

Mechanical Fruit-Zone Leaf Removal and Deficit Irrigation Practices Interact to Affect Yield and Fruit Quality of Cabernet Sauvignon Grown in a Hot Climate

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Abstract Background and goals

The hot climate in the San Joaquin Valley (SJV) makes it difficult to achieve the optimal berry color for Cabernet Sauvignon (*Vitis vinifera* L.), and manual cultural practices are cost prohibitive because of low price paid per ton. A field study was conducted to investigate the interactive effects of irrigation and mechanical fruit-zone leaf removal on grapevine physiology, yield components, and berry anthocyanins of Cabernet Sauvignon grapevines grown in the SJV.

Methods and key findings

A two-way factorial split-plot design with two irrigation treatments and three timings of mechanical leaf removal, each replicated five times, were studied over three years. The irrigation treatments included regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI). RDI was 50% crop evapotranspiration (ET_c) from fruit set to veraison and 80% ET_c from veraison to harvest, whereas SDI was 80% ET_c from fruit set to harvest. Three mechanical leaf removal treatments were tested: bloom, fruit set, and no leaf removal. RDI can increase berry anthocyanins at harvest by 14% in comparison to SDI with the sacrifice of 15% yield. Bloom and fruit-set mechanical leaf removal can increase anthocyanins by 19 and 13%, respectively, compared to no leaf removal control, with no effect on yield.

Conclusions and significance

Bloom leaf removal is more effective than RDI at improving berry total anthocyanin concentration without adversely affecting yield. Given the significant reduction on yield from RDI and the low economic return per ton of fruit in the SJV, bloom mechanical leaf removal coupled with SDI should be the preferred practice.

Key words: anthocyanin, irrigation, leaf removal, yield

Introduction

The San Joaquin Valley (SJV) is a viticultural area where \geq 70% of California winegrapes are grown (USDA NASS 2021). The grapevines must be irrigated because of the arid and hot climate of this area, but there are incentives for minimizing water use. For example, most vineyards are irrigated with groundwater, which is becoming increasingly costly to pump because of the depletion of groundwater, in contrast to surface water in the past, which is nearly no cost. Groundwater depletion has also led to new regulations that restrict agricultural water supply. In the SJV, deficit irrigation is a useful agronomic strategy to reduce applied water use while maximizing yield and fruit quality (Williams 2012, Martínez-Luscher et al. 2017).

Successful management of deficit irrigation requires knowledge of crop evapotranspiration (ET_c) (Williams and Trout 2005, Williams et al. 2010a, 2010b, 2012, Terry and Kurtural 2011). Sustained deficit irrigation (SDI) of 70 to 80% ET_c was found to balance economically sustainable yield, fruit quality, and water-savings goals (Williams 2010). Overirrigation causes grapevines to grow excessively, shading the fruit, which can directly reduce quality and favor the development of fungal diseases and insects (Daane and Williams 2003, Mendez-Costabel et al. 2014). Severe preveraison water deficits significantly reduce grapevine vegetative and reproductive growth, reduce photosynthesis, and delay fruit maturity by reducing net carbon assimilation (A_{net}) (Williams et al. 2010a, 2010b, Levin et al. 2020),

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whereas imposing a moderate postveraison water deficit can sustain grape yield and improve fruit quality while improving irrigation efficiency and reducing vineyard water input in a hot climate (Williams 2014, Levin et al. 2020). The best severity and timing of water deficit depends on production goals and climatic conditions.

In addition to deficit irrigation, many growers remove leaves in the fruit zone to increase fruit exposure, which may directly improve fruit quality and create a microenvironment that discourages powdery mildew and bunch rots (Austin and Wilcox 2011, Zhuang et al. 2014). Leaf removal is commonly practiced in cool climates to mainly reduce disease damage on tight cluster varieties (Sabbatini and Howell 2010), however, studies on leaf removal in hot climates also showed similar benefits (Stapleton et al. 1990, Williams 2012, Cook et al. 2015). Timing and extent of fruit-zone leaf removal determine the potential effect on grapevine yield and berry chemical composition at harvest (Poni et al. 2023). In a cool climate, basal leaf removal prior to bloom may reduce fruit set, thus lowering yield (Acimovic et al. 2016). Effects on fruit set depend on the extent of leaf removal (Acimovic et al. 2016) and are also modulated by weather (Frioni et al. 2017). In hot climates, mechanical fruit-zone leaf removal at bloom had no effect on fruit set or yield (Cook et al. 2015). Along with the potential to reduce fruit set, leaf removal prior to bloom can increase berry total soluble solids (TSS) and anthocyanin content, as well as other secondary metabolites (Ryona et al. 2008, Gatti et al. 2012, VanderWeide et al. 2018). Recently, mechanical fruit-zone leaf removal has gained popularity because of labor shortages and increased labor costs (Hed and Centinari 2018, Zhuang et al. 2019, Kurtural and Fidelibus 2021), and mechanical fruit-zone leaf removal in a cool climate can yield similar or even better results compared to manual leaf removal on final berry chemical composition (VanderWeide et al. 2018).

A few studies investigated the interactive effects of irrigation and mechanical fruit-zone leaf removal on grapevine yield and berry primary and secondary metabolites. In previous studies on Merlot, the irrigation and leaf removal did not interact to affect yield or fruitquality parameters (Williams 2012, Cook et al. 2015). Therefore, deficit irrigation and mechanical leaf removal could be good management strategies for Cabernet Sauvignon, which generally has suboptimal fruit-quality in hot climate regions. However, data to support this hypothesis are lacking. Therefore, the objective of this trial was to determine how irrigation and mechanical fruit-zone leaf removal affect yield and berry chemical composition of Cabernet Sauvignon grown in the SJV.

Materials and Methods

Vineyard site

The experiment was conducted in a commercial vineyard located in Madera County, CA (37°02′N; 120°25′W). The vineyard was established in 2013 on Pachappa fine sandy

loam soil, described as well-drained (minimal), noncalcic brown soils developed from moderately coarse-textured alluvium (www.nrcs.usda.gov). The vines were 10-yearold Cabernet Sauvignon (Vitis vinifera L., clone FPS 08) on Freedom [(solonis × Othello) × Dogridge] rootstock, with the average crop ranging from 22.5 to 27.0 t/ha. The grapevine plant spacing was $1.2 \text{ m} \times 3.0 \text{ m}$ (grapevine × row) with the rows oriented northeast-southwest. The grapevines were quadrilateral cordon trained, with a 55 cm crossarm, to 1.2 m high above the vineyard floor with a pair of catch wires 30 cm above the cordons. Grapevines were spur pruned in the dormant season leaving 10, two-bud spurs per meter of cordon, and shoots were positioned between the catch wire at shoot lengths of ~0.90 to 1.00 m. The vineyard was dripirrigated with pressure-compensating emitters spaced at 76 cm apart, delivering 1.60 L/hr. All cultural practices except irrigation and leaf removal were carried out according to the University of California Cooperative Extension (Zhuang et al. 2019).

