ABSTRACT

THE INTERACTIVE EFFECTS OF FRUIT ZONE LIGHT MANAGEMENT AND APPLIED WATER AMOUNTS ON MERLOT GRAPEVINE PRODUCTIVITY AND PHENOLIC COMPOSITION

A field trial was conducted in the San Joaquin Valley of California on Merlot (Vitis vinifera, L.) to determine the interaction of mechanical leaf removal (control, pre-bloom, post-fruit set) and applied water amounts [sustained deficit irrigation (SDI) at (0.8) and regulated deficit irrigation (RDI) at {0.8 (bud break-fruit set) – 0.5 (fruit set-veraison) – 0.8 (veraison-leaf fall)} of estimated evapotranspiration (ET_c)] on productivity, berry flavonoid content, composition, and unit cost per hectare. The pre-bloom leaf removal treatment consistently maintained at least 20% of ambient photosynthetically active radiation transmittance into fruit zone, while post-fruit set treatment could not. The RDI treatments reduced berry mass, while the post-fruit set treatment reduced berry skin mass. The pre-bloom treatment did not affect yield per meter of row in either year. Flavonoid concentration increased with pre-bloom leaf removal in both years while irrigation treatments had no effect. However, a shift in proportion towards tri-hydroxylated anthocyanins occurred with RDI treatment. Total skin anthocyanins (TSA) were maximized with combination of pre-bloom leaf removal and RDI treatment resulting in ~35% reduction of TSA production cost when compared to no leaf removal and SDI. This study provides fundamental information to red wine grape growers in hot climate regions on how to manage light microclimate to enhance flavonoid concentration and proportion while reducing input costs through mechanization and irrigation amounts.

Michael George Cook
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THE INTERACTIVE EFFECTS OF FRUIT ZONE LIGHT MANAGEMENT AND APPLIED WATER AMOUNTS ON MERLOT GRAPEVINE PRODUCTIVITY AND PHENOLIC COMPOSITION

by

Michael George Cook

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APPROVED

For the Department of Viticulture and Enology

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INTRODUCTION

The majority of wine grapes grown in San Joaquin Valley of California (SJV) are used for bulk wine production. Fruit used to make red wines from this region are characterized by low phenolic accumulation, and receive the lowest price per ton compared to other growing regions in the state. Approximately 34% of the Merlot grapes crushed in the state were grown in SJV with an average grower return of $443/ton compared to $753/ton state average (Cal. Dept. Food. Agric. 2013). In recent years more efforts have been directed towards applying principles of canopy management with the aid of vineyard mechanization and deficit irrigation practices (Kurtural et al. 2013, Terry and Kurtural 2011, Wessner and Kurtural 2013, Williams et al. 2012) to enhance the phenolic profile of red wine grapes grown in the region and improve the grower returns per ton.

The group of flavonoids, consisting of anthocyanins, flavonols, and proanthocyanidins, has considerable implications on berry composition and are collectively synthesized via the flavonoid pathway in red winegrape cultivars. The concentration and relative abundance of monomeric and polymeric flavonoid constituents are variable among red wine grape cultivars due to genetic control and developmental regulation. However, there is general agreement in literature that when amount of diffuse light is increased a beneficial effect is observed with skin anthocyanins, flavonols, and proanthocyanidins of red wine grapes grown in hot climates (Cortell and Kennedy 2006, Dokoozlian and Kliewer 1995). Furthermore, increase in cluster temperature associated with concomitant increase in diffuse light quantity often occurs with amelioration of canopy microclimate (Spayd et al. 2002). Therefore, the effect of sun exposure results from the interaction of several factors that are hardly uncoupled under vineyard conditions.

Leaf removal is a practice that can ameliorate canopy microclimate through improvement of light transmittance into fruiting zone of canopy (Diago et al. 2012, Poni et al. 2006, Wessner and Kurtural 2013, Williams 2012). When leaf removal was applied pre-bloom it was shown to decrease berry set and hence grapevine yield, but improved total skin flavonoid concentration of red wine grapes (Diago et al. 2012, Kemp et al. 2011, Pastore et al. 2013). The results of these studies suggested that yield control was partly responsible for the increase in flavonoid accumulation. Therefore, since growers in SJV are paid in tons produced per hectare, previous work in the hot climate of SJV focused on post-fruit set leaf removal, but was conducted pre-veraison in order to not adversely affect yield (Wessner and Kurtural 2013, Williams 2012). The leaf removal studies conducted in SJV resulted in improved photosynthetically active radiation exposure to canopy interior but no physiological gain for the cultivars studied, some deleterious effects were noted due to overexposure of clusters to direct solar radiation or vegetative compensation response (Geller and Kurtural 2012, Kurtural et al. 2013, Williams 2012).

Water deficits were shown to consistently promote higher concentrations of anthocyanins and flavonols in red wine grapes (Ojeda et al. 2002, Kennedy et al. 2002, Romero et al. 2010, Terry and Kurtural 2011). However, water deficits were shown to have milder effects on proanthocyanidin concentration (Castellarin et al. 2007a, Kennedy et al. 2002, Roby et al. 2004). In any case, there were conflicting results as to whether or not there were any direct effects of water deficit on berry metabolism other than inhibition of berry growth. Matthews and Anderson (1989) reported that growth of berries was inhibited more and concentrations of flavonoids in fruit and wine increased when water deficits were imposed before veraison rather than after veraison. Based on the observation of

similar flavonoid content per berry, Kennedy et al. (2002) and Terry and Kurtural (2011) concluded that post-veraison water deficits only inhibited fruit growth in Cabernet Sauvignon and Syrah vines, respectively. Gene expression studies investigating the regulation of flavonoid biosynthesis in the grapevine concluded that both pre- and post-veraison water deficits directly increased gene expression and accumulation of flavonoids. What is more, water deficits can progressively modify the canopy microclimate by defoliating the basal leaves subtending the fruiting zone, with greater exposure to solar radiation (Terry and Kurtural 2011, Williams 2012). Castellarin et al. (2007b) reported that an increase in flavonol concentration in Merlot following water deficit was due to up-regulation of FLS1 gene possibly as an indirect response to modified microclimate; however, anthocyanin concentration was increased primarily due to direct response of water deficit leading to overexpression of flavonoid synthesis genes, in particular UFGT, CHS2, CHS3, GST, and F3H.

While canopy and crop load management studies (Geller and Kurtural 2012, Kurtural et al. 2013, Terry and Kurtural 2011, Wessner and Kurtural 2013) and irrigation studies, particularly those implementing deficit irrigation, have been conducted in the coastal grape growing regions of California (Matthews and Anderson 1989, Williams 2010, 2014), no such studies have combined both factors on wine grapes cultivated in the hot climate of the SJV of California. The overachieving objective of this trial was to manipulate phenolic profile in order to quantitatively increase the flavonoid concentration of Merlot by investigating the interactive effects of fractions of solar radiation and fractions of water amounts applied in hot climate. The specific objectives of the trial were to improve the light microclimate without adversely affecting yield components while reducing

applied water amounts to quantitatively and qualitatively improve flavonoid composition of Merlot in a resource limited environment.

LITERATURE REVIEW

Grapevine Canopy-Climate Interaction

Numerous factors including soil, climate, and cultural decisions play roles in altering vine physiology and productivity, with consequential effects on wine quality (Smart 1985). However, there is perhaps none more fundamentally critical as climate (Downey et al. 2006, Smart 1985). The term climate can be categorized into three distinct levels: macro, meso, and micro (Smart 1985). Macroclimate is described as the climate of a region such as San Joaquin Valley of California (SJV) while mesoclimate is restricted to a particular vineyard site (Keller 2010). In order to define microclimate the grapevine canopy must first be described. The canopy of a grapevine consists of the above ground portion of the vine formed by the shoot system, which consists of all vegetative and reproductive tissues including the trunk, cordon or canes, shoots, petioles, leaves, tendrils, and fruit (Smart and Robinson 1991). The spatial area where the grapevine canopy and immediately surrounding climate interacts is termed canopy microclimate and is dependent on both canopy architecture configuration and above-ground climate (Keller 2010). As grapevine productivity (i.e. growth, yield, and fruit composition) is contingent on canopy microclimate and because growers are able to manipulate microclimate through viticultural practices, researchers have heavily directed their attention on canopy-climate interaction within the past few decades (Keller 2010).

Canopy Mitigation to Optimize Productivity

Specific guidelines indicating ideal canopy parameters have been developed, which yield an optimum microclimate leading to enhanced vine productivity and maximal berry quality (Smart and Robinson 1991). First,

Reynolds and Vanden Heuvel (2009) determined that canopy leaf layer number should be maintained at 3.0 for non-positioned vines in California. Although Percival et al. (1994) forewarned that this value is adjustable due to phenotypical differences in canopy structure (e.g. leaf size). Second, Smart and Robinson (1991) stated that mean gap percentage should reach 20-40% with 1-1.5 external leaves for optimum light infiltration and cluster exposure. Third, the fruit zone should be near the top or outside of the canopy so that nearly 100% of the fruit is exposed to morning sun in order to maximize anthocyanin and tannin accumulation (Keller 2010).

For canopies that do not meet ideal criterion, an array of viticultural practices have been developed, many of which are currently being improved upon through active research, to ameliorate unfavorable conditions and elicit a desired outcome such as improved phenolic composition, enhanced basic chemical composition, and mitigation of vine imbalance (Downey et al. 2006, Goldammer 2013, Smart and Robinson 1991, Teixeira et al. 2013).

Cultural decisions and canopy management practices are two such categorical strategies that should be utilized collectively to achieve ideal vine performance by not only ameliorating canopy density and distribution of leaf area with the objective of improving microclimate but also by helping to enhance wine quality through the alteration of source-sink relationship or even by inducing expression of particular genes responsible for encoding enzymes related to biosynthesis of secondary metabolites (Downey et al. 2004, Pastore et al. 2013, Poni et al. 2006, Taradaguila et al. 2010, Teixeira et al. 2013). Furthermore, particularly in hot climates such as the SJV, special attention should be taken to allow for optimum microclimate while simultaneously protecting fruit from overexposure to solar radiation and excessive temperatures (Bergqvist et al. 2001,

Poni et al. 2006). Conventional canopy management practices include trellising, dormant pruning, shoot positioning, shoot and cluster thinning, leaf removal, and summer pruning (Smart and Robinson 1991), whereas fertilization management, vine density, irrigation regime, and pest and disease management strategies would be considered cultural decisions (Smart 1985).

Phenology Based Timing and Methodology of Leaf Removal

Leaf removal is a common canopy management practice that is implemented to correct problems such as excessive shading often associated with dense canopies (Bledsoe et al. 1988, Poni et al. 2006, Taradaguila et al. 2010). Traditionally, leaf removal was conducted only after fruit set but before veraison; however, current research has focused on the application of both pre-bloom and post-veraison defoliation (Bledsoe et al. 1988, Gatti et al. 2012, Pastore et al. 2013, Poni et al. 2005). In addition, leaf removal may be conducted manually or through means of mechanization depending on available resources and economic feasibility (Goldammer 2013, Poni et al. 2006). As will be further discussed the diverse configurations of timing, method, and severity of leaf removal have been shown to elicit varying responses affecting both vine physiology and wine quality.

Canopy Architecture and Microclimate

There is agreement in literature that mitigating canopy density can improve microclimate through collective enhancement of light transmission to fruiting zone and appropriate modification of temperature, wind speed, humidity, and evaporation (Keller 2010, Percival et al. 1994, Smart 1985). As would be expected, Pisciotta et al. (2013) demonstrated that solar transmittance into fruit zone increased due to an improvement in canopy porosity by 15 and 3.8% with

traditional (i.e. post-fruit set) leaf removal, conducted by hand or mechanically, respectively. In addition, Pastore et al. (2013) determined that leaf removal carried out at either pre-bloom or veraison reduced total leaf are per vine compared to non-defoliated vines. Intrieri et al. (2008) confirmed this when both hand and mechanical leaf removal significantly reduced total leaf area per vine compared to control. Taradaguila et al. (2010) reported similar results with Graciano and Carignan where pre-bloom and fruit set leaf removal treatments resulted in improved cluster and leaf exposure as well as canopy porosity, regardless of defoliation method.

The magnitude of amelioration of microclimate at time of defoliation is primarily dependent on severity and frequency of leaf removal (Bledsoe et al. 1988, Percivel et al. 1994, Taradaguila et al. 2010). Bledsoe et al. (1988) found that as severity of leaf removal increased, canopy density decreased, allowing more sunlight to penetrate the fruit zone. Typically, hand defoliation is considered more precise and severity much greater than that of mechanical leaf removal (Intrieri et al. 2008, Pisciotta et al. 2013, Taradaguila et al. 2010). For example, Intrieri et al. (2008) noted that mechanical leaf removal only removed 45 and 41% of the total leaf area pulled by hand at pre-bloom and fruit set, respectively. Percivel et al. (1994) found similar results and explained that the reduction in severity compared to hand defoliation was due to the mechanical defoliator's propensity to exclusively strip external leaves. However, manual defoliation is costly and may not be economically feasible therefore necessitating the use of mechanization (Intrieri et al. 2008).

In addition, the lasting effects of defoliation throughout the growing season are further dependent on the response of vegetative compensation. Diago et al. (2012) and Poni et al. (2006) collectively noted an intrinsic vegetative

compensation response following defoliation, with vines recovering post-defoliation and therefore minimizing the duration of improved microclimate throughout growing season. However, Intrieri et al. (2008), Percival et al. (1994), Pisciotta et al. (2013), and Taradaguila et al. (2010) observed that vegetative compensation failed to fully replenish total leaf area per vine in all leaf removal treatments, allowing for improved microclimate for an extended period of time. These differences in vegetative compensation response are contingent on both severity and timing of leaf removal at time of defoliation. For instance, vegetative response was found to be exacerbated with hand defoliation as more leaves were removed per vine compared to that of mechanical defoliation (Pisciotta et al. 2013). Additionally, Diago et al. (2012) noted that reduction in lateral regrowth of mechanical leaf removal compared to that of manual defoliation could be explained by the leaf-removers blowing effect on growing or incipient lateral tips at time of defoliation, preventing further development throughout season. Moreover, results of the effects of phenological timing of leaf removal and vegetative compensation have been corroborated. Taradaguila et al. (2010) did not find any difference regarding vegetative development between pre-bloom and fruit set leaf removal treatments. However, Pastore et al. (2013) concluded that timing was indeed a factor responsible for vegetative regrowth as a compensatory response occurred with their manual pre-bloom leaf removal treatment following defoliation but not with leaf removal implemented at veraison. This was to be expected due to physiological state of vine where vegetative growth was still active at time of pre-bloom and fruit set defoliation, however during veraison lateral growth ceases (Keller 2010).

Yield Components

It is evident that method, severity, and timing of leaf removal have varying effects on yield components and subsequently on berry quality. This is because carbohydrate supply (i.e. source availability) during anthesis is the primary determinate of fruit-set and thus final yield at harvest (Poni et al. 2006). Furthermore, it has been proposed that during anthesis the leaves are the principal source of assimilates rather than overwintering structures (Diago et al. 2012). As a result, the extent to which yield is reduced by leaf removal varies greatly due to the magnitude of altering source-sink relationship (Poni et al. 2006). In general, it has been established that early leaf removal typically elicits a reduction in yield and consequently cluster compactness as sensitivity towards source imbalance is greatest at this phenological stage, while post-fruit set and post-veraison leaf removal treatments often circumvent yield loss (Pastore et al. 2013).

Poni et al. (2006) found that differences in yield due to source imbalance were directly related to severity of leaf removal. This was demonstrated when six basal leaves were removed in Sangiovese resulting in a 5.7% decrease in yield compared to control while in Trebbiano eight basal leaves were removed followed by a 19% reduction in yield (Poni et al. 2006). While in another study, Poni et al. (2005) determined that there was no significant reduction in yield with either pre-bloom or post-bloom leaf removal when every other leaf was removed from nodes one through eight. It should be mentioned that when yield was reduced in the aforementioned studies it was due to a reduction in berry number per cluster (i.e. fruit-set) and berry size. Although results were erratic in the study by Taradaguila et al. (2010), the reduction in berry size was similarly observed in a study by Pallioti et al. (2011) who explained that early leaf removal imposed a temporary stress on canopy foliage, which may have reduced cell division rates in the green

stage of berry growth in addition to decreased berries per cluster. Furthermore, when comparing method of leaf removal, Intrieri et al. (2008) found similar results where early mechanical defoliation reduced yield by only 50% of that of hand leaf removal because of the limited severity associated with mechanical defoliation.

It should be noted that source imbalance may be avoided regardless of severity of leaf removal. This is especially true in warm climates where grapevines often produce far more leaves than required (Percival et al. 1994). In addition, the compensatory response of the vine to defoliation may be adequate in negating potential long-term source inhibition. For example, Poni et al. (2006) stated that even vines defoliated pre-bloom can support berry maturation by the apical leaves and lateral shoots if canopies are large enough in size at time of defoliation. Furthermore, Pisciotta et al. (2013) stated that source inhibition may be avoided since leaves remaining after leaf removal often increase their photosynthetic activity in order to recover from the reduction in total leaf area, meeting the photo-assimilate demand imposed by sink. Nevertheless, it was concluded by Poni et al. (2006) that early leaf removal, when conducted with sufficient severity and under specific climatic conditions, was a prime candidate for yield control and a potential replacement for cluster thinning as it displayed similar improvements in overall berry composition.

As mentioned previously, post-bloom defoliation typically does not decrease percent fruit set and final yield as source imbalance is often avoided (Bledsoe et al. 1988, Poni et al. 2005). However, in hot climates growers are cautioned that a sudden increase in exposure to solar radiation and thus higher temperature may lead to sun burning of clusters and therefore a reduction in yield components (Bergqvist et al. 2001, Palliotti et al. 2011, Pastore et al. 2013, Pisciotta et al. 2013, Poni et al. 2006). This was confirmed by Pastore et al.

(2013) who observed that cluster damage due to sunburn was 5 and 6% higher with post-bloom leaf removal than pre-bloom or control treatments, respectively. Moreover, Williams (2012) cautioned that berry size may also be negatively affected in hot climates, such as the SJV, due to a severe decrease in cluster water potential. It is therefore recommended that precautions be taken in hot climates to protect clusters from overexposure by conducting leaf removal only on the side of canopy which receives morning sun and removing leaves with minimally adequate severity (Keller 2010, Williams 2012).

Finally, skin mass has been shown to possibly play a role in phenolic accumulation and subsequent protection of berry integrity (Diago et al. 2012, Pastore et al. 2013). Pastore et al. (2013) revealed that skin mass increased significantly with pre-bloom leaf removal but not with control or leaf removal conducted at veraison. Furthermore, Diago et al. (2012) and Pallioti et al. (2011) both reported an increase in berry skin mass by 0.7 and 3.6%, respectively, with early defoliation. This was explained by Diago et al. (2012) as a long-term adaptive mechanism as a response to precocious and prolonged exposure of solar radiation on infructescence and clusters. Poni et al. (2008) and Gatti et al. (2012) suggested that alterations in skin mass were heavily dependent on light and temperature, which prevailed over any inhibitory effect of source limitation. The effect of temperature can be directly confirmed by Kliewer (1977) who demonstrated in the cultivar Tokay that skin thickness was reduced during fruit-set when temperatures were held at 40^{o}C compared to 25^{o}C. Consequently, it can be said that under more favorable climates an increase in light and temperature may favor skin development (Diago et al. 2012, Gatti et al. 2012, Poni et al. 2008), however, in hot climates or when microclimate is unfavorable, skin mass may be inhibited (Kliewer 1977, Pastore et al. 2013).

Berry Composition

Although results regarding the effects of leaf removal on total soluble solid content (TSS) have been rather unpredictable, it is widely understood that pre-bloom leaf removal often hastens ripening while leaf removal conducted post-bloom has minimal effects on soluble solid content. Poni et al. (2008) observed a substantial increase in TSS associated with early leaf removal and determined several mechanisms responsible for such a response. The first was linked to the alteration in source-sink relationship of defoliated vines where leaf area to fruit ratio increased therefore indicating that the temporary source limitation caused by leaf removal in their study was offset by subsequent lateral regrowth and decline in yield per shoot. Poni et al. (2008) further stated that source activity of defoliated vines may have also increased due to both an improvement in temporary leaf photosynthetic compensation capacity and lower leaf age following defoliation. Similarly, Intrieri et al. (2008) reported that both early manual and mechanical defoliation improved TSS compared to control with identical reasoning. However, Intrieri et al. (2008) also concluded that changes in source-sink balance due to early leaf removal were shown to promote translocation and assimilation of carbohydrates towards clusters. On the other hand, Taradaguila et al. (2010) reported an improvement in TSS with pre-bloom leaf removal but determined that response to leaf removal was heavily dependent on cultivar and season as TSS was only improved with Graciano and not Carignan and only in one of two years.

