

ABSTRACT

PRUNING SYSTEMS MANAGED DURING PROLONGED DROUGHT AFFECT PRODUCTIVITY, WATER FOOTPRINT, AND PHENOLIC COMPOSITION OF MERLOT GRAPEVINE

A trial was conducted in the central San Joaquin Valley of California to quantify canopy architecture, yield components, yield efficiency, water footprint, fruit chemistry, and phenolic composition of Merlot/Freedom in response to three pruning systems and two applied water amounts. Pruning systems were cane pruned (CP) manually pruned to four, 8-node canes, spur pruned (HP) manually pruned to 22, two node spurs and mechanical box pruned (SHMP) which consisted of hedging to a 100 mm spur height. Two irrigation treatments were applied: sustained deficit irrigation (SDI) where 2.31, 1.67, and 0.39 megaliters/Ha was applied from bud-break to harvest in 2013, 2014, and 2015, respectively and regulated deficit irrigation (RDI) where 1.76, 1.25, and 0.34 megaliters/Ha was applied. Drought conditions within the experiment mitigated canopy development and yield in subsequent years. However, the SHMP system was successful in maintaining higher yields with similar quality metrics, flavonoid content, and phenolic composition as well as successfully mitigating water footprint compared to the HP and CP systems. The results from this study provides applied information to growers about successful management practices that help maintain yield and fruit composition during the transition between manual and mechanically managed vineyards under severe water deficits.

Andrew Beebe
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AFFECT PRODUCTIVITY, WATER FOOTPRINT, AND PHENOLIC
COMPOSITION OF MERLOT GRAPEVINE

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INTRODUCTION

The San Joaquin valley is the highest producing region of winegrapes in California. Growers in the Central San Joaquin valley district 13 alone produced 32% of the total wine grapes harvested in 2014. However, the average return per ton for red winegrapes in the area is \$327/ton (NASS 2014), just over one-third of the states average grower returns. These low grower returns along with increased labor costs, tight profit margins and seasonal drought have decreased the economic sustainability of production of winegrapes in this area. Therefore, it is of utmost importance to investigate methods that will help to reduce labor costs in the vineyard concurrently with maintaining or increasing yields and fruit quality. Mechanization of winter pruning and the application of water deficits have been shown to be useful tools in decreasing vineyard inputs and maintaining vineyard profitability.

Several studies have come to the conclusion that retained node number during winter pruning procedures is not an accurate or a precise indicator of the harvested yield in vineyards (Poni et al. 2004, Bernizzoni et al. 2011, Terry and Kurtural 2011, Geller and Kurtural 2013). There is agreement in recent literature that shows that increasing pruning severity (leaving less buds during winter pruning procedures) to balance grapevine yield alone fails because of the unpredictable vegetative and reproductive compensating responses by grapevines (Kurtural et al. 2006, Bernizzoni et al. 2011, Geller and Kurtural 2013). These compensating responses from the grapevine include enhanced bursting of secondary shoots (Main and Morris 2004) along with fruit bearing shoots that stem from latent buds along the cordon and basal buds that are not normally counted during winter pruning procedures (Kurtural et al. 2006, Poni et al. 2004).

Therefore, growers attempt to combat these compensating responses by using canopy management practices such as shoot and cluster thinning through manual and mechanical means (Geller and Kurtural 2013, Kurtural et al. 2006, Terry and Kurtural 2011). However, these canopy management practices require rigorous crop estimation, even when done mechanically. This can make production with these methods economically prohibitive (Kurtural et al. 2012) as their application increases labor operation costs in the vineyard along with the added cost of lost revenue from fruit removed during these management practices.

The practice of canopy management has been an active area of research since its conception by Dr. Nelson Shaulis decades ago. Research in canopy management has been performed across a number of climates and has made advances in vineyard management that has increased production efficiency along with the ability to manipulate yield and quality. Recent research performed in *Region V* of California suggests that a shoot density target of 35 shoots per meter of row in hypocumbent cultivars was desirable to produce sustainable yields, improve canopy microclimate and achieve recommended vine balance values (Geller and Kurtural 2013). However, decreasing shoot densities provided no further benefit to canopy function. The lack of physiological response from increased severity of shoot thinning is due to vegetative compensation of grapevines generating a much larger leaf area on the remaining shoots on a sparsely populated canopy (Geller and Kurtural 2013). Furthermore, another study performed by Bernizzoni and colleagues (2011) suggested a shoot density of 15 shoots per meter of row maximized efficiency of grapevine canopies of potted Barbera grapevines by inducing a fast recovery of whole vine photosynthesis post-shoot thinning. This recommended shoot density also led to improved berry phenolics (Bernizzoni et al. 2011). Despite shoot thinning, and other canopy

management practices, proving to be valuable canopy management tools in reaching crop balance along with maintaining yield and quality, growers do not normally implement these practices because of their additional cost and time constraints (Geller and Kurtural 2013, Terry and Kurtural 2011). Therefore, instead of implementing these costly practices, many growers are attempting to convert current trellises to a high, single-wire sprawling system and managing the spur height with mechanical box pruning. Canopy size is then mitigated with the application of differential regulated deficit irrigation (RDI). There is currently anecdotal evidence that the combination of a mechanically box-pruned single high-wire system managed with RDI methods can maintain ideal crop load values while maintaining or improving fruit quality.

