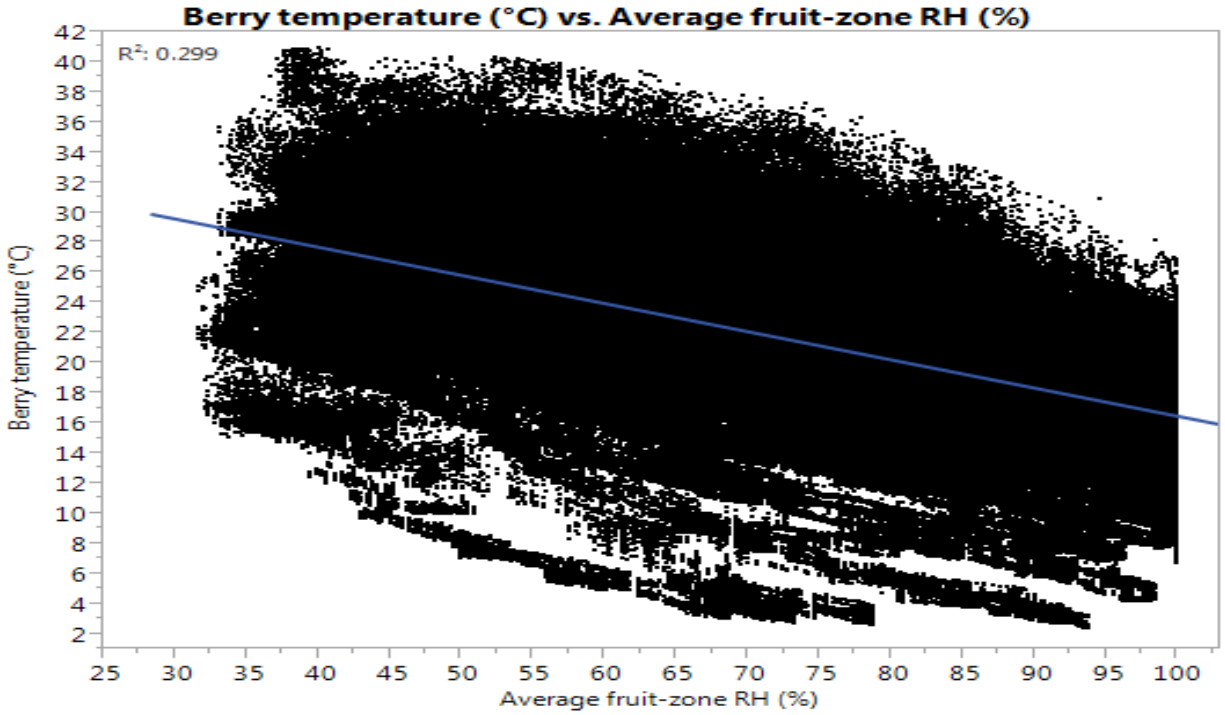

Fig. 22. The linear relationship between fruit-zone RH and berry temperature over 2014-2015.

Average fruit-zone RH and ambient air temperature: The above section showed that the fruit-zone RH was the only logged meteorological metric that was *negatively* related to berry temperature. However, the relationship of fruit-zone RH to berry temperature (Fig. 22) was very similar to the relationship of fruit-zone RH to ambient air temperature (Fig. 23). There was also a strong inverse relationship between fruit-zone RH and ambient air temperature throughout the

day (Fig. 24). Further, the diurnal fruit-zone RH-berry temperature and fruit-zone RH-air temperature relationships paralleled each other, excepting from 0800-1800, when R^2 values were relatively greater in the fruit-zone RH-berry temperature relationship (Fig. 25). The same factor was assumed to be responsible for the relatively greater R^2 values in the fruit-zone RH-berry temperature relationship, and the relatively lower R^2 values of the air-berry temperature relationship, as these occurred at the same times of day. Collectively, these relationships showed that fruit-zone RH was not necessarily inversely related to berry temperature. Rather, fruit-zone RH decreased as air temperature, and consequently berry temperature, increased throughout the day, and vice-versa.

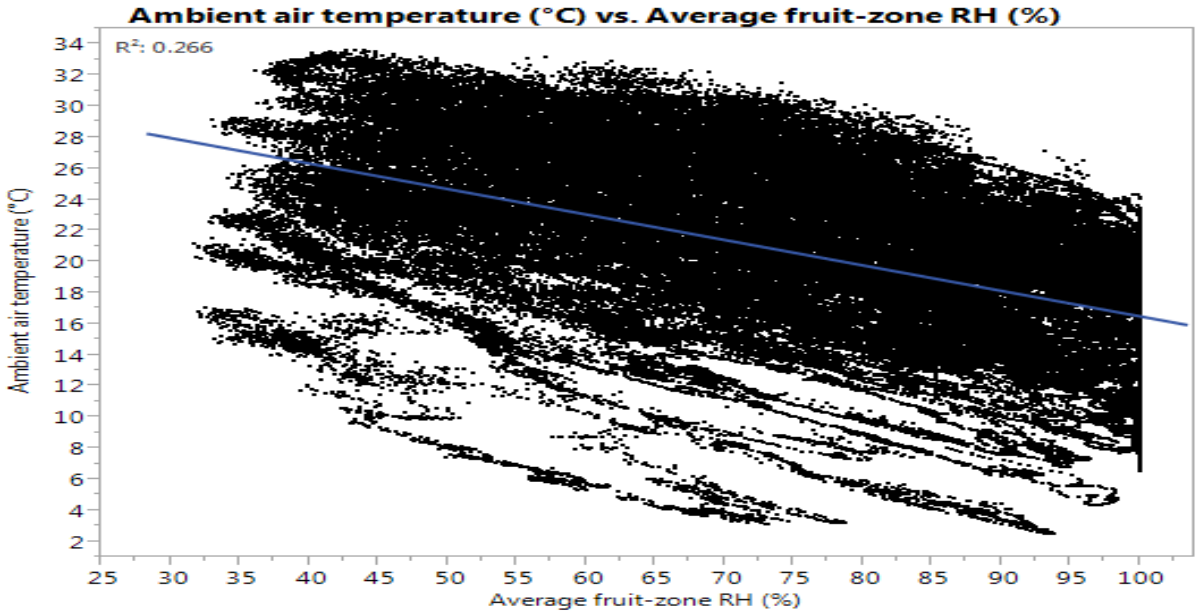

Fig. 23. The linear relationship between fruit-zone RH and ambient air temperature over 2014-2015.

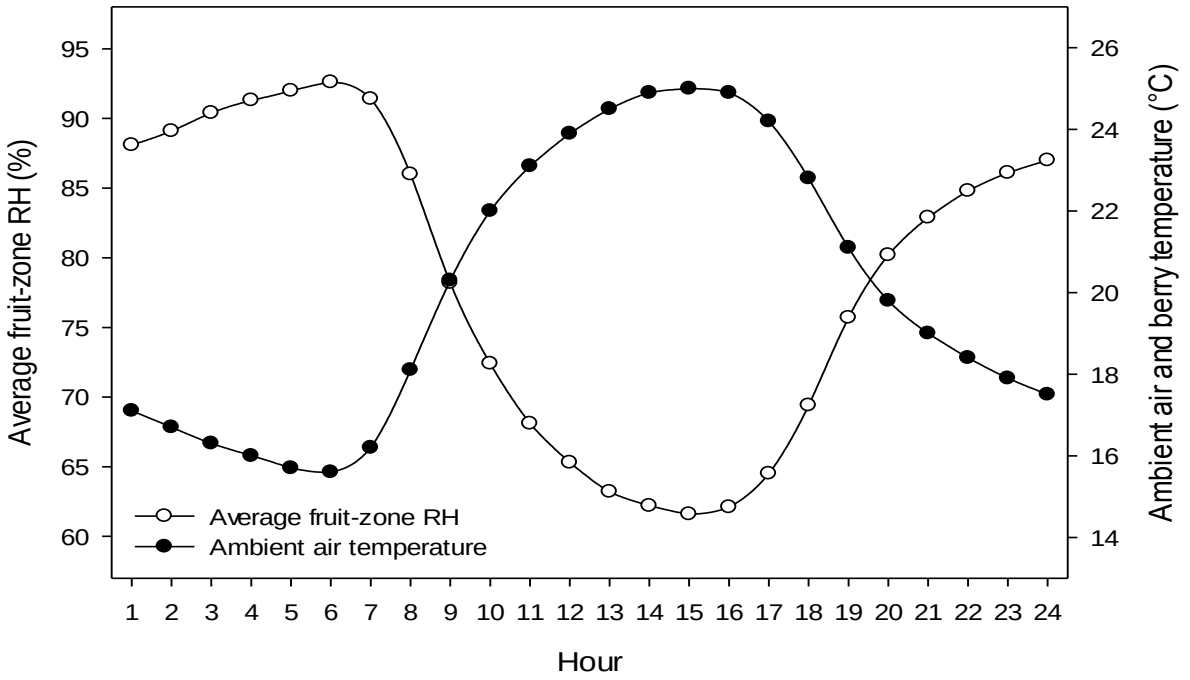

Fig. 24. The diurnal trend of average fruit-zone RH, and ambient air and berry temperature over 2014-2015.

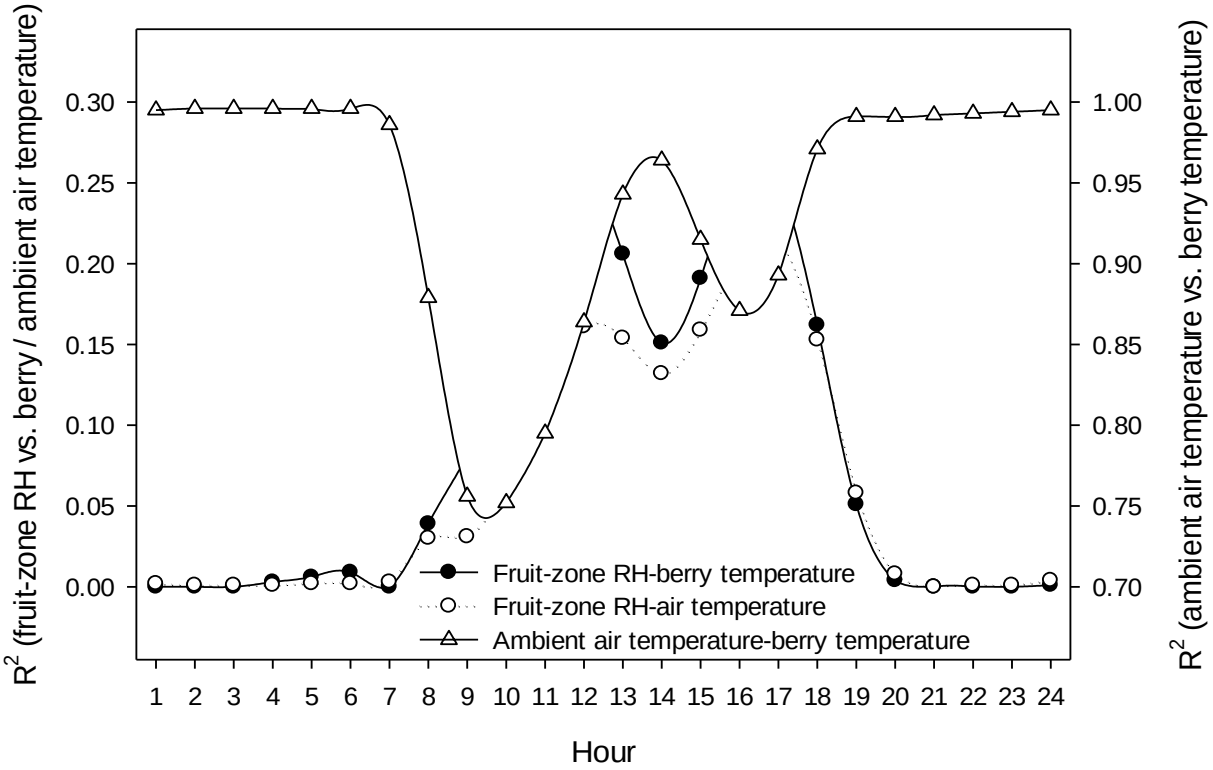

Fig. 25. The hourly R^2 of the diurnal relationship between fruit-zone RH and ambient air temperature, fruit-zone RH and berry temperature, and ambient air temperature and berry temperature over 2014-2015.

Ambient air temperature vs. green and red berry temperature: The linear air-berry temperature relationship was strong when evaluated by combining logged green and red berry temperature (Fig. 26). The temperature of red berries tended to be marginally greater than green berries at a given ambient air temperature. Accordingly, the air-berry temperature relationship was slightly stronger in green berries ($R^2 = 0.914$) compared to red berries ($R^2 = 0.900$) (data not shown). This trend was maintained in both years, as the air-red berry temperature relationship had 0.014-0.016 lower R^2 values when compared to the air-green berry temperature relationship over the course of 2014 and 2015 (data not shown). When evaluated by canopy side, the air-berry temperature relationship did not greatly differ between green and red berries, although, as shown above, this relationship was stronger on the west canopy side ($R^2 = 0.954$-0.955 across red and green berries, respectively) compared to the east canopy side ($R^2 = 0.855$-0.872 across red and green berries, respectively) (data not shown). This data revealed that the air-berry temperature relationship was marginally affected by berry color, was maintained in both years, and that canopy side did not differentially affect this relationship.