The effects of late season leaf removal on soluble solid content is more challenging to elucidate. Main and Morris (2004) concluded with Cynthiana in warm climate that TSS was unaffected by leaf removal treatment conducted 25 days post-bloom. Similarly, Pastore et al. (2013) observed no difference in TSS with leaf removal carried out during veraison. In contrast, Bledsoe et al. (1988)

noted a mean increase in TSS of ~0.45 with leaf removal treatments conducted during fruit set and at veraison compared to control, having explained that a positive improvement in microclimate may have improved photosynthetic assimilation rate or elicited a change in the pattern of assimilate movement. From the complimentary results of Kliewer (1977) and Bergqvist et al. (2001), however, it has been cautioned that in hot climates TSS may indeed increase concomitantly with that of light transmission into fruit zone but once solar radiation levels increase to where ambient temperatures surpass ~37^{o}C, sugar accumulation can be drastically inhibited resulting in decreased TSS.

Results regarding the effects of leaf removal treatment on must pH and titratable acidity (TA) content have been more consistent than that of TSS. Diago et al. (2012) and Pastore et al. (2013) collectively noted that both early and late leaf removal elicited a reduction in TA content and increase in pH. It was cautioned that low TA levels below 6-7 g/L often produce bland wine and will thus need to be adjusted with the addition of tartaric or citric acid (Jackson and Lombard 1993). With these studies it was postulated that respiration rate of berry, being a function of temperature, was the primary factor influencing TA and pH levels, where an increase in temperature to a specific yet unidentified threshold resulting from leaf removal encouraged malic acid degradation. While it was true that Intrieri et al. (2008) found no difference in TA or pH between any treatments, this may be expected because the study was conducted in a cooler climate and therefore an increase in temperature associated with leaf removal may not have been sufficient in significantly degrading acid content. Similarly, Williams (2012) found no difference in TA or pH even though the study was conducted in a hot climate; again, it is possible that as temperatures were already high in this region an increase due to leaf removal may not have significantly altered TA or pH level.

Vine Balance

Vine balance has been defined as the appropriate relationship between vegetative and reproductive growth (Reynolds and Vanden Heuvel 2009), which will allow for consistent production of a healthy yield of high quality fruit (Howell 2001). Two common approaches for assessing vine balance have been utilized with success: ratio of yield to pruning weight (i.e. Ravaz index) and ratio of leaf area to fruit (Howell 2001).

Crop load. Vine balance has been suggested to be optimized when Ravaz index values are in between the range of 5-10 kg/kg for medium vigor vines; however ideal values may vary by cultivar, vigor status, and climate (Smart and Robinson 1991). Furthermore, Ravaz index values higher than the suggested range may be acceptable for *Vitis vinifera* vines if proper canopy microclimate is provided in order that leaf exposure is enhanced sufficient enough to support crop load (Reynolds and Vanden Heuvel 2009). According to Howell (2001), a low or high Ravaz index could lead to poor yields the following season and/or fruit quality not reaching its full potential. Leaf removal has the potential to alter crop load and therefore vine balance but it has been stressed that leaf removal is a temporary seasonal tool which must be incorporated as a part of yearly vineyard management since the positive effects of leaf removal usually last only one season (Smart and Robinson 1991).

Bledsoe et al. (1988) applied leaf removal post-bloom and even though leaves were removed at three different severity levels they were all ineffective in eliciting any source imbalance and therefore yield and pruning weights were unaffected by defoliation treatment and thus no adverse effect on Ravaz. Pallioti et al. (2011) applied leaf removal pre-bloom by removing 80% (i.e. high severity) of leaf area per vine and although final leaf area at harvest remained unaffected

compared to control, due to vegetative compensation, there was a significant delay in vegetative regrowth and a temporary yet severe source imbalance leading to a restriction in cane growth by approximately 19%. This observed reduction in cane growth was successful in moderating vine vigor and optimizing vine balance as vegetative sink competition was lessened (Pallioti et al. 2011). Additionally, Williams (2012) observed in hot climate, that neither pre- nor post-bloom leaf removal reduced pruning weight or Ravaz index, compared to control with values remaining within an optimal range below 12 kg/kg even though yield was slightly reduced due to a brief limitation of source material associated with pre-bloom leaf removal. Consequently, it can be said that when source imbalance is affected severely enough by means of leaf removal to where yield decreases over that of pruning weight, Ravaz will decrease and vice versa. Moreover, Intrieri et al. (2008) alleviated the concern that the negative effect on bud initiation due to source limitation of early leaf removal was fully offset by the improved light microclimate of the basal cane nodes retained at winter pruning for next seasons cropping.

Yield efficiency. The second method of assessing vine balance is taking the ratio of leaf area to fruit, also known as yield efficiency (Howell 2001). Similar to Ravaz, an ideal set of values have been assessed in order to optimize vine balance. Kliewer and Dokoozlian (2004) recommended that a leaf area of 0.8-1.2 m^2 per kilogram fruit is necessary to obtain high yields of ripe fruit while having no detrimental effect on vegetative growth. Likewise, Terry and Kurtural (2013) determined that for a single plane non-positioned canopy a ratio of 1.2 m^2/kg yielded proper vine balance. Yield efficiency is critical for wine quality since low values are inadequate to ripen high quality fruit and high values result in increased

pH (Smart and Robinson 1991). Leaf removal has been shown to alter yield efficiency due to alterations in both final leaf area per vine and yield. Intrieri et al. (2008) determined that neither manual nor mechanical pre-bloom leaf removal had an effect on yield efficiency parameters since both yield and final leaf area were reduced equally, when compared to control. Pastore et al. (2013) found that yield efficiency was raised with pre-bloom leaf removal but not with veraison treatment because although both leaf removal treatments in their study displayed a reduction in final leaf area compared to control, the pre-bloom treatment also decreased yield. On the other hand, Poni et al. (2008) noted that final leaf area per vine was actually higher in pre-bloom leaf removal treatment compared to control due to lateral regrowth but with yield still lower than control, resulting in an increased yield efficiency ratio. Regardless of treatment in the aforementioned studies, yield efficiency was always well within the optimal ranges suggested by Kliewer and Dokoozlian (2005) and Terry and Kurtural (2013). Therefore, the timing, frequency, and severity of leaf removal can be tailored by the grower to not only promote optimal high berry quality for a single season but, through secondary amelioration of vine balance, can be sustained from season to season.

Phenolic Composition

Phenolic compounds, categorized as either flavonoids or non-flavonoids, make up only a small fraction of berry constituents but have enormous implications on organoleptic properties, pigmentation, wine stability, and protection against challenging environments (Teixeira et al. 2013). The flavonoids are comprised of three groups, the flavanols, anthocyanins, and flavonols; all of which follow the flavonoid biosynthetic pathway (Tarara et al. 2008).

Anthocyanins. Anthocyanins are the second most abundant flavonoid compound found in red wine and contribute to color, antioxidative activity, and play a role in photo-protection (Keller 2010). Anthocyanin accumulation and profile in skin of *Vitis vinifera* are determined by synergistic combination of solar radiation and berry temperature, both of which can be directly manipulated by canopy and irrigation management (Cohen and Kennedy 2010, Spayd et al. 2002, Tarara et al. 2008).

Total skin anthocyanins (TSA) consistently improved with increased fruit exposure aided by pre-bloom and/or post-bloom leaf removal treatment (Diago et al. 2012, Pastore et al. 2013). Yield control is one mechanism responsible for improved anthocyanin content (Pallioti et al. 2011). Given the yield restriction often associated with early leaf removal, it has been shown that the seasonal amount of assimilates per unit of crop made available for ripening is often higher for defoliated vines, and has been proposed as a primary cause for improved grape composition as biosynthesis of secondary metabolites are favored (Pallioti et al. 2011, Pastore et al. 2013, Poni et al. 2008). This was confirmed by Intrieri et al. (2008) who suggested that a positive reduction of sinks promoted the translocation of assimilates towards the remaining bunches. In addition, the subsequent response of vegetative regrowth and increase in photosynthetic activity of younger leaves due to defoliation may also increase assimilation rates, further aiding anthocyanin accumulation (Pallioti et al. 2011, Pastore et al. 2013).

The second and perhaps most influential mechanism affecting accumulation and proportion of anthocyanins are the direct and synergistic effects of solar radiation and temperature on clusters resulting from ameliorated canopy microclimate. The general consensus is that low light reduces anthocyanin content of fruit through inhibition of biosynthesis while increased light triggers the up-

regulation of genes necessary for enzymatic processes responsible for anthocyanin biosynthesis (Downey et al. 2006). However, Bergqvist et al. (2001) also noted that when light exposure exceeded 1000 µmol/m^2/sec anthocyanin concentration began to decrease, though this was due to the linear increase in heat gain associated with higher solar radiation, leading to biosynthesis inhibition and possibly even anthocyanin degradation. Similarly, with berries that were well exposed to sunlight a shift in anthocyanin proportion from acylated to non-acylated compounds was encouraged as well as a transition from di-hydroxylation to tri-hydroxylation (Downey et al. 2004, Gao and Cahoon 1994). Haselgrove et al. (2000) further identified a shift within acylated anthocyanins where under elevated light conditions 3-Acetyl-glucoside constituents increased over 3-Coumaroyl-glucosides.

While many studies attribute improvements in grape composition solely to enhanced light filtration into fruit zone, it was Spayd et al. (2002) who separated the effects of light and temperature with Merlot, demonstrating that accumulation of anthocyanin is more of a function of temperature rather than light. In this study, cooling exposed fruit increased anthocyanin content while heating less exposed fruit reduced anthocyanin concentration. It was reported that warm temperatures were necessary for anthocyanin biosynthesis since rate of metabolic processes was encouraged but that excessively high berry temperatures exceeding 30^{o}C were detrimental to synthesis and may have also led to anthocyanin degradation similar to Bergqvist et al. (2001). Furthermore, Tarara et al. (2008) noted that as cluster temperature increased in both shaded and sunlit fruit, acylated anthocyanins represented an increasing proportion of TSA, however when temperatures became extreme, acylation proportion decreased. It is known that acylated anthocyanins are more stable than their non-acylated counterparts and

thus it was theorized that berry temperature may influence the aliphatic and aromatic acyltransferases responsible for acylation to where under increased temperature the vine shunts more available anthocyanins towards acylation to improve stabilization under temperature stress (Tarara et al. 2008). Likewise, Downey et al. (2004) and Spayed et al. (2002) also reported that as temperatures increased, 3-Coumaryol-glucosides increased over that of 3-Acetyl-glucosides. Therefore, in contrast to the response of other higher plants, the accumulation of anthocyanins in *Vitis spp*. was determined not to be completely dependent on light and although influential, is not an absolute requirement (Cohen and Kennedy 2010, Downey et al. 2004, Spayd et al. 2002). Downey et al. (2004) further concluded that two systems are responsible for regulating anthocyanin accumulation in grapes, an initial and constitutive system that generates a base level of anthocyanins and an inducible system that is light-requiring. In summary, amelioration of canopy microclimate yielding positive improvements in solar radiation and temperature through precocious defoliation may have both a positive and direct effect on anthocyanin biosynthesis and shift in proportion as well as an indirect effect on accumulation resulting from alterations in the physiological development of yield components.

There was a third mechanism which may have influenced anthocyanin concentration. Diago et al. (2012) suggested that an increase in relative skin mass of the grapevine as a response to precocious exposure of the flower cluster infructescence and clusters to solar radiation in both pre- and post-bloom leaf removal treatments could have possibly aided in anthocyanin accumulation potential. Poni et al. (2008) concluded similar reasoning with Barbera, noting that the increase in relative skin mass associated with pre-bloom defoliation matched the increase in anthocyanin concentration ($R^2 = 0.70$) regardless of overall berry

size. Comparably, skin mass was enhanced with pre-bloom leaf removal in a study by Pastore et al. (2013), however, skin mass of veraison leaf removal treatment was lower than control and pre-bloom due to overexposure and sunburn incidence. The increase in skin mass was significant in influencing anthocyanin accumulation potential as it is understood that anthocyanins are stored after glycosylation in the vacuoles of the outer and inner hypodermal layers and therefore a reduction of vacuolar space may lead to a subsequent decrease in anthocyanin accumulation potential, as was observed in the veraison treatment of Pastore et al. (2013).

Flavonols. Contrary to the synergistic relationship observed with anthocyanin accumulation, it has been well established that UV-protecting flavonols are synthesized primarily in response to the incidence of visible and ultra-violet radiation (Downey et al. 2004, Spayd et al. 2002, Tarara et al. 2008). Thus it has been proposed by Downey et al. (2004) that only the branch of the flavonoid pathway leading to flavonol biosynthesis is light-dependent. Spayd et al. (2002) verified the significance of ultra-violet radiation by implementing UV barriers on clusters, concluding that ultra-violet radiation, while not an absolute requisite, greatly influenced the content of aglycones and glycosylated forms of flavonols in skin, especially those of quercetin.

Activation of respective flavonol genes leading to enzymatic biosynthesis typically occurs during anthesis and again at veraison under normal growing conditions (Cortell and Kennedy 2006). Therefore, it was to be expected that when a positive increase in *PAR* transmittance, and subsequently ultra-violet radiation, followed pre-bloom and post-bloom defoliation, an activation of transcription factors led to the expression of genes encoding the two isoforms of the enzyme flavonol synthase (FLS), as was reported by Pastore et al. (2013).

Furthermore, while fluctuations in berry skin mass were positively correlated to anthocyanin accumulation, flavonol accumulation was not (Diago et al. 2012, Downey et al. 2004). This is because of the specific locations where anthocyanins and flavonols are stored. Diago et al. (2012) stated that since flavonols accumulate in the vacuoles of the epidermal and outer hypodermal layer of the skin they are more localized compared to anthocyanins, making them less sensitive to modifications in skin mass. These findings were validated by Pastore et al. (2013) who indicated that even though skin mass increased with pre-bloom leaf removal and decreased with post-bloom leaf removal compared to control, flavonol concentration was comparably higher in both defoliation treatments.

Flavanols. Proanthocyanidins (i.e. condensed tannins) are comprised of flavan-3-ol subunits and are perhaps the most abundant and stable flavonoids under diverse growing conditions (Teixeira et al. 2013). They accumulate both in berry skin and seed, being collectively responsible for mouthfeel characteristics and stability of wine matrix (Pastore del Rio and Kennedy 2006). Biosynthesis of skin proanthocyanidin is heightened during flowering with accumulation continuing until two weeks post-veraison while accumulation of seed tannins immediately follows fruit-set and lasts until veraison (Downey et al. 2006).

Just as solar radiation and/or temperature affected anthocyanin and flavonol concentration, a handful of studies have elicited a similar effect on monomeric and polymeric proanthocyanidins in skin. For example, positive correlations between solar radiation and skin proanthocyanidin concentration were observed with Cortell and Kennedy (2006) and Scafidi et al. (2013) where concomitant increase in exposure to solar radiation enhanced proanthocyanidin content at veraison and successfully carried through harvest. On the other hand, Downey et al. (2004)

noticed increased proanthocyanidin concentration of berry skin with exposed treatment at veraison but differences between shaded and exposed fruit diminished at harvest. Similarly, Pallioti et al. (2011) observed a significant increase in total tannin concentration in wine with pre-bloom leaf removal treatment due to mitigation of unfavorable microclimate. Kemp et al. (2011) further differentiated responses between final monomeric and polymeric proanthocyanidin concentration in wine where monomers were enhanced with early defoliation whereas polymeric proanthocyanidins were not. Kemp et al. (2011) explained that monomeric but not polymeric proanthocyanidin biosynthesis could have been dependent on cluster exposure and that early season cluster exposure favored catechin and epicatechin biosynthesis by increasing leucoanthocyanidin-reductase (LAR) and/or anthocyanin-reductase (BAN) activity. Therefore, obtaining any benefit through leaf removal must be timed appropriately to coincide with tannin biosynthesis (Kemp et al. 2011).

While the effects of solar radiation may be fairly straightforward, the response of proanthocyanidin accumulation to temperature has been more difficult to elucidate. Cohen et al. (2008) concluded that proanthocyanidin content was not affected by temperature differences (i.e. ambient, heated, or cooled) across three years when analyzed at veraison and again during harvest with Merlot. Although concentration was not affected in their study, composition was where fewer degree days favored a shift towards tri-hydroxylated skin proanthocyanidins. Pastore del Rio and Kennedy (2006) suggested that temperature may indeed affect biosynthesis and tannin interactions by pointing out with Pinot noir that final skin proanthocyanidin content was higher between seasons as a result of higher heat-summation. Additionally, while it was previously made known that Scafidi et al. (2013) observed an increase in tannin concentration of berry skin with

concomitant increase in solar radiation, the positive and linear increase in temperature associated with solar radiation was also attributed to having improved proanthocyanidin content of the white cultivar Grillo.

It was illustrated by Teixeira et al. (2013) that skin proanthocyanidins were more sensitive to changes in environmental conditions compared to those located in seed. This finding is corroborated by both Cortell and Kennedy (2006) and Downey et al. (2004) who determined that light had no quantifiable effect on seed concentration or composition of either free monomeric or polymeric proanthocyanidins at harvest. Furthermore, Cohen et al. (2008) reported that seed proanthocyanidins were not affected by variations in diurnal temperature. However, when comparing temperature variations among seasons, Pastore del Rio and Kennedy (2006) observed a reduction in seed flavan-3-ol monomer amount in warmer seasons compared to cooler seasons. Interestingly, the cumulative decrease in seed monomeric and polymeric proanthocyanidin content associated with these studies may be beneficial to winemakers as extraction potential of bitter tannins would be reduced (Cortell and Kennedy 2006). Nevertheless, it would be expected that an improvement in microclimate resulting from leaf removal may have minimal effects on seed proanthocyanidin content and composition.

Phenology Based Timing and Methodology of Deficit Irrigation

Vine water requirements are dependent upon evaporative demand at location of vineyard, stage of vine development, and percent shaded area of vine canopy, regardless of grape cultivar (Williams 2001). Good irrigation management is required for efficient and profitable use of water that will yield high quality fruit by ensuring that the vines are neither over nor under irrigated (Goldammer 2013). A major part of any management program is the decision

making process for determining irrigation scheduling (Goldammer 2013). Scheduling methods are soil-based, vine-based, or weather-based and are often utilized in various combinations by growers to determine irrigation rate and timing (Goldammer 2013). Irrigation should only applied when precipitation is inadequate, enabling the control of soil water availability, and therefore the vine water potential at various developmental stages (Bravdo 2001).

Deficit irrigation is a cultural decision that exposes the grapevine to varying degrees of water deficit during critical phenological stages (Romero et al. 2010). The effect of water deficit depends on vine phenological stage and the severity of the stress imposed (Williams 2012). Furthermore, grape response to deficit irrigation regime is also cultivar-dependent as *Vitis vinifera* varieties have been shown to respond differently to water stress (Teixeira et al. 2013). Water deficits are commonly applied at two phenological periods in order to reduce water consumption and enhance berry quality. Water deficits early in season, from fruit set to veraison, can control berry size and reduce vine vigor (Keller 2010). Water deficits implemented after veraison can increase the biosynthesis of anthocyanins and other phenolic compounds (Kennedy et al. 2002). A disadvantage of deficit irrigation is that it requires water status to be maintained within a narrow tolerance range and over- or under-irrigating can undermine associated benefits, even leading to severe yield and quality loss (Romero et al. 2010). Therefore, prudent irrigation scheduling is necessary (Romero et al. 2010, Williams 2012).

Canopy Architecture and Microclimate

Soil water deficits have the ability to reduce vegetative growth of grapevines, resulting in smaller canopies with less leaf area per vine and a fruiting zone less congested with foliage (Williams 2012). Thus, some of the effects of

deficit irrigation may result from an improved microclimate within the fruiting zone similar to those resulting from leaf removal. However, comparable to that of leaf removal, timing and severity of regulated deficit irrigation has been shown to affect the magnitude of these responses (Romero et al. 2010). Hamman and Dami (2000) implemented three irrigation regimes supplying either 100% (control), 50%, or 25% of evaporative demand, which was sustained from bloom through harvest, with the latter two treatments eliciting a reduction in shoot growth and canopy height. Similarly, Shellie (2006) detected a decline in main shoot growth with Merlot as leaf water potential surpassed -1.0 MPa. In their study the increase in severity of water deficit consistently corresponded with a linear increase in light transmission into fruit zone as a result of reduced vegetative growth. Shellie (2006) concluded that the sensitivity of main shoot growth to water deficit of Merlot, even under varying soil and climatic conditions, suggested that early-season water deficits could be used in regions with little or no precipitation during growing season to reduce vegetative growth and improve microclimate. Williams (2012) went a step further and compared the responses of sustained water stress (40% and 80% of evaporative demand) to a surplus treatment of 120% estimated crop evapotranspiration (ET_c) implemented throughout the entire season with Merlot in SJV. He concluded that the surplus irrigation favored excessive vegetative growth, as measured by percent shaded area, leading to the lowest light transmission into fruiting zone while their most severe deficit treatment of 40% ET_c displayed the lowest percent shaded area and the highest light transmission. Conversely, Terry and Kurtural (2011) determined that early RDI carried out from fruit set to veraison and replacing 50% of evaporative demand was most effective in reducing leaf layer number in hot climate. Regardless of timing, Romero et al. (2010) warned that when water deficits were severe enough, in their case being

reduced to 15% ET_c, leaf number decreased deleteriously in response to the acute decline in long-term photosynthetic capacity resulting from a qualitative loss in photosynthetic apparatus and/or damage to the biochemical photosynthetic machinery.