The application of regulated deficit irrigation in vineyards has a number of positive effects for production. One of the primary uses of RDI is to reduce the vegetative growth of canopies (Shellie 2006) early in the season in order to achieve a desirable leaf area that will ripen a given crop size. There is agreement within literature that RDI enhances fruit ripening by increasing cluster light exposure and berry temperature (Arozarena 2002, Dokoozlian and Kliewer 1995, Spayd et al. 2002) as well as improving fruit and wine quality through an increase of the skin to pulp ratio and increased phenolic and aroma precursor compounds (Ferreyra et al. 2002, Kennedy et al. 2002, Matthews and Anderson 1989, and Romero et al. 2010). There is also evidence that water stress initiates specific biochemical events that influence the biosynthesis of anthocyanins and precursor compounds in maturing fruit (Kennedy 2008, and Kennedy et al. 2002). Furthermore, little is known on the impact of differential water deficits on the growth, yield, and capacity of vines in subsequent growing seasons.

While there have been a number of pruning system and deficit irrigation studies performed in red winegrapes all throughout California and the western United States, the majority of them have researched these factors separately. Furthermore, few have included a trellis conversion and mechanization of winter pruning in concert with the application of regulated deficit irrigation. Therefore, the objectives of this study are to a) investigate the management of mechanically box pruned systems and see if they can be effectively managed under water deficits while maintaining ideal crop load values and increase or maintain quality, and b) investigate the effects differential water deficits have on growth, yield, and capacity of vines in subsequent seasons and c) investigate how pruning systems and water deficits interact on the phenolic composition of Merlot in the hot climate.

LITERATURE REVIEW

Canopy Microclimate

Canopy microclimate of vineyards was first defined by German scientist Rudolph Geiger as the climate immediately surrounding and within the plant canopy (Geiger 1961). The canopy as part of the microclimate, from a viticultural perspective, is all the parts of the vine located above the soil plane that includes shoots, leaves, fruit, trunk, and cordon (Keller 2015). This particular climate within vineyards is unique as it can be manipulated by vineyard cultural practices. The canopy microclimate is dependent on the leaf area produced by the vine and the spatial distribution of the leaves within an allotted space (Smart 1985). Therefore, trellis systems and training methods are important in the distribution of leaf area and aids in creating canopies of varying dimensions and densities. The way in which the leaves are distributed within canopies creates an area that interacts with a number of environmental factors such as light, temperature, humidity, wind speed, and evaporation (Smart and Robinson 1991). These factors have showed to have similar characteristics of attenuation throughout the grapevine canopy where maximal values are found at the canopy surface exposed to the ambient environment and the lowest values are found in the middle near the fruiting zone (Dokoozlian and Kliewer 1995, Smart and Robinson 1991).

Canopy Microclimate Interaction with Environment

Grapevine canopies interact with a number of climatic factors to create unique microclimates. Light is arguably the most important environmental factor in grape production as it has such a profound effect on vine development and has been shown to affect a number of grape quality parameters. Grapevine canopies

utilize sunlight in the visible range of light that ranges from 400 to 700 nm. The light that is absorbed from this range is called photosynthetically active radiation (*PAR*). About 85-90% of this light is absorbed by the first layer of leaves of canopies while 4-9% of the residual light is transmitted through the first layer and 6% is reflected from the surface (Smart 1985). Hot climates such as the SJV are characterized by very high light intensities throughout the majority of the growing season. The majority of days throughout the growing season reach intensity values greater than $1000 \mu\text{Em}^{-2}\text{s}^{-1}$ with many of them reaching values closer to $2000 \mu\text{Em}^{-2}\text{s}^{-1}$ (Smart 1985, Smart and Robinson 1991). These high intensity values are a significant contribution to vine development as they allow photosynthesis to reach the saturation point and maximize production potential, although this can be a potential problem when matched with extreme temperatures.

Following light, temperature is another significant climatic factor interacting with grapevine canopies. Temperature is an even bigger factor to consider for warmer climates such as that of the SJV because of its known contribution to mitigating color in red cultivars (Winkler et al. 1974). The increase in the temperature of the fruit to high levels has been shown to mitigate the accumulation of sugars (Kliewer 1977) as well as reduce TA by increased degradation of malic acid (Lakso and Kliewer 1978). A more important aspect to consider in regards to canopies interacting with the environment is the interaction between light exposure and temperature. Recently, Bergqvist et al. (2001) studied the effects of different sunlight exposure levels and temperatures on fruit composition and berry growth. This research concluded that the effects of light are highly dependent upon how much the temperature is mediated by increased light exposure. Indirect light has little effect on temperature while exposure to direct light leads to concomitant increases in temperature to a certain point. Therefore, it

is of utmost importance to utilize canopy management strategies that reduce the incidence of direct light into the fruit zone and maximize the exposure to indirect light to maximize fruit quality.