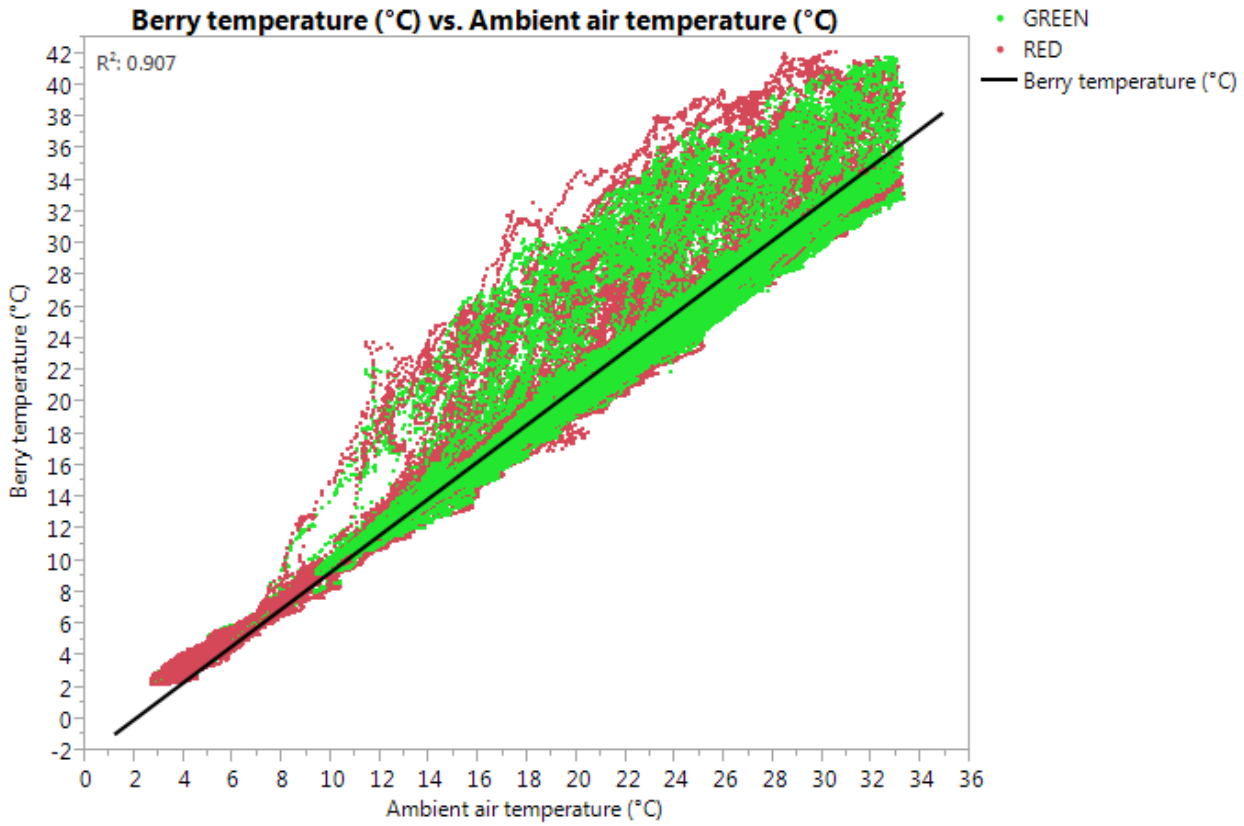

Fig. 26. The linear relationship between ambient air temperature and berry temperature, accounting for green and red berry color over 2014-2015.

Ambient PAR vs. green and red berry temperature: There was a positive, linear relationship between ambient PAR and green and red berry temperature (Fig. 27), although this relationship was much weaker than the air-berry temperature relationship. However, unlike in the air-berry temperature relationship, there was no clear trend in separation of data points between green and red berries. The ambient PAR-berry temperature relationship was slightly stronger in red (R^2 = 0.479) berries compared to green berries (R^2 = 0.463), and this trend was maintained in both 2014 (R^2 = 0.274-0.302) and 2015 (R^2 = 0.522-0.537) (data not shown). When evaluated by canopy side, the ambient PAR-berry temperature relationship did not greatly differ between green and red berries, although, as shown above, this relationship was stronger on the east canopy side (R^2 = 0.539-0.553 across green and red berries, respectively) compared to the west canopy side (R^2 = 0.401-0.409 across red and green berries, respectively) (data not shown).

Collectively, this data showed that the ambient PAR-berry temperature relationship was not greatly affected by berry color, was maintained in both years, and that canopy side did not differentially affect this relationship.

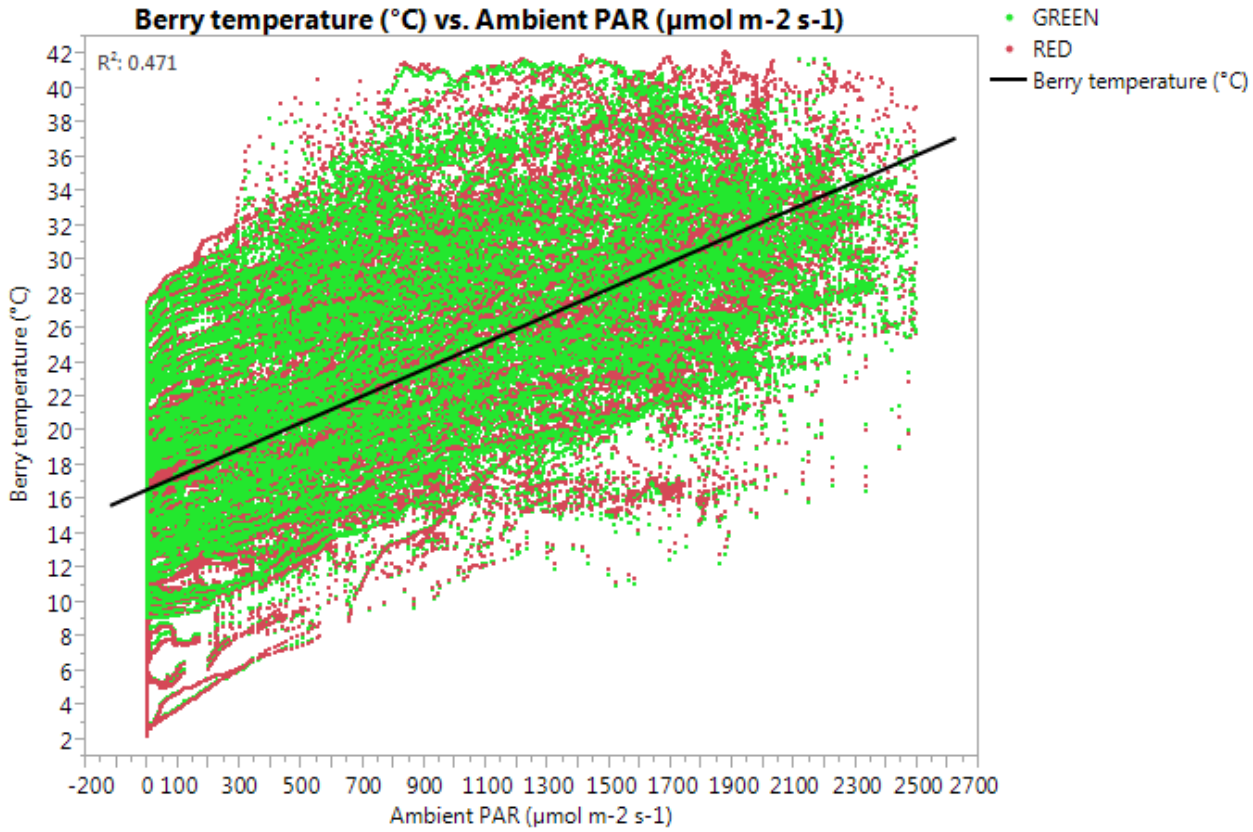

Fig. 27. The linear relationship between ambient PAR and berry temperature, accounting for green and red berry color over 2014-2015.

Diurnal patterns of the relationship of berry temperature with ambient air temperature, ambient PAR, and fruit-zone PAR: Ambient air temperature had the strongest linear relationship to berry temperature compared to all other measured meteorological metrics, and there was strong evidence that data shifted above the trend line, as well as evidence of a consistent, canopy-side specific response to the air-berry temperature relationship. Ambient and fruit-zone PAR had weaker, positive, relationships to berry temperature. However, the ambient/fruit-zone PAR-berry temperature relationships were stronger in opposite scenarios compared to the air-berry temperature relationship; this was suggestive that radiation was the factor that changed the relationship between ambient air and berry temperature. For example, the ambient/fruit-zone PAR-berry temperature relationship was relatively stronger on the east canopy side and in leaf removal plots, while the air-berry temperature relationship was stronger on the west canopy side and in no leaf removal plots. While RH appeared to be negatively related to berry temperature, no trend was observed that suggested it was a factor that affected air-berry temperature relationship. Further investigation revealed that RH merely decreased as ambient air temperature, and thus berry temperature, increased; and vice-versa. Therefore, it was concluded that berry temperature was not impacted by RH. Berry color did not change the way that berry temperature was affected by ambient air temperature and PAR, regardless of canopy side. It was similarly concluded that berry temperature was not impacted by berry color.

Collectively, these findings suggested that berry temperature was primarily a function of ambient air temperature, but that radiation could affect this relationship by increasing berry temperature when ambient air temperature was not concomitantly increased; this was particularly true when leaves were removed from the fruit-zone. The linear relationships did not take time into account, but ambient air temperature and PAR are known to change throughout the day.

Thus, it was suspected that the air-berry temperature relationship would change over the course of the day, and this would be different between leaf removal treatments and canopy sides. Thus, the relationship of east- and west-side berry temperature with ambient air temperature, ambient PAR, and fruit-zone PAR were diurnally investigated.

The diurnal air-berry temperature relationship showed that berry temperature was always highly predictable with air temperature in no leaf removal plots ($R^2 \geq 0.946$), regardless of canopy side or time of day (Fig. 28 C). By contrast, berry temperature prediction with air temperature was dependent on time of day and canopy side in leaf removal plots (Fig. 28 A, B). East-side berry temperature was highly predictable with air temperature until 0700, predictability decreased until 1400, and was again highly predictable with air temperature thereafter. West-side berry temperature was highly predictable with air temperature until 1400, after which predictability decreased until 1900, and was again highly predictable with air temperature thereafter. There were slight decreases in the air-berry temperature relationship on the west-side in the morning and the east-side in the afternoon, and these were relatively greater in eight leaf removal plots (Fig. 28 A) compared to four leaf removal plots (Fig. 28 B).

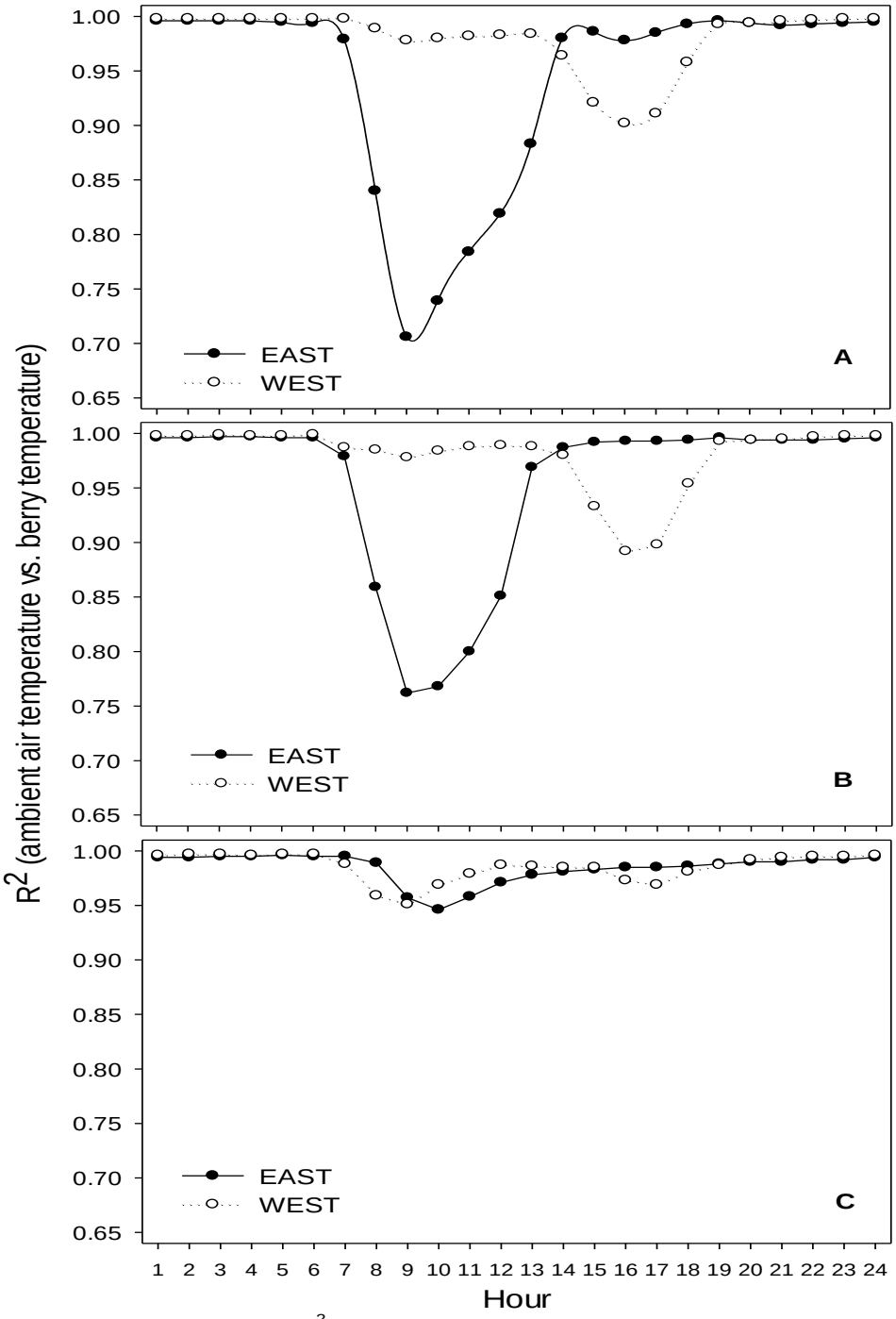

Fig. 28. The hourly R^2 of the canopy-side specific diurnal relationship between ambient air and berry temperature, as affected by pre-bloom removal of eight (A), four (B), and no (C) leaves over 2013-2015.