Yield Components

Reproductive growth is also sensitive to vine water status, again being dependent on timing and severity of water deficit regime. Hamman and Dami (2000) noticed a dramatic reduction in berry and cluster mass leading to lower yields. In their study the severe water deficit treatment that supplied 48 L/vine/week prompted the lowest yield while the moderate water deficit treatment (96 L/vine/week) had yields similar to control (Hamman and Dami 2000). Similarly, yield was reduced by up to 48% under deficit irrigation with Shellie (2005) who determined that lower yields were associated with smaller berries, lower cluster mass, and fewer clusters per vine. Again, the more severe water deficits reduced yield components more drastically than the moderate irrigation regime (Shellie 2005). Furthermore, their regulated deficit treatment that supplied 35% of ET_c until veraison and 70% of ET_c following harvest performed similarly to the moderate deficit treatment; thus it was determined that alleviating water stress severity post-veraison may compensate for undesirable yield reductions (Shellie 2005). Ojeda et al. (2002) further separated effects of water deficit based on phenological timing, where a decrease in berry mass was more pronounced in their early deficit treatment applied from anthesis to veraison than for late water deficit treatment applied during veraison to maturity. These findings were corroborated by Castellarin (2007a) and Keller et al. (2008). Moreover, pulp mass was reduced in all water deficit treatments leading to a higher skin-to-pulp weight

ratio, however, skin mass was only reduced when the water deficit was applied during anthesis and at severe enough level (Ojeda et al. 2002). In hot climate, Williams (2012) noted a reduction in berry mass by 4 and 31% with the 80% ET_c and 40% ET_c irrigation treatments compared to surplus treatment of 120% ET_c, respectively. Yield followed a similar pattern and was thought to be due to lower cluster water potential, with the 40% ET_c treatment having the lowest cluster water potential and surplus treatment the highest (Williams 2012).

Berry Composition

Water deficits had no significant effect on juice pH in studies conducted by Castellarin et al. (2007b), Keller et al. (2008), Romero et al. (2010), and Williams (2012), however, Terry and Kurtural (2011) noted with Syrah that juice pH of their late water deficit treatment was 5% lower compared to early deficit and control treatments. Similarly, severe water deficit lowered pH level in wine in a study conducted by Hamman and Dami (2000). The decrease in juice and wine pH levels could have possibly been a response of shade mitigation caused by severe water deficit (Hamman and Dami 2000, Terry and Kurtural 2011, Smart 1985).

While TA content in must remained unaffected by water deficit in a study by Hamman and Dami (2000) and Romero et al. (2010), Shellie (2006) and Williams (2012) noted a reduction in must TA as severity of water stress increased. Shellie (2006) attributed this to malic acid degradation due to an increase in light transmission, temperature, and therefore respiration rate, whereas Williams (2012) stated that differences may have been due to variability in berry maturity when sampled on similar dates because at harvest the TA of must was similar in two of three years.

The effect of water deficit on soluble solid content was similar to the often unreliable response of pH and TA content. Whilst Castellarin (2007b) and Keller et al. (2008) did not observe any difference in TSS with deficit irrigated Cabernet Sauvignon vines, Castellarin (2007a), Shellie (2006), and Williams (2012) observed a slight increase in TSS with Merlot vines at harvest under moderate and severe water deficits. Castellarin (2007a) attributed the increase in TSS of both early and late deficit irrigation treatments to a perpendicular reduction in berry mass. Similarly, Shellie (2006) determined that a difference in TSS content among irrigation regimes was only apparent in their first year since it was the only season which had a substantial variation in yield per vine. Furthermore, Shellie (2006) concluded that their most severe treatment (35% ET_c) had the highest soluble solid content because sugar accumulation was promoted by the substantial reduction in yield and increase in canopy light transmission. However, Roby et al. (2004) proposed that the increase in sugar accumulation may also reflect a partitioning response to water deficit and was not necessarily dependent on berry size. This was substantiated by Romero et al. (2010) who noted that TSS decreased with moderate and severe water deficit treatments post-veraison even though berry size was reduced suggesting that the substantial loss in canopy leaf area due to intense leaf abscission superseded any benefit of reduced berry size as photosynthetic capacity was heavily limited. Moreover, Hamman and Dami (2000) noticed that while previous studies suggested that late season reductions in irrigation could increase TSS, results from their study indicated that their most severe irrigation treatment actually reduced TSS compared to moderate water deficit regime or control. Hamman and Dami (2000) continued to explain that the vines under their greatest deficit irrigation regime (T-3) likely experienced acute water stress causing a physiological adjustment that resulted in stomatal closure,

which in their case led to reduced photosynthesis and decreased levels of soluble solids. Therefore, it could be stated that moderate water deficits often promote sugar accumulation; however, water deficits may illicit stomatal closure or excessive leaf abscission leading to an overall reduction in TSS content if too severe.

Vine Balance

Water deficits have the potential to positively affect vine balance in arid climate as both vegetative and reproductive development can be manipulated; however the magnitude of vine balance amelioration is heavily dependent on proper timing and appropriate severity level (Romero et al. 2010).

Crop load. In a study conducted by Hamman and Dami (2000) it was determined that both water deficit treatments reduced pruning weight, as canopy density was mitigated in season, leading to an increased Ravaz index, as would be expected. Williams (2012) noted a similar linear reduction in pruning weight as severity of water deficit increased to 40% of ET_c, however, Ravaz index slightly decreased with their severe water deficit treatment because of an associated reduction in yield. Furthermore, Terry and Kurtural (2011) determined that neither water deficit treatment in their study affected pruning weight when analyzed as a main affect. Nevertheless, early and late regulated deficit irrigation (RDI) decreased Ravaz index by 68 and 18%, respectively. The substantial decrease in Ravaz index correlated with early RDI was due to a 27% reduction in yield. Regardless of the effect of RDI on Ravaz index, all irrigation treatments in their study remained within the optimal crop load range for warm climate. Conversely, in a five year study by Keller et al. (2008) it was noted that in three of five years early RDI reduced pruning weight but had no effect on final yield

obtaining a higher Ravaz index compared to late RDI treatment or control. Still, similar to Terry and Kurtural (2011) vines in their study were within optimal crop load range and were able to successfully support and ripen a crop, even under relatively severe water deficits (Keller et al. 2008). Therefore, it is apparent that pruning weight is most affected by early water deficit stress and is even further reduced when severity of water deficit is increased (Keller et al. 2008). In addition, since vine balance is dependent on both vegetative and reproductive growth, the effect of water deficit on yield components are critical and its influence on yield may supersede that of vegetative growth (Romero et al. 2010).

Yield efficiency. Effects of water deficit have been shown to be less significant regarding yield efficiency. For instance, Terry and Kurtural (2011) stated that neither early nor late water deficits affected the ratio of leaf area to fruit in either year of their study since RDI had no significant effect on total leaf area per vine and although yield was reduced with early RDI, it was not sufficient in altering yield efficiency. Again, regardless of irrigation treatment, all vines were well within the optimal yield efficiency range of 0.8-1.2 m^2/kg, indicating there was sufficient leaf area to ripen the crop. These results were nearly identical to Keller et al. (2008) who determined that in four of five years water deficits had no effect on yield efficiency as final yield per vine remained unaffected and even though final leaf area at harvest was slightly reduced with early and late RDI treatments it was not enough to alter the yield efficiency ratio.

Berry Phenolic Composition

The accumulation of phenolic compounds can be impaired or ameliorated by imposing plant water stress (Castellarin et al. 2007b). Therefore, irrigation management, particularly in arid areas, has been shown to be one of the largest

and most controllable determinants of secondary metabolite accumulation and composition (Romero et al. 2010).

Anthocyanins. Water deficits have consistently promoted higher anthocyanin concentration in red winegrapes; however, the compounding factors of timing and severity have varying direct and indirect effects on anthocyanin accumulation and composition (Castellarin et al. 2007a, Kennedy et al. 2002, Romero et al. 2010). For example, Bucchetti et al. (2011) noted that water deficit (WD) increased anthocyanin concentration of Merlot in all four years of their study, attributing this to an increase in content per berry resulting from a triggering of biosynthesis as well as an increase in concentration per fresh weight due to reduced fruit growth. Similarly, Castellarin et al. (2007b) compared water deficit treatment (WS) to control, noting that WS vines not only attained higher anthocyanin concentration but also a proportional shift from di- to tri-hydroxylated anthocyanins. Moreover, color hue of WS berries became more purple-blue as a result of increased hydroxylation and methoxylation of anthocyanins. It was explained that the improvement in anthocyanin content and composition associated with WS berries was not strictly due to a reduction in berry growth, partial water loss or concentration of dry matter, but was substantially enhanced by active induction of anthocyanin biosynthesis. Castellarin et al. (2007b) went on to state that because plant water stress progressively modified canopy architecture by a reduction of basal foliage of shoots were vines bore bunches, one could have argued that the alteration in anthocyanin biosynthesis was partly due to light-mediated effects. However, as the biosynthetic up-regulation and accumulation of anthocyanins occurred before leaf layer number and percentage of cluster exposure became significantly different between water deficit and control

treatments, it was concluded that plant water stress also increased anthocyanins directly through genetic up-regulation. Therefore, the effects of direct and indirect genetic up-regulation and the increase in skin-to-pulp ratio collectively contributed to improved anthocyanin concentration and shift in composition in their study.

Additionally, Romero et al. (2010) observed an increase in color intensity with both moderate and severe RDI treatments compared to control having also contributed this to an improvement in cluster exposure rather than solely to an increase in skin-to-pulp ratio associated with reduced berry mass. However, differences in anthocyanin content were observed between water deficit treatments. The more severe RDI treatment yielded lower extractable anthocyanins compared to moderate RDI even though canopy microclimate was similar and berry mass remained lower. The ineffectiveness of the more severe water deficit was reported to be due to a combination of intense leaf abscission and diminished photosynthetic capacity which superseded any benefit obtained by a reduction in berry mass or amelioration of canopy microclimate. Thus, it was concluded that moderate water deficits were shown to be more effective in improving anthocyanin content in skin compared to severe water deficit (Romero et al. 2010). Interestingly, Roby et al. (2004) confirmed the findings of aforementioned studies, stating that water deficit may have more than just an indirect effect on anthocyanin content through reduction of berry mass. In their study the group compared berries of similar size reducing the effects of dilution, concluding that anthocyanin concentrations were nearly always higher with water deficit treatments and lower with excess irrigation. In addition, Zarrouk et al. (2012) suggested that anthocyanin accumulation is also environment dependent, having cautioned that while their sustained deficit and regulated deficit treatments produced high anthocyanin content in Tempranillo vines, the non-irrigated

treatment, being their most severe water deficit treatment, yielded poor color development in the hot climate. This was because the non-irrigated treatment was too severe, leading to over-defoliated vines that deleteriously amplified sunlight and temperature exposure into fruiting zone causing biosynthetic inhibition and possibly anthocyanin degradation.

When comparing timing of water deficit, Castellarin et al. (2007a) stated that anthocyanin concentration was increased by both early and late water deficits; however, the concentration was greatest with early water deficit (ED) vines. This was because ED vines induced genetic expression and up-regulation of F3'H, F3'5'H, DFR, and LDOX enzymes earlier than late water deficit or control treatments. Moreover, both water deficit treatments increased expression of F3'5'H enzyme resulting in a shift from di- to tri-hydroxylated anthocyanins. However, it was not determined whether these alterations in content and composition were due to the direct effect of water deficit on biosynthesis or through indirect effects such as improved microclimate or reduced berry mass.

Finally, Ojeda et al. (2002) stated that berry skin mass is another contributing factor possibly responsible for determining final anthocyanin accumulation, and while many water deficit studies did not quantify skin mass, it was cautioned that reductions in skin mass due to severe water deficit, particularly during the green growth stage from anthesis to veraison, could restrict anthocyanin accumulation potential.

Flavonols. It was determined that water deficit generally had a much milder effect on flavonol accumulation compared to that of anthocyanin accumulation (Castellarin et al. 2007a). Moreover, while flavonol concentration often increased

under drought stress, overall response is greatly reliant on cultivar (Teixeira et al. 2013).

Ojeda et al. (2002) showed that water deficit influenced biosynthesis and concentration of flavonols in skin, where both their moderate water deficit treatment applied from anthesis to veraison (early) and strong water deficit treatment imposed from veraison to maturity (late) prompted the greatest flavonol accumulation. Ojeda et al. (2002) suggested that this increase was partly due to an increase in skin-to-pulp ratio as similarly observed with anthocyanins. In addition, as flavonol biosynthesis is very sensitive to light exposure (Downey et al. 2004), it was theorized that perhaps water deficit elicited an indirect response by reducing canopy leafiness and improving sunlight exposure rather than a direct response on biosynthesis (Ojeda et al. 2002). This theory was corroborated by Castellarin et al. (2007b) who determined that genetic expression of FLS enzyme was linearly correlated with total radiation ($R^2 = 0.88$) where both solar radiation and flavonol accumulation was greatest with WS vines compared to control. Intriguingly, in a study conducted by Kennedy et al. (2002), flavonol concentration was not affected by irrigation regime when expressed on a per berry basis or on a fresh weight basis; however a connection is difficult to make as light transmittance was not assessed.

Flavanols. Few studies have reported that water deficit may modify skin proanthocyanidins (Teixeira et al. 2013). Bucchetti et al. (2011) determined with Merlot that water deficit treatment (WD) was much more effective in increasing anthocyanins than tannins since WD only increased skin tannin concentration as a result of reduced berry growth and not content per berry due to biosynthetic up-regulation. Similarly, Kennedy et al. (2002) observed an increase in catechin and

mean polymeric proanthocyanidins on a per weight basis with minimally irrigated vines compared to standard irrigation practices, concluding that the pronounced effect of irrigation stress on berry size indicated it was the predominant factor affecting tannin concentration. However, it was also proposed that irrigation stress may have a positive effect on reducing vine vigor, which may consequentially improve canopy microclimate, indirectly promoting biosynthesis (Downey et al. 2006, Kennedy et al. 2002). Roby et al. (2004) continued to note that increased skin tannins accompanying water deficits in their study appeared to result more from differential growth sensitivity of the inner mesocarp and exocarp than from indirect effects on reduced berry size or direct effects on phenolic biosynthesis. Still, Roby et al. (2004) did not dismiss the possibility of a direct or indirect stimulation of biosynthesis resulting from water deficit. Furthermore, Ojeda et al. (2002) determined that moderate pre-veraison and strong post-veraison water deficits were effective in increasing monomeric and polymeric proanthocyanidins and mean degree of polymerization, accompanied by a small loss in berry size. On the contrary, Castellarin et al. (2007a) showed that while early water deficit enhanced the expression of LAR2 enzyme prior to veraison, changes were minor and during ripening there were no differences in mean proanthocyanidin concentrations between treatments. In conclusion, Teixeira et al. (2013) alluded that while water deficits have often been shown to increase skin proanthocyanidins, more studies are necessary to further clarify the mechanisms that are responsible for such an increase.

The effects of water deficit on seed proanthocyanidins have been difficult to elucidate (Cohen and Kennedy 2010). Roby et al. (2004) concluded that water deficit had no clear effect on seed tannin concentration even though there was a linear function between seed tannin content and seed number, as well as seed mass

per berry. Roby et al. (2004) further described the relationship between seed tannin concentration and berry size by affirming that there was a two phase response, an initial decrease in tannin content and then an increase as berry size increased. Comparably, Kennedy et al. (2000) also noted that seed growth and polyphenols per berry were less sensitive to water deficits. However, although there were no significant effects of water deficits on seed proanthocyanidins or their polymerization, with all treatments following second-order kinetics, minimal water deficit did decrease the amount of monomeric proanthocyanidins, greatly increasing their rate of loss during fruit ripening (Kennedy et al. 2000). Moreover, the indirect effects of ameliorated microclimate resulting from water deficit may behave similarly to that of leaf removal, as previously discussed.

MATERIALS AND METHODS

Site Description and Climatology

The experiment was conducted at a commercial vineyard planted with 'Merlot' (clone 1) × Freedom (27% *V. vinifera* hybrid) rootstock in 1998. The vine spacing was 2.13 m × 3.35 m (vine × row) in North-South orientation. The research site was located in Merced County, CA (37°33.181'N 120°39.665'W, elevation 71 m) and was planted on Whitney and Rocklin Sandy-Loam soil, a fine-loamy, mixed, active, thermic Mollic Haploxeralf and described as a well-drained soil formed in alluvium derived from granite (www.nrcs.usda.gov/). The vines were head trained 1.1 m above vineyard floor with two catch wires at 1.3 m and 1.6 m and cane pruned to six, eight-node long canes, and allowed to sprawl. The vineyard was drip-irrigated with pressure-compensating emitters spaced at 1.1 m with two emitters per vine delivering 1.89 L/h each.

Vineyard crop evapotranspiration (ET_c) was estimated as the product of reference evapotranspiration (ET_o) and seasonal crop coefficients (K_c) (Allen et al. 1998). The reference ET_o was obtained from the California Irrigation Management Information System (CIMIS) weather station (#206) in Denair, CA. The amount precipitation received, and the additional irrigation amounts were recorded weekly. The seasonal K_cs used to schedule irrigation at this site were developed by measuring the shade cast on the vineyard floor beneath the canopy of vines irrigated at 0.8 ET_c (SDI) treatments at solar noon weekly. The shaded area beneath the canopy was determined by counting the number of equi-distant 0.01 m^2 cells on an 18 m^2 grid and summing their area. The growing degree days (GDD) were calculated using the sine method with a threshold of 10°C with data

obtained from CIMIS. All other cultural practices were carried out according to commercial industry standards for that area.

Experimental Design

The experiment was a three (leaf removal) × two (deficit irrigation) factorial with a split-plot design with four replicated blocks. Three rows of 190 vines each comprised one block and four guard rows separated each block. The three leaf removal treatments were randomly applied as main plot to three rows each. Each main plot of three rows was split into two deficit irrigation treatments as sub-plot at random, in the geographic middle on the East-West axis of the vineyard. Each experimental unit consisted of 285 vines of which 48 were sampled from an equi-distant grid per treatment-replicate.

Leaf Removal Treatments

There were three leaf removal treatments applied. An untreated control consisted of no leaf removal. A pre-bloom leaf removal treatment was applied on the East side of the canopy mechanically, at 200 GDD (EL-Stage 17) with a roll-over type leaf remover with a perpendicularly mounted sickle-bar clipper adapted for sprawling-type canopies (Model EL-50, Clemens Vineyard Equipment, Woodland, CA) in both years. The leaf remover was also equipped with a centrifugal fan that dislodged defoliated leaves with air-assistance. The leaf remover opened a 50 cm window in the fruiting zone of the canopy. The post-fruit set leaf removal treatment was applied at 540 GDD and 644 GDD (EL-Stage 29) in 2013 and 2014, respectively.

Deficit Irrigation Treatments

There were two irrigation treatments applied. A control treatment of sustained deficit irrigation (SDI) at 0.8 of estimated ET_c was applied from anthesis until harvest (EL-Stage 38) with a mid-day leaf water potential (Ψ_l) threshold of -1.2 MPa. A regulated deficit irrigation (RDI) treatment was applied at 0.8 ET_c from anthesis to fruit set (EL-Stage 28) with a Ψ_l threshold of -1.2 MPa, 0.5 ET_c from fruit set to veraison (EL-Stage 35) with a Ψ_l threshold of -1.4 MPa and at 0.8 ET_c from veraison until harvest with a Ψ_l at -1.2 MPa. Irrigation treatments in each year were not initiated until Ψ_l reached -1.0MPa for vines in the 0.8 ET_c treatments.

Data Collection and Laboratory Analysis

Water Status Determination

The water status of the grapevines throughout the growing season was monitored weekly by measuring the Ψ_l. One fully expanded leaf, exposed to the sun showing no sign of disease or damage was selected. A zip-top plastic bag was placed over the single leaf and sealed before the petiole was excised in order to suppress transpiration. Ψ_l was then directly determined with the use of a pressure chamber (Model 610 Pressure Chamber Instrument., PMS Instrument Co., Corvallis, OR).