Application of Regulated Deficit Irrigation

Regulated deficit irrigation (RDI) is a vineyard management tool that aims to impose set periods of irrigation stress at key phenological stages of grapevine development to cause a desirable physiological response (Bravdo et al. 1985; Hepner et al. 1985, Romero et al. 2010). Intended physiological responses are dependent on the phenological period in which the water deficit is imposed on. A general agreement in the use of RDI is that whole plant water demand should be applied during the early stages of flower development before any water stress is induced as this is a period that is highly sensitive to water stress and could lead to poor fruit set and berry development. This is due to water stress reducing the effectiveness of pollination and fertilization within grapevines and in most extreme cases, the abscission of inflorescences from the vine (Hardie and Considine 1976, Smart and Coombe 1983). The application of water stress during the period between bloom and fruit set across a number of crops has also been shown to mitigate the transport and accumulation of starch and sugars to the developing pollen grains reducing their effectiveness in pollination (Saini 1997). Moreover, vines that are exposed to high temperatures, such as those experienced in the SJV have been shown to have decreased pollen viability due to gametophyte malformations (Pereira et al. 2014). Therefore, the application of any water stress between the period of bloom and fruit set is undesirable for commercial production.

The proper utilization of regulated deficit irrigation requires a general knowledge of site specific parameters such as soil type, local climatic weather patterns, evapotranspiration rates, and the ability to quantify plant water status (Keller et al. 2008). There are a number of ways to quantify plant water status such as predawn leaf water potential (Ψ_{PD}), midday leaf water potential (Ψ_L) and midday stem water potential (Ψ_{stem}). These measurements are common factors used in scheduling RDI. Previous research has shown that these different water potential factors are highly correlated with one another as well as closely linked to soil water content (SWC) and applied water amounts (Williams and Araujo 2002). These water potential measurements were also found to be linearly correlated with net CO₂ canopy assimilation rates (A) and stomatal conductance (g_s) (Williams and Araujo 2002).

The proper implementation of RDI methods requires knowledge in the reproductive development of grape berries, more specifically, the increase in fresh weight throughout the season. The development of seeded grape berries was first described by Winkler and Williams (1935), as a double-sigmoid pattern that is split into three distinct phases. The first stage begins right after anthesis and is characterized by cell division and expansion along with the accumulation of organic acids (Harris et al. 1968). Stage two is commonly referred to as the “lag phase” of berry development. Berry growth pauses during this phase and berries enter metabolic reprogramming accompanied by seed embryo formation and growth. Cell division no longer occurs past this stage. Stage three occurs at the onset of veraison and is characterized by color changes, berry softening, rapid cell expansion, accumulation of sugars, metabolizing of acids, and accumulation of phenolic compounds (Keller 2015, Pirie and Mullins 1980). Having knowledge in

this berry development cycle and how applied water amounts effect each phase aids in irrigation management decisions to help meet quality and production goals.

Post fruit set is the period in which deficit irrigation is routinely applied. The amount of irrigation water to apply is calculated using daily evapotranspiration (ET_o) measurements that can be found using weather station data along with a vine based K_c developed for fully irrigated grapevines (Williams 2001). Basing irrigation applications on calculated K_c values rather than just ET_o helps to increase the accuracy of irrigation in vineyards, as vine water demand is dynamic throughout the season as the canopy develops. K_c values change throughout the season as the canopy expands and the fraction of ground covered by vegetation increases (Allen et al. 2007). Moreover, K_c values and percent ground cover are highly correlated with one another (Lopez et al. 2012).

Canopy Architecture

The training of grapevines onto trellis systems is an essential function for commercial production as it helps to facilitate cultural practices in the vineyard. A number of factors that affect vine vigor must be considered prior to the selection of a trellis system in order to meet production goals such as rootstock selection, climate, soil type, and water availability. According to Reynolds and Vanden Heuvel (2009), the commercial training of grapevines must accomplish a set of objectives in order to maximize production efficiency. First, the permanent wood or canes (for the case of cane pruned vines) must be placed in a way to maximize the exposure of canopy leaf area to light in order to maximize potential yield, optimize the leaf area to fruit ratio for vine balance, increased fruit quality and disease control. Second, the bearing wood must be placed in such a way that allows movement of equipment through vineyards to facilitate mechanization.

Third, vines are planted in such a way as to reduce the competition for light between vines. And fourth, a renewal zone must exist to ensure that the vine retains its shape throughout its life. Completing these previous objectives when implementing trellis systems for commercial grape production will help to facilitate vineyard cultural practices in the most efficient manner. Furthermore, the training of grapevines is the most effective means of setting up the framework of canopy microclimate.

Grapevine canopies can be described as divided or non-divided canopies where canopy division is a modification to a trellis systems configuration that separates the canopy into two or more distinct curtains of vegetative growth (Reynolds and Vanden Heuvel 2009). These curtains can be divided vertically or horizontally creating a number of different canopy configurations. Canopies can also be described as shoot-positioned or non shoot-positioned. Shoot-positioning is an element of vine training where shoots are tucked into foliage wires to keep shoots within a specific configuration to improve fruit exposure and to prevent adjacent vines from growing into each other and increasing canopy shading (Jackson and Lombard 1993, Smart et al. 1982), whereas non-shoot positioned systems are those where canopies are allowed to develop freely, creating a distinct canopy architecture that is associated with the California Sprawl system.