The diurnal ambient PAR-berry temperature relationship was similar in leaf removal and no leaf removal plots (Fig. 29 A-C). East-side berry temperature was unaffected by ambient PAR until 0600, after which R^2 values increased, reached a morning maximum between 0900-1100, dropped until 1400, reached an afternoon maximum at 1700, then dropped off thereafter until berry temperature was no longer influenced by ambient PAR at 2100. West-side berry temperature was similarly influenced by diurnal ambient PAR when compared to east berry temperature. However, the maximum afternoon R^2 peak was greater on the west canopy side, whereas the maximum morning R^2 peak was greater on the east canopy side. The east-side morning R^2 peaks were slightly greater than the west-side afternoon R^2 peaks, suggestive that radiation was consistently greater in the morning compared to afternoon hours.

Berry temperature was affected by ambient PAR to a greater extent in leaf removal compared to no leaf removal plots, as shown by the relatively greater R^2 values in leaf removal plots. There was a greater difference between the morning and afternoon R^2 peaks on each canopy side in the leaf removal compared to no leaf removal plots. These last two points suggested that there was relatively greater direct and indirect radiation in leaf removal compared to no leaf removal plots. The diurnal ambient PAR-berry temperature relationship was essentially inverse to that of the diurnal air-berry temperature relationship – as R^2 values were reduced in the air-berry temperature relationship, R^2 values increased in the ambient PAR-berry temperature relationship, and vice-versa. Collectively, these trends provided further evidence that radiation was the primary factor that changed the way air temperature was related to berry temperature.

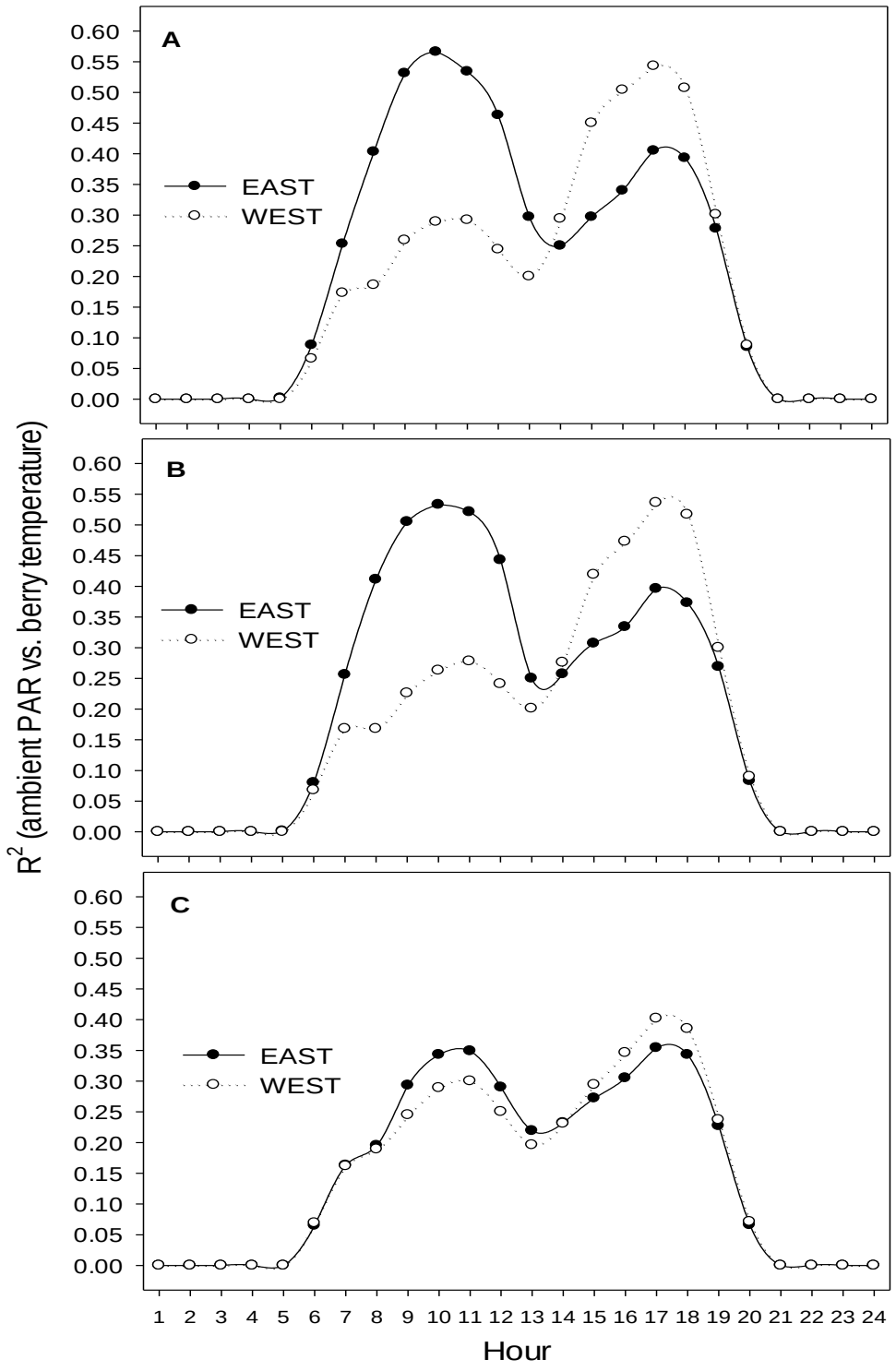

Fig. 29. The hourly R2 of the canopy-side specific diurnal relationship between ambient PAR and berry temperature, as affected by pre-bloom removal of eight (A), four (B), and no (C) leaves over 2013-2015.

The diurnal pattern of the fruit-zone PAR-berry temperature relationship was similar to the diurnal pattern of ambient PAR-berry temperature relationship (Fig. 30). East-side berry temperature tended to be affected by fruit-zone PAR to a greater extent in the morning, and vice-versa for west-side berries in the afternoon, as evidenced by higher R^2 values in these respective periods. Berry temperature was affected by fruit-zone PAR to a greater extent in the pre-bloom of eight compared to four leaf removal plots, and to a greater extent in the pre-bloom removal four compared to no leaf removal plots (Fig. 30 A-C). The R^2 values were lower in the fruit-zone PAR-berry temperature relationship compared to the ambient PAR-berry temperature relationship. Further, the midday depression in the R^2 values of the fruit-zone PAR-berry temperature relationship were greater and lasted longer than those of the ambient PAR-berry temperature relationship. These last two points were suspected to be a function of the above-cordon (i.e. center of fruit-zone) placement of the fruit-zone quantum sensors. For the former point, the logged temperature of outside-facing berries was uncoupled from the logged radiation transmission to the center of the fruit-zone. For the latter point, radiation transmission to the center of the fruit-zone was largely blocked by the canopy during midday. Though weaker than the ambient PAR-berry temperature relationship, the diurnal trend of the fruit-zone PAR-berry temperature relationship was inverse to that of the diurnal air-berry temperature relationship. This, again, supported that radiation was the factor that decreased the ability to predict berry temperature with ambient air temperature.

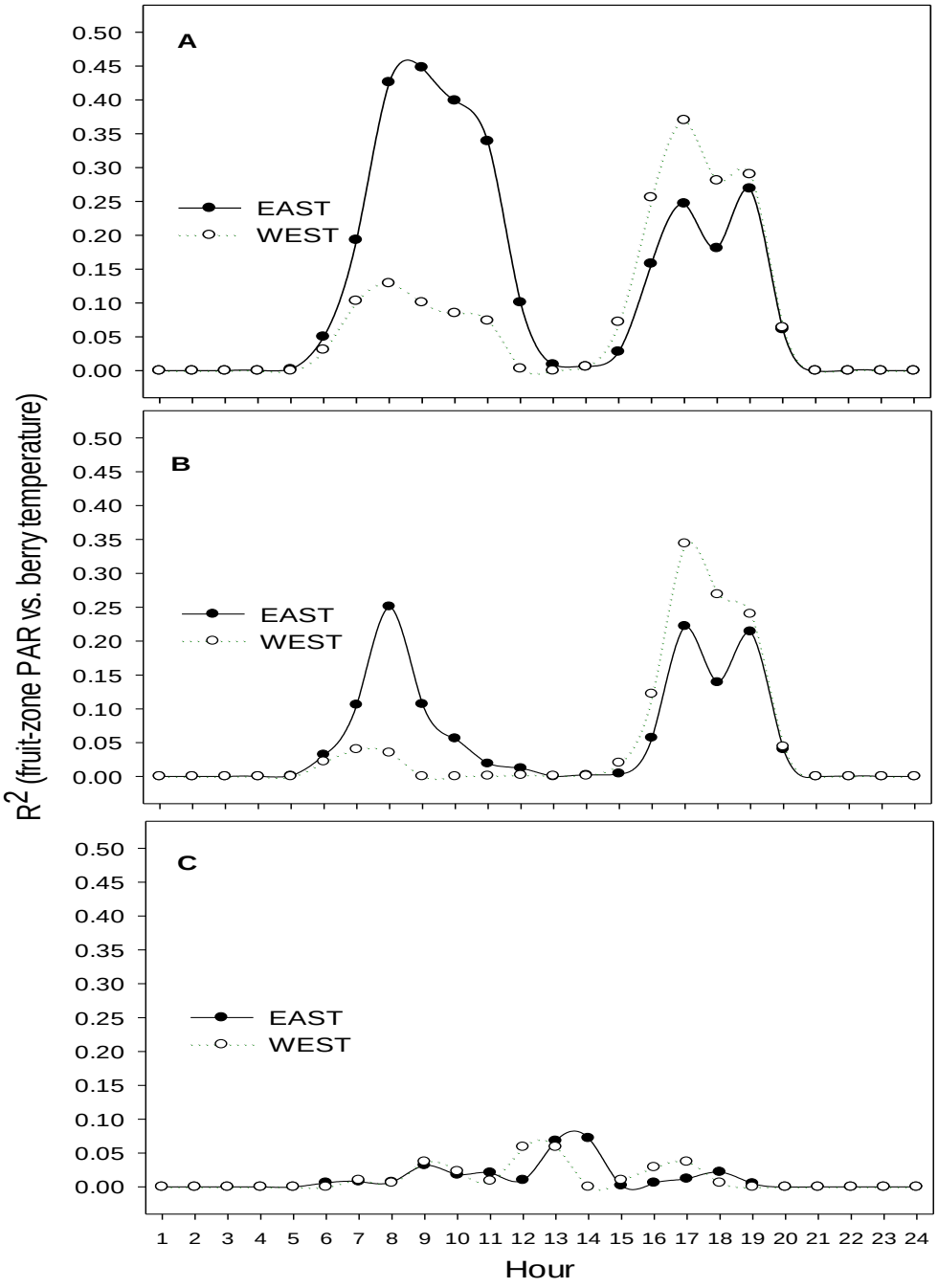

Fig. 30. The hourly R^2 of the canopy-side specific diurnal relationship between fruit-zone PAR and berry temperature, as affected by pre-bloom leaf removal of eight (A), four (B), and no (C) leaves.

Diurnal patterns of ambient air temperature, berry temperature, and ambient and fruit-zone PAR: Collectively, the above plots showed that berry temperature is highly predictable with, and thus mainly affected by, ambient air temperature. However, berry temperature was also affected by other factors, such as temporal changes in hour angle over the course of the day, fruit-zone leaf and lateral shoot removal, and the canopy side that the berry was exposed to. Diurnal patterns of ambient air temperature, ambient and fruit-zone PAR, and berry temperature are displayed as a function of both leaf removal treatment and canopy side (Fig 31). The diurnal relationship plots presented above, and the hourly mean values presented in Fig. 31, showed that there are only certain times of the day that berry temperature is different from ambient air temperature. Further, that berry temperature is primarily affected by radiation when leaves are removed from the fruit-zones, when the above-head canopy is not blocking radiation (i.e. surrounding solar noon), and when that canopy side is receiving direct radiation.