Canopy Architecture and Microclimate

Four point quadrat measurements were collected three times during the growing season at 225 GDD (EL-Stage 18), 950 GDD (EL-Stage 34), and 1400 GDD (EL-Stage 36) in both 2013 and 2014. Canopy architecture was quantified by measuring exterior leaf, interior leaf, and gap number with five insertions per

sample vine at 30 cm intervals as reported elsewhere by Wessner and Kurtural (2013). Calculation of leaf layer number was described by Smart and Robinson (1991). The percentage of photosynthetically active radiation (*PAR*) intercepted in the fruiting zone was measured for sprawling type canopies as reported by Kurtural et al. (2013) with a handheld ceptometer (AccuPAR-80, Decagon Devices, Pullman, WA).

Yield Components

Yield components were measured on a single harvest (EL-Stage 38) date on 26 August 2013 (1660 GDD) and 19 August 2014 (1740 GDD) as the fruit reached 24 Brix. Each treatment replicate was harvested manually. Yield/m and clusters/m were collected and weighed. The mean cluster mass was calculated by dividing yield/m by clusters/m. One hundred berries were randomly collected and weighed using a Mettler-Toledo analytical top-loading scale (ML 104, Mettler-Toledo International Inc., Columbus, OH) to calculate mean berry mass. The mean berry number per cluster was calculated by dividing mean cluster mass by mean berry mass.

Yield Efficiency, Crop Load, and Labor Costs

Measurement of exposed leaf area/m was conducted once during the growing season at EL-Stage 36. A one-meter section of the canopy was randomly selected (Terry and Kurtural 2011) where shoots were counted and destructively harvested. Exposed leaf area/m was measured with a leaf area meter (LI-3000; LI-COR, Lincoln, NE) and was calculated based on previous methods as described elsewhere (Kurtural et al. 2012). Yield efficiency was calculated by dividing leaf area of each sample vine by yield per vine and expressed as m^2/kg. Dormant

pruning was conducted on 9 January 2013 and 10 January 2014 with pruning mass from each data vine measured in kg. The Ravaz index was expressed as the ratio of yield per vine to the dormant pruning mass in kg/kg.

Labor operation costs were extrapolated to determine the cost to produce one gram of total skin anthocyanin per hectare, which was calculated by taking total labor operations cost of cultural practices and cost of irrigation amount for one megaliter (ML) per hectare (Kurtural et al. 2012) divided by total skin anthocyanin content on a per hectare basis.

Berry Composition Analysis

The berry total soluble solids (TSS), measured as degree Brix, juice pH, and titratable acidity (g/L, as tartaric acid, TA) were analyzed from a one-hundred randomized berry sample collected from each treatment replicate during harvest. The protocol of sample collection and chemical analysis was described by Terry and Kurtural (2011). The TSS was measured with a digital refractometer (ATAGO PR-32 Pallette digital refractometer; ATAGO USA, Bellevue, WA). Juice pH was determined with a glass electrode pH meter (Accumet 13-620-183A AB15; Fisher Scientific, Pittsburg, PA). The TA was analyzed by titrating to an endpoint pH of 8.2 with 0.1N sodium hydroxide and values expressed in grams per liter with a Mettler-Toledo DL15 endpoint titrator (Mettler-Toledo International Inc., Columbus, OH).

Berry Phenolic Composition

The phenolic composition of berry tissue was determined with exhaustive extraction method modified from Pastor del Rio and Kennedy (2006). At harvest twenty random berry samples were collected per data vine, weighed, and stored at -80^{o}C until analyzed in order to preserve berry integrity. Skin and seeds were then

independently expressed from the berries manually, rinsed with deionized water, and counted. Samples were lyophilized (Triad Freeze Dry System; Labconco, Kansas City, MO) and re-weighed in order to obtain dry mass. The average dry mass of single berry skin and seed per sample vine were determined by dividing the total dry mass of skins and seeds by the number of skins and seeds, respectively. The samples were then extracted in 20 mL 66% (v/v) acetone solution in darkness for a 24 hour period. Samples were filtered through a Whatmann #1 90mm filter under vacuum, the grape marc was discarded, and a one mL sample was collected. The acetone was then evaporated from the samples under vacuum with a centrivap (model: 7810010; Labconco, Kansas City, MO) attached to a -103^{o}C cold trap (model: 7385020; Labconco, Kansas City, MO) and brought up to a volume of five mL with water using a type 'A' volumetric flask. Samples were then centrifuged for fifteen minutes at 1400 g in order to remove precipitate. The supernatant was then pasture pipetted into a two mL HPLC vial and was then subjected to HPLC analysis.

HPLC Analysis and Procedure

Phenolic analysis was conducted with reversed-phase high performance liquid chromatography (HPLC) using an Agilent 1100 series (Santa Clara, CA) HPLC system. The Agilent system included a system controller, degasser (Model: G1379A), quaternary pump (Model: G1311A), autosampler (Model: G1313A), column compartment (Model: G1316A), and a DAD/UV-vis detector (Model: G1315A). Data analysis was processed using ChemStation version A.10.02 designed for an LC system. Separation of phenolic compounds was performed with a LiChrospher 100 RP-18 (4 × 250 mm, 5 μm particle size) column (Agilent

Technologies, Santa Clara CA); a guard column of the same material was also installed and column temperature maintained at 40^{o}C.

The procedure utilized three mobile phase solutions for analysis. The solvents were (A) 50mM ammonium di-hydrogen phosphate adjusted to a pH of 2.6, (B) 20% Mobile A + 80 % Acetonitrile (v/v), and (C) 0.2 M ortho-phosphoric acid adjusted to pH of 1.5. Solvents established the following gradient: isocratic 100% A in 5 min, from 100 to 92% A and from 0 to 8% B in 3 min, from 92 to 0% A, from 8 to 14% B and from 0 to 86% C in 12 min, from 0 to 1.5% A, from 14 to 16.5% B and from 86 to 82% C in 5 min, from 1.5 to 0% A, 16.5 to 21.5% B and 82 to 78.5% C in 10 min, from 21.5 to 50% B and from 78.5 to 50% C in 35 min, from 0 to 100% A, 50 to 0%B and 50 to 0% C in 15 min at a flow rate of 0.5 mL/min. Analytical grade water was purified with (Siemens Labostar Ultrapure Water Systems) 0.2 µm charged sterile filter before use. The mobile phase components were of HPLC-grade and were purchased from Fischer Scientific (Pittsburg, PA). Spectra were recorded from 280 to 520 nm.

Quantification of flavonoid compounds was conducted with the use of peak area measurements at 280 nm for flavan-3-ols, 365 nm for flavonols, and 520 nm for anthocyanins. The commercial standards used were (+)-catechin, rutin, and malvidin-3-*O*-glucoside (Extrasynthése, Genay, France). Individual phenolic compounds were tentatively identified according to their order of elution, retention times of pure compound, and previous research conducted by Ritchey and Waterhouse (1999). The protocol of measuring total tannin content of skin and seed tissues was described by Kurtural et al. (2013). Briefly, total tannins were quantified spectrophotometrically (Lambda 25 UV/VIS; PerkinElmer, Waltham, MA). Tannin content was assayed using protein precipitation (bovine serum albumin, Sigma-Aldrich, St. Louis, MO), ferric chloride reagent (Fisher Scientific,

Pittsburg, PA), buffer solutions (Hagerman and Butler, 1978; Harbertson et al., 2003), and were quantified from a standard curve for catechin (catechin hydrate, Sigma-Aldrich).

Statistical Analysis

Interactions between year and treatments were tested and, whenever these interactions were significant ($P<0.05$), analysis was conducted separately for each year. The results were subjected to a two-way (leaf removal x deficit irrigation) analysis using MIXED procedure of SAS (v.9.3, SAS Institute, Cary, NC) appropriate for split-plot with a factorial arrangement of treatments. All data were tested for normality using Shapiro-Wilk's test, some phenolic data required a combination of log and square root transformations where deemed necessary and *PAR* values were log transformed in both 2013 and 2014. Treatment means were considered significantly different by Tukey's honestly significant difference adjustment at $P< 0.05$ in the LSMEANS option of MIXED procedure.

RESULTS

Climatology

The cumulative growing degree days (GDD) in both years of the study, were higher than the five year (2010-2014) average of 2037 (data not shown). The daily mean temperature, monitored from bud break to harvest, was higher in 2014 than in 2013. In 2013, the number of days that exceeded 32^{o}C and 37^{o}C were 61 and 10, respectively (data not shown). While in 2014, the number of days reaching above 32^{o}C and 37^{o}C were 71 and 11, respectively. Hence, the dates of each phenological stage in 2014 were approximately four to seven days earlier than 2013 (Table 1). Compared to 2013, the majority of precipitation in 2014 was received between bud break and fruit set (Figure 1). The amount of rainfall received at the site in 2014 affected the estimated crop coefficient (K_c). In 2013, the maximum estimated K_c was estimated at only 0.31 reached on 6 May 2013, while in 2014 the maximum estimated K_c was 0.43 reached on 7 July 2014. Therefore the estimated K_c in each year affected crop evapotranspiration (ET_c) in each respective year (Figure 1).

There was a consistent and reliable separation of irrigation treatment means in both years of the study (Figure 1). Sustained deficit irrigation (SDI) and regulated deficit irrigation (RDI) were maintained at approximately the prescribed levels of leaf water potential (Ψ_l). This was more evident in the seven-week period when RDI was imposed on the split irrigation treatments where the means separated from the SDI consistently. There was no interaction of leaf removal and irrigation treatments on Ψ_l in either year (data not shown).

Effects of Mechanical Leaf Removal and Deficit Irrigation on Canopy Architecture and Microclimate

The external leaf number and leaf layer numbers of the canopy were reduced and canopy gap number increased in both years of the study by the application of pre-bloom leaf removal treatment, regardless of measurement date (Figures 2, 3, and 4). Likewise, the post-fruit set leaf removal treatment reduced external leaf number and leaf layer numbers after its application. However, canopy gap number was only increased by post-fruit set on application date but not thereafter. Irrigation treatments did not have an effect on the canopy variables measured in either year of the study (data not shown).

In both years, pre-bloom leaf removal treatment had the highest photosynthetically active radiation (*PAR*) transmittance into canopy interior compared to control (Figure 5). Likewise, the post-fruit set leaf removal treatment increased *PAR* transmittance into canopy interior after its application. However, in 2013, the canopy of pre-bloom leaf removal treatment responded by vegetatively compensating to post-fruit set leaf removal treatment levels. A similar response to post-fruit set leaf removal treatment was not observed in 2014.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Yield Components

Leaf removal consistently affected berry skin mass in this two-year study. The post-fruit set leaf removal treatments reduced berry skin mass by 18% in 2013 and 13% in 2014, compared to control (Table 2). Berry skin mass of pre-bloom leaf removal treatment remained similar to control in both years. In 2013, leaf removal also affected berry mass, seed mass, and cluster mass. The berry mass, berry seed mass, and cluster mass were reduced by approximately 7, 4, and 13% by both pre-bloom and post-fruit set in 2013, respectively. However, there was no

effect of leaf removal on these components in 2014, indicating a year effect on yield components. Leaf removal had no impact on berry number/cluster in either 2013 or 2014. Yield/m at harvest was not affected by leaf removal treatments in 2013, but in 2014 post-fruit set leaf removal treatment reduced yield/m by 32% compared to control and pre-bloom leaf removal.

The irrigation regime consistently affected berry mass in both years. RDI reduced berry mass by 6 and 9% in 2013 and 2014, respectively, compared to SDI (Table 2). In 2014, RDI reduced the cluster mass and yield/m by approximately 13%, compared to SDI. No similar effect of irrigation regime was observed in 2013. Irrigation regime did not affect berry skin mass, berry seed mass, berry number/cluster, or clusters/m.

Experimental year had an effect on all yield components (Table 2). All variables, with the exception of clusters/m, were slightly reduced in 2014 compared to 2013. Additionally, leaf removal treatment increased clusters/m in 2013 but reduced it in 2014.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Crop Load and Yield Efficiency

Leaf removal consistently affected exposed leaf area/m in this two-year study (Table 3). The exposed leaf area/m was reduced by pre-bloom and post-fruit set leaf removal by 18 and 21% in both 2013 and 2014, respectively, compared to control. Additionally, the leaf area to fruit ratio was reduced in 2013 by 14 and 22% by pre-bloom and post-fruit set leaf removal, respectively, compared to control. Nonetheless, leaf removal did not have any effect on yield efficiency in 2014.

Furthermore, pruning weight was reduced by pre-bloom and post-fruit set leaf removal by 25 and 21% in 2013 and 2014, compared to control, respectively

(Table 3). Leaf removal consistently affected Ravaz index in both years. The Ravaz index was increased in 2013 by 35% with pre-bloom and post fruit-set leaf removal, compared to control. However, Ravaz index was only increased by pre-bloom leaf removal in 2014. Irrigation treatment had no effect on exposed leaf area/m or crop load components in either year of this study (Table 3). Nevertheless, the leaf area to fruit ratio was increased by RDI by 33% when compared to SDI but only in 2014.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Berry Composition

A considerable effect between years on berry composition at harvest was observed. Juice pH was slightly higher in 2013 than 2014 while the inverse occurred with TA (Table 4). Both leaf removal and irrigation regime from year to year affected TSS but not juice pH or TA. There was an effect of leaf removal on TSS in 2013. Post-fruit set leaf removal decreased TSS by 3% when compared to both control and pre-bloom leaf removal. In 2014, leaf removal had no effect on TSS. Differences between TSS values due to irrigation regime were observed in both growing seasons. RDI increased TSS by 2 and 2.5% in both 2013 and 2014, respectively, compared to SDI.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Phenolic Composition of Grape Tissue Extracts

Anthocyanins

There were 11 anthocyanins identified in the berry skins of Merlot in the hot climate. The 3-glucosides of anthocyanins were all present. The acylated forms of delphinidin and coumarates of cyanidin and peonidin were not seen in the skin of Merlot (Table 5). Skin anthocyanins were strongly affected by year, where

concentrations were lower in 2014 than in 2013. The total skin anthocyanin concentration (TSA) of Merlot was affected by the interaction of year and leaf removal treatments. The TSA were higher in 2013 with the pre-bloom leaf removal treatment. In both years of the study the pre-bloom leaf removal treatment increased TSA concentration by 25% compared to control. The irrigation treatments did not affect TSA concentration in either year. The 3-glucosides of Merlot skin anthocyanins were strongly affected by year and leaf removal interaction where their concentrations were higher in 2013 compared to 2014. In 2013, pre-bloom leaf removal treatment increased concentrations of delphinidin-3-glucoside (d-3-g), cyanidin-3-glucoside (c-3-g), petunidin-3-glucoside (pt-3-g), peonidin-3-glucoside (pe-3-g) and malvidin-3-glucoside (m-3-g) when compared to control and post fruit-set leaf removal treatments. The irrigation treatments did not affect the concentrations of 3-glucosides in 2013. The increases in the concentrations of 3-glucosides were consistent with pre-bloom leaf removal treatment in 2014, as well. However, in 2014, post fruit-set leaf removal treatment also increased the concentration of 3-glucosides. As in the initial year, irrigation treatments did not affect the concentration of 3-glucosides in 2014. The 3-Acetyl glucosides were affected by the interaction of year, and leaf removal treatments. The concentrations of 3-Acetyl glucosides measured in the Merlot skins were higher with pre-bloom leaf removal treatment in 2013, compared to 2014.

The acylated concentrations of c-3-g, pt-3-g, po-3-g, and m-3-g increased in both years of the study with the application of pre-bloom leaf removal treatment compared to control (Table 5). In 2014, post fruit-set leaf removal treatment also increased the concentration of acylated forms of anthocyanidins found in Merlot skin tissue. The irrigation treatments did not affect the concentrations of 3-Acetyl

glucosides in 2014 either. The coumarate of pt-3-g was not affected by the leaf removal treatments in 2013. However in 2014, pre-bloom and post-fruit set leaf removal treatments increased its concentration compared to control. The concentration of the coumarate of m-3-g increased consistently in 2013 and 2014 with the application of leaf removal treatments. The irrigation treatments did not affect the concentration of 3-coumaryl-glucosides in either year of the study.

A year affect was observed with proportion of anthocyanidin composition, except with petunidin (Table 6). In 2014, proportions of delphinidin and peonidin decreased while cyanidin and malvidin proportion increased. Malvidin was always the largest constituent, followed by peonidin, petunidin, delphinidin, and then cyanidin. Leaf removal had an effect on all anthocyanidins, with the exception of peonidin, in both years. In 2013, pre-bloom leaf removal increased proportion of delphinidin, cyanidin, and petunidin, while decreasing proportion of malvidin, when compared to the control. Post-fruit set leaf removal responded similarly to the control. In 2014, post-fruit set leaf removal resulted in the highest delphinidin, cyanidin, and petunidin proportion, but had the lowest malvidin proportion, when compared to both pre-bloom leaf removal and the control. Irrigation regime affected both cyanidin and peonidin proportion in 2014, where SDI increased it compared to RDI.

The partitioning of percent acylated versus non-acylated TSA was comparable in both years (Table 6). A higher proportion of TSA were non-acylated as opposed to acylated. No effect of leaf removal or irrigation regime was observed in either year regarding percent acylated versus non-acylated TSA. Proportion of di- and tri-hydroxylated of skin anthocyanins was strongly affected by year, with concentrations of both di-hydroxylated and tri-hydroxylated anthocyanins being lower in 2014. Tri-hydroxylated anthocyanins were always

found in higher proportion than di-hydroxylated anthocyanins regardless of treatment. Leaf removal did not affect hydroxylation in either year. Irrigation had an effect on hydroxylation proportion in both years. The SDI treatment increased the proportion of di-hydroxylated anthocyanins in both years when compared to RDI.

Flavonols

Leaf removal had a consistent effect on flavonol concentration in berry skin (Table 7). In both years of this study concentration of quercetin and myricetin were increased with pre-bloom leaf removal and post-fruit set leaf removal, compared to control. In addition, a year affect occurred with the concentration of quercetin but not myricetin where overall concentration was higher in 2014 than in 2013. Irrigation regime had no effect on flavonol concentration in either year of this study.

Flavanols

Leaf removal had an effect on monomeric and polymeric proanthocyanidin concentration in this study (Table 7). Both flavan-3-ol monomers, (+)-catechin and (-)-epicatechin, were increased by pre-bloom leaf removal treatment compared to post-fruit set leaf removal and control in 2013. However, in 2014, the concentration of (+)-catechin and (-)-epicatechin were increased with pre-bloom leaf removal and post-fruit set leaf removal treatment, compared to control. Furthermore, total polymeric proanthocyanidin concentration was increased by leaf removal treatments in 2013 when compared to control. However, in 2014 leaf removal had no effect on tannin concentration in berry skin. Moreover, a year affect occurred with (-)-epicatechin and tannin concentration where overall content in skin increased in 2014 compared to 2013.

Additionally, leaf removal had an effect on monomeric proanthocyanidin concentration in berry seed but no such effect on polymeric proanthocyanidin concentration in either year (Table 8). In 2013, (+)-catechin and (-)-epicatechin concentrations were reduced with post-fruit set leaf removal compared to pre-bloom leaf removal and control. However, in 2014 both leaf removal treatments increased monomeric proanthocyanidin concentration when compared to control. Note that a year effect did occur where overall flavanol concentration in seed decreased in all treatments 2014 compared to 2013. Finally, irrigation treatments had no effect on flavanol concentration in either year of this study.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Labor Operation Costs

Adding mechanical leaf removal to cultural operations in a traditionally managed SJV vineyard increased labor operations cost by only $30/ha (Table 9). Shifting the amount of irrigation applied by utilizing the RDI treatment compared to SDI saved 0.34 ML/ha and 0.48 ML/ha of water in 2013 and 2014, respectively. Consequently, the irrigation water cost declined proportionally. The TSA produced per hectare was consistently greatest with the pre-bloom and RDI or SDI treatment combinations in both years of the study. Compared to control irrigated with SDI, pre-bloom leaf removal treatment irrigated with RDI increased TSA productivity per hectare by 45 and 34% in 2013 and 2014 respectively.

The most expensive treatment to farm for one g/ha of TSA was control irrigated with SDI treatment (Table 9). More TSA was produced at a lower cost per unit with the pre-bloom leaf removal treatment when combined with RDI or SDI treatments. Therefore, the cost to grow anthocyanin (g/ha) was affected by the interaction of leaf removal and irrigation treatments.