Canopy Architecture and Training Systems

When selecting a training system, one of the most important implications to consider is the amount of leaf area that can be consistently exposed to sunlight (Gladstone and Dokoozlian 2003, Reynolds and Vanden Heuvel 2009). The amount of leaves that intercept light plays a large role in yield expression and fruit composition, as these factors are reliant on the photosynthetic activity of the

canopy. This brings to light the importance of the concept of canopy radiation microclimate where the architecture or grapevine canopies plays a large role. As mentioned previously, as much as 10% of PAR is transmitted through the first leaf layer of grapevine canopies (Smart 1985). Studies have shown that diffused light into the canopy can still be utilized effectively by interior leaves (Reynolds and Vanden Heuvel 2009). Furthermore, any modifications to trellis systems therefore can improve the radiation microclimate by increasing the interception of this diffuse radiation (Smart 1973).

A study performed by Gladstone and Dokoozlian (2003) included an in depth investigation on a number of training systems and their effects on canopy microclimate. Non-positioned systems were found to have a unique distribution of leaf area where a higher leaf area density formed around the outside of the canopy with a relatively lower leaf area density in the canopy interior (Gladstone and Dokoozlian 2003). Conversely, positioned systems were characterized by an increase in leaf area density from the top down to the fruit zone of these canopies. These characteristically different canopy densities have an effect on the light attenuation within canopies. High density canopies such as vertical shoot positioned (VSP) showed photosynthetic photon flux (PPF) values <5% of ambient in the fruit zone while low density canopies, such as the single curtain non-positioned system, had PPF values >10% (Gladstone and Dokoozlian 2003). This has important implications to wine grape production, as it is believed that decreasing canopy densities has a positive effect on bud fruitfulness, yield, and fruit quality.

Most vineyards across California are still planted onto the California Sprawl Trellis system, which is characterized as a two to three wire single curtain, non-shoot positioned system (Gladstone and Dokoozlian 2003). This system has

remained the gold standard due to its relatively low cost of installation and production along with its ability to have high productivity as well as the low cost of spur pruning of the system. However, this system is often utilized improperly, particularly with procumbent cultivars such as ‘Merlot’ that lead to overly dense canopies causing minimal light exposure in the fruit zone (Dokoozlian and Kliewer 1995, Terry and Kurtural 2011). Moreover, single curtain, non-shoot positioned systems such as the California Sprawl tend to produce canopies which produce larger leaf layer number (LLN) values compared to other systems (Reynolds and Vanden Heuvel 2009). This in turn can be problematic as shading within canopy interiors has a number of detrimental effects on production and quality parameters as previously discussed.

Although hand pruning the California Sprawl system has become economically questionable in the hot climate, growers are taking advantage of its flexibility by converting it to a mechanically pruned system (Dokoozlian 2009). Mechanized systems are pruned in a non-selective nature that leaves a greater number of buds along established cordons. The retention of higher bud counts in this system tends to produce higher shoot densities (Terry and Kurtural 2011, Zabadal et al. 2002). In agreement, Andersen and colleagues (1996) concluded that a mechanically box-pruned system retaining twice as many buds over that of a manually pruned system produced twice as many shoots per vine. A more recent mechanical pruning study that retained as many as four times the amount of buds over a hand pruning treatment only had a shoot count that was double that of the spur pruned system (De Toda and Sancha 1999). This shows one of the many self-regulating mechanisms mechanized systems have in response to increased bud loads. Despite these larger shoot counts, mechanically box-pruned vines were found to have fewer leaf layers and increased canopy gaps that led to more

desirable fruit exposure (Reynolds 1988) and can primarily be attributed to producing shorter shoots. In agreement, Wessner and Kurtural (2013) have concluded that mechanically pruned systems produce a more ideal canopy microclimate by increasing PAR transmittance into the fruit zone.

Canopy Architecture and Deficit Irrigation

One of the most significant outcomes of the use of RDI is mitigation of canopy growth. When applying water amounts that are lower than what is calculated with ET_c , water within the soil profile will dry down during the period in which RDI is implemented and shoot growth can be controlled. Shellie (2006) concluded that reduction in shoot growth was apparent in Merlot vines as they reached -1.0 MPa. In agreement, Greenspan (2005) made similar conclusions in grafted and ungrafted Cabernet sauvignon and Pinot gris where shoot length was reduced at leaf water potentials reaching -1.0 MPa. Studies have also concluded a reduction in vine vegetative growth after the implementation of RDI compared to standard irrigation practices which increased *PAR* transmittance into the fruiting zone and improving the canopy microclimate (Romero et al. 2010, Terry and Kurtural 2011). However, care must be taken when applying severe water stress during the post-veraison period of berry development as leaf abscission can be advanced and the development of leaf area reduced (Romero et al. 2010). Conversely, Opazo and colleagues (2010) concluded that applied water deficits did not significantly reduce vegetative growth, likely due to the imposed restrictions not being sufficient enough to impose low enough stem water potentials to mitigate vegetative expression. These findings show the importance of using plant based water potentials as a means to schedule water deficits in a precise manner in order to stimulate desired canopy responses.