Experimental design

This experiment employed a two (irrigation) × three (leaf removal) factorial split-plot design for three seasons, 2018 through 2020, with the main plot treatments being regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI), and the subplot treatments being bloom leaf removal, fruit-set leaf removal, or no leaf removal. Main-plot treatments were randomly assigned to whole rows in a randomized complete block design, replicated five times. Each main plot was equally divided into three subplots, which were randomly assigned to the different subplot treatments. There were six experimental plots per block and each plot was composed of six experimental units (grapevine). A total of 180 grapevines were used for this field study.

Weather data

Weather data were collected for 2018 through 2020 from the nearby California Irrigation Management Information System (CIMIS) station of Los Banos, Merced County, CA (37°05′N; 120°45′W). The CIMIS station was located ~29 km northwest of the field site. Ambient temperature, precipitation, and solar light radiation data were recorded hourly. Growing degree days (GDD) were calculated based on the single sine curve method (Baskerville and Emin 1969) after budbreak at 10°C.

Plant water status

The midday leaf water potential (Ψ) was assessed after each weekly irrigation cycle between 1230 to 1430 hrs (solar noon) on a recently fully expanded leaf exposed to the sun and showing no sign of disease or damage. A zip-top plastic bag was placed over a single leaf and sealed around the petiole before it was severed. Then, midday leaf Ψ was directly determined using a pressure chamber (Model 610 Pressure Chamber Instrument, PMS Instrument Co.). Two leaves per experimental unit were measured weekly during the growing season, as described (Terry and Kurtural 2011).

Irrigation treatments

The grapevines were first irrigated when the Ψ reached -1.0 MPa. Thereafter, irrigation was maintained at 80% of weekly ET_c for all irrigation treatments before fruit set. The ET_c was calculated using the equation of $ET_c = ET_o \times K_c$ (Williams 2010), where ET_0 is the reference transpiration and K_c is the crop coefficient. The amount of water applied in each irrigation cycle was determined by multiplying irrigation duration by the average flow rate of the emitters. The average flow rate was determined by measuring emitter output over time with a graduated cylinder. ET_o was obtained from the nearby CIMIS station of Los Banos, and Kc was calculated using additional grapevines by measuring (weekly at solar noon) the shade cast on the vineyard floor beneath the grapevine canopy irrigated at ~100% of weekly ET_c (Cook et al. 2015). Those grapevines were not water stressed, and therefore, were suitable for use to develop a nonstressed baseline K_c (Williams 2010). This irrigation regime was achieved by installing three emitters per grapevine under the same irrigation duration as other irrigation treatments. Weekly midday leaf Ψ was measured to maintain the Ψ < -1.0 MPa. After fruit set, SDI and RDI treatments were applied differentially as main plot factors: SDI maintained 80% of weekly ET_c from fruit set to harvest, and RDI maintained 50% of weekly ET_c from fruit set to veraison; after veraison, RDI was switched back to 80% of weekly ET_c until harvest. SDI represented the grower's standard for this area, and SDI and RDI were implemented by adjusting different emitters per grapevine under the same irrigation duration. Irrigation was shut off two weeks after harvest and additional postharvest irrigation might be applied based on the precipitation and soil moisture level.

Leaf removal

Leaf removal treatments were applied in subplots at two different times during the growing season (Table 1): at bloom (Eichhorn-Lorenz [E-L] stage 23), and at fruit set (E-L stage 31), e.g., berry pea-size; no leaf removal was the control (Coombe 1995). Grapevine phenological stages were assessed based on the E-L system. Bloom timing was on average three to four weeks prior to fruit set when the RDI and SDI were initiated. Leaf removal was applied to both sides of the canopy at ~400 GDD (E-L stage 19, ~five to seven days before full bloom) using a roll-over leaf plucker with a sickle-bar sprawl clipper adapted for a sprawling-type canopy (Model EL-50, Clemens Vineyard Equipment). The leaf plucker defoliated a 60 cm window in the fruiting zone of the canopy to increase photosynthetically active radiation (PAR) in the fruiting zone. Leaf removal at fruit set was applied one time at ~630 GDD, approximately seven to 14 days after full bloom, following the same procedure.

Light and canopy measurements

Midday PAR in the fruit zone was measured per grapevine basis using a line quantum sensor (Li-191R, LI-COR Biosciences) at prebloom, postbloom, prefruit set, postfruit set, and veraison. Six PAR readings were averaged from one experimental unit per canopy side and the ambient PAR was simultaneously measured to calculate the percent of PAR transmitted to the fruit zone as described (Cook et al. 2015). In 2019 and 2020, midday leaf gas exchange-including leaf Anet and transpiration-was also measured biweekly, selecting a recently fully expanded leaf exposed to direct sunlight, and two leaves per experimental unit were measured using a portable gas exchange analyzer (Li-Cor 6400, LI-COR Biosciences). Total leaf area per vine was measured destructively at veraison by defoliating a 1 m section of the canopy from a treated grapevine adjacent to the data collection grapevines for a total of 30 replicates, and all the leaves collected in the plastic bag were stored in a cooler before being transported to the laboratory. In the laboratory, leaf area was measured using the leaf area meter (LI-3100C Area Meter, LI-COR Biosciences), and the total leaf area per grapevine was calculated as reported (Cook et al. 2015).

Yield components

When the berry TSS reached the commercial harvest standard of ~24 Brix for SJV Cabernet Sauvignon, yield and yield components (number of clusters, average cluster weight, average berry weight, and number of berries per cluster) were determined at harvest from each vine using methods similar to those described (Fidelibus et al. 2009). Mean cluster weight was calculated as the ratio of yield divided by number of clusters per grapevine. One hundred berries from each side of the canopy were randomly collected into zip-top bags from the top, middle, and bottom of clusters. The mean berry weight was calculated as the total berry weight divided by 100. Then the cluster samples collected from both sides of the canopy were placed in coolers and taken to a laboratory to measure basic berry composition. The number of berries per cluster was calculated as the ratio of the mean cluster weight divided by the mean berry weight. Rot incidence at harvest was determined by counting the number of clusters that had four or more adjoining

 Table 1
 Calendar date (CD) and growing degree days (GDD based on 10°C) of grapevine phenological stages in 2018, 2019, and 2020 in Los Banos, California.