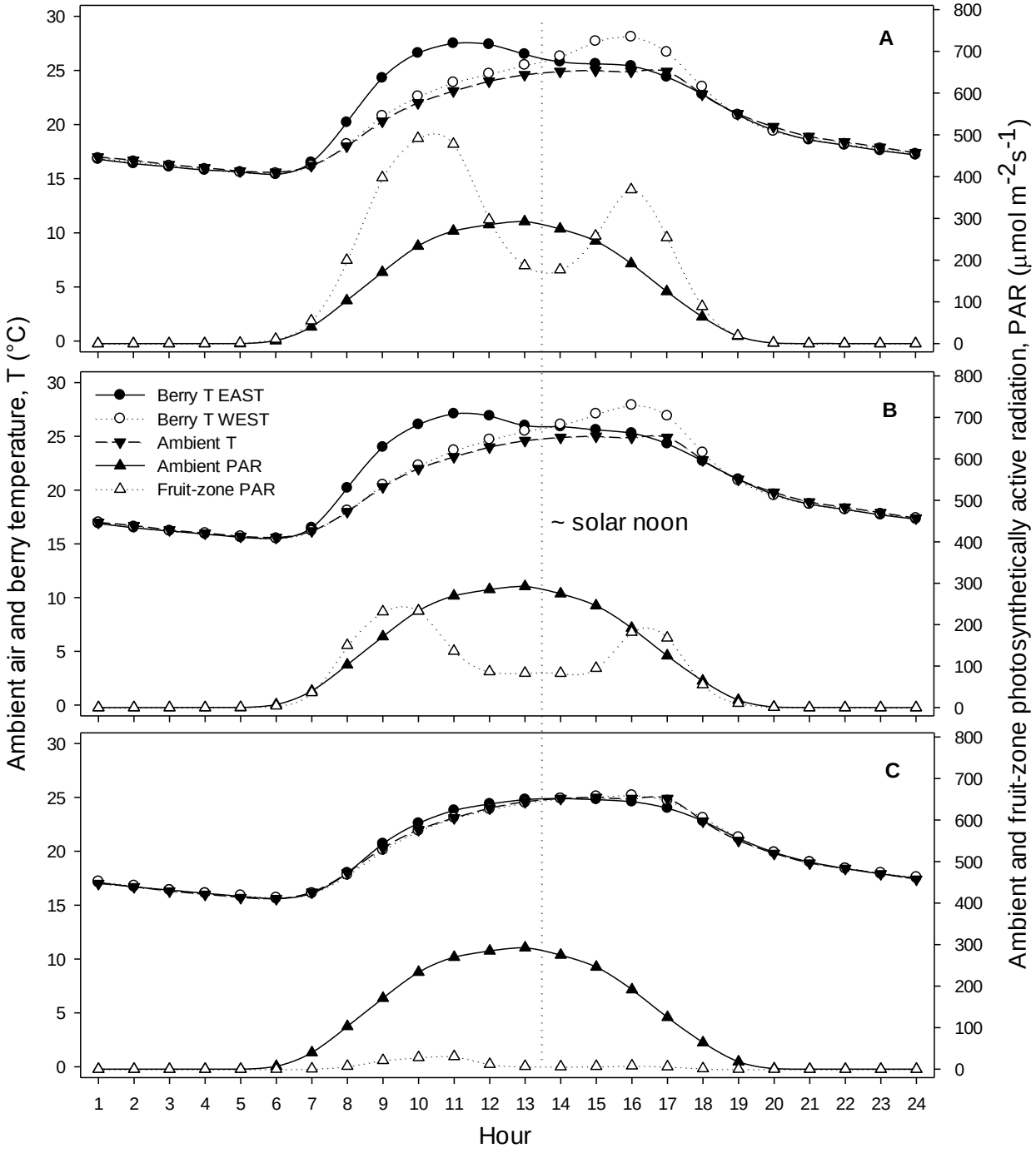

Fig. 31. Diurnal pattern of ambient air temperature, ambient and fruit-zone PAR, and berry temperature as affected by pre-bloom removal of eight (A), four (B), and no (C) fruit-zone leaves/laterals. Data logged on 15- and 1-min intervals over 2013-2015 seasons. Ambient PAR is presented as 20% of actual value, and was logged on 15- and 1-min intervals over 2014-2015 seasons.

Berry temperature prediction models: The data henceforth presented used 15° hour angles as a solar radiation-time reference, as time of day per se was not responsible for affecting the air-berry temperature relationship. Rather, hour angle changed over the course of the day and season, which changed the ability to predict berry temperature with ambient air temperature. The use of hour angle was included to make the models more applicable across geographic regions. Since berry temperature could be predicted with ambient air temperature, except during the times of day that radiation also affected berry temperature, linear berry temperature prediction models were developed with both ambient air temperature and radiation. While both ambient and fruit-zone PAR affected berry temperature, ambient PAR was used as the independent variable to develop models. Compared to fruit-zone PAR, ambient PAR (1) had a stronger relationship to berry temperature, (2) could be used with the hour angle time reference, and (3) can often be retrieved from public weather stations. As such, ambient PAR was expected to be more applicable for use by growers and researchers alike. Most forthcoming models were developed separately by canopy side because radiation differentially affected the temperature of berries exposed to east and west canopy sides. NOTE: the difference between berry temperature and ambient air temperature will henceforth be referred to as the "berry temperature differential."

Canopy side-specific, leaf removal treatment non-specific, berry temperature-prediction models: The ability to predict east-side berry temperature differential when averaged across all leaf removal treatments was greater from -105 to -15° hour angles (R^2 = 0.2423-0.4244) compared to 15 to 105° hour angles (R^2 = 0.0000-0.0340) (Appendix A). Mean, maximum, and standard deviation in berry temperature differential tended to be greater from -75 to 15° hour angles compared to hour angles outside of this range. Mean berry temperature differential was 1.12 to 3.53 °C from -75 to 15° hour angles. The ability to predict west-side berry temperature differential when averaged across all leaf removal treatments was greater from 30 to 75° hour angles (R^2 = 0.2443-0.3841) compared to hour angles outside of this range (R^2 = 0.0000-0.1233) (Appendix B). Mean, maximum, and standard deviation in berry temperature differential all tended to be greater from 30 to 75° hour angles compared to hour angles outside of this range. Mean berry temperature differential was 1.19 to 2.55 °C from 30 to 75° hour angles.

Canopy side-specific, leaf removal treatment-specific, berry temperature-prediction models: The ability to predict east-side berry temperature differential in PB-NO plots was greater from -60 to 0° hour angles (R^2 = 0.1119-0.1938) compared to hour angles outside of this range (R^2 = 0.0012-0.1220) (Appendix C). Mean and standard deviation in berry temperature differential tended to be greater from -60 to 0° hour angles compared to hour angles outside of this range, while maximum berry temperature differential had inconsistent trends across hour angles. Mean berry temperature differential was 0.70 to 1.10 °C from -60 to 0° hour angles. The ability to predict west-side berry temperature differential in PB-NO plots was greater from -75 to -45° hour angles (R^2 = 0.1971-0.4895) compared to hour angles outside of this range (R^2 = 0.001-0.1409) (Appendix D). Maximum and mean berry temperature differential had inconsistent trends across hour angles, and were at most 6.15 and 0.83 °C, respectively. Standard deviation in berry

temperature differential was greater from -75 to -45° hour angles compared to hour angles outside of this range.

The ability to predict east-side berry temperature differential in PB-4 plots was greater from -105 to -15° hour angles (R^2 = 0.3539-0.5815) compared to hour angles outside of this range (R^2 = 0.004-0.0877) (Appendix E). The predictability of east-side berry temperature differential in PB-4 plots from -105 to -15° hour angles was greater than the predictability of east-side berry temperature differential in PB-NO plots from -60 to 0° hour angles. Mean, maximum, and standard deviation in berry temperature differential tended to be greater from -75 to 0° hour angles compared to hour angles outside of this range. Mean berry temperature differential was 1.86 to 4.28 °C over the -75 to 0° hour angles, and these temperatures were greater than the mean east-side berry temperature differential over the -60 to 0° hour angles in the PB-NO plots. The ability to predict west-side berry temperature differential in PB-4 plots was greater from 30 to 90° hour angles (R^2 = 0.2749-0.4961) compared to hour angles outside of this range (R^2 = 0.0009-0.0620) (Appendix F). Mean, maximum, and standard deviation in berry temperature differential were greater from 30 to 75° hour angles compared to hour angles outside of this range. Mean berry temperature differential was 1.36 to 3.21 °C over the 30 to 75° hour angles, and these temperatures were greater than mean west-side berry temperature differential at any hour angle in the PB-NO plots.

Similar to the hour angle range that east-side berry temperature differential in the PB-4 plots was best predicted, there was a greater ability to predict east-side berry temperature differential in the PB-8 plots from -105 to -15° hour angles (R^2 = 0.3854-0.5164) compared to hour angles outside of this range (R^2 = 0.0012-0.3594) (Appendix G). Maximum, mean, and standard deviation in berry temperature differential tended to be greater from -75 to 0° hour

angles compared to hour angles outside of this range. Mean berry temperature differential was 1.68 to 4.64 °C over the -75 to 0° hour angle range; these temperatures were similar to mean east-side berry temperature differential over the -75 to 0° hour angle range in the PB-4 plots, and greater than the mean east-side berry temperature differential over the -60 to 0° hour angles in the PB-NO plots. There was a greater ability to predict west-side berry temperature differential in the PB-8 plots from 30 to -75° hour angles (R^2 = 0.4082-0.5329) compared to hour angles outside of this range (R^2 = 0.0003-0.1373) (Appendix H). Maximum berry temperature differential tended to be inconsistent across hour angles, but was consistently high from 15 to 90° hour angles. Mean and standard deviation in berry temperature differential were greater over the 30 to 90° hour angles compared to hour angles outside of this range. Mean berry temperature differential was 1.40 to 3.37 °C over the 30 to 75° hour angle range; these temperatures were similar to the mean west-side mean berry temperature differential in the PB-4 plots over the 30 to 75° hour angle range, and greater than mean west-side berry temperature differential at any hour angle in the PB-NO plots.

Manually measured berry temperature for model validation: Ambient air temperature tended to be greater after the 0° hour angle compared to before the 0° hour angle over the three dates that manually measured berry temperature was compared to predicted berry temperature (Fig. 32). The standard deviation of ambient air temperature was particularly high at the -60 to 0° hour angles on 31 Jul 2015 compared to other hour angles on this date, as well as on 29 Jun and 25 Aug 2015. Ambient PAR tended to be greater at the 0 and 15° hour angles, albeit ambient PAR at the 0° hour angle on 31 Jul 2015 was atypically low (Fig. 33). Similar to the standard deviation of ambient air temperature, the standard deviation of ambient PAR was particularly high at the -60 to 0° hour angles on 31 Jul 2015 compared to other hour angles on this date, as

well as on 29 Jun and 25 Aug 2015. Thus, it was inferred that ambient air temperature and PAR was highly variable on 31 Jul compared to other dates.

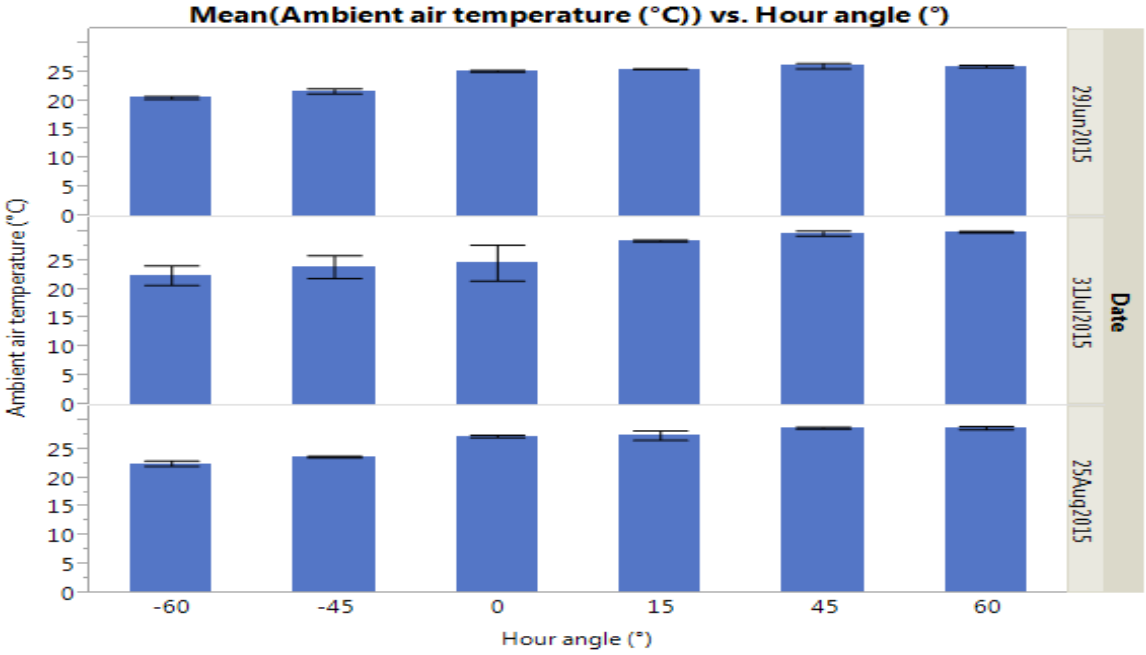

Fig. 32. The mean ambient air temperature at each hour angle date that manual berry temperature was measured for purposes of comparison to predicted berry temperature. Error bars are ± one standard deviation from the mean.