Yield Components

Yield Components and Training Systems

The selection of trellis systems has a direct effect on yield production, as it is the primary technique in the distribution of the vine canopy. One of the most significant contributions made to wine grape production was the development of divided canopies. Divided canopies increase yields over that of non-divided canopies generally by increasing the number of clusters per vine by way of increased shoots per vine or per set distance of row, assuming they are vines of high-capacity (Reynolds and Vanden Heuvel 2009). The increase in yield of divided canopies can also be attributed to their increase in exposed leaf area and increased canopy surface area (Reynolds and Vanden Heuvel 2009) thereby increasing the photosynthetic capacity and photosynthate accumulation of vines (Illand et al. 2011). It is important to note that higher shoot numbers per given length of row leads to concomitant increases in interior canopy shading (Smart 1985). Studies performed by Shaulis et al. (1966) concluded that excessive shading of canopy interiors was a primary cause of yield reduction and could be overcome by this division of grapevine canopies. The most notable contribution from Shaulis's work is that of the Geneva Double Curtain (GDC). The GDC is a horizontally divided canopy that consists of parallel bilateral cordons with spurs retained along them. The shoots are trained outward and downward to create multiple canopies (Reynolds and Vanden Heuvel 2009). Shaulis et al. (1966) compared GDC to a single curtain system and concluded that GDC increased yields by 90% when grafted onto a high capacity rootstock such as 3309. Similarly, a study performed by Reynolds and Wardle (1994) in Canada with Seyval blanc concluded that GDC systems increased yields over non-divided

canopies by an average of 42%. Another study found that GDC and a V-trellis system (another style of divided canopy) increased yields in Riesling due to higher number of clusters per distance of vine row due to the increase in shoot numbers (Reynolds et al 1996). Despite GDC increasing yields over non-divided canopies, vines trained to this system tend to produce excessive Ravaz Index values causing out of balance vines due to the reduction of vigor of downward growing shoots (Reynolds and Wardle 1994). Similarly, Bordelon et al. (2008) concluded that Traminette trained onto a divided Scott Henry system increased yields by 22% over that of those trained onto VSP. The increase in yield in this experiment for the divided canopy was primarily due to increased shoot numbers.

Mechanization of winter pruning has become an integral part of production in the San Joaquin Valley of California due to its ability to reduce pruning costs tremendously along with the potential to increase yields. A recent study performed in Fresno County found a labor cost savings of up to 80% over hand pruning procedures by mechanizing winter pruning while maintaining yield and quality (Kurtural et al. 2012). Typically, mechanization of box pruning tends to leave a greater amount of buds on the vine due to its reduced level of pruning severity. As mentioned previously, increases in node numbers typically results in increases in number of clusters therefore leading to higher yields (Reynolds and Vanden Heuvel 2009). However, there is still lack of agreement within the current body of literature as to the effects of mechanical pruning on yield components, primarily due to grapevines varied reproductive compensation responses.

One of the biggest concerns that growers have about the use of mechanical pruning is the possibility of overcropping due to the high number of retained nodes. It is thought that this leads to alternate bearing yield production, poor fruit quality, and subsequent yield decline. Due to the non-selective nature of machine

pruning, a sudden increase in yield can be expected the first year due to the increase in node number. This may concern some growers but it is important to note that a vines ability to self-regulate its yield in response to mechanical pruning takes several years as shown by a few long-term mechanical pruning studies (Gatti et al. 2011, Keller et al. 2004, De Toda and Sancha 1999). The suitability of mechanical pruning to grapevines is highly dependent on the vines degree of adjustment to increases in retained node numbers. Research has shown that this ability to compensate reproductively, due to increased node numbers, is expressed as reduced bud break (Poni et al. 2004), reduced clusters per shoot (De Toda and Sancha 1999), reduced berry set (Jackson et al. 1984), and reduced berry and cluster weight (Intrieri et al. 1988, Reynolds 1988). The implementation of mechanical winter pruning is feasible if there is sufficient reproductive compensation from the above responses in addition to a high level of canopy efficiency (canopies that develop full canopies much earlier in the season via increased shoot numbers). When these criteria are met, mechanically pruned systems can produce crops that are higher or similar in yield of that to hand pruned systems while maintaining grape quality (Keller et al. 2004, Zabadal et al. 2002).

Yield Components and Deficit Irrigation

There is agreement within the literature that the application of water deficits reduces yields across a number of cultivars (Santesteban et al. 2011, Shellie 2014, Terry and Kurtural 2011). However, there have also been some studies showing little to no yield reduction as a consequence of applied water deficits (Cook et al. 2015 and Keller et al. 2008). Yield reductions as a result of water deficits can be seen when deficits are applied before or after veraison or from fruit set to harvest (Terry and Kurtural 2011, Matthews and Anderson 1989). Yield reductions are

primarily due to smaller berry size, fewer berries per cluster, and in some cases, fewer clusters per vine. Shellie and Bowen (2014) have reported that a moderate level of water deficit (23% of estimated crop evapotranspiration) applied from fruit set to veraison, lowering midday leaf water potential to as low as -1.39 MPa, in ungrafted Cabernet Sauvignon reduced yields by 37%. This particular yield reduction was in most part due to reduced berry fresh weight (Shellie and Bowen 2014). Shellie (2014) also reported yield reductions as high as 58% with applied water deficits with smaller berry weights being the largest contributing factor. Furthermore, it has also been reported that severe deficits applied to potted 'Cabernet franc' vines during the early reproductive development phase, reducing leaf water potential at dawn to as low as -0.8 MPa, reduced fruit yield by way of reduced fruit set (Hardie and Considine 1976) showing another mechanism of yield reduction induced by applied water deficits.