DISCUSSION

Influence of Climate on Vine Physiology

Certain climatic factors are characteristic for San Joaquin Valley of California (SJV), categorized by Winkler et al. (1974) as Region IV and described previously, which elucidate varying responses to vine physiology, canopy development, and subsequent quality performance indices as compared to other distinct macroclimates. Both 2013 and 2014 seasons exhibited an increase in mean temperature, accumulation of growing degree days, and extreme temperature events when compared to the five-year mean. Additionally, 2014 was an especially challenging year, giving rise to the highest recorded mean temperatures in all months except April and extreme temperature events. This explained the successive increase in reference evapotranspiration (ET_o) values observed throughout seasons (Williams 2001). Poni et al. (2013) explains that increase in mean temperature from year to year may trigger a shift in phenological growth resulting in earlier occurrence of bud break, as was the case in our study. Furthermore, green water during winter was inadequate in both years and although 2014 received a net gain of 15mm, precipitation events remained concentrated within 40 days post bud break similarly witnessed in 2013. This difference in rainfall affected the estimated crop coefficient (K_c) used in irrigation amount calculations where in 2014 the maximum estimated K_c was higher than in 2013. Because of an increased K_c due to precipitation events in addition to a rise in ET_o associated with seasonal temperature, vines required additional supplemental irrigation in 2014 where differences between sustained deficit irrigation (SDI) and regulated deficit irrigation (RDI) application rate were exacerbated, rising from 219 L/vine/week in 2013 to 328 L/vine/week in 2014, respectively. It should be

noted that the attained estimated K_c values in this study are low compared to recommendations for a hot climate region. Allen et al. (1998) recommended a maximum K_c of 0.70 for vineyards with a row width of 3.35 m. However, Williams et al. (2005) recommend a maximum K_c of 0.98 for vineyards with a row width of 3.35 m. Since K_c is dependent on trellis height, pruning system, and row spacing a common maximum K_c cannot be appropriate for all trellis, canopy, and vineyard configurations, and hence our results presented in this study. The short canopy height (1.6 m) and wide vineyard row spacing (3.12 m) was the contributor to the collectively lower K_c values. In addition, K_c values reported by Allen et al. (1998) were for non-stressed crops cultivated under standard conditions achieving maximum crop yield. Reduced soil moisture at bud break due to lack of winter precipitation and reduced stored soil moisture have been shown to severely restrict shoot growth and canopy development, regardless of in-season irrigation application (Mendez-Costabel et al. 2013). Although the canopy was slower to develop and close in 2013, similar canopy architecture values were achieved in both years of the study. The K_c in both years remained at the maximum amount reached until the end of the season (31 October in both years) since vineyard was irrigated to that day in both years. This is in line with previous reports from the SJV that K_c would not decrease after harvest if irrigation is not decreased or terminated after that time (Williams 2012). The validation of the estimated seasonal K_c attained in this study was achieved using comparisons of weekly leaf water potential (Ψ_l) values and berry mass at harvest. The mean Ψ_l measured throughout and at the end of the season for the SDI treatment was greater than that of RDI as reported elsewhere by Girona et al. (2006) and Williams and Baeza (2007). Berry mass at harvest of the RDI treatment was 92% of SDI when averaged over the two years experiment was conducted. Achieving a

similar response of berry mass to fractions of ET_c application to previous work by Williams (2012) is probably fortuitous, but demonstrates the reliability of utilizing K_c to estimate vineyard water use.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Canopy Architecture and Microclimate

It was determined by Smart that shade conditions are ameliorated by reducing leaf area with a proportional increase in canopy gaps, thus minimizing canopy density (1985). Consistent with these findings, canopy architecture was positively manipulated by reducing canopy density with the implementation of leaf removal. Exposed (functional) leaf area/m decreased through a paralleled reduction in external leaf and total leaf layer number (Goldammer 2013). As a result, canopy gap number increased with both leaf removal treatments when compared to control as similarly reported by Pisciotta et al. (2013). According to Reynolds and Vanden Heuvel (2009), vine efficiency is optimized with a leaf layer number of 3.0 for non-positioned vines in California. Although Percival et al. (1994) forewarned that this value is adjustable due to phenotypical differences in canopy structure (e.g. leaf size). In 2013, both leaf removal treatments achieved a more optimal leaf layer number as compared to control. In 2014, however, all treatments were found to be near optimum levels. This can be explained by a general decrease in exposed leaf area/ m, thought to have been moderated due to adverse climatic conditions witnessed in second year (Goldammer 2013, Guidoni et al. 2008, Spayd et al. 2002). Nevertheless, leaf removal, in particular pre-bloom leaf removal treatment, consistently lowered vegetative growth indices resulting in a less dense canopy when compared to control (Intrieri et al. 2008, Percival et al. 1994, Taradaguila et al. 2010).

In this study, minimal differences between canopy architecture indices from early to mid-season indicated that vine canopies were relatively well formed by early May compared to cooler climate regions. This follows the current understanding that canopy development occurs much more rapidly in warm climates as opposed to cooler regions where growth is much more gradual (Howell 2001, Winkler et al. 1974). Percent shaded area is also a good indicator of canopy growth where Williams (2012) determined with Merlot in SJV that all vegetative growth was completed roughly 750 GDD post bud break with a K_c maxing out at 0.70 and with percent shaded area increasing from 20 to 40% from early to mid-season. Our K_c value never exceeded 0.43 because of a restrictive North Valley Cane Pruned trellis system compounded by limited precipitation events common in SJV. As such, percent shaded area only increased from 18 to 25% during the same time frame, demonstrating further that canopy growth did indeed increase throughout season but that sufficient quantities of leaves were already present early in season before defoliation was initiated. Thus, while shoot morphology (e.g. lateral shoot tip sensitivity to mechanical defoliator) may have been dissimilar between timing of defoliation treatments, the proportion and therefore severity of mechanical leaf removal within fruiting zone remained consistent in both years (Diago et al. 2012). Mechanical leaf removal reduced exposed leaf area/m by approximately 20% at defoliation, which was found to be less severe than Gatti et al. (2012), Poni et al. (2006), and Williams (2012), while a similar response was reported by Pisciotta et al. (2013) and Percival et al. (1994) who explained that the reduction in severity compared to hand defoliation was due to the mechanical defoliator's propensity to exclusively strip external leaves.

It has been widely understood that mitigating canopy density can improve microclimate through the collective enhancement of light transmission to fruiting

zone and appropriate modification of temperature, wind speed, humidity, and evaporation (Keller 2010, Percival et al. 1994, Smart 1985, Spayd et al. 2002). As to be expected (Bledsoe et al. 1988), photosynthetically active radiation (*PAR*) transmission into fruit zone increased significantly following a reduction in exposed leaf area/ m and an increase in canopy gaps. Furthermore, *PAR* levels measured in the control leaf removal canopies in both years were similar to those reported by Williams (2012) and Dokoozlian and Kliewer (1995) for dense, sprawling type canopies commonly seen in the SJV. Although not empirically quantified, the complimentary response of humidity dissipation and increased evaporation rate due to improvement in air circulation and solar radiation interception would be expected based off of previous research (Keller 2010, Smart 1985).

Comparable to Diago et al. (2012) and Poni et al. (2006), an intrinsic vegetative compensation response towards defoliation was observed when tracking growth throughout season, with vines recovering post-defoliation. However, analogous to Intrieri et al. (2008), Pisciotta et al. (2013), Percival et al. (1994), and Taradaguila et al. (2010), vegetative compensation did not fully replenish exposed leaf area/ m in leaf removal treatments as external leaf and leaf layer number remained slightly lower and *PAR* transmission higher compared to control in both years. Note however that differences in vegetative compensation response between pre-bloom and post-bloom leaf removal treatments were observed. The effects of post-fruit set leaf removal were not as long lived as pre-bloom leaf removal where canopies were quick to recover achieving similar *PAR* transmission as control but still had fewer leaf layers than control. The predilection of pre-bloom leaf removal treatment to maintain an improved canopy microclimate through inhibition of vegetative growth can be attributed to reduced

lateral growth and the leaf removal blower effects on growing or incipient lateral tips at the time of defoliation preventing further development during the growing season as reported by Diago et al. (2012). In 2014, post-fruit set leaf removal responded in a similar fashion to pre-bloom leaf removal treatment where *PAR* transmittance into fruit zone and the leaf layer numbers were similar at harvest. *PAR* transmittance of both pre- and post-fruit set leaf removal treatments increased at veraison and harvest, which was similar to that of Terry and Kurtural (2011) who explained that the weight of fruit on canes pulled the basal portion of shoots apart from one another, therefore providing more light into the center of canopy. Because of these findings, and since pre-bloom leaf removal treatment was applied 40 days prior to post-fruit set treatment, the pre-bloom treatment successfully maintained a more efficient microclimate, by sustaining 20% of ambient *PAR* into fruit zone throughout growing season, compared to control as analogously observed by Diago et al. (2012) and Taradaguila et al. (2010), and even post-fruit set leaf removal (Pastore et al. 2013).

Interestingly, irrigation regime had no consistent effect on canopy architecture or microclimate indices in either year. This is in stark contrast with other deficit irrigation studies (Hamman and Dami 2000, Matthews and Anderson 1989, Shellie 2006, Terry and Kurtural 2011, Williams 2012), where reduction of basal leaves subtended the fruiting zone leading to a concomitant increase in solar radiation. Because vegetative growth and hence vine vigor is sensitive in its response to water stress during specific phenological times, at different application rates and frequencies, under diverse climatic conditions, between different varieties, and within various soil profiles, it can be challenging to make connections between reported findings (Goldammer 2013, Matthews and Anderson 1989). Regardless, the evident distinction between our work and other

research is the variation in phenological timing of water stress and application rate of irrigation. For example, Hamman and Dami (2000) implemented three irrigation regimes supplying either 100% (control), 50%, or 25% of evaporative demand, which was sustained from bloom through harvest, with the latter two treatments eliciting a reduction in shoot growth and canopy height. Similarly, Shellie (2006) detected a decline in main shoot growth with Merlot as vine ψ_l surpassed -1.0 MPa. Granted Ψ_l of SDI and RDI treatments in this study were maintained at -1.2 and -1.4 MPa, respectively, the duration of moderate stress was implemented strictly from fruit-set to veraison. Williams (2012) went a step further and compared the responses of sustained water stress (40% and 80% of evaporative demand) to a surplus treatment of 120% with Merlot in SJV. As might be expected, an unequivocal response between irrigation regimes was noted due to wide variations in water stress levels. Hence, due to marginal differences between water application rate and ultimately water stress between SDI and RDI regime, an alteration in canopy architecture and/or microclimate was not prompted in this study.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Yield Components

Carbohydrate supply (i.e. source availability) during anthesis is the primary determinate of fruit-set and thus final yield at harvest (Poni et al. 2006). Therefore, the extent to which yield is reduced by the less traditional method of early leaf removal varies greatly due to the magnitude of altering source-sink relationship (Poni et al. 2006). The principal factors involved in source inhibition include variances in timing and severity of leaf removal, genotype, canopy size, vegetative compensation response, and growing conditions throughout season (Poni et al. 2005, 2006, Williams 2012). Percival et al. (1994) stated that

grapevines often produce far more leaves than required, especially in warm climates, and thus a reduction in leaf area may not be adequate in eliciting a negative response in yield. Furthermore, Poni et al. (2006) found that differences in yield were directly related to severity as demonstrated when six basal leaves were removed in Sangiovese resulting in a 5.7% decrease in yield compared to control while in Trebbiano eight basal leaves were removed followed by a 19% reduction in yield. Similarly, Gatti et al. (2012) removed 33% of total leaf area before anthesis in Sangiovese vines noting a significant reduction in yield. In another study, Poni et al. (2005) found that there was no significant reduction in yield with either pre-bloom or post-bloom leaf removal when every other leaf was removed from nodes one though eight. In both years of our study berry set and yield/m of row of pre-bloom leaf removal treatment remained comparable to control. This can be explained due to a synergistic combination of the aforementioned factors. Namely, pre-bloom leaf removal treatment did not elicit severe enough source imbalance on fruit-set and yield/m because canopies had sufficient source material pre- and post-defoliation as a compounding result of canopy development characteristics in hot climate, minimal severity of defoliation, and vegetative compensation not being deleteriously inhibited.

It has been found that post-bloom defoliation typically does not decrease yield components as source imbalance is often avoided (Bledsoe et al. 1988, Poni et al. 2005). It is important to note that although berry number and yield/m was not affected by either leaf removal treatment in 2013, berry mass and cluster mass were slightly reduced as reported by Poni et al. (2006) and Wessner and Kurtural (2013) and was attributed as the result of solar radiation exposure (Bergqvist et al. 2001) and possibly differences in lower cluster water potential of exposed berries (Williams 2012). However, the effect of leaf removal on berry and cluster mass

was not repeatable in 2014. Furthermore, Pastore et al. (2013) ascribed a reduction in yield per vine to an increase in proportion of sun burn damage per cluster. It has been warned by many that a sudden increase in exposure to solar radiation and thus higher temperature may lead to sun burning and partial or entire failure of clusters, especially in hot climates (Bergqvist et al. 2001, Palliotti et al. 2011, Pastore et al. 2013, Pisciotta et al. 2013, Poni et al. 2006). While precautions were taken to guarantee an advantage in improved microclimate while protecting clusters from adverse climatic conditions (i.e. leaf removal conducted on East side of canopy and with minimal severity), the sudden increase in temperature due to enhanced sunlight exposure may have been sufficient enough to cause sun burning of the non-acclimated clusters of post-set leaf removal in 2014, leading to the reduction in yield. Note however that sun burning may not always occur with post-bloom defoliation in hot climates, as seen in 2013, where climatic conditions and canopy architecture and microclimate were more favorable, thus aiding in the protection of clusters during the hottest time of the season. Intriguingly, as berry number carried to harvest and yield/m of pre-bloom leaf removal treatment remained similar to that of control, it could be concluded that sunburn never occurred with clusters of pre-bloom defoliation since leaf removal occurred early in season where seasonal temperatures were less severe allowing berries sufficient time to acclimate to the linear increase in exposure throughout season (Pastore et al. 2013).

Finally, berry skin mass may play an important role in phenolic accumulation and subsequent protection of berry integrity (Diago et al. 2012, Palliotti et al. 2011), therefore the marked dissimilarities between treatments found in this study should be discussed. The increase in skin mass of Merlot berries following pre-bloom leaf removal application can possibly be explained by the

long term adaptation mechanism of berry thickening (Diago et al. 2012, Pallioti et al. 2011, Poni et al. 2008). The increase in skin mass associated with pre-bloom leaf removal treatment was 15 and 9% in 2013 and 2014 respectively when compared to post-fruit set leaf removal. These values are higher than those reported in previous work (3.6% by Palliotti et al. 2011, 0.7% by Diago et al. 2012). Furthermore, Poni et al. (2008) and Gatti et al. (2012) concluded that alterations in skin mass were heavily dependent on light and temperature, which prevailed over any inhibitory effect of source limitation. The effect of temperature can be confirmed by Kliewer (1977) who demonstrated in the cultivar Tokay that skin mass was reduced during fruit-set when temperatures were held at 40^{o}C compared to 25^{o}C. Consequently, under more favorable climates an increase in light and temperature may favor skin development (Diago et al. 2012, Gatti et al. 2012, Poni et al. 2008), however, in hot climates skin mass may be inhibited (Kliewer 1977). From this it can be concluded that the long term adaptation mechanism of skin mass enhancement is a response to precocious and prolonged infructescence and cluster exposure caused by pre-bloom leaf removal. While the abrupt increase in light and elevated temperature, regardless of whether sunburn occurred or not, inhibited skin tissue formation due to undue stress imposed on clusters with post-fruit set leaf removal (Pastore et al. 2013, Williams 2012).

It was found that reproductive growth was more sensitive to vine water status than that of vegetative growth. Irrigation stress was successful in reducing berry mass as found in other studies (Hamman and Dami 2000, Matthews and Anderson 1989, Ojeda et al. 2002, Roby et al. 2004, Shellie 2006, Williams 2012). As would be expected, skin mass was unaltered due to phenological timing of deficit irrigation treatments (Ojeda et al. 2002). As both berry skin and seed mass remained unaffected by irrigation regime in our study, it would be plausible that

the reduction in berry mass was primarily due to a decline of inner mesocarp cell sap (Roby et al. 2004) and inhibition of cell expansion over cell division (Keller 2010, Matthews and Anderson 1989). RDI decreased berry mass by 6 and 9% in 2013 and 2014, respectively. The reduction of berry mass by the RDI treatment was lower in Merlot compared to the reduction seen in Syrah in the same growing region with similar irrigation treatments (Terry and Kurtural 2011). Interestingly enough, yield/m was not significantly altered by irrigation regime in 2013 possibly due to the mitigation of vegetative compensation through sustained deficit or regulated deficit irrigation having invigorated lateral shoot regeneration just enough to set similar berries per cluster. In 2014, a reduction in berry mass was more pronounced with RDI, which further reduced cluster mass and ultimately a 14% decrease in yield/m, possibly as a result of lowered cluster water potential (Williams 2012). Additionally, the lowered cluster water potential was thought to be due to climatic conditions associated with second season where higher mean temperature and more extreme temperature events negatively affected berry development (Pastore et al. 2013).

Effects of Mechanical Leaf Removal and Deficit Irrigation on Crop Load and Yield Efficiency

Ravaz index (i.e. crop load) values in this study suggested that all treatment vines were out of balance in 2013, indicating there was insufficient vegetative growth to sustain a high crop (Goldammer 2013, Kliewer and Dokoozlian 2005, Smart and Robinson 1991). In addition, leaf removal treatments promoted higher crop load indices compared to control in 2013. This would be expected since vegetative compensation was not sufficient in completely re-filling the canopy as seen with Intrieri et al. (2008), Percival et al. (1994), and Taradaguila et al. (2010), and pruning weight remained lower than control. This is confirmed by Williams

(2012) where the inverse occurred, he did not see any difference between Ravaz index values with leaf removal treatments because pruning weights remained similar due to substantial re-filling of the canopy post-defoliation, which was able to successfully sustain cane growth. Furthermore, Ravaz index of pre-bloom leaf removal treatment remained higher than control in 2014, however, post-fruit set leaf removal treatment reduced Ravaz index to similar level as control. This occurred due the additional loss of yield/m associated with post-fruit set leaf removal. According to Howell (2001), a high Ravaz index could lead to a reduction in yield the following season and/or fruit quality not reaching its full potential. It should be noted however that Ravaz index does not reflect the improved microclimate associated with defoliation treatments and thus a higher index value may be possible if leaf and cluster exposure is enhanced sufficiently enough to support a high fruit load, which did indeed occur in this study (Reynolds and Vanden Heuvel 2009). This is in agreement with findings from the allometric method of yield efficiency where ratios of defoliation treatments in our study were actually more efficient compared to control in 2013 because they were brought closer to optimal value of 1.2 m^2/kg for a single plane, non-shoot positioned canopy (Terry and Kurtural 2011). These findings are a result of exposed leaf area/m remaining lower and yield remaining unaltered.

To conclude, although Ravaz index indicated that all defoliation treatments were over-cropped in 2013, yield efficiency implied that leaf removal treatments were more efficient at ripening a single kg of fruit. In 2014, all yield efficiency ratios of leaf removal treatments were found to be in the optimum range because of overall reduction of leaf area/m over yield/m from season to season due to dissimilarities in climate. However, the difference among treatments means were

retained and comparatively only pre-bloom leaf removal treatment had the balanced vegetative to reproductive growth ratio.

Irrigation regime had no significant effect on pruning weight, Ravaz index, or yield efficiency in 2013, as would be expected since vegetative growth remained unaltered and reproductive growth was negligibly affected (Terry and Kurtural 2011). Again, this is confirmed by Hamman and Dami (2000) and Williams (2012) who noticed that when the magnitude of irrigation stress was intensified, a marked decline in vegetative growth and pruning weight occurred. Note however, that in both studies Ravaz index either increased or remained similar because yield was also influenced. In 2014, irrigation regime had a more pronounced effect on yield/m; therefore, both the overall reduction in vegetative growth seen in second season and reduction in yield increased ratio of yield efficiency with RDI compared to SDI treatment. Comparably, even though not statistically significant, RDI slightly reduced pruning weight and Ravaz index compared to SDI treatment. Nevertheless, it should be reaffirmed that although RDI increased yield efficiency, both values are still well within the recommended optimal range (Kliewer and Dokoozlian 2005).

Effects of Mechanical Leaf Removal and Deficit Irrigation on Berry Composition

All vines successfully reached commercial maturity of 24 TSS, expressed as degree Brix, in both years, regardless of treatment. However, in 2013 the post-fruit set leaf removal treatment reduced TSS by 3% when compared to control and pre-bloom treatments. Yet, these results were inconsistent from year to year as reported by Pisciotta et al. (2013), Taradaguila et al. (2010), and Williams (2012). Nevertheless, a possible explanation as to why leaf removal reduced TSS in 2013 could be due to the incidence of temperature due to sunlight overexposure that is

often associated with post-fruit set leaf removal in hot climate. Bergqvist et al. (2001) confirmed this with Grenache and Cabernet Sauvignon grown in the Central Valley of California by concluding that soluble solid content initially increased as light exposure increased but decreased as light exposure continued to increase because of the concomitant elevation in temperature. In a previous study conducted by Kliewer (1977) sugar accumulation was drastically inhibited as temperatures began to exceed 37^{o}C, supporting the results of Bergqvist et al. (2001). Other studies found TSS to be higher with leaf removal, particularly when conducted pre-bloom (Diago et al. 2012, Gatti et al. 2012, Intrieri et al. 2008, Palliotti et al. 2011, Poni et al. 2008), however, differences in canopy size, climate, and severity of leaf removal once again distinguish our results from the results of conflicting research, where smaller canopies become more sensitive to source limitation, and therefore assimilation and translocation into fruit, as severity in defoliation increases.