Year	Budbreak		Bloom		Fruit set		Veraison		Harvest	
rear	CD	GDD ^a	CD	GDD	CD	GDD	CD	GDD	CD	GDD
2018	29 March	10	15 May	355	8 June	590	14 July	1084	5 Oct	2220
2019	2 April	5	18 May	357	18 June	710	27 July	1243	25 Oct	2311
2020	25 March	1	16 May	370	10 June	664	3 Aug	1405	20 Oct	2433

^aGDD is calculated starting from budbreak date.

berries showing decay from molds. The percentage of clusters with rot were then calculated. The leaf area to fruit ratio (m^2/kg) was calculated as the ratio of total leaf area per grapevine divided by the yield per grapevine. During the winter dormant season, pruning weights of one-year-old canes were recorded as well as the total shoot number per grapevine, and the Ravaz index (kg/kg) was calculated as the ratio of yield divided by the pruning weight. Bud fertility was calculated as the ratio of total inflorescences per grapevine divided by the total shoot number 20 days after budbreak. The number of inflorescences and shoots were counted when the average shoot length was ~25 to 35 cm.

Berry primary and secondary metabolites

Starting at veraison, 100 berries per experimental unit were randomly collected biweekly for a total of four sampling dates prior to harvest. Berries from the top, middle, and bottom of the clusters were sampled and put into ziptop bags and stored in a freezer until processed. Berries were hand crushed in the zip-top bags and the resulting juice was used to measure TSS (measured as Brix), pH, and titratable acidity (TA; g/L of tartaric acid). TSS (Brix) was determined using a digital refractometer (Atago PR-32 Palette digital refractometer, ATAGO USA) and juice pH was measured by a pH meter (Orion 2 star, Thermo Fisher Scientific). The TA, in g of tartaric acid/L of juice, was measured by titrating to an end point pH of 8.2 with 0.1 N sodium hydroxide and expressed as g/L. Berry total anthocyanins and phenolics were measured using a UV-vis spectrophotometer (Lambda 25 ultraviolet/VIS, Perkin Elmer) as described (Zhuang et al. 2014).

Statistical analysis

Two-way analysis of variance (ANOVA) (irrigation × leaf removal) was conducted for fruit-zone PAR, leaf gas exchange, and midday leaf Ψ . Three-way ANOVA (irrigation × leaf removal × year) was run for yield components, and fourway ANOVA (irrigation × leaf removal × canopy side × year) was run for harvest fruit chemistry using the PROC Mixed Procedure of SAS (v. 9.4, SAS Institute, Inc.). Only interaction irrigation × leaf removal on TSS was tested significantly with p < 0.05. All data were tested for normality using the Shapiro-Wilk's test. When the normality test failed, data were log or square root transformed to pass the test. Differences among treatment means were tested by Tukey's honestly significant difference at p < 0.05 using least squares means under the Mixed Procedure.

Results

GDD accumulation was similar in 2018 and 2019, whereas in 2020 a new heat record in CA was set, followed by cooling due to wildfire smoke (Table 1). Canopy closure occurred at approximately fruit-set stage each year (Tables 1 and 2), although maximum K_c was higher in 2019 than in 2018 or 2020 because of the abundant precipitation in the early spring of 2019, which promoted a large spring canopy (Table 2). High

precipitation also postponed the first irrigation in 2019 until approximately fruit set, which was 710 GDD. The annual irrigation amount for RDI was 2.97 ML/ha (2276 liters per vine [LPV]) in 2018, 2.42 ML/ha (1849 LPV) in 2019, and 3.00 ML/ ha (2293 LPV) in 2020, and the annual irrigation amount for SDI was 3.58 ML/ha (2736 LPV) in 2018, 3.00 ML/ha (2290 LPV) in 2019, and 3.73 ML/ha (2850 LPV) in 2020 (Figure 1). The higher irrigation amount in 2020 was mainly because of the lower winter precipitation and record summer heat.

Midday leaf Ψ was maintained at >-1.0 MPa before irrigation was initiated, and maintained with 80% ET_c before fruit set. After fruit set, Ψ from the RDI treatment was consistently lower than Ψ from the SDI treatment until veraison, when irrigation was imposed at the same level of 80% ET_c (Figure 2). Leaf removal treatments had no effect on leaf Ψ (data not shown).

Fruit-zone PAR was significantly affected by mechanical fruit-zone leaf removal in three seasons (Figure 3). Leaf removal at bloom (>50% of ambient PAR) almost doubled the fruit-zone PAR compared to the no leaf removal (20% of ambient PAR) in 2018 and 2020, and the elevated levels of PAR in the fruit zone lasted for approximately two weeks; at that point, PAR from bloom leaf removal was similar to the no leaf removal control. Fruit-set leaf removal also increased fruitzone PAR in comparison to the no leaf removal. Although its increase of fruit-zone PAR was less pronounced than it was from bloom leaf removal, the elevated fruit-zone PAR from fruit-set leaf removal was maintained from fruit set to veraison. In two out of three years, this elevation even stayed after veraison, while fruit-zone PAR from bloom leaf removal started to decline after bloom and stayed the same as the no leaf removal control through the rest of the season. The effect of leaf removal on fruit-zone PAR was consistent across three years. However, we did notice a significant variation between years, particularly in 2019. While bloom leaf removal increased the fruit-zone PAR by up to 50% or more of ambient PAR in 2018 and 2020, it only increased PAR by up to 15% of ambient PAR in 2019. The same results were also found on fruit-zone PAR from the no leaf removal control at bloom, which was much lower in 2019 (<10% of ambient) than in 2018 and 2020 (~20% of ambient).

Fruit-zone PAR was also affected by irrigation treatments. Across three years, RDI increased the fruit-zone PAR after fruit set (Figure 4), in comparison to SDI. Effects of irrigation treatments on fruit-zone PAR were consistent and unchanged until veraison.

Table 2 Maximum crop control in 2018	pefficient (K _c , 2019, and 2	Sauvignon	
	2018	2019	2020
Maximum K _c ^a	0.84	0.87	0.82
Growing degree days ^b	685	655	856

 ${}^{a}K_{c}$ is calculated based on the canopy casting from fully irrigated grapevines with ~100% crop evapotranspiration at solar noon. ${}^{b}G$ rowing degree days is calculated between the budbreak date and the date when maximum K_c was achieved; the calculation is based on 10°C.