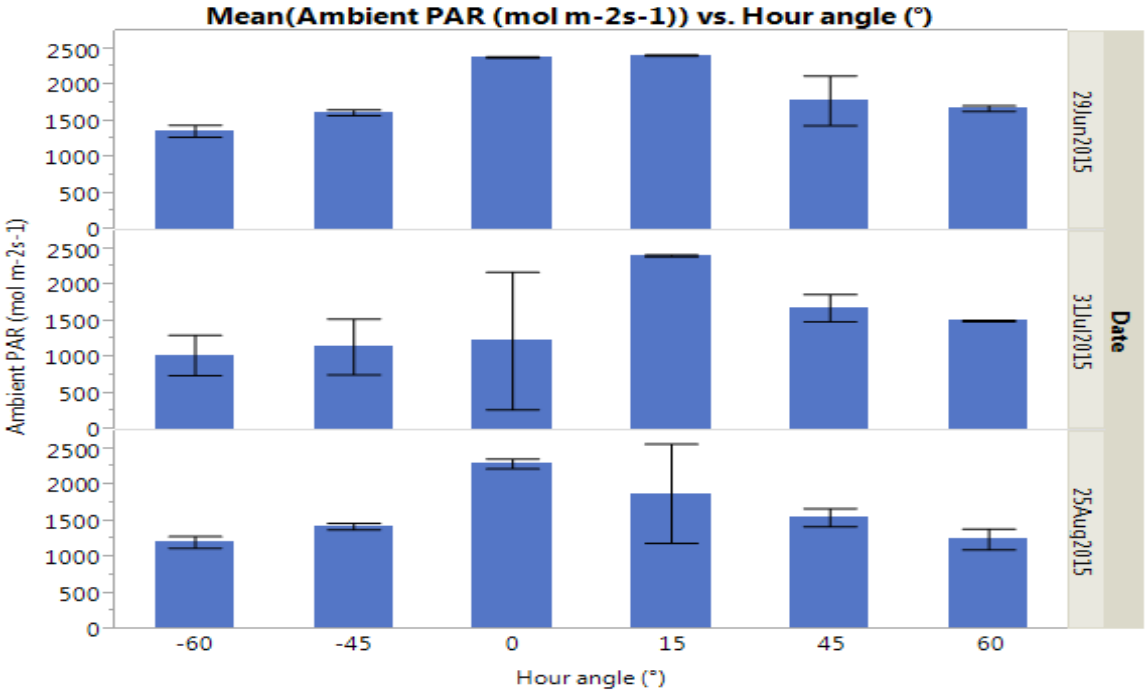

Fig. 33. The mean ambient PAR at each hour angle date that manual berry temperature was measured for purposes of comparison to predicted berry temperature. Error bars are ± one standard deviation from the mean.

The linear relationships between predicted and manually measured east side berry temperature was stronger in PB-NO plots (R^2 = 0.756) compared to PB-4 (R^2 = 0.438) and PB-8 plots (R^2 = 0.348) when data were combined across all hour angles (Fig. 34). Measured berry temperature was consistently greater than predicted berry temperature in leaf removal plots on 25 Aug 2015. The linear relationships between predicted and manually measured west side berry temperature was relatively similar in strength between all pre-bloom leaf removal treatments (R^2 = 0.907-0.941) when data was combined across all hour angles (Fig. 35). The ability to predict west side berry temperature was not affected by date as it was for the east side.

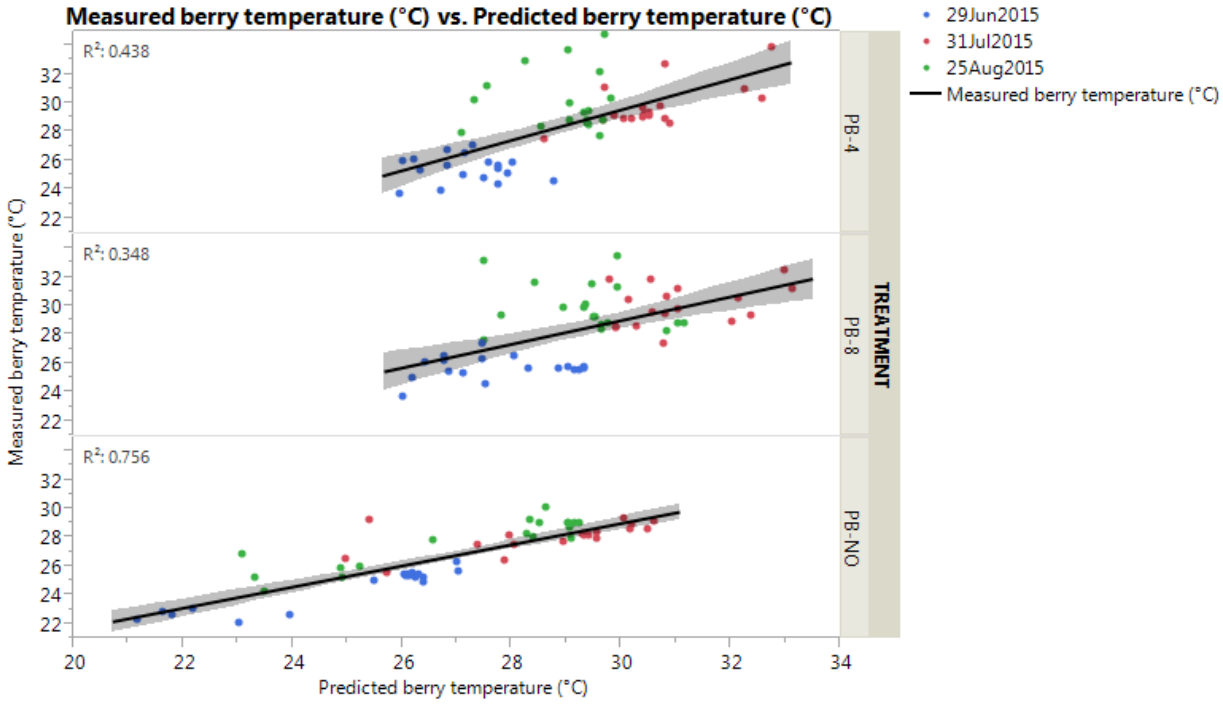

Fig. 34. The linear relationship between predicted berry temperature and manually measured berry temperature on the east canopy side of pre-bloom leaf removal plots.

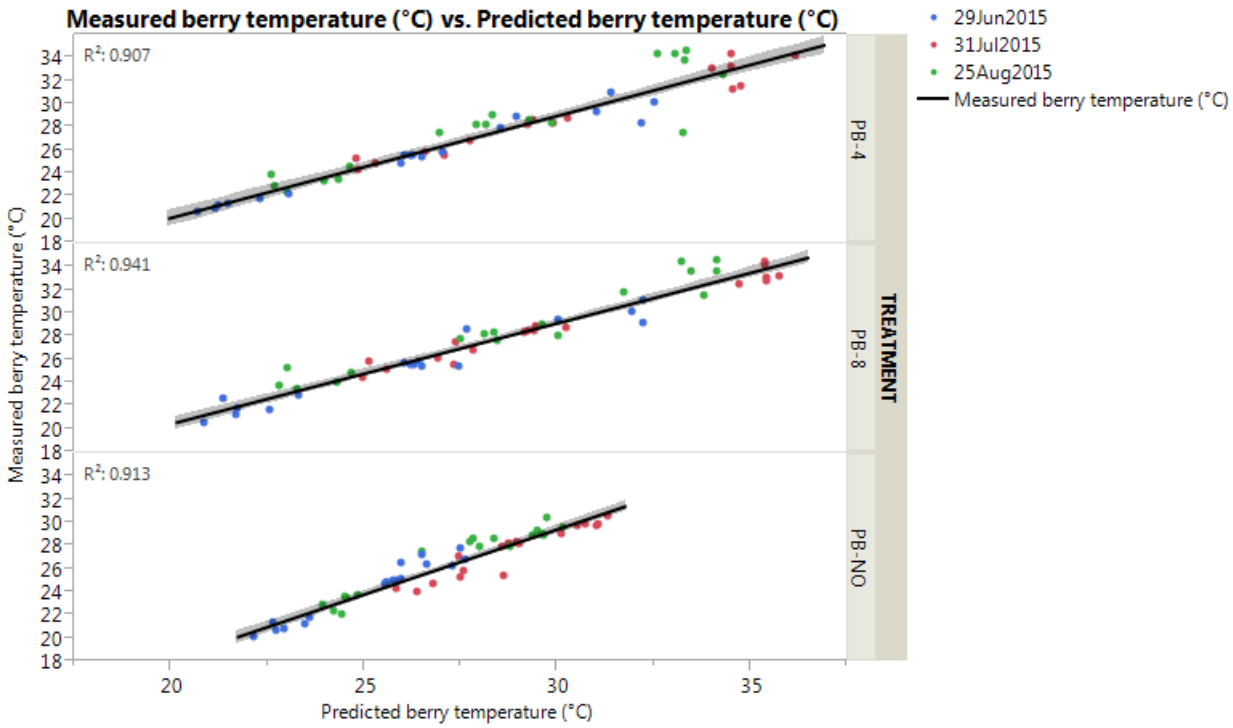

Fig. 35. The linear relationship between predicted berry temperature and manually measured berry temperature on the west canopy side of pre-bloom leaf removal plots.

There was generally a stronger linear relationship (R^2) between predicted and manually measured east side berry temperature in PB-NO plots when compared to PB-4 and PB-8 plots at -60 and -45° hour angles; this was attributed to a relatively lower standard deviation in the difference between east side manually measured and predicted berry temperature (MBT-PBT) in the PB-NO compared to PB-4 and PB-8 plots at -60 and -45° hour angles (Table 10). The linear relationship between predicted and manually measured berry temperature was similar between leaf removal and no leaf removal plots after the -45° hour angle, attributed to the comparable standard deviation in MBT-PBT across treatments. The east side mean MBT-PBT tended to be negative, and, thus, measured berry temperatures were typically lower than predicted berry temperatures. The greatest absolute value of the east side mean MBT-PBT was 1.43 °C at the -60° hour angle in PB-NO plots, and -1.65 and -2.84 °C at the 0° hour angle in PB-4 and PB-8

plots, respectively. East side mean MBT-PBT was ≤ [0.87 °C] across all treatments from the 15 to 60° hour angle.

There was generally a stronger linear relationship (R^2) between predicted and manually measured west side berry temperature in PB-NO plots when compared to PB-4 and PB-8 plots at 45 and 60° hour angles; this was attributed to a relatively lower standard deviation in the MBT-PBT in the PB-NO compared to PB-4 and PB-8 plots at 45 and 60° hour angles (Table 10). The linear relationship between predicted and manually measured berry temperature was similar between leaf removal and no leaf removal plots before the 45° hour angle, attributed to the comparable standard deviation in MBT-PBT across treatments. As with the east side, the west side mean MBT-PBT tended to be negative, and, thus, measured berry temperatures were typically lower than predicted berry temperatures. The absolute value of the west side mean MBT-PBT tended to be relatively greater at the -60 and -45° hour angles in PB-NO plots, and at the 15° hour angle in the PB-4 and PB-8 plots, as well as the 60° hour angle in the PB-8 plots. West side mean MBT-PBT was ≤ [0.91 °C] across all treatments at the 0 and 45° hour angles.

Table 2. The relationship, difference, and standard deviation between predicted and measured berry temperature in pre-bloom leaf removal plots.

EAST												
	PB-NO				PB-4				PB-8			
Hour angle	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]
-60	0.671	1.43	1.31	11	0.545	0.85	2.91	12	0.457	0.64	2.61	12
-45	0.783	-0.22	1.00	7	0.216	-0.64	3.50	6	0.351	-0.94	2.50	5
0	0.789	-0.68	0.75	13	0.778	-1.65	0.88	12	0.851	-2.84	0.76	13
15	0.553	0.14	1.22	5	0.591	-0.36	0.98	6	0.655	-0.66	1.01	5
45	0.896	-0.87	0.58	14	0.912	-0.67	0.52	14	0.912	-0.82	0.53	14
60	0.905	-0.73	0.54	4	0.992	-0.31	0.30	4	0.993	0.71	0.69	4
WEST												
	PB-NO				PB-4				PB-8			
Hour angle	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]
-60	0.929	-2.08	0.54	11	0.902	-0.14	0.58	12	0.774	0.19	0.92	12
-45	0.941	-1.47	0.48	7	0.942	-0.85	0.53	6	0.873	-0.60	0.82	5
0	0.901	-0.46	0.56	13	0.866	-0.58	0.58	12	0.928	-0.66	0.37	13
15	0.647	-0.29	0.86	5	0.699	-1.05	0.83	6	0.560	-1.17	1.00	5
45	0.866	-0.43	0.71	14	0.496	-0.80	1.74	14	0.681	-0.91	1.35	14
60	0.988	-0.74	0.27	4	0.644	-0.14	1.90	4	0.548	-1.63	1.39	4

aMBT-PBT and StdDev MBT-PBT = the mean difference between measured and predicted berry temperature, and the standard deviation of the difference between measured and predicted berry temperature, respectively.

[b]N = total number of logged observations of manual measured berry temperature at each hour angle over the course of 29 Jun, 31 Jul and 25 Aug 2015; each N = an average of six individual berry temperature measurements.