Lastly, leaf removal had no significant effect on juice pH or titratable acidity (TA) within either season in our study as similarly observed by Pallioti et al. (2011), Pisciotta et al. (2013), Intrieri et al. (2008), and Williams (2012). It was postulated by Williams (2012) that in regions where ambient temperatures are already high, a subsequent increase in temperature due to leaf removal may have no noticeable effect on acid degradation as TA or pH levels are already significantly lower and higher, respectively. Taradaguila et al. (2010) further noticed that temperature fluctuations between seasons altered TA and pH levels, where in the hotter and drier season TA was lowered and pH increased but in a cooler season the inverse occurred. This principle was corroborated in our study where 2014 was a hotter year and consequently TA was slightly lower and pH higher compared to the more favorable conditions of 2013.

Irrigation regimes in our study had no significant effect on juice pH or TA similar to that of Castellarin et al. (2007b) and Hamman and Dami (2000). On the contrary, Shellie (2006) and Williams (2012) noted a reduction in must TA as severity of water stress increased. Shellie (2006) attributed this to the degradation of malic acid due to an increase in light transmission and therefore respiration rate, whereas Williams (2012) stated that differences may have been due to variability in berry maturity when sampled on similar dates because at harvest the TA of must was similar in two of three years. Therefore, because variances in irrigation stress were much less severe compared to other studies, the results of our work are to be expected.

Although irrigation regime did not affect juice pH or TA, it reliably affected total soluble solid content in both years where clusters of RDI ripened relatively earlier than SDI. This outcome has been verified by several studies (Castellarin et al. 2007a, Roby et al. 2004, Shellie 2006, Williams 2012) where it has been theorized that irrigation stress may improve sugar accumulation through a perpendicular reduction in berry mass as seen in our study. Additionally, Roby et al. (2004) stated that such an increase may also reflect a partitioning response to water status where a moderate but not severe water stress may have increased the allocation of photosynthate to developing fruit. These findings are further strengthened by Hamman and Dami (2000), who found that increased water stress actually reduced total soluble solids and explained that this was due to the fact that stomatal closure was amplified with their most severe deficit treatment.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Phenolic Composition of Grape Tissue Extracts

Anthocyanins

Anthocyanin accumulation and profile in skin of *Vitis* are determined by synergistic combination of solar radiation and berry temperature, both of which can be directly manipulated by canopy and irrigation management (Cohen and Kennedy 2010, Spayd et al. 2002, Tarara et al. 2008). The 3-glucosides, 3-Acetyl glucosides, 3-Coumaroyl-glucosides, and total skin anthocyanins (TSA) of Merlot berries in this study consistently increased with increased fruit exposure early in the growing season aided by pre-bloom leaf removal treatment. There was a strong and a positive linear relationship between TSA and *PAR* transmittance into the fruit zone in 2013 ($R^2 = 0.7889$, $P < 0.0001$) and 2014 ($R^2 = 0.6994$, $P < 0.0001$). However, this relationship was only evident when *PAR* measurements were taken one week following pre-bloom leaf removal application and only when it exceeded 20% of ambient *PAR*. Therefore, in contrast to the response of other higher plants, the accumulation of anthocyanins in *Vitis* spp. was determined not to be completely dependent on light and therefore, although influential, is not an absolute requirement (Cohen and Kennedy 2010, Downey et al. 2004, Spayd et al. 2002). However, in our study, the absolute amount of *PAR* exposure to canopy interior directly affected anthocyanins. The most obvious differences in anthocyanin composition between treatments that received pre-bloom leaf removal were an increase in tri-hydroxylated and methoxylated anthocyanins (i.e. the glucosides of malvidin, petunidin, and delphinidin). Similar results were reported with Syrah by Downey et al. (2004). Conversely, di-hydroxylated anthocyanins (i.e. peonidin and cyanidin) were not as consistently affected by leaf removal treatments. This result suggests that increasing *PAR* transmittance to at least 20%

of ambient in the fruit zone by pre-bloom leaf removal increased the activity of F3'5'H, or expression of the gene encoding that enzyme, or alternatively decreased activity of F3'H or down-regulated expression of that gene.

Acylation proportion was lower compared to non-acylated anthocyanins as would be expected (Tarara et al. 2008); however, the composition pattern of acylated anthocyanins under the hot climate was not affected by treatments imposed in this study. Gao and Cahoon (1994) reported that the proportion of non-acylated cyanidin glucosides decreased with shading while the proportion of acylated cyanidin glucosides increased. Haselgrove et al. (2000) suggested that increased light exposure led to a decreasing proportion of coumaroyl derivatives in the fruit. However, our results were not consistent with these findings. There is agreement with other literature that higher temperature rather than light results in a shift toward coumaroylated anthocyanins, and that higher temperature results in decreased total anthocyanins (Bergqvist et al. 2001, Downey et al. 2004, Spayd et al. 2002). Although we did not see the shift in coumaroylated anthocyanins in 2014, a warmer year than 2013; the TSA was lower in 2014 as evidenced by previous work. For example, there was a 25% decrease in TSA for the control leaf removal treatment from 2013 to 2014. The control leaf removal treatment in both years had less than 15% of the ambient *PAR* transmitted to fruit zone and at least one more leaf layer than the leaf removal treatments. The difference however, was in the ambient temperature amongst years where 2014 had higher mean temperatures in all months except April and had ten more days above 32^{o}C compared to 2013. Note that this decrease in TSA, as a response to temperature from year to year, was not due to a decrease in the content of malvidin-3-glucoside or its acylated derivatives. This too is in agreement with previous research which had established that malvidin based anthocyanins were less sensitive to

biosynthetic inhibition or even degradation due to higher temperatures because of the associated maximization of methoxyl moieties on B ring (Mori et al. 2005, Tarara et al. 2008). Therefore, malvidin proportion may actually increase in the presence of higher temperatures in a warm climate as was the case in our study where there was a 10% shift in 2014.

It has been shown that anthocyanin accumulation is not only affected by high temperature during daytime but also by fluctuations and magnitude of diurnal temperatures (Cohen and Kennedy 2010, Mori et al. 2005). For example, Mori et al. (2005) determined with Pinot noir that when nocturnal temperatures were continuously maintained at 30^{o}C compared to low night (15^{o}C) with high daytime temperatures, nearly all 3-glucosides except for malvidin were greatly reduced. Thus, based on canopy architecture dynamics associated with leaf removal it can be theorized that as the fruiting zone remained more open with defoliation treatments an increase in air flow and decrease in humidity may have occurred, which would allow the clusters to have possibly remained cooler at night due to amplified heat loss when compared to control (Smart and Robinson 1991). These findings coincide with those of Downey et al. (2004) who determined that there were two primary systems required to regulate anthocyanin accumulation. An initial and constitutive system generated the base level anthocyanins and an inducible system that required concomitant increase in light amount into the fruit zone to affect the hydroxylation pattern of anthocyanins.

An additional mechanism possibly responsible for the increase in anthocyanin concentration was a paralleled increase in skin mass as a response to precocious exposure of the flower cluster infructescence, and the cluster to solar radiation as reported by Diago et al. (2012), Pallioti et al. (2011), and Poni et al. (2008). The increase in skin mass was thought to be important as it is understood

that anthocyanins are stored after glycosylation in the vacuoles of the outer and inner hypodermal layers and therefore a reduction of vacuolar space may lead to a subsequent decrease in anthocyanin accumulation potential (Diago et al. 2012, Pastore et al. 2013). This aids in the explanation as to why post-fruit set leaf removal treatment performed similarly to control in 2013. In addition to this, the sudden application of the post-fruit set treatment not only led to undue stress reducing skin mass but an abrupt increase in temperature may have also compounded the reduction in anthocyanin content through a further inhibition of anthocyanin regulation and development, and even color degradation (Cohen and Kennedy 2010, Pastore et al. 2013). In 2014, however, post-fruit set leaf removal treatment performed similarly to pre-bloom leaf removal. Note that this was attributed to 23% decline in yield/m making available more source material per unit sink during the season (i.e. reproductive structures), thus promoting the biosynthetic up-regulation and therefore accumulation of anthocyanin content (King et al. 2012, Pallioti et al. 2011), and ultimately overriding the deleterious effect of diminished skin mass. These observations are similar to that of pre-bloom defoliation in other studies where yield control occurred (Intrieri et al. 2008, Poni et al. 2006).

Contrary to previous research, concentrations of 3-glucosides, 3-Acetyl glucosides and 3-Coumaroyl-glucosides of anthocyanins and TSA were not affected by irrigation treatments applied in our study (Castellarin et al. 2007b, Hamman and Dami 2000, Ojeda et al. 2002, Roby et al. 2004, Romero et al. 2010). Moreover, unlike leaf removal treatment, irrigation regime did not consistently have the same effects on anthocyanin composition. Interestingly, increasing the irrigation amount with the SDI treatment by even 0.34 to 0.48 ML compared to RDI over the course of the season in the hot climate shifted the

hydroxylation pattern of anthocyanins from tri- to di-hydroxylated anthocyanins. This result suggests that when Merlot was subjected to the SDI treatment the activity of F3'H increased or the expression of that gene was up-regulated. The results presented here prove that the effect of pre-bloom leaf removal and RDI are to increase the proportion of malvidin, petunidin and delphinidin glucosides relative to cyanidin and peonidin glucosides (Downey et al. 2004; Castellarin et al. 2007b). On an oenological basis, these findings have great implications towards improved quality of Merlot fruit in hot climate as a shift towards tri-hydroxylated and methoxylated anthocyanins, especially malvidin based derivatives, promote color stability and age-ability of wine (Ribereau-Gayon et al. 2006).

Flavonols

Comparable to Pastore et al. (2013), leaf removal treatments reliably affected flavonol concentration in skin in both years of this study whereas flavonol accumulation in seed was negligible as anticipated (Cortell and Kennedy 2006). It has been widely understood that ultra-violet protecting flavonols are synthesized in response to the incidence of solar radiation, including ultra-violet radiation, rather than temperature (Downey et al. 2004, Spayd et al. 2002, Tarara et al. 2008). Spayd et al. (2002) implemented ultra-violet (UV) barriers on clusters and concluded that UV radiation, while not an absolute requisite greatly influenced the content of aglycones and glycosylated forms of flavonols, especially those of quercetin. Furthermore, it was found that UVB is the primary UV constituent influencing flavonol accumulation (Spayd et al. 2002). This was due to the inclination of UVB to illicit severe tissue damage in addition to being the most dominant constituent, after UVA radiation that passes through the stratosphere, reaching Earth's surface (Keller 2010, Martinez-Luscher et al. 2014). This

relationship is in contrast to that of anthocyanins, which require a synergistic combination of light and temperature to attain maximum concentration (Downey et al. 2004). Thus it has been proposed by Downey et al. (2004) that only the branch of the flavonoid pathway leading to flavonol biosynthesis is light-dependent.

Typically, there are two principal phenological stages where genes of flavonols are activated and enzymatic synthesis commences, once during anthesis and again at veraison (Cortell and Kennedy 2006). Therefore, it would be expected that a positive increase in *PAR* transmittance, and subsequently UV radiation, following pre-bloom or post-bloom defoliation would activate transcription factors leading to the expression of genes encoding the enzyme flavonol synthase (FLS) in the flavonoid pathway (Downey et al. 2004, Pastore et al. 2013, Spayd et al. 2002). Similar to previous research, it was determined in our study that concentrations of quercetin and myricetin were enhanced as a response towards defoliation. However, although pre-bloom and post-fruit set leaf removal treatments had significantly higher flavonol content in skin compared to control, it should be noted that even though statistically comparable, pre-bloom treatment had a higher concentration when compared to post-fruit set treatment in 2013, analogous to Pastore et al. (2013). This can be explained as a response to the improvement in *PAR* transmittance earlier and throughout season. In 2014, flavonol concentration of leaf removal treatments remained more identical as a result of increased *PAR* transmittance at veraison with post-fruit set treatment.

Incongruous to the aforementioned positive correlation observed between berry skin mass and final anthocyanin accumulation, flavonol accumulation is much less sensitive to changes in berry skin mass (Diago et al. 2012, Downey et al. 2004). This is because of the location where flavonols are stored. Diago et al.

(2012) stated that because flavonols accumulate in the vacuoles of the epidermal and outer hypodermal layer of the skin they are more localized compared to anthocyanins, making them less sensitive to modifications in skin mass. In summary, our research reaffirms that amelioration of canopy microclimate is a consistent and straightforward method in enhancing flavonol concentration in skin tissue (Downey et al. 2004).

Ojeda et al. (2002) determined that water deficit strongly influenced the biosynthesis and concentration of flavonols in skin tissue. Similarly, Castellarin et al. (2007a) noted that water deficits affected flavonol accumulation but not to the degree of anthocyanins. In contrast, Kennedy et al. (2002) determined with Cabernet Sauvignon that water deficits were inadequate in altering final flavonol concentration. In our study, irrigation regime had no effect on flavonol accumulation in either year. Again, the variability observed between previous research and our own can be attributed to the difference in irrigation treatments, where timing and magnitude of irrigation rate between SDI and RDI was insufficient at elucidating a significant response.

Flavanols

Flavan-3-ol monomers and polymeric proanthocyanidins found in skin and seed (i.e. condensed tannins) are perhaps the most stable flavonoids under diverse growing conditions (Teixeira et al. 2013). Just as solar radiation and/or temperature affected anthocyanin and flavonol concentration, a handful of studies have elicited a similar effect on monomeric and polymeric proanthocyanidins in skin. For example, positive correlations between solar radiation and skin proanthocyanidin concentration were observed with Cortell and Kennedy (2006) where concomitant increase in exposure to solar radiation enhanced

proanthocyanidin content at veraison and at harvest. On the other hand, Downey et al. (2004) noticed increased proanthocyanidin concentration with exposed treatment at veraison but differences between shaded and exposed fruit diminished at harvest. Kemp et al. (2011) further differentiated responses between final monomeric and polymeric proanthocyanidin concentration in wine where monomers were affected by defoliation timing whereas polymeric proanthocyanidins were not. He further explained that monomeric but not polymeric proanthocyanidin biosynthesis could have been dependent on cluster exposure. These findings were corroborated with results from our study where monomeric proanthocyanidins were consistently affected by pre-bloom leaf removal treatment, with concentrations being enhanced due to improved microclimate, while the impact of both defoliation treatments on final proanthocyanidin content were not as consistent. The enhancement of monomeric proanthocyanidins may have been due to the up-regulation of the gene controlling enzymatic production of leucoanthocyanidin-reductase (LAR) and/or anthocyanin-reductase (BAN) (Castellarin et al. 2007a, Cortell and Kennedy 2006). In addition, monomeric proanthocyanidin concentrations of post-fruit set leaf removal treatment were similar to that of control in 2013. Based on our findings, sensitivity to modifications in skin mass with flavan-3-ol monomers may be comparable to that of anthocyanin accumulation, while total proanthocyanidin content may behave similar to that of flavonols as validated by Cortell and Kennedy (2006). Nevertheless, although behavior may seem compatible the underlying mechanisms may be dissimilar (Teixeira et al. 2013). It is interesting to note that in 2014 flavan-3-ol monomer concentration increased with post-fruit set leaf removal treatment, which can be explained as a result of reduced yield as previously seen with anthocyanin accumulation.

While effects of solar radiation may be fairly straightforward, the response of proanthocyanidin accumulation to temperature has been more difficult to elucidate. Cohen et al. (2008) concluded that proanthocyanidin content was not affected by temperature differences (i.e. ambient, heated, or cooled) across three years when analyzed at veraison and during harvest. Although content was not affected, composition was where fewer degree-days favored a shift in tri-hydroxylated proanthocyanidins. Therefore, differences in concentration of flavan-3-ol monomers in our study can be attributed to improved *PAR* transmittance into fruit zone and improved microclimate associated with pre-bloom leaf removal treatment rather than temperature variation within season. However, Pastore del Rio and Kennedy (2006) pointed out that final skin proanthocyanidin content was higher between seasons as a result of warmer mean temperatures, as was the case in our study where 2014 had noticeably higher total skin tannin content at harvest compared to 2013.

It is noted by Teixeira et al. (2013) that skin proanthocyanidins are more sensitive to changes in environmental conditions compared to those located in seed as was the case in our study. This finding is corroborated by both Cortell and Kennedy (2006) and Downey et al. (2004) who determined that light had no quantifiable effect on seed concentration or composition of either free monomeric or polymeric proanthocyanidins at harvest. Furthermore, Cohen et al. (2008) reported that seed proanthocyanidins were not affected by variations in diurnal temperature. However, when comparing temperature variations among seasons, Pastore del Rio and Kennedy (2006) observed a reduction in flavan-3-ol monomer amount in warmer seasons compared to cooler seasons. These findings are in agreement with our study where overall catechin and epicatechin amount at harvest was lower in the hotter season of 2014. In addition, the general reduction

in total seed proanthocyanidin concentration from year to year may have been due to diminution of extractability linked to growing conditions of 2014 as suggested by Downey et al. (2004). The cumulative decrease in seed monomeric and polymeric proanthocyanidin content associated with 2014 may be beneficial to winemakers as extraction potential of bitter tannins would be reduced (Cortell and Kennedy 2006). On the other hand, contrary to previous research, free monomers were affected by leaf removal treatments within both growing seasons. Interestingly in 2013, catechin and epicatechin levels were lowered by post-fruit set leaf removal treatment compared to control and pre-bloom treatment, whereas both defoliation treatments had higher monomer concentration in 2014 compared to control. Although reasoning behind this behavior is uncertain, the perceived shift in concentration can possibly be explained by the reduction in seed mass associated with post-fruit set leaf removal treatment in 2013, where sudden undue environmental stress coupled with the reduction in seed mass led to the lowest concentration of flavan-3-ol monomers (Pastore del Rio and Kennedy 2006). In 2014, there were no restrictions in seed mass among leaf removal treatments yet monomeric concentration increased with both defoliation treatments compared to control. Thus, in the hot climate of SJV, underlying environmental conditions may have exceeded thresholds affecting the expression and up-regulation of genes encoding the LAR enzyme leading to the minor improvement in monomeric accumulation within seed.

Irrigation regime had no effect on skin or seed proanthocyanidin concentration in either year of our study. This is in contrast to Kennedy et al. (2002) who observed that minimal irrigation (MI) treatment, which was maintained at -1.6 MPa, increased both catechin and proanthocyanidin content compared to standard irrigation treatment. This improvement in skin tannin

concentration was thought to be primarily a function of decreased berry mass due to water deficit and therefore an increase in skin-to-pulp ratio. However, Castellarin et al. (2007a) detected a much less noticeable effect on skin tannin, perhaps because vine water status did not surpass -1.4 MPa and reduction in berry mass was less severe, which was comparable to that of our study. Castellarin et al. (2007a) concluded that water deficits only had minimal effect on biosynthesis and accumulation of proanthocyanidins, where the genetic expression responsible for encoding LAR2 was slightly enhanced with early deficit treatment. Ojeda et al. (2002) discussed timing of irrigation in more detail with early deficit (anthesis to veraison) reducing berry mass more severely than that of late deficit. In fact, their strong early water deficit treatment (S1) actually inhibited biosynthesis of skin tannin due to a 50% reduction in berry mass, whereas medium early water deficit (S2) reduced berry mass by only 32% followed by an increase in skin tannin concentration. Ojeda et al. (2002) summarized their findings by stating that two types of responses to water deficits were shown, an indirect "concentration" response due to reduced berry size and a second direct response on tannin biosynthesis that could be either positive or negative. Lastly, Roby et al. (2004) concluded that water deficit had no clear effect on seed tannin concentration even though there was a linear function between seed tannin content and seed number, as well as seed mass per berry. Roby et al. (2004) further described the relationship between seed tannin concentration and berry size by affirming that there was a two phase response, an initial decrease in content and then an increase as berry size increased. Therefore, because of the dissimilarities between irrigation rate and timing, the outcomes in our study are to be expected. Briefly, results from our study were similar to that of Castellarin et al. (2007a) where vine water status of our most severe deficit treatment (i.e. RDI) was maintained at -1.4

MPa compared to -1.6 MPa with Kennedy et al. (2002) and Roby et al. (2004). In addition, the reduction in berry mass due to RDI was much less pronounced compared to the greater reduction found in the aforementioned studies where berry mass was reduced by as much as half. Thus, an improvement in tannin content due to enhanced skin to pulp ratio would be much less appreciable in our study. Moreover, as previously mentioned the minimal variation in magnitude of irrigation rate between SDI and RDI treatments in this study were minimal compared to previous research further explaining why mean separation was not elicited.