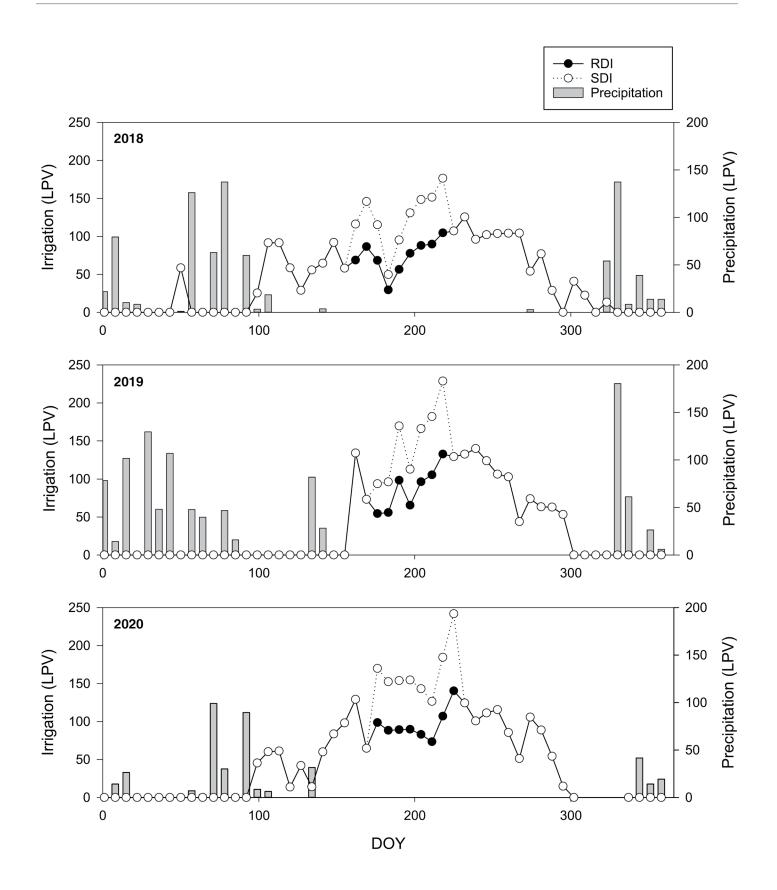


Figure 1 Weekly irrigation from regulated deficit irrigation (RDI), sustained deficit irrigation (SDI), and precipitation (liter per vine [LPV]) from growing seasons of 2018, 2019, and 2020. 2018 yearly irrigation amount: RDI, 2276 LPV (2.97 ML/ha); SDI, 2736 LPV (3.58 ML/ha). 2019 yearly irrigation amount: RDI, 1849 LPV (2.42 ML/ha); SDI, 2290 LPV (3.00 ML/ha). 2020 yearly irrigation amount: RDI, 2293 LPV (3.00 ML/ha); SDI, 2850 LPV (3.73 ML/ha). DOY, day of year.

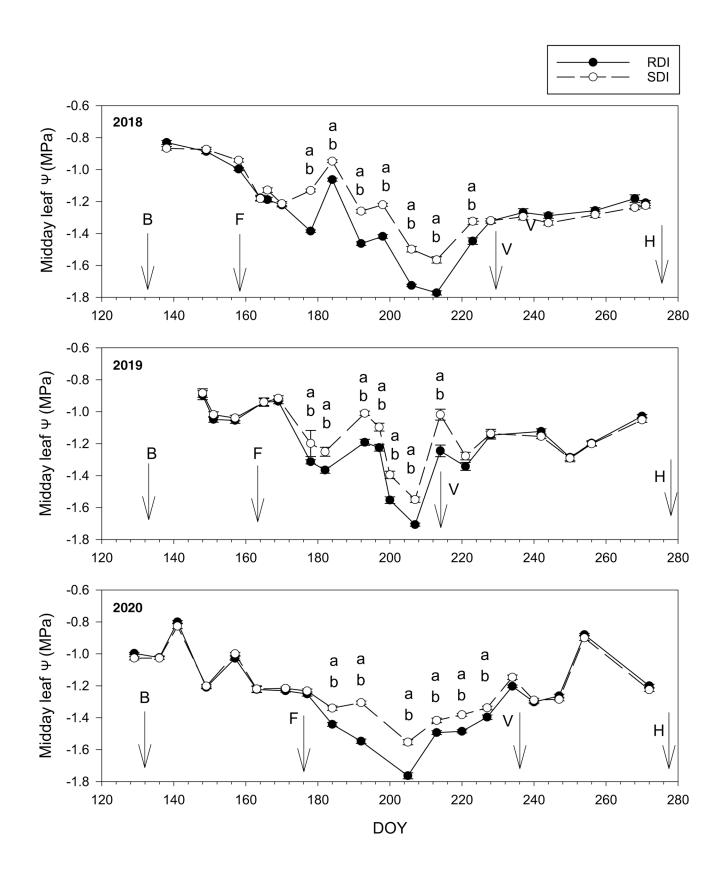


Figure 2 Midday leaf water potential (ψ) from regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) in 2018, 2019, and 2020. Arrows indicate the phenological stages of bloom (B), fruit set (F), veraison (V), and harvest (H) of each growing season, and different letters represent significant differences between treatments according to Tukey's honestly significant difference at p < 0.05. DOY, day of year.

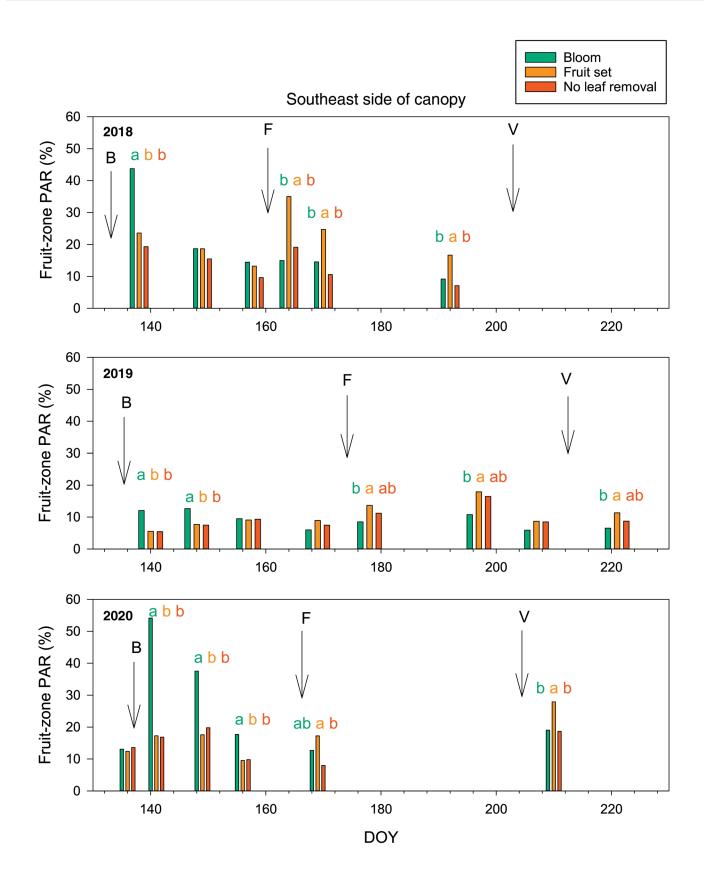


Figure 3 Midday fruit-zone photosynthetically active radiation (PAR, % of ambient) daily average on the southeast side of canopy from bloom leaf removal (Bloom), fruit set leaf removal (Fruit set), and no leaf removal in 2018, 2019, and 2020. Arrows indicate the phenological stages of bloom (B), fruit set (F), and veraison (V) of each growing season. Different letters represent significant differences between treatments according to Tukey's honestly significant difference at p < 0.05. DOY, day of year.