As mentioned previously, berry development takes place during two distinct growth phases divided by a lag phase. Water deficits are regularly applied as an early deficit, those that are applied from fruit set until veraison. This is done in order to mitigate shoot and berry growth early in the season in an attempt to improve fruit quality. The water deficit is then lifted after veraison in order to maintain canopy function in order for the fruit to reach maturity (Intrigliolo and Castel 2010). Implementing water deficits at this stage of berry development has been shown to mitigate cell division and expansion within the berry mesocarp thereby limiting the size of the berries (Roby and Matthews 2004). It has also been shown that cell expansion rather than cell division is much more sensitive to water deficits (Ojeda et al. 2001). This characteristic reduction in berry size is what generally contributes to yield reduction from water deficits applied before veraison.

Water deficits applied after veraison all the way through harvest can also contribute to a reduction in yield. After the onset of veraison, the flow of the xylem vessels within the grapevine reverses directions so the xylem flow moves out from the berry and the phloem begins to flow inward (Keller 2015). Water deficits applied at this time can contribute to berry shriveling if the deficit is severe or prolonged enough. The shrinkage of berries takes place when xylem efflux along with the transpiration of berries exceeds the level of phloem influx (Keller et al. 2006), which can be possible in hot climates such as the San Joaquin valley of California. Although there is much agreement within the literature that supports the idea that applied water deficits reduce yield, there are still some experiments that have shown no effects from water deficits. This lack of universal agreement shows that yield reduction is not entirely dependent on plant water status and may involve a number of other factors such as climate, soil type, rootstock and scion interactions, and possible differences between cultivars.

Fruit Chemistry

One of the largest problems that growers face in wine grape production in the central San Joaquin Valley is producing uniformly ripe fruit while maintaining adequate levels in other quality components such as phenolic content, pH and TA. This is primarily due to the excessive sunlight exposure and high temperatures that are characteristics of the Central Valley's uniquely hot climate. The effects of sunlight and temperature on fruit composition have been a significant area of study in the past few decades. Shading of fruit has been shown to reduce soluble solid content (Francesco et al. 1994, Reynolds et al. 1986, Smart et al. 1988). Reynolds and colleagues (1986) have also shown that shaded clusters of Seyval Blanc had lower TA than that of exposed clusters primarily due to the activity of acid

metabolism involving malic acid where exposed clusters experienced higher activity of enzymatic degradation. Phenolic content of grapes is also an important quality parameter that is affected by both light and temperature. Spayd and colleagues (2002) found that exposed clusters had lower anthocyanin concentrations due to both degradation of compounds and inhibition of the synthetic pathway while temperature had very little effects on flavonol concentrations. High temperatures, such as those found in the San Joaquin Valley, have also been shown to inhibit and degrade total soluble solids whether clusters were exposed or not (Spayd et al. 2002). The independent and interactive effects of light and temperature on fruit chemistry creates a difficulty for vineyard management in the hot climate as growers must balance the need for maximizing sunlight interception by the canopy while also allowing some fruit exposure to aid in reaching adequate fruit quality parameters.

Fruit Chemistry and Training Systems

As previously discussed, the relationship between sunlight exposure and temperature can be complicated but is important to fruit composition. These factors and their effects are largely dependent on canopy microclimate and fruit environment (Spayd et al. 2002). Because of the variability in sunlight interception across different vineyard configurations (Smart 1973), recent research has been performed to characterize light quantity and quality within grapevine canopies of varying canopy densities and microclimate (Dokoozlian and Kliewer 1995, Gladstone and Dokoozlian 2003). These studies concluded varying attenuations of light quantity and quality across different canopies. Therefore, different canopies can be expected to affect fruit quality parameters at harvest due to the effects of varying light microclimates.

In general, research has shown that more exposed clusters have higher soluble solid content than their shaded counterparts (Bergqvist et al. 2001, Spayd et al. 2002, Reynolds et al. 1986). However, care must be taken in a hot climate where clusters that are exposed to extreme temperature variations above 37°C have shown inhibition of sugar accumulation (Bergqvist et al. 2001). Trellis systems that support increases in exposed leaf area have been shown to have concomitant increases in fruit composition parameters (Howell et al. 1987, Reynolds et al. 1995) where increases in exposed leaf area improve the efficiency of photosynthate production of grapevine canopies.

Mechanized pruning systems have become a popular area of study due to their ability to save a significant amount of money for growers. Reynolds (1988) concluded that simulated mechanical pruning systems in Riesling vines slightly reduced Brix compared to that of hand pruned systems, found to be primarily due to higher crop loads. Reynolds (1988) also concluded that the mechanized system had reduced pH and TA compared to that of hand pruned. In agreement, De Toda and Sancha (1999) concluded that mechanized systems slightly reduced Brix and pH compared to hand pruned. This could be explained by enhanced fruit microclimate by mechanized systems in this study setting due to the mechanized system producing shorter shoots, a self-regulating mechanism of vegetative growth in mechanized systems, which mitigated canopy congestion. Conversely, studies across a number of varieties have also concluded that mechanically managed systems do not affect fruit quality when compared to hand pruned systems (Kurtural et al. 2006, Morris 2007). In agreement, a number of recent studies studying the effects of mechanizing canopy management practices have concluded no difference in fruit chemistry components of mechanized systems (Geller and Kurtural 2013, Terry and Kurtural 2011). These findings demonstrate

the importance of mechanized pruning systems in their possibility to maintain or improve fruit quality while simultaneously reducing inputs in the vineyard, thereby improving profitability of production.