The linear relationships between predicted berry temperature on the east side of pre-bloom leaf removal plots and measured berry temperature on the east side of post-fruit set leaf removal plots was stronger in PFS-NO plots (R^2 = 0.493) compared to PFS-6 plots (R^2 = 0.010-0.019) when data was combined across all hour angles (Fig. 36). Measured berry temperature tended to be consistently greater than predicted berry temperature on 25 Aug 2015, but only in leaf removal plots. The linear relationships between predicted berry temperature on the west side of pre-bloom leaf removal plots and measured berry temperature on the west side of post-fruit set leaf removal plots was relatively stronger in PFS-6 plots (R^2 = 0.810-0.814) compared to PB-NO plots (R^2 = 0.589) when data was combined across all hour angles (Fig. 37). The ability to predict west side berry temperature was not differentially affected by date, but measured berry temperature was greater than predicted berry temperature on 31 Jul 2015.

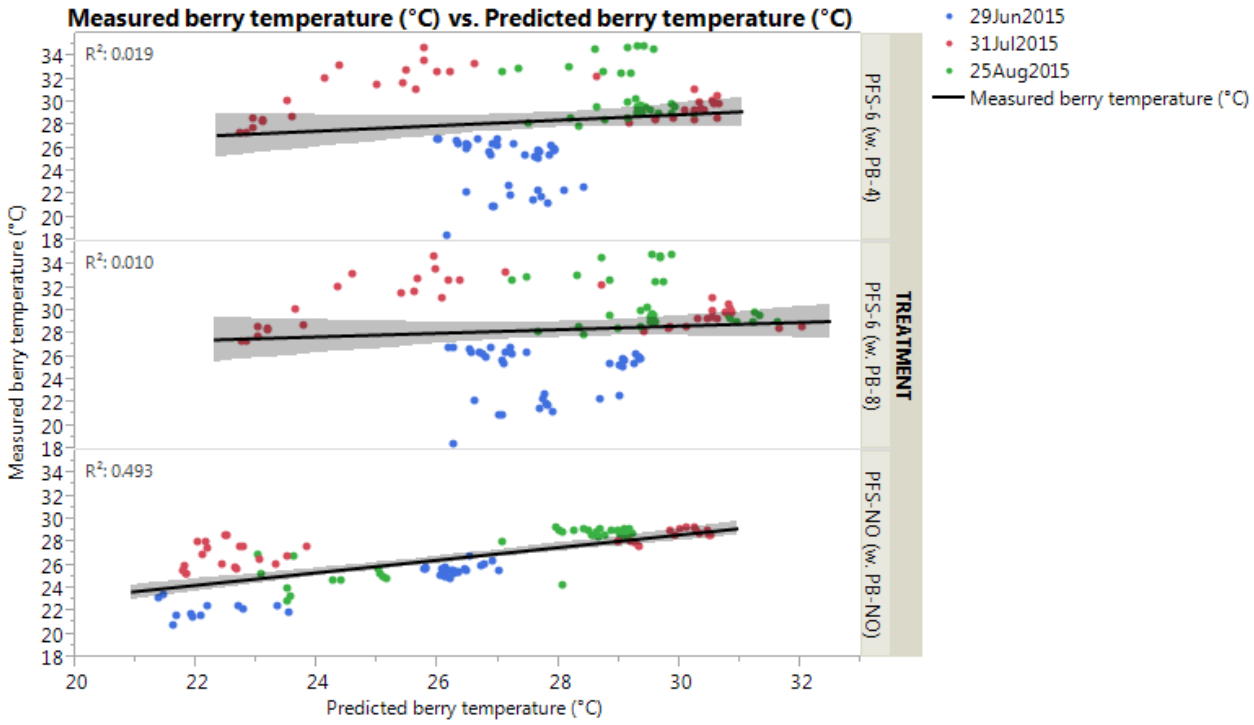

Fig. 36. The linear relationship between predicted berry temperature on the east canopy side of pre-bloom leaf removal plots and manually measured berry temperature on the east canopy side of post-fruit set leaf removal plots.

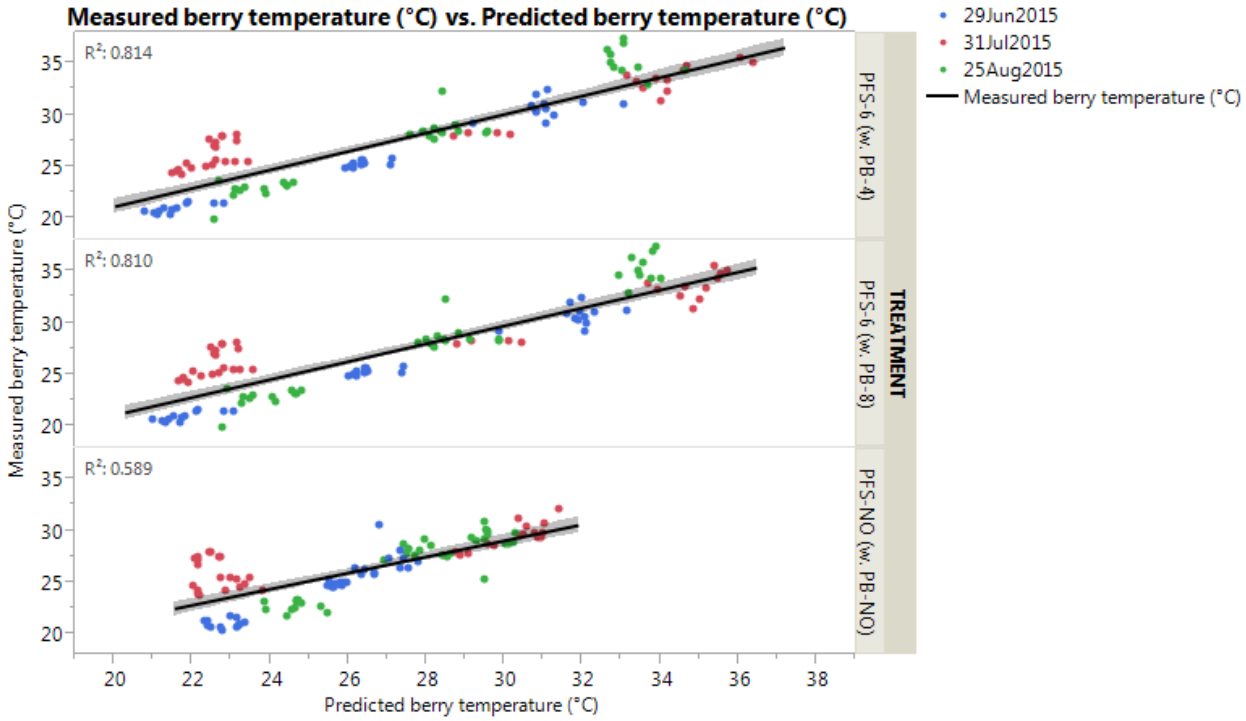

Fig. 37. The linear relationship between predicted berry temperature on the west canopy side of pre-bloom leaf removal plots and manually measured berry temperature on the west canopy side of post-fruit set leaf removal plots.

Though linear relationships were weak, there was a comparatively stronger linear relationship (R^2) between predicted and manually measured east side berry temperature in PFS-NO plots when compared to PFS-6 plots at -60 and -45° hour angles, and, again, this was observed to be a function of lower standard deviation in the MBT-PBT in PFS-NO compared to PFS-6 plots (Table 11). From the 0° hour angle on, the linear relationship between predicted and manually measured east side berry temperature, and the standard deviation in the MBT-PBT, were similar between PFS-NO and PFS-6 plots. The east-side mean MBT-PBT tended to be negative, have an absolute value that was relatively close to zero, and was similar across PFS-NO and PFS-6 plots at 15 to 60° hour angles. By contrast, the east side mean MBT-PBT tended to be positive and relatively high at the -60° hour angle, but relatively greater in PFS-6 plots compared to PFS-NO plots.

Though linear relationships were weak, there was a comparatively stronger linear relationship (R^2) between predicted and manually measured west side berry temperature in PFS-6 plots when compared to PFS-NO plots at -60 and -45° hour angles; as for the east side data, this was observed to be a function of slightly lower standard deviation in the MBT-PBT in PFS-6 compared to PFS-NO plots (Table 11). From the 15° hour angle on, the linear relationship between predicted and manually measured west side berry temperature was relatively greater in the PFS-NO compared to the PFS-6 plots, and this was associated with relatively lower standard deviation in the MBT-PBT in PFS-NO compared to PFS-6 plots. The west-side mean MBT-PBT tended to be positive, relatively low, and similar across PFS-NO and PFS-6 plots at the 0° hour angle. At the 15° hour angle, west side mean MBT-PBT tended to be negative, and have a greater absolute value in the PFS-6 compared to PFS-NO plots.

Table 3. The relationship, difference, and standard deviation between predicted berry temperature in the pre-bloom leaf removal plots and measured berry temperature in post-fruit set leaf removal plots.

EAST												
	PFS-NO with PB-NO				PFS-6 with PB-4				PFS-6 with PB-8			
Hour angle	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]
-60	0.072	1.76	1.95	24	0.000	2.12	5.97	25	0.000	1.98	5.94	25
-45	0.105	0.88	1.91	12	0.019	1.4	5.45	11	0.012	0.87	5.50	11
0	0.007	1.06	2.82	27	0.001	0.52	3.24	27	0.001	-0.48	3.84	27
15	0.681	-0.35	0.89	8	0.662	-0.46	0.87	9	0.653	-0.65	0.88	9
45	0.875	-0.69	0.61	30	0.884	-0.29	0.57	29	0.883	-0.50	0.57	29
60	0.959	-0.59	0.28	6	0.901	0.14	0.61	6	0.895	-0.13	0.62	6
WEST												
	PFS-NO with PB-NO				PFS-6 with PB-4				PFS-6 with PB-8			
Hour angle	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]	R^2	MBT-PBT[a]	StdDev MBT-PBT[a]	N[b]
-60	0.008	-0.88	1.90	23	0.088	0.57	1.84	25	0.075	0.36	1.86	25
-45	0.011	-0.17	1.87	12	0.140	-0.10	1.60	11	0.112	-0.31	1.64	11
0	0.024	1.23	2.53	28	0.045	1.13	2.59	27	0.043	1.07	2.62	27
15	0.868	-0.87	0.48	8	0.613	-1.12	0.80	9	0.572	-1.27	0.85	9
45	0.539	-0.36	1.27	30	0.402	0.37	1.80	27	0.360	-0.47	1.86	27
60	0.806	-0.81	0.82	6	0.563	-0.65	1.10	6	0.621	-0.03	1.01	6

aMBT-PBT and StdDev MBT-PBT = the mean difference between measured and predicted berry temperature, and the standard deviation of the difference between measured and predicted berry temperature, respectively.

[b]N = total number of logged observations of manual measured berry temperature at each hour angle over the course of 29 Jun, 31 Jul and 25 Aug 2015; each N = an average of six individual berry temperature measurements.

Discussion

Berry temperature was linearly related to and governed to some extent by ambient air temperature. The close relationship of ambient air and berry temperature was shown before in the arid environment of the central San Joaquin Valley of California (Bergqvist et al. 2001). Yet, in that study, there was no evidence of data points from fully exposed straying above the ambient air-berry temperature trend line. In fact, the R^2 value between ambient air temperature and berry temperature was *greater* in the fully exposed treatment compared to the moderately exposed and shaded treatments (Bergqvist et al. 2001). Nonetheless, modelling berry temperature was not a goal of that study, and berry temperature was only measured at points-in-time. Thus, the relatively low *n* did not provide insight into long-term berry temperature trends. The current study observed that removing leaves from fruit-zones resulted in a *weaker* air-berry temperature relationship compared to shaded fruit-zones, particularly due to the straying of data points *above* the air-berry temperature relationship trend line. This revealed that ambient air temperature did not solely govern berry temperature, particularly when leaves were removed from fruit-zones.

Ambient and fruit-zone radiation were also related to berry temperature in the current study, although to a comparatively lesser extent than ambient air temperature was. Bergqvist et al. (2001) reported greater R^2 values of the ambient PAR and berry temperature relationship on both north and south canopy sides when compared to the R^2 values of the ambient PAR and berry temperature relationship on east and west canopy sides in the current study. However, again, because Bergqvist et al. (2001), only collected berry temperature and ambient PAR at points in time, long term trends were not determined. However, ambient radiation in the central San Joaquin Valley of California was likely more intense and consistent throughout the entire day compared to the variable radiation patterns experienced throughout the day in the humid

region of the current study. In the current study, grapes were heated above ambient air temperature when radiation was not blocked by the overhead canopy, and radiation heated east and west exposed grapes above ambient air temperature during certain negative and positive hour angle ranges, respectively. Since direct sunlight heats tissues more intensely than diffuse light does (Smart et al. 1976), it is probable that radiation is more consistently related to berry temperature in drier/arid climates that experience clear skies throughout the day.