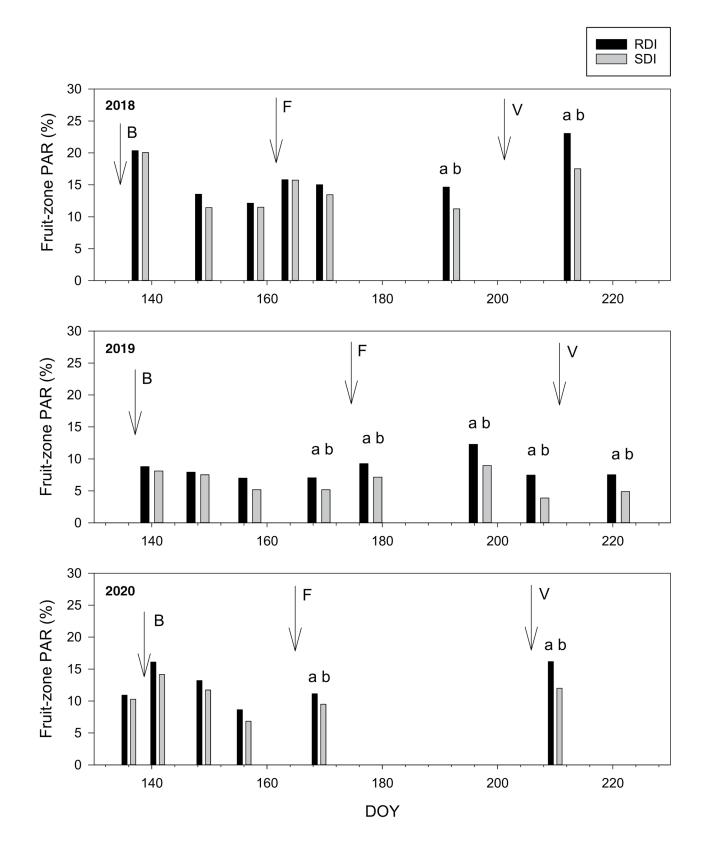


Figure 4 Fruit-zone photosynthetically active radiation (PAR, % of ambient) from regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) during the growing seasons of 2018, 2019, and 2020. PAR value was averaged on the southeast side of canopy. Arrows indicate the phenological stages of bloom (B), fruit set (F), and veraison (V) of each growing season. Different letters represent significant differences between treatments according to Tukey's honestly significant difference at p < 0.05. DOY, day of year.

Grapevine A_{net} showed consistent results from leaf removal treatments in 2019 and 2020 (Figure 5). Bloom leaf removal increased leaf A_{net} from leaf removal through fruit set. Leaf removal treatments did not affect leaf A_{net} after fruit set. RDI also significantly decreased leaf A_{net} between fruit set and veraison, compared to SDI, as water stress increased (Figure 6).

RDI reduced yield by 15% compared to SDI, on average, across three years, and the reduction of yield from RDI was mainly due to smaller berries and clusters (Tables 3 and 4). Leaf removal did not significantly affect yield (Table 3).

Irrigation treatment did not significantly reduce leaf area in any given year, because both RDI and SDI were applied at fruit set and canopy closure was achieved around the same time (Table 3). Leaf removal reduced whole-vine leaf area by 9% at bloom and 11% at fruit set, in comparison to no leaf removal. In contrast to the whole vine's leaf area, irrigation treatment significantly influenced the leaf area to fruit ratio, and the leaf area to fruit ratio was increased from 0.62 m^2/kg for SDI to 0.71 m^2/kg for RDI. Pruning weight was also significantly reduced by RDI (Table 3). Leaf removal did not affect the leaf area to fruit ratio or pruning weight in any given year during this experiment. No effects of either irrigation treatment or leaf removal on shoot number per grapevine were found (Table 3).

Berry weight at harvest was mainly affected by irrigation treatment as it was previously reported, and RDI reduced the final berry weight by 8% in contrast to SDI. Leaf removal did not influence berry weight in any year (Table 4). The canopy side also affected berry weight (Table 4), and berries were heavier from the northwest versus the southeast side of the canopy.

RDI reduced TSS by 0.7 Brix, but leaf removal had no effect on TSS. Leaf removal at fruit set increased juice pH, but irrigation did not. Neither irrigation nor leaf removal affected juice TA (Table 4). The effect of canopy side on soluble solids and TA were inconsistent. Interestingly, we found a significant interactive effect of leaf removal and irrigation on berry TSS (Table 5). Leaf removal either at bloom or fruit set significantly increased berry TSS when SDI was applied, whereas leaf removal generally reduced the harvest TSS when RDI was applied.

Anthocyanins were significantly affected by both leaf removal and irrigation treatments. The increase of berry anthocyanins resulting from leaf removal at either bloom or fruit set was observed immediately after veraison (Figure 7). Increased berry anthocyanins were maintained through harvest each year (Figure 7), although the patterns of accumulation showed annual variations. Berry total anthocyanin accumulation peaked at ~240 DOY (day of year) at ~21 Brix then declined before harvest in 2020, while anthocyanin accumulation increased at the onset of veraison and never peaked before harvest in 2018 and 2019. Similarly, RDI increased berry anthocyanins after veraison and they remained elevated until harvest (Figure 8). As a summary, across three years, RDI increased berry anthocyanins at harvest by 14% compared to SDI, and bloom and fruit set leaf removal increased anthocyanins by 19 and 13%, respectively, compared to no leaf removal.

Discussion

Williams and Trout (2005) showed that a targeted midday **v** can be achieved by irrigating the vines with a fraction of ET_c. Nelson et al. (2016) further confirmed that by using a fraction (SDI versus RDI) of ET_c at different growth stages, grapevine water status can be maintained at a certain range of midday Ψ. Our results validated the use of a fraction (50 and 80%) of ET_c to induce moderate grapevine water stress at the targeted phenological stage to limit canopy growth, reduce berry size, increase fruit-zone PAR, and achieve higher anthocyanin content (Figure 2). RDI reduced leaf Anet in our study, but before the RDI was imposed at fruit set, leaf removal generally increased leaf Anet (Figures 5 and 6). Martínez-Lüscher and Kurtural (2021) have shown that manual leaf removal prebloom increased leaf Anet, apparently to help compensate for the loss of leaf area. Leaf removal either through mechanical or manual means around the fruit zone did not have a significant effect on the midday leaf Ψ from previous studies (Williams 2012, Cook et al. 2015) because the removal of basal leaves around the fruit zone did not have a significant effect on the canopy light interception, which affected the midday leaf Ψ .