Fruit Chemistry and Deficit Irrigation

There is a lack of agreement within the literature regarding the effects of applied water deficits on fruit composition. Shellie (2006) found that applying 35% of ET_c or early RDI deficits of 35% increased pH and decreased TA. In agreement, Keller and colleagues (2008) concluded that TA was reduced with early deficits (those applied up till veraison) compared to late season deficits with minimal increases in pH. Conversely, Terry and Kurtural (2011) found that late deficits reduced pH compared to early deficits and sustained deficits. Furthermore, a number of studies have found no significant effects of water deficits on juice pH (Cook 2015, Keller et al. 2008, Romero et al. 2010). Earlier research has linked reductions in TA in response to applied water deficits to reductions in malate (Esteban et al. 1999, Matthews and Anderson 1989). Early deficits have also been linked to reductions in accumulation of malic acid before veraison (Matthews and Anderson 1988). Cultivar may play a role in responses of fruit composition to applied water deficits. Shellie (2011) found increased pH in Merlot with water deficits while the same response was not evident in Cabernet sauvignon. In agreement, Keller and colleagues (2008) showed no change in pH in Cabernet Sauvignon with applied deficits. The lack of agreement seen in the literature hints that water stress plays a more indirect role in TA and pH. As discussed previously, water deficits in most cases reduce shoot growth and increases fruit exposure and temperature, which has been linked to increased malic acid degradation (Shellie 2006). Concomitant decreases in canopy shade with increasing water stress

severity have been linked to decreases in pH (Smart 1985, Terry and Kurtural 2011).

Phenolic Composition

The major classes of flavonoid compounds that make up the phenolic composition of grapes are flavan-3-ols, proanthocyanidins, anthocyanins, and flavonols (Soquet et al. 1996). These groups of compounds are the most important constituents to wine grape quality as they are the primary contributors to color, flavor, texture, and astringency of wine along with antioxidant properties that have been a popular topic when considering their possible contributions to human health (German and Walzem 2000). The biosynthesis of these compounds occurs in the phenylpropanoid and flavonoid pathways, which originates from the aromatic amino acid phenylalanine, which is a product of the shikimate pathway (Conde et al. 2007). The synthesis of these compounds are closely linked to the different stages of berry development. Grape berries have two development phases that are divided by a lag phase and follows a double sigmoid growth pattern (Keller 2015). Flavonols, flavan-3-ols, and proanthocyanidins are synthesized during the first stage of berry development while anthocyanins are synthesized during the fruit ripening period of development (Cortell and Kennedy 2006). The predominant classes of flavonoids found within berry skins are anthocyanins, proanthocyanidins and to a lesser extent, flavan-3-ols and flavonols while seeds predominantly contain flavan-3-ols and proanthocyanidins (Kennedy et al. 2002; Teixeira et al. 2013).

Flavonols are a group of phenolic compounds that consists of quercetin, myricetin, and kaempferol along with their methylated forms (Mattivi et al. 2006). It appears that flavonols have a role as a UV protectant as well as behaving as a

factor in co-pigmentation with anthocyanins (Braidot et al. 2008), which is important for reinforcing pigmentation in wine during the aging process. There is agreement within a number of light mitigating experiments that the biosynthesis of flavonols is a light-dependent reaction (Downey et al. 2004, Price et al. 1995, Spayd et al. 2002) where berries that are more exposed to light have higher concentrations in tissues in which they are developed. A recent study on Cabernet Sauvignon showed a complete reduction of flavonol concentration within berries skins by light exclusion (Koyama et al. 2012). Koyama and colleagues (2012) have also concluded that the exclusion of UV light remarkably reduces the concentration of flavonols, which indicates that the quality of light also has a dramatic effect on flavonol biosynthesis in grape skins.

Proanthocyanidins (PAs) are the most abundant group of phenolics found within grape seeds and skins and are polymers made up of the flavon-3-ol compounds catechin, epicatechin, epicatechin-3-O-gallate, and epigallocatechin. These compounds are responsible for the bitter and astringent properties of wine and are important in the consumer perception of quality (Kennedy et al. 2002) as bitterness is taste and astringency contributes to the drying sensation that is felt on the tongue. In addition, they play an important role in the long term color stability of wine (Somers 1971) as well as acting as a deterrent to herbivores during the early development stage of grape berries. The molecular size of PAs has a significant effect on their perception of bitterness and astringency (Peleg et al. 1999) where smaller monomer constituents have a higher perception of bitterness. Conversely, larger derivatives such as epicatechin-3-O-gallate and epigallocatechin, contribute more to the perception of astringency.