While radiation is an important determinant of berry temperature, berry temperature is more difficult to model under highly variable compared to consistent radiation patterns (Cola et al. 2009). The results of the current study confirm that variable cloudiness can greatly reduce the ability to the accurately predict berry temperature using ambient air temperature, especially in fruit-zones that are well exposed to radiation. For example, greater maximum berry temperature differential from ambient air temperature occurred in leaf removal plots at hour angles in which radiation was directed on the fruit-zone (Tables 6-9). In leaf removal plots, models were relatively stronger at -75 to -15° hour angles on the east canopy side, and from 30 to 75° hour angles on the west canopy side because (1) models were built with ambient radiation as the independent variable, and (2) a change in berry temperature was prompted by a change in fruit-zone radiation. However, models never reached R^2 values above 0.5815 at those hour angles, a probable consequence of relatively greater standard deviation in berry temperature differential from ambient air temperature at the -75 to -15° and 30 to 75° hour angles when compared to other hour angles. The greater standard deviation in berry temperature differential from ambient air temperature in leaf removal plots was likely caused by the high variability in ambient radiation in the current study. It was speculated that the predictability of exposed berry temperature with both ambient air temperature and PAR would be much greater in arid/drier

regions, as observed in Bergqvist et al. (2001). Models were relatively weak throughout the diurnal period in no leaf removal plots, and this was attributed to using ambient radiation as an independent variable in the models. However, a change in ambient radiation did not prompt a change in berry temperature in no leaf removal plots as it did in leaf removal plots. As such, ambient air temperature alone may be the best predictor of berry temperature in relatively well-shaded fruit-zones.

Model validation affirmed that greater standard deviation in the difference between measured and predicted berry temperature was the reason that berry temperature was predicted *less consistently* in leaf removal compared to no leaf removal plots, particularly at hour angle ranges that coincided with direct fruit-zone radiation. However, there did not appear to be a leaf removal effect on the *magnitude of difference* in measured and predicted berry temperature, other than at the 0 and 15° hour angles, when there was typically a greater difference in measured and predicted berry temperature in leaf removal compared to no leaf removal plots. This suggested that the model did not account enough for the amount of incident radiation that reached the fruit-zone in leaf removal plots at hour angles that were close to solar noon. Model validation also revealed that measured berry temperature was *less* than predicted berry temperature on 52 of the 72 total occasions that these measurements were compared. This suggested that the model did not adequately account for radiant heating of berries to actual temperatures. Thus, the current model may benefit from refining the ambient PAR range used for model development (0-2500 µmol $m^{-2}s^{-1}$) to account for a (unknown) lower threshold at which berry temperature is affected by PAR (i.e. *500*-2500 µmol $m^{-2}s^{-1}$). Measured berry temperature was consistently greater than predicted berry temperature on the east canopy side at the -60° hour angle, but this trend was not evident for the 45° hour angle on the west canopy side. Further insight into the consistently

greater measured compared to predicted east side berry temperature in leaf removal plots on 25 Aug 2015 (Figs. 34 and 36) revealed that this occurred at the -60° hour angle. These examples revealed that berries were heated by radiation to a greater extent on the east canopy side in the morning compared to the west canopy side in the afternoon. Since air temperature, and thus berry temperature, is relatively cooler in the morning compared to the afternoon, it was reasoned that cool berries are subject to greater radiant heating compared to warmer berries. Thus, the current models developed for predicting east side berry temperature in leaf removal plots at the -60° hour angle could be further refined if the difference in the magnitude of radiant heating-induced temperature increase could be defined between relatively cooler and warmer berries.

Factors such as berry color and relative humidity were less important in determining berry temperature than ambient air temperature, radiation, and leaf removal practice. Wind speed was suggested to be a factor that could affect berry temperature (Cola et al. 2009). While not measured in the current study, it was hypothesized that relatively high wind speeds would have reduced berry temperature, and to a greater extent in well-exposed compared to shaded fruit-zones. Cluster position within the canopy of a given training systems is an important determinant of how much radiation that cluster will receive, and, thus the extent that grapes in that cluster will be heated (Bergqvist et al. 2001, Spayd et al. 2002). As such, model validation was may have been particularly complicated by the manual measurement of berries that were not positioned in the fruit-zone in the exact manner of those that remained in the same position throughout data logging over the course of each season. Further, the current study developed berry temperature prediction models using a vertically-shoot positioned training system. While practical for the eastern US, diurnal berry temperature would likely differ for vines trained to a high wire system with trailing shoots due to different patterns of fruit-zone radiation penetration

at a given hour angle. Berries in more compact clusters are radiantly heated to a relatively greater degree compared to looser clusters, due to heat transfer between neighboring berries (Keller 2010). The results of the current study showed that measured east-side berry temperature at the -60° hour angle was an average of 0.75 °C greater in *pre-bloom* leaf removal plots, but an average of 2.05 °C greater in *post-fruit set* leaf removal plots, when compared to predicted east-side berry temperature in pre-bloom leaf removal plots. This data supported that berries can be radiantly heated to a greater extent in tighter clusters (post-fruit set leaf removal plots) compared to looser clusters (pre-bloom leaf removal plots). Thus, though likely changing berry temperature by minute extents, physical differences in berry and cluster architecture may further complicate the ability to accurately predict berry temperature across *Vitis* varieties.

The current study highlighted 15° hour angles that berry temperature was particularly susceptible to radiant heating in leaf removal plots. As such, if it was of desire to estimate berry temperature *extremes*, it would be advised to do so using the models from -75 to -15° hour angles on the east canopy side, and from 30 to 75° hour angles on the west canopy side. The current models require metrics anticipated to be available from many weather stations across the country – ambient air temperature and radiation. As the models currently stand, one would need only to estimate their average fruit-zone leaf layer number, and input those two metrics into the appropriate hour angle linear equation in order to estimate berry temperature differential from ambient air temperature. Thereafter, one could refine future canopy management strategies based on assessed risk for "overheating" of grapes on their site. However, in the humid region that the current study was conducted in, extreme grape heating was an unlikely occurrence, as ambient air temperature ≥ 30 °C was logged for only 72 total hrs in the most extreme season, and was never logged ≥ 33.6 °C. A limitation to the models is that they were developed using hour

angle, an astronomical metric that is unlikely common vernacular. Another limitation to the models is that they were developed in generally north-south oriented rows and, thus, do not account for east-west oriented rows. While models may benefit by accounting for changes in hour angle and row orientation, accounting for these factors would complicate the ability to model the effect of radiation on berry temperature.

Conclusion: Ambient air temperature was highly related to berry temperature, but ambient radiation affected this relationship by heating berries above ambient air temperature. Model validation revealed that the greatest difference between manually measured and predicted berry temperature ranged [0.17] to [2.84] °C across canopy sides of differentially leaf-thinned fruit-zones. This was considered a negligible range considering all potential factors that could affect berry temperature in a field setting. There was a more *consistent* ability to predict berry temperature when radiation penetration to the fruit-zone was blocked by foliage, or when radiation was directed on the opposite side of the canopy from which berry temperature was being measured. However, a consistent absolute difference between predicted and manually measured berry temperature was not observed between exposed and shaded fruit-zones across hour angles. These trends suggested that the model was robust across fruit-zone leaf removal treatments and canopy sides, but that the highly variable radiation patterns experienced in a humid region complicated berry temperature prediction. As the models currently stand, they marginally predict berry temperature differential from ambient air temperature at specific 15° hour angles, but provide insight into hour angles at which berry temperature extremes (i.e. ≥ 30-35 °C) would most likely occur. Models would greatly benefit by accounting for row orientation, and be refined by developing models using only the ambient radiation levels known to elicit an increase in berry temperature.