Irrigation treatments had more effect on berry weight and yield than leaf removal treatments in our study. Because RDI reduced yield more than the leaf area, the leaf area to fruit ratio was significantly increased by RDI, unlike previous studies (Williams et al. 2010a, 2010b). Differences between studies were probably due to differences in the timing, duration, and severity of water stress. A leaf area to fruit ratio between 0.5 to 1.0 m²/kg is considered to be in balance, whereas <0.5 to >1.0 m²/kg is generally regarded as overcropped and undercropped, respectively (Kliewer and Dokoozlian 2005). In our study, leaf area to fruit ratios were all in the optimum category.

In summary, RDI decreased berry TSS compared to SDI (Table 4). However, the significant interactive effect of leaf removal and irrigation treatments on berry TSS (Table 5) suggests that under the circumstance of high leaf A_{net}, as observed under SDI, leaf removal generally increased berry TSS. As cluster temperature increases because of the light exposure from the leaf removal treatment, the berry primary metabolism increases until the midday PAR exceeds 50 to 100 μ mol/m²/sec, when higher cluster temperature starts to inhibit the sugar accumulation (Bergqvist et al. 2001). This conclusion agreed with previous reports (VanderWeide et al. 2018, 2020). When leaf area was not limiting, timing of leaf removal did not show any differential effect on berry TSS. However, when vine carbon starvation occurred because of the restricted leaf A_{net} , as shown by results from Figure 6, or preharvest leaf senescence resulted from the severe water stress or other abiotic factors, leaf removal could cause further carbon starvation, therefore reducing the berry TSS (Martínez-Lüscher and Kurtural 2021).

The effect of irrigation treatments on berry TSS was dependent on the severity of water stress and leaf removal treatment in our study. Similar studies in the same region have demonstrated that preveraison water stress may upregulate the anthocyanin biosynthetic pathway (Yu et al. 2016).

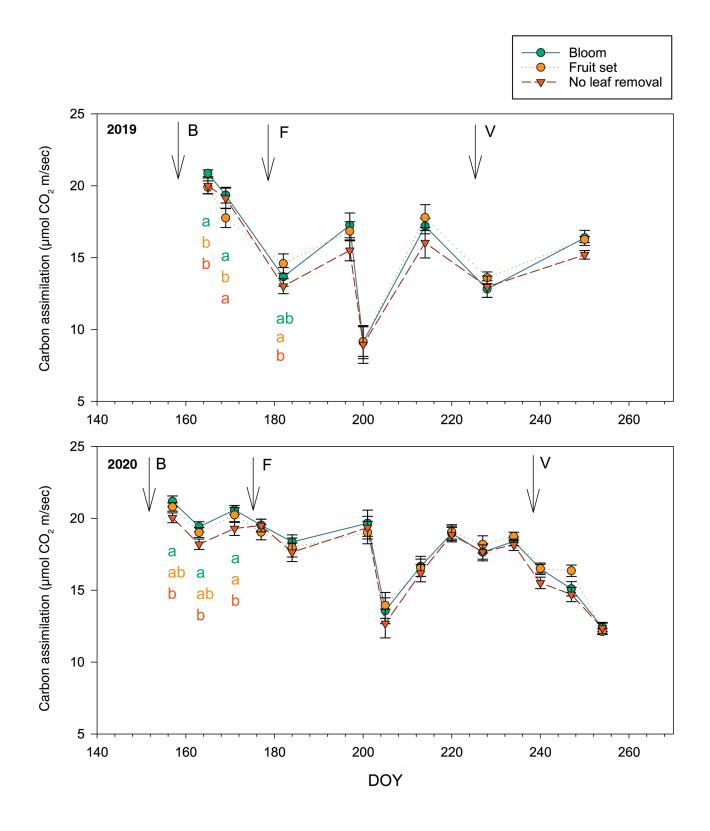


Figure 5 Midday leaf carbon assimilation rate in 2019 and 2020 from bloom leaf removal (Bloom), fruit set leaf removal (Fruit set), and no leaf removal. Arrows indicate the phenological stages of bloom (B), fruit set (F), and veraison (V) in 2019 and 2020. Different letters represent significant differences between treatments according to Tukey's honestly significant difference at p < 0.05. DOY, day of year.

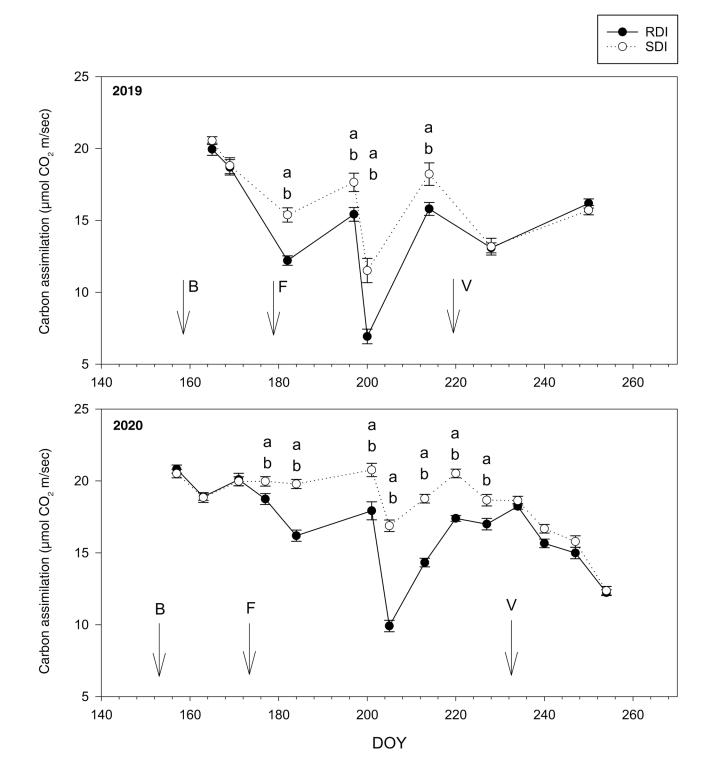


Figure 6 Midday leaf carbon assimilation rate in 2019 and 2020 from regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI). Arrows indicate the phenological stages of bloom (B), fruit set (F), and veraison (V) in 2019 and 2020. Different letters represent significant differences between treatments according to Tukey's honestly significant difference at p < 0.05. DOY, day of year.

A key biochemical change occurring at veraison is the degradation of berry organic acids and the accumulation of berry sugars and anthocyanins (Coombe 1992). Water stress after veraison increases berry anthocyanins by stimulating abscisic acid biosynthesis (Castellarin et al. 2007). However, the two irrigation treatments did not impose differential water stress after veraison, as evidenced by similar midday leaf Ψ for grapevines subjected to SDI or RDI from veraison to harvest (Figure 2).