Effects of Mechanical Leaf Removal and Deficit Irrigation on Labor Operation Costs

Labor operation and irrigation costs in addition to TSA production on a gram per hectare basis were calculated and extrapolated to determine the cost to produce one unit of color per unit area, allowing us to compare economic impact of the interactive effects of mechanized leaf removal and irrigation regime. Based on these findings it is clear that in 2013 pre-bloom leaf removal treatment yielded the most efficient production costs and when utilized in combination with SDI the cost to produce one gram of color was reduced by 30%, compared to control. Furthermore, when pre-bloom leaf removal was combined with RDI, production costs were reduced by another 5%, due to the decrease in irrigation rate associated with RDI. Post-fruit set leaf removal treatment performed similar to control in 2013 as a result of comparable TSA content. In 2014, pre-bloom leaf removal treatment reliably obtained the most efficient production costs, even though efficiency proportion was less pronounced than in 2013. This was primarily due to the increase in irrigation rate and slight decrease in TSA production as was affected by temperature. Still, pre-bloom leaf removal in combination with SDI

reduced production costs compared to control by 15% and when RDI was implemented, production costs were reduced an additional 4%. Post-fruit set treatment on the other hand, regardless of irrigation regime, was the least efficient treatment combination because of reduced yields in 2014. Therefore, pre-bloom leaf removal in conjunction with either SDI or RDI consistently attained the most efficient production costs for growers in both years and thus was the most economically feasible treatment combination in this study.

CONCLUSION

In the hot climate of the San Joaquin Valley of California (SJV) labor and water are limiting factors. Growers are paid by the tons they produce and receive the lowest price per ton for red wine grapes due to low flavonoid and in particular low anthocyanin accumulation in berries. In this study, we identified three mechanisms that explained the synergistic effects that leaf removal and applied water amounts in the hot climate had on both total skin anthocyanin concentration (TSA) and anthocyanidin proportion. The first mechanism was the increased activity of F3'5'H or expression of the gene encoding that enzyme due to increased photosynthetically active radiation (*PAR)* transmittance into fruit zone leading to the concomitant increase in total skin anthocyanin (TSA) concentration and shift favoring tri-hydroxylated anthocyanins. The second mechanism was an increase in skin mass as a response to precocious exposure of the flower cluster infructescence to at least 20% of ambient *PAR* with the pre-bloom leaf removal treatment. The third mechanism identified enables growers to shift proportion from di- to tri-hydroxylated anthocyanins based on the fraction of irrigation applied and phenological stage of berry. Furthermore, the mechanism influencing flavonol and monomeric proanthocyanidin accumulation was the increased activity of FLS and LAR or expression of the genes encoding the enzymes, respectively, as a response to increased *PAR* leading to improved concentrations. Skin polymeric proanthocyanidin concentration was considerably affected by season but increased with defoliation treatment in first year of our study possibly as a response of ameliorated microclimate and a more favorable growing season associated with pre-bloom leaf removal. Finally, corresponding to recent

literature, seed polymeric proanthocyanidins were negligibly affected by leaf removal and water deficits.

Since there was no decrease in yield or berry composition and a proportionally preferred increase in berry flavonoids of Merlot, mechanical pre-bloom leaf removal is recommended in the SJV. If the vineyard is vertically integrated into a winery receiving fruit grown, RDI can be considered due to preferred proportional shift to tri-hydroxylated anthocyanins and the grower is compensated for the yield loss. Otherwise, for the majority of red wine grape growers a combination of pre-bloom leaf removal and irrigating to 80% of ET_c provides commercially acceptable yield with increased TSA, flavonols, monomeric and polymeric proanthocyanidins of skin, and reduced labor operations cost.

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APPENDICES

APPENDIX A: TABLES

Table 1. Phenological progression of 'Merlot 01/Freedom' in 2013 and 2014, in northern San Joaquin Valley of California.

		2013		2014	
Phenological stage	Modified Eichhorn-Lorenz Stage[z]	Date	GDD[y] accumulated	Date	GDD accumulated
Bud break	4	22-March	33	17-March	15
Anthesis	19	4-May	311	1-May	273
Fruit set	27	27-May	509	21-May	448
Veraison	35	15-July	1155	11-July	1107
Harvest	38	26-August	1715	19-August	1672
Dormant Pruning	-	9-January	-	9-January	-

[z] Modified Eichhorn-Lorenz stage = Modified E-L system for identifying major and intermediate grapevine growth stages (Coombe, 1995).
[y] GDD = growing degree day accumulation based on 100C since 15 March.

Table 2. Effects of mechanical leaf removal and applied water amounts on components of yield of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	Berry skin mass (mg)	Berry seed mass (mg)	Berry mass (g)	Berry/cluster (no)	Cluster mass (g)	Clusters/m	Yield (kg/m)
Leaf removal[y]				2013			
Control	55.0[z] a	26.7 a	1.36 a	125	167.7 a	41.2 b	6.64
Pre-bloom	51.7 a	25.5 b	1.27 b	117	147.4 b	44.5 ab	6.34
Post-fruit set	45.0 b	25.7 b	1.28 b	114	143.5 b	48.7 a	6.78
Pr>F	0.0020	0.0166	0.0216	0.2066	0.0029	0.0451	0.4996
ET_c fraction[x]							
SDI	51.3	25.7	1.34 a	116	153.5	46.9	6.86
RDI	47.8	26.2	1.26 b	121	152.3	42.7	6.31
Pr>F	0.5103	0.1687	0.0068	0.3176	0.8379	0.0876	0.0748
LR × ET_c fraction	0.9074	0.2473	0.9004	0.5804	0.5665	0.5855	0.8684
Leaf removal				2014			
Control	45.3 a	24.9	1.09	102	111.6	55.3 a	6.17 a
Pre-bloom	42.9 a	24.9	1.07	101	109.9	53.9 a	6.10 a
Post-fruit set	39.5 b	25.7	1.11	93	106.8	44.9 b	4.46 b
Pr>F	0.0310	0.1406	0.5314	0.1717	0.6426	0.0022	0.0016
ET_c fraction							
SDI	42.7	25.4	1.14 a	100	116.4 a	52.5	6.08 a
RDI	42.3	25.0	1.04 b	97	102.3 b	51.1	5.27 b
Pr>F	0.6963	0.3482	0.0021	0.4886	0.0011	0.9589	0.0135
LR × ET_c fraction	0.5892	0.1792	0.4878	0.8706	0.9943	0.0949	0.0778
Year	<0.0001	0.00039	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
Year × Leaf removal	0.0006	0.1174	0.0725	0.2119	0.0866	0.0002	0.0053
Year × ET_c fraction	0.7289	0.5608	0.0002	0.6396	0.0033	0.4565	0.0115
Year × LR × ET_c fraction	0.8891	0.2395	0.7788	0.9766	0.9986	0.1223	0.2039

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.

[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy at EL-Stage 17 or EL-Stage 29, or no leaf removal.

[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.

Table 3. Effects of mechanical leaf removal and applied water amounts on crop load of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	Pruning wt. (kg/vine)	Ravaz index (kg/kg)	Exposed leaf area (m^2/m)	Leaf area: fruit (m^2/kg)
Leaf removal[y]			2013	
Control	0.63[z] a	25 b	7.42 a	1.78 a
Pre-bloom	0.48 b	37 a	6.05 b	1.53 ab
Post-fruit set	0.50 b	35 a	5.83 b	1.38 b
Pr>F	0.0138	0.0399	0.0052	0.0389
ET_c fraction[x]				
SDI	0.52	34	6.44	1.52
RDI	0.55	30	6.40	1.60
Pr>F	0.5429	0.3872	0.8567	0.6068
LR × ET_c fraction	0.5814	0.7693	0.8789	0.9274
Leaf removal			2014	
Control	0.89 a	15 b	5.37 a	0.941
Pre-bloom	0.66 b	19 a	4.41 b	0.872
Post-fruit set	0.78 ab	14 b	4.20 b	0.948
Pr>F	0.0011	0.0105	0.0140	0.3050
ET_c fraction				
SDI	0.80	17	4.58	0.79 b
RDI	0.76	15	4.76	1.05 a
Pr>F	0.4157	0.0795	0.6730	0.0176
LR × ET_c fraction	0.5963	0.9188	0.7502	0.6048
Year	<0.0001	<0.0001	<0.0001	0.0421
Year × Leaf removal	0.0012	0.0003	0.0020	0.5477
Year × ET_c fraction	0.1206	0.5403	0.8818	0.0431
Year × LR × ET_c fraction	0.9960	0.7943	0.9655	0.8888

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.

[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.

[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.

Table 4. Effects of mechanical leaf removal and applied water amounts on berry composition of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	TSS (%)[w]	Juice pH	TA (g/L)[v]
Leaf removal[y]		2013	
Control	24.6[z] a	3.57	5.26
Pre-bloom	24.7 a	3.59	4.78
Post-fruit set	24.0 b	3.58	5.06
Pr>F	0.0171	0.6783	0.0779
ET_c fraction[x]			
SDI	24.2 b	3.59	5.04
RDI	24.7 a	3.57	5.02
Pr>F	0.0206	0.2531	0.8853
LR × ET_c fraction	0.8882	0.7035	0.7264
Leaf removal		2014	
Control	24.3	3.60	4.83
Pre-bloom	24.1	3.62	4.66
Post-fruit set	24.2	3.64	4.69
Pr>F	0.7905	0.2110	0.4152
ET_c fraction			
SDI	23.9 b	3.63	4.83
RDI	24.5 a	3.61	4.62
Pr>F	0.0199	0.2880	0.0786
LR × ET_c fraction	0.6608	0.5241	0.9698
Year	0.1108	0.0005	0.0036
Year × Leaf removal	0.0308	0.3772	0.0842
Year × ET_c fraction	0.0024	0.2874	0.3781
Year × LR × ET_c fraction	0.7704	0.7251	0.9197

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.
[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.
[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.
[w] TSS = percent total soluble solids expressed as degree Brix.
[v] TA = titratable acidity expressed as g tartaric acid/L.

Table 5. Effects of mechanical leaf removal and applied water amounts on berry skin anthocyanins (mg/kg) of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	3-glucosides					3-Acetyl-glucosides				3-Coumaroyl-glucosides		
	d-3-g	c-3-g	pet-3-g	peo-3-g	m-3-g	cy-3-g-a	pe-3-g-a	po-3-ga	m-3-g-a	pe-3-g-c	m-3-g-c	TSA[w]
Leaf removal[y]						2013						
Control	100[z] b	37.2 b	112 b	195 b	690 b	17.9 b	12.2 b	26.1 b	316 b	46.0	274 b	2066.4 b
Pre-bloom	168 a	74.0 a	169 a	288 a	943 a	27.9 a	17.6 a	38.8 a	462 a	49.2	387 a	2763.9 a
Post-fruit set	111 b	49.0 b	109 b	203 b	757 ab	18.1 b	15.0 ab	27.9 b	398 b	45.9	367 a	2381.5ab
Pr>F	0.0020	0.0017	0.0042	0.0135	0.0328	0.0014	0.0135	0.0059	0.0208	0.9721	0.0419	0.0055
ET_c fraction[x]												
SDI	115	61.5	121	232	795	17.5	14.2	30.0	402	43.8	316	2284.7
RDI	133	53.5	135	217	864	20.1	13.6	33.1	420	49.2	364	2527.2
Pr>F	0.2586	0.3892	0.4239	0.5644	0.5343	0.3236	0.6885	0.3640	0.8921	0.1031	0.2220	0.3190
LR × ET_c fraction	0.0836	0.1356	0.1681	0.1723	0.1212	0.0221	0.0638	0.0967	0.2354	0.8243	0.1249	0.1267
Leaf removal						2014						
Control	83 b	29.7 b	85.4 b	95.4 b	758 b	14.0 b	5.1 b	19.9 b	335 b	10.4 b	198 b	1554.1 b
Pre-bloom	94 a	38.6 a	94.1 a	121.4 a	915 a	15.2 a	7.4 a	22.1 ab	415 a	12.0 ab	269 a	2135.3 a
Post-fruit set	110 a	43.8 a	106.2 a	137.4 a	916 a	17.4 a	6.5 ab	24.5 a	394 ab	13.3 a	243 ab	2044.9 a
Pr>F	0.0178	0.0483	0.0363	0.0125	0.0421	0.0369	0.0206	0.0379	0.0439	0.0460	0.0220	0.0014
ET_c fraction												
SDI	94	40.9	92.9	128.7	830	15.1	6.9	22.6	379	11.4	219	1902.1
RDI	100	33.7	99.2	109.1	908	16.3	5.6	22.3	391	12.6	252	2012.6
Pr>F	0.6016	0.1453	0.4625	0.1017	0.1444	0.2965	0.0969	0.8600	0.6630	0.1921	0.1491	0.1093
LR × ET_c fraction	0.7999	0.7996	0.7722	0.7413	0.1666	0.2898	0.6280	0.1359	0.1295	0.1191	0.2599	0.2871
Year	0.0025	0.0018	0.0001	0.0001	0.0001	0.0032	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Year × Leaf removal	0.0005	0.0030	0.0012	0.0051	0.0465	0.0001	0.0253	0.0075	0.0491	0.5442	0.0031	0.0065
Year × ET_c fraction	0.2577	0.0725	0.2794	0.1501	0.2634	0.7751	0.2275	0.4655	0.6429	0.3021	0.2264	0.6230
Year × LR × ET_c fraction	0.0679	0.3604	0.1793	0.2523	0.1984	0.1959	0.2597	0.0835	0.1668	0.9450	0.2704	0.1221

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.

[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.

[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.

[w] TSA = total skin anthocyanin content..

Table 6. Effects of mechanical leaf removal and applied water amounts on proportion of skin anthocyanin of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	Anthocyanidin (%)[w]					Acylation (%)[v]		Hydroxylation (%)[t]	
	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin	Acylated TSA	Nonacylated TSA	Di-hydroxylated	Tri-hydroxylated
					2013				
Leaf removal[y]									
Control	4.7[z] b	2.8 b	9.1 a	12.1	70.2 ab	38.4	61.6	21.1	78.9
Pre-bloom	6.0 a	3.8 a	8.8 a	12.4	67.8 b	37.1	62.9	22.7	77.3
Post-fruit set	4.6 b	3.0 b	7.9 b	10.7	72.0 a	40.3	59.7	21.5	78.8
Pr>F	0.0006	0.0144	0.0145	0.1333	0.0281	0.2560	0.2570	0.6440	0.6435
ET_c fraction[x]									
SDI	4.9	3.4	8.4	12.3	69.5	38.2	61.8	22.9 a	77.1 b
RDI	5.2	2.9	8.7	10.9	70.2	38.9	61.1	20.5 b	79.5 a
Pr>F	0.3880	0.1075	0.3493	0.0591	0.5371	0.4205	0.4877	0.0011	0.0483
LR × ET_c fraction	0.3715	0.1358	0.7990	0.5364	0.4739	0.5494	0.1460	0.4300	0.1443
Leaf removal					2014				
Control	3.5 b	3.6 b	8.2 b	10.5	79.4 a	36.2	66.4	12.9	87.1
Pre-bloom	3.9 ab	3.9 ab	8.4 ab	10.9	78.8 ab	36.6	66.3	13.5	86.5
Post-fruit set	4.6 a	4.6 a	9.0 a	10.5	75.7 b	34.3	68.6	15.4	84.6
Pr>F	0.0435	0.0374	0.0421	0.2089	0.0381	0.1028	0.1127	0.2162	0.0889
ET_c fraction									
SDI	6.7	4.1 a	7.5	11.2 a	58.7	35.4	67.6	15.9 a	84.1 b
RDI	7.5	3.3 b	7.9	9.1 b	61.2	35.5	64.5	12.1 b	87.9 a
Pr>F	0.1093	0.0433	0.1540	0.0038	0.0650	0.5716	0.4751	0.0012	0.0011
LR × ET_c fraction	0.9325	0.5685	0.4843	0.8334	0.6993	0.5082	0.4495	0.8306	0.8169
Year	0.0001	0.0001	0.2460	0.0021	0.0001	0.5478	0.5512	0.0020	0.0001
Year × Leaf removal	0.0101	0.4328	0.9974	0.0144	0.0001	0.2219	0.3547	0.0001	0.0001
Year × ET_c fraction	0.0001	0.5412	0.5425	0.1254	0.1248	0.8512	0.8142	0.3671	0.3941
Year × LR × ET_c fraction	0.0012	0.4124	0.5478	0.2514	0.6578	0.5441	0.2578	0.3587	0.1465

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.
[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.
[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.
[w] Anthocyanidin = proportion of individual anthocyanin compounds of TSA.
[v] Acylation = acylated: acylated portion of the TSA; nonacylated: nonacylated portion of the TSA.
[t] Hydroxylation = di-hydroxylated: proportion of all cyanidin- and peonidin-based anthocyanins; tri-hydroxylated: proportion of all delphinidin-, petunidin-, and malvidin-based anthocyanins.

Table 7. Effects of mechanical leaf removal and applied water amounts on berry skin flavonoids (mg/kg) of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	(+)-catechin	(-)-epicatechin	quercetin	myricetin	tannins
Leaf removal[y]			2013		
Control	103[z] b	89 b	180 b	16.4 b	158 b
Pre-bloom	196 a	169 a	335 a	23.7 a	192 ab
Post-fruit set	144 ab	120 b	262 a	22.9 a	228 a
Pr>F	0.0016	0.0007	0.0003	0.0133	0.0140
ET_c fraction[x]					
SDI	136	116	242	19.5	193
RDI	155	128	266	21.9	188
Pr>F	0.3607	0.4465	0.4211	0.2910	0.7781
LR × ET_c fraction	0.2725	0.5666	0.9957	0.2629	0.8020
Leaf removal			2014		
Control	141 b	149 b	325 b	17.9 b	258
Pre-bloom	160 ab	166 ab	390 ab	22.0 a	260
Post-fruit set	186 a	184 a	432.1 a	22.3 a	265
Pr>F	0.0255	0.0481	0.0132	0.0395	0.9633
ET_c fraction					
SDI	157	160	378	19.6	269
RDI	172	175	384	21.9	253
Pr>F	0.3704	0.2079	0.8475	0.1726	0.4757
LR × ET_c fraction	0.9114	0.8767	0.6226	0.2128	0.7225
Year	0.4142	0.0096	<0.0001	0.4496	0.0030
Year × Leaf removal	0.0406	0.0019	0.0023	0.0240	0.0573
Year × ET_c fraction	0.2155	0.2305	0.6437	0.1778	0.7690
Year × LR × ET_c fraction	0.3029	0.6719	0.3879	0.3471	0.9545

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.

[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.

[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.

Table 8. Effects of mechanical leaf removal and applied water amounts on grape seed flavanols (mg/kg) of 'Merlot 01/Freedom' at harvest in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	(+)-catechin	(-)-epicatechin	tannins
Leaf removal[y]		2013	
Control	233[z] a	306 a	518
Pre-bloom	229 ab	288 a	542
Post-fruit set	201 b	241 b	547
Pr>F	0.0594	0.0022	0.3017
ET_c fraction[x]			
SDI	228	284	536
RDI	210	269	534
Pr>F	0.1342	0.3838	0.8741
LR × ET_c fraction	0.3919	0.1798	0.2331
Leaf removal		2014	
Control	125 b	122 b	292
Pre-bloom	149 a	149 a	308
Post-fruit set	153 a	139 a	331
Pr>F	0.0440	0.0294	0.1381
ET_c fraction			
SDI	145	141	314
RDI	140	132	305
Pr>F	0.6035	0.1569	0.5586
LR × ET_c fraction	0.0255	0.0106	0.0030
Year	<0.0001	<0.0001	<0.0001
Year × Leaf removal	0.0385	0.0065	0.4368
Year × ET_c fraction	0.4731	0.7829	0.9444
Year × LR × ET_c fraction	0.0579	0.0632	0.0708

[z] Columns followed by a different letter are significantly different within year and between treatment according to Tukey's HSD at Pr>F 0.05.

[y] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal.

[x] ET_c fraction = SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 ET_c from veraison – leaf fall.

Table 9. Labor operations cost and economic impact of mechanical leaf removal and applied water amounts of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 and 2014 (n = 4).