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Appendices

Appendix **Page**

Appendix A. The prediction of **east-side** berry temperature differential from ambient air temperature with data logged from **all leaf removal treatments** over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.23	n/a	0	0.97	0.23	0.16	4383	0
-165	n/a	0.24	n/a	0	1.19	0.24	0.17	8926	0
-150	n/a	0.25	n/a	0	1.13	0.25	0.16	9463	0
-135	-0.02930	0.24	0.0008	0	1.01	0.24	0.15	9733	5
-120	0.00238	0.23	0.0407	0	2.24	0.23	0.16	10175	153
-105	0.00267	0.21	0.2976	0	3.49	0.26	0.27	10876	3984
-90	0.00208	0.14	0.3526	0	4.87	0.42	0.53	13041	11094
-75	0.00454	-0.32	0.4244	0	11.6	1.46	1.87	15098	15062
-60	0.00398	-0.08	0.2994	0	12.2	2.85	2.84	16353	16353
-45	0.00321	0.03	0.2855	0	13.76	3.45	3.01	16927	16927
-30	0.00256	0.26	0.2678	0	12.63	3.53	3.06	16820	16820
-15	0.00178	0.50	0.2423	0	11.56	2.89	2.53	16225	16225
0	0.00082	0.73	0.0983	0	9.25	1.84	1.77	16176	16176
15	0.00015	0.92	0.0144	0	6.56	1.12	0.81	16091	16091
30	-0.00002	0.77	0.0004	0	3.98	0.75	0.51	15452	15452
45	0.00001	0.60	0.0000	0	16.48	0.60	0.55	13765	13765
60	0.00002	0.46	0.0003	0	4.88	0.47	0.43	12110	12110
75	0.00007	0.31	0.0044	0	5.24	0.33	0.26	12226	12214
90	-0.00026	0.30	0.0197	0	2.41	0.28	0.23	11006	8696
105	-0.00247	0.30	0.0340	0	1.51	0.29	0.24	8838	2006
120	-0.03668	0.26	0.0002	0	1.71	0.26	0.20	8147	1
135	n/a	0.24	n/a	0	1.24	0.24	0.17	8514	0
150	n/a	0.24	n/a	0	1.45	0.24	0.18	8092	0
165	n/a	0.24	n/a	0	1.42	0.24	0.17	8449	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.
[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.
[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix B. The prediction of **west-side** berry temperature differential from ambient air temperature with data logged from **all leaf removal treatments** over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.21	n/a	0.00	0.93	0.21	0.18	5115	0
-165	n/a	0.21	n/a	0.00	1.16	0.21	0.17	10861	0
-150	n/a	0.22	n/a	0.00	1.06	0.22	0.16	11289	0
-135	-0.00655	0.21	0.0000	0.00	1.23	0.21	0.16	11945	6
-120	-0.00020	0.21	0.0002	0.00	1.13	0.21	0.15	11876	108
-105	-0.00050	0.22	0.0132	0.00	1.75	0.21	0.16	12968	4338
-90	0.00031	0.21	0.0307	0.00	4.01	0.25	0.23	13814	11590
-75	0.00089	0.12	0.1233	0.00	6.73	0.40	0.59	12386	12350
-60	0.00056	0.26	0.1073	0.00	5.27	0.63	0.67	12012	12012
-45	0.00028	0.46	0.0520	0.00	5.33	0.73	0.60	13232	13232
-30	0.00011	0.61	0.0161	0.00	5.54	0.75	0.54	14163	14163
-15	0.00004	0.74	0.0030	0.00	2.87	0.79	0.51	14442	14442
0	0.00006	0.80	0.0050	0.00	3.86	0.88	0.56	14821	14821
15	0.00033	0.69	0.0685	0.00	6.61	1.13	0.83	15640	15640
30	0.00114	0.34	0.2443	0.00	7.69	1.76	1.47	15729	15729
45	0.00217	0.32	0.2984	0.00	9.94	2.55	2.15	16398	16398
60	0.00310	0.25	0.3335	0.00	11.03	2.46	2.26	16726	16726
75	0.00278	0.08	0.3841	0.00	8.35	1.19	1.30	15755	15743
90	0.00004	0.37	0.0003	0.00	8.59	0.37	0.35	11281	9367
105	-0.00270	0.35	0.0495	0.00	2.53	0.32	0.29	7587	1854
120	-0.02853	0.25	0.0008	0.00	1.14	0.25	0.19	7467	6
135	n/a	0.22	n/a	0.00	1.25	0.22	0.18	8414	0
150	n/a	0.22	n/a	0.00	1.12	0.22	0.18	9062	0
165	n/a	0.21	n/a	0.00	1.38	0.21	0.18	10176	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.
[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.
[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix C. The prediction of **east-side** berry temperature differential from ambient air temperature with data logged in the **PB-NO** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.30	n/a	0.00	0.97	0.30	0.18	2066	0
-165	n/a	0.31	n/a	0.00	1.19	0.31	0.20	4227	0
-150	n/a	0.32	n/a	0.00	1.13	0.32	0.18	4512	0
-135	-0.04281	0.29	0.0012	0.00	1.01	0.29	0.17	4626	2
-120	-0.00155	0.28	0.0058	0.00	1.06	0.28	0.17	4770	37
-105	0.00166	0.28	0.1220	0.00	1.86	0.30	0.21	4974	1402
-90	-0.00024	0.31	0.0251	0.00	1.44	0.29	0.20	3996	3168
-75	0.00021	0.22	0.0467	0.00	2.05	0.30	0.26	3190	3178
-60	0.00074	0.17	0.1581	0.00	3.63	0.70	0.73	3995	3995
-45	0.00077	0.29	0.1742	0.00	4.36	1.09	0.92	4516	4516
-30	0.00053	0.44	0.1328	0.00	3.92	1.10	0.92	4634	4634
-15	0.00046	0.35	0.1938	0.00	3.43	0.96	0.75	4387	4387
0	0.00028	0.38	0.1119	0.00	2.68	0.75	0.56	4175	4175
15	0.00010	0.45	0.0338	0.00	4.40	0.58	0.36	3953	3953
30	-0.00004	0.49	0.0043	0.00	3.34	0.45	0.37	3577	3577
45	-0.00005	0.38	0.0041	0.00	8.01	0.34	0.34	2934	2934
60	-0.00008	0.37	0.0112	0.00	4.51	0.33	0.26	3059	3059
75	-0.00004	0.41	0.0013	0.00	1.61	0.40	0.25	4012	4008
90	-0.00066	0.47	0.0491	0.00	2.41	0.42	0.27	4441	3408
105	-0.00423	0.43	0.0371	0.00	1.51	0.41	0.27	4556	805
120	n/a	0.34	n/a	0.00	1.71	0.34	0.24	4291	0
135	n/a	0.30	n/a	0.00	1.24	0.30	0.20	4265	0
150	n/a	0.31	n/a	0.00	1.45	0.31	0.21	3983	0
165	n/a	0.31	n/a	0.00	1.42	0.31	0.19	3920	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix D. The prediction of **west-side** berry temperature differential from ambient air temperature with data logged in the **PB-NO** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.30	n/a	0.00	0.93	0.30	0.19	2310	0
-165	n/a	0.31	n/a	0.00	1.16	0.31	0.18	4667	0
-150	n/a	0.32	n/a	0.00	1.06	0.32	0.18	4858	0
-135	n/a	0.30	n/a	0.00	1.23	0.30	0.17	5081	0
-120	-0.03918	0.29	0.0005	0.00	1.13	0.29	0.16	5000	1
-105	-0.00179	0.31	0.0573	0.00	1.75	0.29	0.17	5265	1444
-90	0.00047	0.28	0.0253	0.00	4.01	0.32	0.35	4339	3477
-75	0.00309	-0.28	0.4895	0.00	5.34	0.54	1.08	2646	2634
-60	0.00193	-0.30	0.4064	0.00	5.03	0.70	1.23	2184	2184
-45	0.00072	0.00	0.1971	0.00	4.27	0.61	0.83	2792	2792
-30	0.00027	0.29	0.0774	0.00	5.54	0.59	0.62	3194	3194
-15	0.00008	0.43	0.0143	0.00	2.54	0.53	0.46	3256	3256
0	0.00000	0.49	0.0000	0.00	2.31	0.49	0.40	3227	3227
15	-0.00005	0.55	0.0050	0.00	6.15	0.49	0.44	3466	3466
30	0.00001	0.50	0.0001	0.00	2.49	0.51	0.38	3707	3707
45	0.00017	0.52	0.0262	0.00	4.72	0.69	0.54	4076	4076
60	0.00069	0.38	0.1409	0.00	4.95	0.83	0.74	4555	4555
75	0.00054	0.55	0.0649	0.00	3.25	0.74	0.55	4755	4751
90	-0.00071	0.60	0.0394	0.00	2.34	0.53	0.41	4766	3729
105	-0.00500	0.46	0.0661	0.00	1.95	0.43	0.31	4645	943
120	-0.04133	0.33	0.0009	0.00	1.14	0.33	0.20	4367	2
135	n/a	0.30	n/a	0.00	1.25	0.30	0.19	4451	0
150	n/a	0.30	n/a	0.00	1.12	0.30	0.21	4465	0
165	n/a	0.30	n/a	0.00	1.38	0.30	0.21	4551	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix E. The prediction of **east-side** berry temperature differential from ambient air temperature with data logged in the **PB-4** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.18	n/a	0.00	0.59	0.18	0.12	1350	0
-165	n/a	0.19	n/a	0.00	0.76	0.19	0.12	2716	0
-150	n/a	0.20	n/a	0.00	0.55	0.20	0.12	2816	0
-135	n/a	0.20	n/a	0.00	0.55	0.20	0.11	2888	0
-120	0.00212	0.19	0.0670	0.00	0.92	0.19	0.13	3086	53
-105	0.00245	0.17	0.3629	0.00	2.60	0.23	0.24	3323	1348
-90	0.00267	0.08	0.4839	0.00	4.87	0.48	0.58	4797	4182
-75	0.00584	-0.50	0.5815	0.00	10.33	1.87	2.05	5994	5982
-60	0.00494	-0.23	0.4654	0.05	11.77	3.45	2.83	6181	6181
-45	0.00387	-0.09	0.4377	0.22	11.81	4.07	2.93	6206	6206
-30	0.00316	0.20	0.4226	0.10	11.13	4.28	2.98	6093	6093
-15	0.00198	0.68	0.3539	0.22	10.29	3.36	2.33	5934	5934
0	0.00064	0.99	0.0877	0.02	8.57	1.86	1.47	6018	6018
15	0.00008	1.13	0.0059	0.00	3.46	1.24	0.64	6088	6088
30	-0.00005	0.89	0.0047	0.00	3.67	0.83	0.44	5978	5978
45	-0.00007	0.67	0.0137	0.00	1.77	0.59	0.33	5444	5444
60	-0.00010	0.51	0.0213	0.00	1.74	0.45	0.28	4540	4540
75	-0.00002	0.29	0.0004	0.00	1.23	0.28	0.20	4073	4069
90	-0.00005	0.19	0.0022	0.00	1.16	0.19	0.13	3664	2941
105	-0.00044	0.17	0.0079	0.00	0.60	0.17	0.11	2453	669
120	n/a	0.18	n/a	0.00	0.55	0.18	0.10	2190	0
135	n/a	0.19	n/a	0.00	0.56	0.19	0.11	2410	0
150	n/a	0.19	n/a	0.00	0.55	0.19	0.11	2386	0
165	n/a	0.19	n/a	0.00	0.56	0.19	0.12	2623	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix F. The prediction of **west-side** berry temperature differential from ambient air temperature with data logged in the **PB-4** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.13	n/a	0.00	0.51	0.13	0.11	1503	0
-165	n/a	0.14	n/a	0.00	0.65	0.14	0.10	3278	0
-150	n/a	0.14	n/a	0.00	0.45	0.14	0.10	3411	0
-135	0.01837	0.14	0.0013	0.00	0.44	0.14	0.10	3562	3
-120	0.00053	0.15	0.0052	0.00	0.51	0.15	0.11	3597	66
-105	0.00014	0.15	0.0026	0.00	1.53	0.15	0.12	4066	1586
-90	0.00026	0.17	0.0620	0.00	0.99	0.20	0.14	4684	4005
-75	0.00034	0.26	0.0343	0.00	4.68	0.37	0.41	4724	4712
-60	0.00016	0.42	0.0182	0.00	5.27	0.52	0.45	4426	4426
-45	0.00005	0.62	0.0025	0.00	5.33	0.67	0.48	4996	4996
-30	-0.00003	0.72	0.0014	0.00	2.07	0.68	0.43	5454	5454
-15	0.00002	0.81	0.0008	0.00	2.47	0.83	0.46	5636	5636
0	0.00003	0.93	0.0012	0.00	3.04	0.97	0.50	5835	5835
15	0.00029	0.86	0.0783	0.00	4.63	1.25	0.68	6076	6076
30	0.00102	0.63	0.2872	0.00	6.00	1.92	1.21	6032	6032
45	0.00233	0.52	0.4003	0.00	9.94	2.95	2.01	6171	6171
60	0.00386	0.37	0.4288	0.00	11.03	3.21	2.52	6108	6108
75	0.00363	-0.20	0.4961	0.00	8.35	1.36	1.53	5506	5502
90	0.00074	0.15	0.2749	0.00	1.82	0.27	0.23	3442	2963
105	-0.00033	0.15	0.0128	0.00	0.45	0.14	0.10	1593	530
120	-0.01297	0.16	0.0009	0.00	0.43	0.16	0.11	1588	2
135	n/a	0.15	n/a	0.00	0.41	0.15	0.10	1971	0
150	n/a	0.14	n/a	0.00	0.44	0.14	0.10	2355	0
165	n/a	0.13	n/a	0.00	0.51	0.13	0.10	2979	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix G. The prediction of **east-side** berry temperature differential from ambient air temperature with data logged in the **PB-8** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	m[a]	b[a]	R^2	Min Diff (°C)[b]	Max Diff (°C)[b]	Mean Diff (°C)[b]	StDev Diff (°C)[b]	N[c]	N (PAR > 0)[c]
-180	n/a	0.15	n/a	0.00	0.41	0.15	0.10	967	0
-165	n/a	0.16	n/a	0.00	0.56	0.16	0.10	1983	0
-150	n/a	0.17	n/a	0.00	0.40	0.17	0.10	2135	0
-135	-0.01611	0.17	0.0014	0.00	0.40	0.17	0.11	2219	3
-120	0.00478	0.17	0.3594	0.00	2.24	0.18	0.14	2319	63
-105	0.00414	0.12	0.5164	0.00	3.49	0.24	0.38	2579	1234
-90	0.00290	0.01	0.5064	0.00	4.80	0.48	0.64	4248	3744
-75	0.00505	-0.39	0.4936	0.00	11.60	1.68	1.93	5914	5902
-60	0.00478	0.09	0.3870	0.00	12.20	3.66	3.00	6177	6177
-45	0.00407	0.17	0.4223	0.01	13.76	4.55	3.14	6205	6205
-30	0.00342	0.21	0.4409	0.13	12.63	4.64	3.17	6093	6093
-15	0.00250	0.47	0.3854	0.00	11.56	3.86	2.80	5904	5904
0	0.00131	0.78	0.1701	0.00	9.25	2.58	2.17	5983	5983
15	0.00015	1.15	0.0104	0.00	6.56	1.36	1.00	6050	6050
30	-0.00006	0.93	0.0045	0.00	3.98	0.86	0.57	5897	5897
45	-0.00001	0.76	0.0000	0.00	16.48	0.76	0.73	5387	5387
60	0.00008	0.54	0.0033	0.00	4.88	0.59	0.59	4511	4511
75	0.00027	0.23	0.0502	0.00	5.24	0.32	0.30	4141	4137
90	0.00020	0.15	0.0529	0.00	1.14	0.17	0.12	2901	2347
105	-0.00062	0.14	0.0270	0.00	0.36	0.14	0.09	1829	532
120	-0.02012	0.14	0.0012	0.00	0.38	0.14	0.10	1666	1
135	n/a	0.15	n/a	0.00	0.48	0.15	0.10	1839	0
150	n/a	0.17	n/a	0.00	0.47	0.17	0.10	1723	0
165	n/a	0.16	n/a	0.00	0.43	0.16	0.10	1906	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.

Appendix H. The prediction of **west-side** berry temperature differential from ambient air temperature with data logged in the **PB-8** plots over 2014 and 2015.

Berry temperature differential (Diff) = m(ambient PAR) + b									
Hour angle (°)	**m[a]**	**b[a]**	**R^2**	**Min Diff (°C)[b]**	**Max Diff (°C)[b]**	**Mean Diff (°C)[b]**	**StDev Diff (°C)[b]**	**N[c]**	**N (PAR > 0)[c]**
-180	n/a	0.13	n/a	0.00	0.58	0.13	0.11	1302	0
-165	n/a	0.13	n/a	0.00	0.67	0.13	0.11	2916	0
-150	n/a	0.14	n/a	0.00	0.62	0.14	0.10	3020	0
-135	-0.01190	0.14	0.0006	0.00	0.61	0.14	0.10	3302	3
-120	-0.00047	0.15	0.0019	0.00	0.62	0.15	0.10	3279	41
-105	-0.00009	0.16	0.0009	0.00	1.40	0.16	0.11	3637	1308
-90	0.00039	0.17	0.1373	0.00	1.07	0.22	0.15	4791	4108
-75	0.00022	0.28	0.0367	0.00	6.73	0.36	0.27	5016	5004
-60	0.00028	0.49	0.0562	0.00	2.48	0.68	0.45	5402	5402
-45	0.00019	0.65	0.0294	0.00	2.70	0.85	0.54	5444	5444
-30	0.00011	0.76	0.0137	0.00	3.11	0.90	0.54	5515	5515
-15	-0.00001	0.92	0.0003	0.00	2.87	0.90	0.53	5550	5550
0	0.00006	0.92	0.0052	0.00	3.86	1.01	0.61	5759	5759
15	0.00046	0.74	0.1028	0.00	6.61	1.37	0.95	6098	6098
30	0.00166	0.24	0.4082	0.00	7.69	2.36	1.66	5990	5990
45	0.00298	0.26	0.5329	0.00	8.66	3.37	2.23	6151	6151
60	0.00338	0.42	0.4651	0.00	8.52	2.92	2.12	6063	6063
75	0.00316	0.05	0.4494	0.00	6.85	1.40	1.40	5494	5490
90	0.00054	0.15	0.1257	0.00	8.59	0.25	0.26	3073	2675
105	-0.00056	0.16	0.0183	0.00	2.53	0.16	0.13	1349	381
120	-0.01039	0.14	0.0006	0.00	0.42	0.14	0.10	1512	2
135	n/a	0.13	n/a	0.00	0.50	0.13	0.09	1992	0
150	n/a	0.13	n/a	0.00	0.50	0.13	0.09	2242	0
165	n/a	0.13	n/a	0.00	0.57	0.13	0.10	2646	0

[a]m and b are the coefficients of determination for the linear regression equation for berry temperature differential at each hour angle over 2014-2015.

[b]Min, Max, Mean, and StdDev are the minimum, maximum, mean, and standard deviation of logged berry temperature differential from ambient air temperature at each hour angle over 2014-2015, respectively.

[c]N = number of logged observations at each hour angle over 2014-2015; N (PAR > 0) = number of logged observations when ambient PAR was greater than 0 at each hour angle over 2014-2015.