Leaf removal after the berry pea-size stage was found to improve the fruit-zone light environment, increase harvest TSS, reduce berry organic acids, increase berry anthocyanins and phenolics, and decrease disease pressure without affecting yield (Williams 2012, Cook et al. 2015). Early leaf removal at prebloom or bloom through either manual application or mechanical application has been recently shown to be more beneficial than traditional leaf removal at fruit set (Cook et al. 2015, Yu et al. 2016, VanderWeide et al. 2018, 2020), and mechanical leaf removal at prebloom or bloom was found to be more superior than manual application at the same time (VanderWeide et al. 2018). Leaf removal at bloom did not reduce the final yield in our study and no effect on the number of berries per cluster was found (Table 3). This result was contradictory to previous studies done in cool climates, in which leaf removal at bloom reduced fruit set and thus, yield (VanderWeide et al. 2018, 2020), whereas similar results have been observed in other studies in the same region (Williams 2012, Cook et al. 2015, Yu et al. 2016). Evidently, grapevines in our study had sufficient photosynthetic capacity without carbon starvation after leaf removal, as shown by leaf Anet (Figures 5 and 6) (Martínez-Lüscher and Kurtural 2021).

RDI increased berry total anthocyanins by 14% compared to SDI (Table 4), and the elevated level of berry total anthocyanins was started at the onset of veraison and maintained until harvest (Figure 8). However, the increase of berry total anthocyanins was proportional to the 15% yield reduction mainly by reduced berry weight (Table 3). Brillante et al. (2018) reported similar results that RDI reduced yield by 15% while increasing berry anthocyanins by 27%. Total anthocyanins per berry (berry weight × berry total anthocyanin content) for RDI was similar to anthocyanins per berry for SDI. Water deficit could also affect fruit-zone microclimate and applied water restriction affects carbon allocation, which can affect the fruit-zone light exposure through the restricted vegetative growth measured by pruning weight; lower pruning weight explained the higher fruit-zone PAR resulting from the RDI treatment (Figure 4) (Torres et al. 2021). In our study, bloom leaf removal only increased fruit-zone PAR until fruit set (a process lasting approximately two weeks); similar results were reported in other studies (Cook et al. 2015). In contrast, leaf removal after fruit set provided a long-lasting increase in fruit-zone PAR, and its elevated fruit-zone exposure lasted even after veraison (Figure 3). Long-lasting elevated fruit-zone PAR (>15% ambient) can be undesirable in a hot climate region (Bergqvist et al. 2001). A recent study further confirmed that canopy porosity >30% was subjected to higher temperatures that dramatically decreased the berry anthocyanins in hot climates (Torres et al. 2020). Thus, bloom leaf removal provided a short-lived fruit-zone exposure without exposing the fruit zone to the extreme heat after fruit set, which was approaching the typical peak of summer heat in the studied area. More recently, bloom leaf removal was found to increase berry anthocyanins through improved leaf area to fruit ratio and relative skin mass (Poni et al. 2009); this could be because leaf removal affected the precursors of anthocyanins through the modulation of

Treatment	Cluster no./ grapevine	Yield (kg/grapevine)	Berry no./ cluster	Leaf area (m²/grapevine)	Leaf area:fruit- weight ratio (m ² /kg)	Pruning weight (kg/grapevine)	Shoot no., grapevine
RDI	89	10.8 b ^a	125	7.5	0.71 a	1.1 b	59
SDI	96	12.7 a	124	7.7	0.62 b	1.2 a	58
p value ^b	0.117	0.001	0.686	0.328	0.021	0.044	0.482
Bloom	92	11.4	122	7.4 b	0.66	1.2	59
Fruit set	93	11.8	126	7.2 b	0.63	1.1	58
No leaf removal	94	12.0	125	8.1 a	0.63	1.2	58
p value	0.559	0.051	0.440	0.029	0.168	0.508	0.174
Year	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Year × irrigation	0.549	0.385	0.888	0.303	0.141	0.893	0.319
Year × leaf removal	0.438	0.487	0.908	0.577	0.405	0.873	0.411
Irrigation × leaf removal	0.346	0.052	0.528	0.363	0.052	0.378	0.169
Year × irrigation × leaf removal	0.560	0.601	0.816	0.156	0.270	0.998	0.825

Table 4 Harvest berry chemistry of Cabernet Sauvignon from 2018, 2019, and 2020. TSS, total soluble solids; TA, titratable acidity;
FW, fresh weight; RDI, regulated deficit irrigation; SDI, sustained deficit irrigation.

Treatment	Berry weight (g)	TSS (Brix)	рН	TA (g/L)	Anthocyanins (mg/g FW)	Total phenolics (au/g FW)
RDI	1.03 b ^a	23.0 b	3.8	4.1	0.74 a	1.02
SDI	1.12 a	23.7 a	3.9	4.1	0.65 b	0.99
p value ^b	0.003	0.008	0.070	0.987	0.005	0.141
Bloom	1.08	23.4	3.8 b	4.1	0.75 a	1.00
Fruit set	1.07	23.3	3.9 a	4.1	0.71 b	1.02
No leaf removal	1.07	23.3	3.8 b	4.1	0.63 c	0.99
p value	0.632	0.567	0.004	0.486	<0.000	0.129
Southeast side	1.07 b	23.4	3.9 a	4.0 b	0.69	1.01
Northwest side	1.13 a	23.3	3.8 b	4.2 a	0.70	0.99
p value	<0.000	0.123	<0.000	<0.000	0.777	0.194
Year	0.000	<0.000	<0.000	<0.000	<0.000	<0.000
Year $ imes$ irrigation	0.463	0.282	0.015	0.020	0.541	0.922
Year × leaf removal	0.868	0.913	0.788	0.186	0.290	<0.000
Irrigation × leaf removal	0.879	<0.000	0.167	0.148	0.422	0.420
Year \times irrigation \times leaf removal \times side	0.529	0.983	0.931	0.837	0.772	0.642

^aDifferent letters represent significant differences according to Tukey's honestly significant difference at p < 0.05. ^bItalic font represents the p values from Type 3 Analysis of Effects.

Table 5Effect of irrigation and leaf removal on harvest berrytotal soluble solids from 2018, 2019, and 2020. RDI, regulateddeficit irrigation; SDI, sustained deficit irrigation.