	Pruning cost ($/ha)	Leaf removal cost ($/ha)	Irrigation applied (ML/ha)	Irrigation cost ($/ha)	TSA[x] production (g/ha)	TSA unit cost ($/g)
			2013			
Control + SDI[z]	748	0	2.37	950	1086	1.56
Control + RDI	748	0	2.03	827	1718	0.92
Pre-bloom + SDI	748	30	2.37	950	1976	0.87
Pre-bloom + RDI	748	30	2.03	827	1958	0.82
Post-fruit set + SDI	748	30	2.37	950	1589	1.09
Post-fruit set + RDI	748	30	2.03	827	1799	0.89
			2014			
Control + SDI	748	0	3.08	1235	1079	1.84
Control + RDI	748	0	2.60	1029	1261	1.41
Pre-bloom + SDI	748	30	3.08	1235	1657	1.21
Pre-bloom + RDI	748	30	2.60	1029	1552	1.16
Post-fruit set + SDI	748	30	3.08	1235	1062	1.90
Post-fruit set + RDI	748	30	2.60	1029	1181	1.53

[z] Leaf removal on east side of the canopy in a 50cm window in the fruiting zone of the canopy pre-bloom at EL-Stage 17 or post-fruit set at EL-Stage 29, or no leaf removal; SDI: sustained deficit irrigation initiated at 0.8 ET_c from bud break – leaf fall; RDI: regulated deficit irrigation initiated at 0.8 ET_c from bud break – fruit set, 0.5 ET_c from fruit-set – veraison, and 0.8 Et_c from veraison – leaf fall.

[x] TSA = total skin anthocyanin content.

APPENDIX B: FIGURES

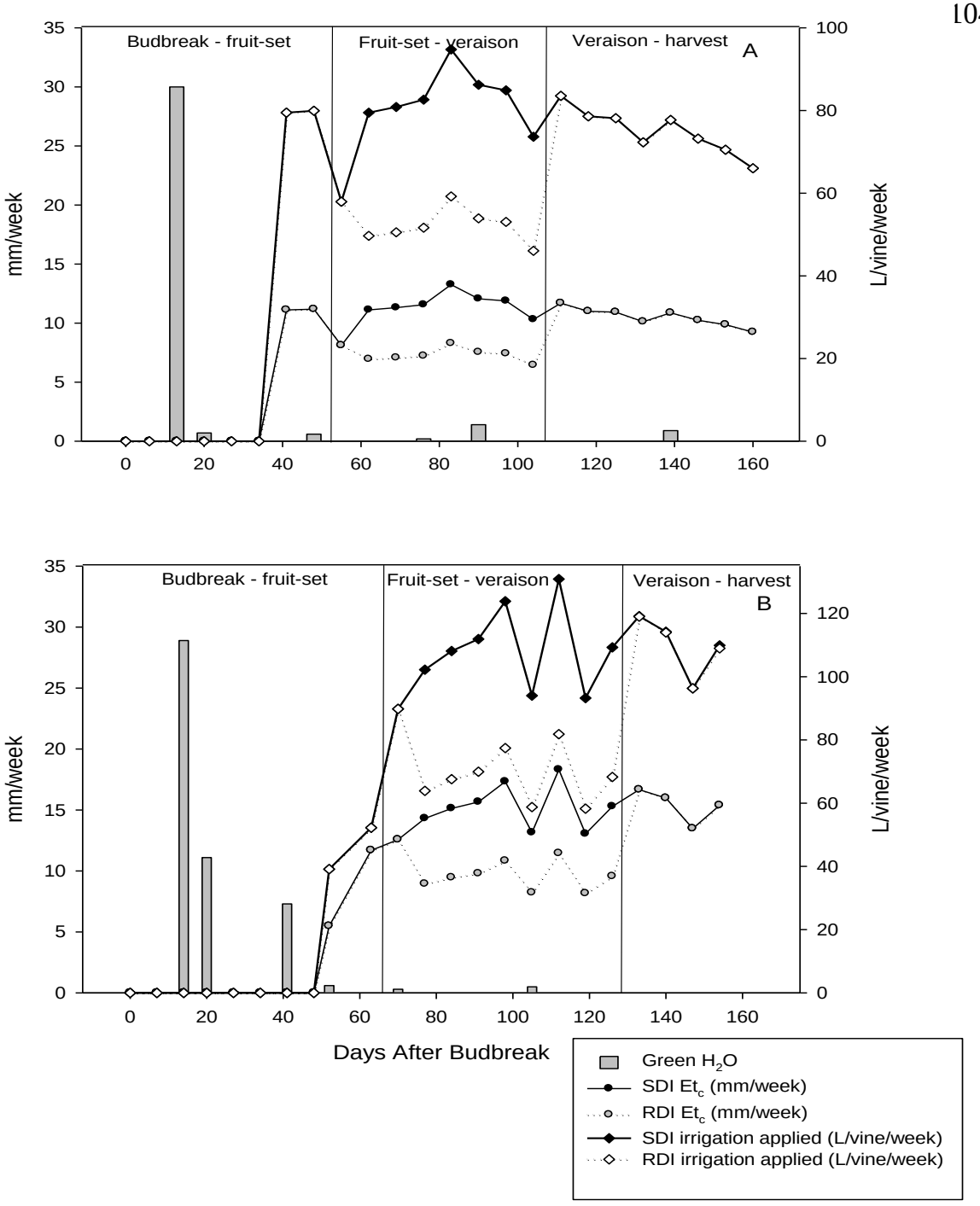

Figure 1. Seasonal water relations of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 (A) and 2014 (B).

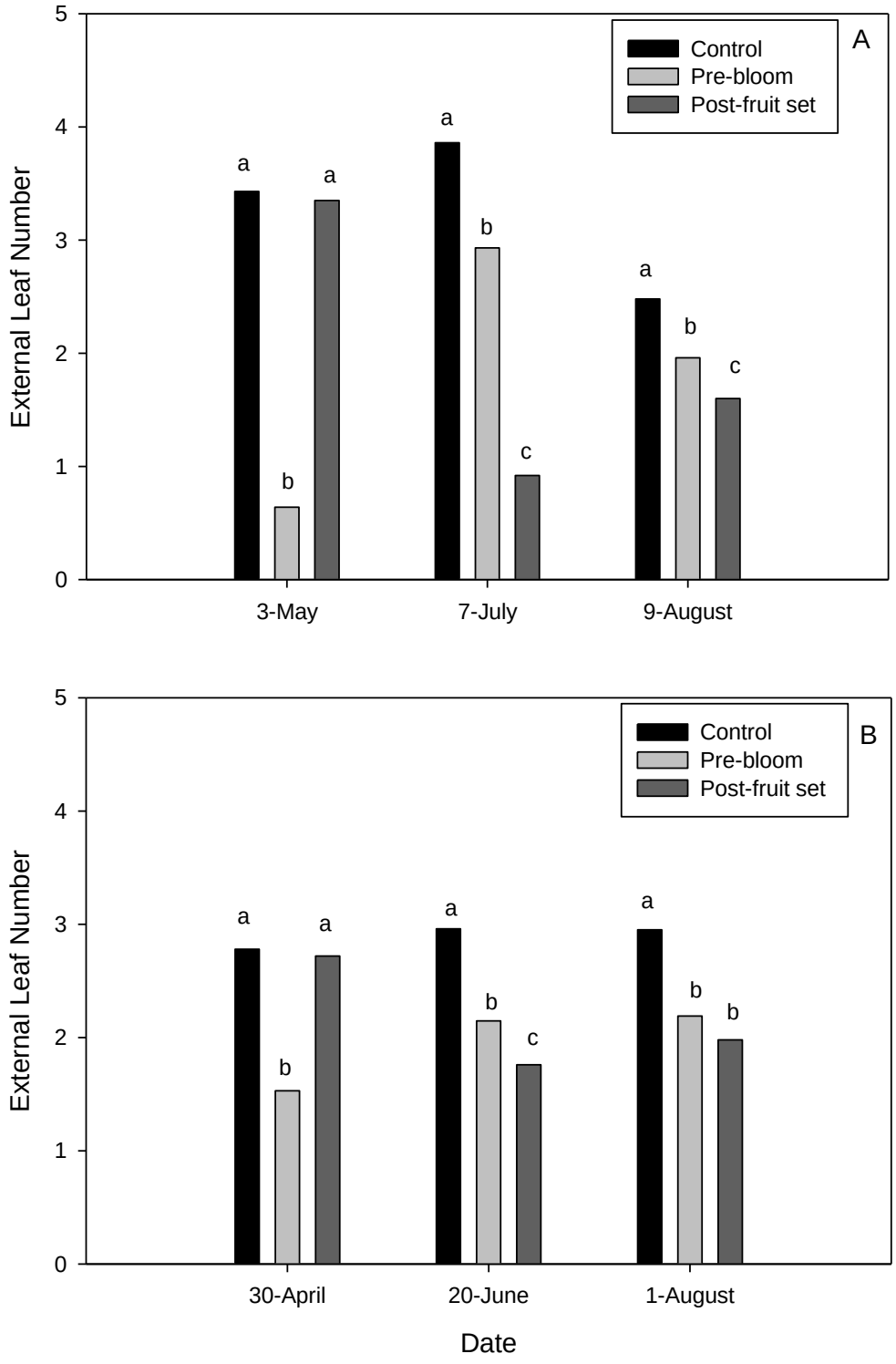

Figure 2. Effects of mechanical leaf removal on external leaf number of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 (A) and 2014 (B). Bars with different letters indicate statistical difference at $P < 0.05$ according to Tukey's HSD test.

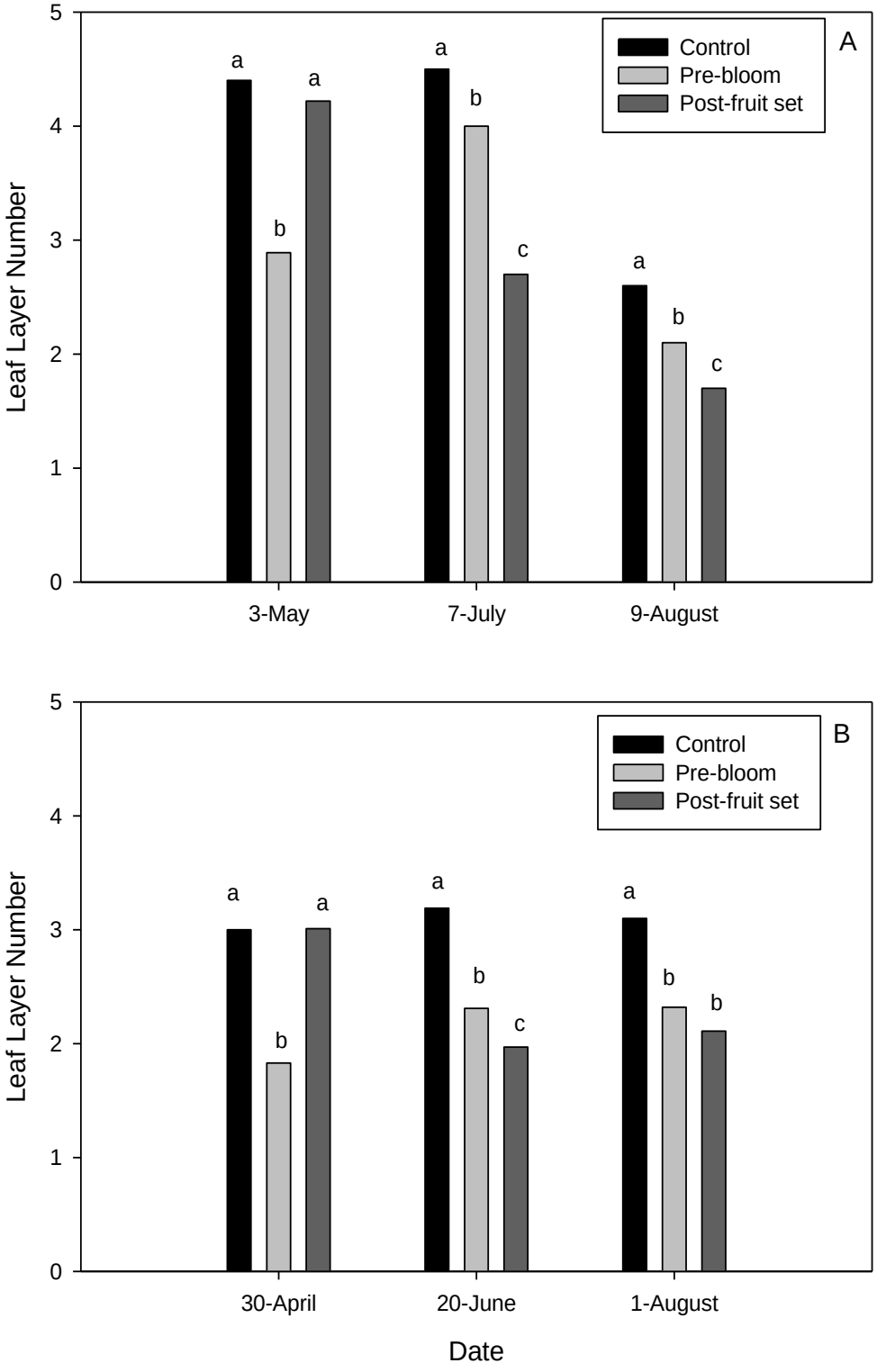

Figure 3. Effects of mechanical leaf removal on leaf layer number of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 (A) and 2014 (B). Bars with different letters indicate statistical difference at P <0.05 according to Tukey's HSD test.

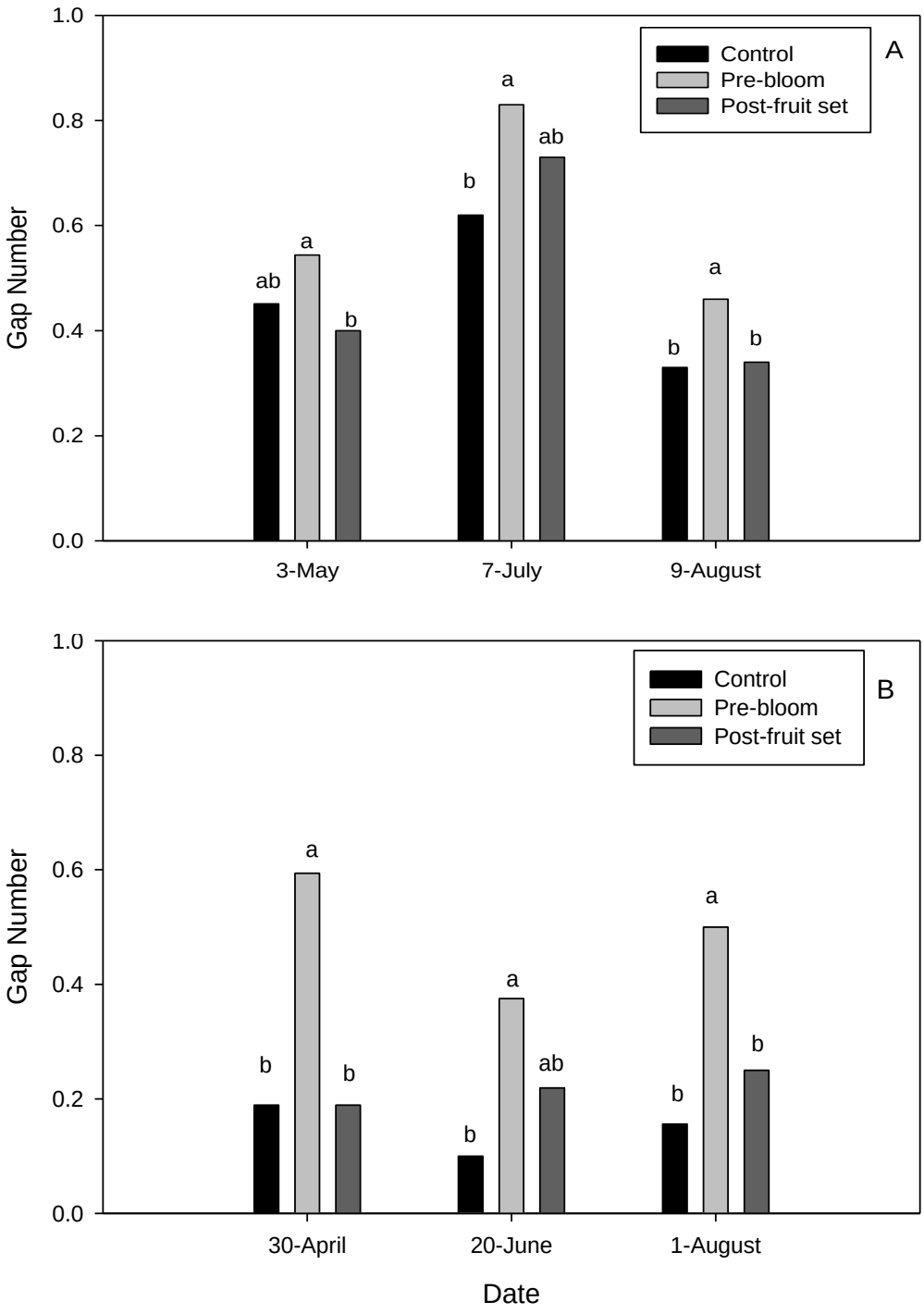

Figure 4. Effects of mechanical leaf removal on canopy gap number of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 (A) and 2014 (B). Bars with different letters indicate statistical difference at P <0.05 according to Tukey's HSD test.

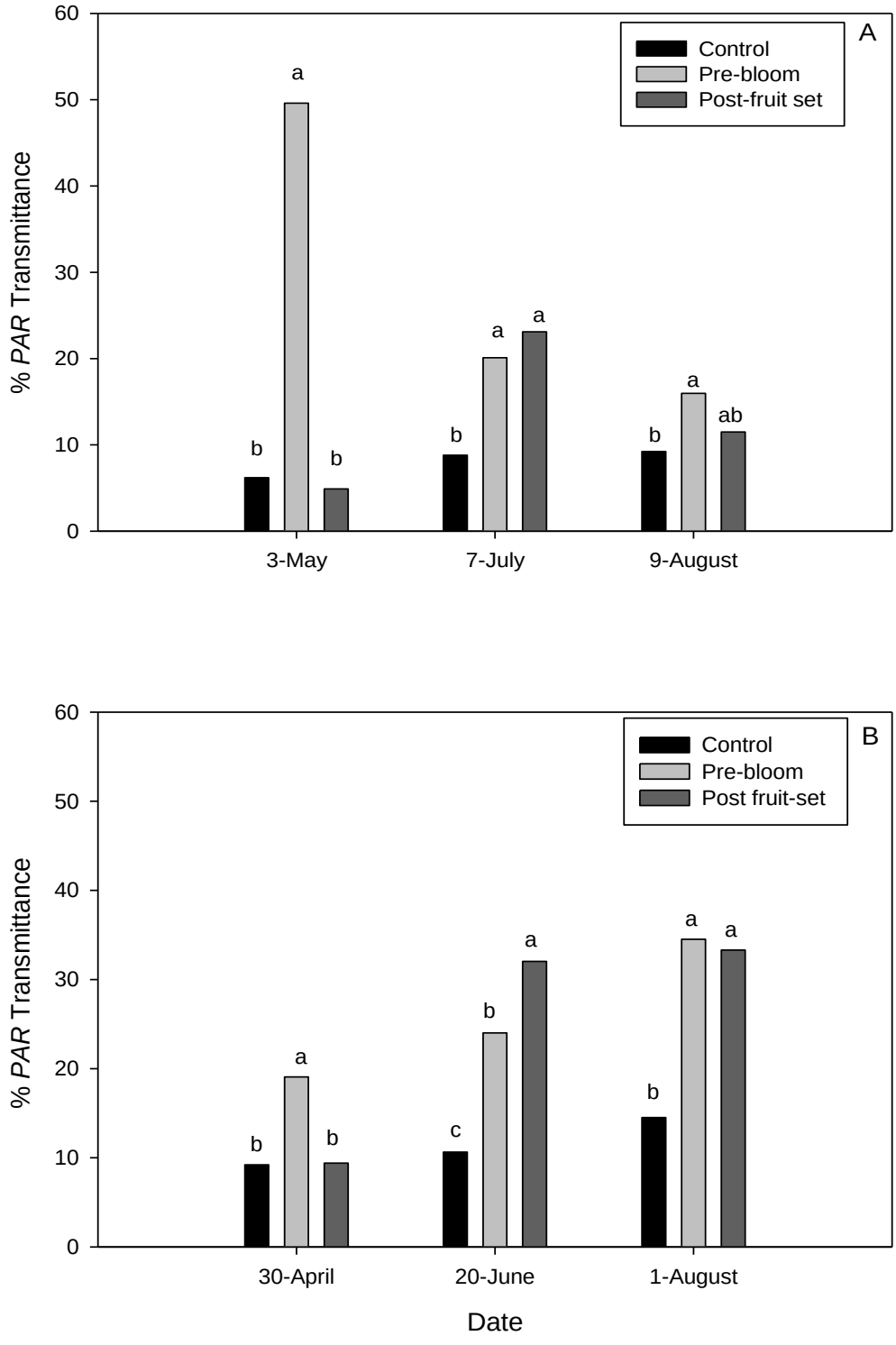

Figure 5. Effects of mechanical leaf removal on temporal progression of percent light transmission (% *PAR*) through the fruiting zone of 'Merlot 01/Freedom' in northern San Joaquin Valley of California in 2013 (A) and 2014 (B). Bars with different letters indicate statistical difference at $P < 0.05$ according to Tukey's HSD test.

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