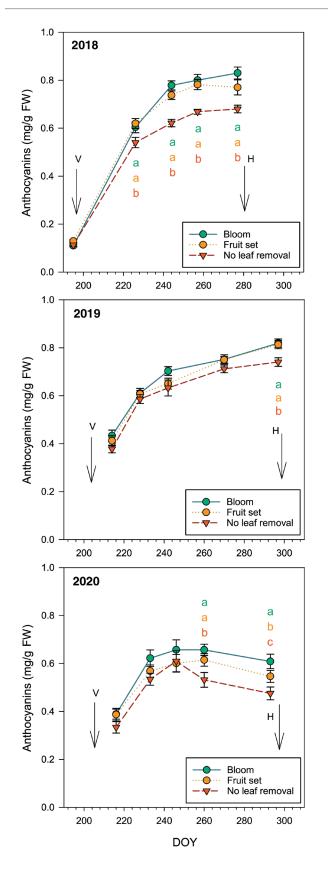
Leaf removal	Irrigation				
	RDI	SDI			
Bloom	22.9 b ^a	23.9 a			
Fruit set	22.8 b	23.9 a			
No leaf removal	23.3 a	23.2 b			
p value ^b	0.008	<0.001			

^aDifferent letters within columns represent significant differences according to Tukey's honestly significant difference at p < 0.05. ^bItalic font represents the *p* values from Type 3 Analysis of Effects.

the grape transcriptional ripening program and flavonoid metabolism (Pastore et al. 2013). To increase berry TSS, VanderWeide et al. (2018) identified that prebloom mechanical leaf removal was superior to mechanical leaf removal at fruit set. The increase of berry TSS was mainly due to the enhanced fruit-zone microclimate and higher carbon partitioning through a younger canopy containing basal leaf fragments proximal to fruit. Our results agreed with previous studies that bloom leaf removal changed the fruit-zone microclimate by increasing sun exposure without extending the exposure well into the summer heat season, and that it increased leaf A_{net} prior to fruit set (Figure 5). As a contrast, fruit-set leaf removal could be detrimental in the hot climate for berry anthocyanin accumulation because of extended fruit-zone sun exposure and berry sunburn with no effect on leaf A_{net} (Figure 3 and 5) (Torres et al. 2020). Enhanced microclimate (through short-lived sun exposure at bloom without overheating the berries after fruit set) and higher leaf carbon assimilation might explain why bloom was superior to fruit set for leaf removal timing. Furthermore, overheat resulted from sun exposure, which led to

the degradation of berry anthocyanins (Bergqvist et al. 2001, Spayd et al. 2002). Our results agreed with previous studies that anthocyanins from fruit-set leaf removal started to decline close to harvest, and the separation of berry anthocyanin accumulation was identified between fruit set and bloom leaf removal as harvest was approached in 2018 and 2020 (Figure 7). The long-lasting fruit-zone openness from fruit-set leaf removal was largely explained by the carbon restriction resulting from the water deficit initiated after fruit set in our study.

A few studies on leaf removal were conducted in hot climates; these generally agree that bloom leaf removal increases berry anthocyanins the most compared to leaf removal at fruit set or veraison (Cook et al. 2015). Our results support the previous studies conducted in hot climates, where the improvement of fruit-zone light levels at bloom increased berry anthocyanins the most at harvest (Figure 7). However, in contrast to the previous studies, our results showed that the increase of berry anthocyanin content from bloom leaf removal was largely due to the shortlived increased fruit-zone light levels without extending the fruit-zone sun exposure after fruit set, because overexposure during the berry development led to anthocyanin degradation from the previous studies in hot climates (Torres et al. 2020). A few studies compared mechanical leaf removal to manual leaf removal; mechanical leaf removal was generally found to be superior to manual leaf removal (Vander-Weide et al. 2018, 2020). Prebloom mechanical leaf removal had higher berry TSS than manual leaf removal at the same time, because mechanical leaf removal improved the carbon partitioning through a younger canopy containing basal leaf fragments proximal to fruits while improving the fruit-zone microclimate. Manual leaf removal is cost prohibitive in the studied area and mechanical leaf removal offers superior results with much lower cost (Zhuang et al. 2019).



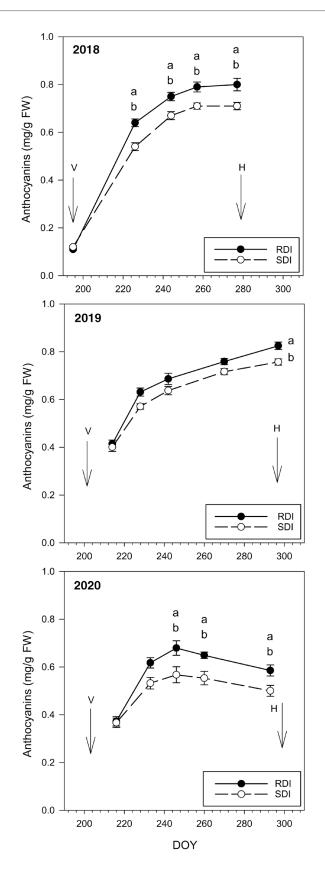


Figure 7 Berry total anthocyanin accumulation starts at veraison in 2018, 2019, and 2020 from bloom leaf removal (Bloom), fruit set leaf removal (Fruit set), and no leaf removal. Arrows indicate the phenological stages of veraison (V) and harvest (H) of each season. Different letters represent significant differences according to Tukey's honestly significant difference at p < 0.05. FW, fresh weight; DOY, day of year.

Figure 8 Berry total anthocyanin accumulation starts at veraison in 2018, 2019, and 2020 from regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI). Arrows indicate the phenological stages of veraison (V) and harvest (H) of each season. Different letters represent significant differences according to Tukey's honestly significant difference at p < 0.05. FW, fresh weight; DOY, day of year.

Conclusion

Irrigation and leaf removal are the main viticultural practices used in arid hot regions to manage canopy growth, yield formation, and fruit quality. In our three-year study, RDI increased berry anthocyanins at harvest by 14% in comparison to SDI, but with a sacrifice of 15% yield. Our research findings underscore the pivotal role of mechanical leaf removal during both bloom and fruit-set stages in augmenting anthocyanin content within grape berries. Notably, our study reveals a substantial enhancement of anthocyanin levels by 19 and 13% during bloom and fruit set, respectively, compared to the control group where no leaf removal was employed. Crucially, this enhancement in anthocyanin production is achieved without incurring any deleterious effect on overall yield. While it is acknowledged that RDI strategies have been associated with a significant reduction in yield, and the economic returns per ton of fruit have remained modest in the SJV, our findings offer a promising alternative. Specifically, the concurrent application of mechanical leaf removal during bloom, complemented by SDI, emerges as the optimal course of action to promote anthocyanin accumulation within berries, while maintaining commercially acceptable yield levels. Thus, this research advocates for the implementation of mechanical leaf removal at bloom in conjunction with SDI as the preferred agricultural practice in the SJV. By doing so, growers can effectively harness the biochemical potential of grapevines to synthesize higher anthocyanin quantities, thereby elevating the overall quality and market value of grape and wine products. This innovative approach ensures a synergy between sustainable viticultural practices and the improvement of bioactive compounds, reaffirming the resilience and adaptability of grape cultivation in response to climatic considerations.

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