Pruning Systems and Phenolic Content

As discussed earlier, light is one of the most significant environmental factors interacting with grapevine canopies that can affect grape composition. Therefore, pruning systems play more of an indirect factor in the production of phenolics in grapes. Improved light microclimate within canopies is known to improve overall grape quality as well as having an effect on phenolic accumulation (Downey et al. 2006, Smart and Robinson 1991). However, the more extreme temperatures that are a major characteristic of the San Joaquin Valley can have a detrimental effect on phenolic development when linked to high light exposure. Bergqvist et al. (2011) concluded that increased exposure to sunlight did indeed improve berry color as long as it was increased exposure to indirect sunlight as exposure to direct sunlight led to concomitant increases in fruit temperature and this led to a reduction in color. Temperatures that register over 35°C are associated with anthocyanin degradation and inhibition of anthocyanin accumulation (He et al. 2010). It was reported that exposure to PAR values greater than 100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ negatively influenced the accumulation of anthocyanins due to increases in berry temperature to undesirable levels (Bergqvist et al. 2001). Maximum values of PAR are a regular occurrence throughout the season in the hot climate so finding a proper balance to fruit exposure within canopies has been difficult. This brings to light the importance of canopy microclimate as those systems that maximize diffuse light within the canopy interior will result in overall improved fruit color (Reynolds and Vanden Heuvel 2009).

Literature on the effects of pruning systems on phenolic content has been variable. Gatti and colleagues (2011) concluded that anthocyanin concentrations in mechanically hedged systems were lower when compared to traditional hand pruned vines. However, it must be noted that the mechanically hedged system in

this experiment had the highest shoot number per vine, which increased canopy density. The higher degree of interior canopy shading in this situation led to the reduction in berry color constituents. Conversely, a study by Wessner and Kurtural (2013) concluded no difference in berry skin phenolics despite mechanized treatments having improved light microclimate variables. This lack of response was attributed to the high day and night temperatures that are routinely experienced in the hot climate of California. Another important indirect effect on berry skin phenolics deals with one of the vines reproductive compensation responses due to increased bud numbers. Mechanized systems that retain a higher bud count tend to decrease berry size, which can indirectly increase phenolic composition (Morris 2007, Zabadal et al. 2002).

Deficit Irrigation and Phenolic Content

It is well established that grapevines managed under restricted water supplies can indeed improve phenolic content of winegrapes (Ferreyra et al. 2002, Kennedy et al. 2002, Matthews and Anderson 1988, Ojeda et al. 2002, Roby and Matthews 2004). It is thought that one of the primary contributors to increased phenolic content as a response to applied water deficits is a reduction in berry size that leads to a higher skin to pulp ratio (Kennedy et al. 2002). However, the concentrating effect of smaller berries may not be a sufficient explanation in some situations as recent reports have shown that higher skin tissue mass under less water stress may accumulate greater amounts of anthocyanins on a per berry basis compared to more severe water stress treatments (Shellie and Bowen 2014). Roby and Matthews (2004) has also investigated the effects of different berry sizes on flavonoid content and concluded that there are effects of vine water status on fruit composition that are independent from the responses from inhibited berry growth.

Furthermore, Ojeda et al. (2002) concluded that extreme water stress leading to the development of smaller skin mass reduces the potential to accumulate anthocyanin based on a limitation of space within tissues.

As previously discussed, the timing and severity of water deficits can have a significantly different impact on vine responses. Ferreyra et al. (2002) reported increases in total phenols and anthocyanins in both early and late applied water deficits. The early deficits in this experiment produced the highest total phenol concentrations while post-veraison deficits induced higher anthocyanin concentrations. In agreement, Kennedy and colleagues (2002) concluded that increasing post veraison water stress increased anthocyanins primarily by the effects of reduced berry size and modification of flavonoid biosynthesis. Recent research has concluded that water stress can transcriptionally regulate the biosynthesis of anthocyanins and flavonols by up-regulating the expression of a number of genes (Castellarin et al. 2007a, Castellarin et al. 2007b). Castellarin and colleagues (2007a) concluded that both early and late severe water deficits, where water was not even applied from fruit set to veraison or from veraison to harvest, up-regulated the expression of *F3H*, *DFR*, *LDOX*, *F3'5'H*, *UFGT*, and *GST*. The extreme early deficit was shown to have increased the onset of anthocyanin biosynthesis therefore increasing anthocyanins more than that of late deficits. This was due to activation of *UFGT* and *GST* much earlier on in fruit development as these genes are strictly associated with the synthesis and storage of anthocyanins instead of playing a role in other flavonoid compounds. More extreme deficits similar to drought conditions however may have a more detrimental effect to development of phenolics. A study performed by Romero et al. (2010) in a semi-arid climate looked at two different severities of regulated deficit irrigation (early deficits). Both water deficits produced higher amounts of anthocyanins, however,

the more severe water treatment that applied 15% of ET_c from fruit set to veraison produced less than that of the less severe deficit. It was concluded that this was in response to more severe water deficits causing less than desirable canopy microclimates due to early canopy defoliation which in turn led to increased levels of fruit exposure. As discussed previously, increasing cluster exposure to direct sunlight can be detrimental to berry composition due to subsequent increases in berry temperatures above harmful thresholds (Bergqvist et al. 2001).

Yield Efficiency and Water Footprint

The concept of vine balance is an important aspect of grapevine production as it helps to understand the balance between the vegetative and reproductive portions of the vine. Vines that are deemed as balanced are those vines that have an optimized ratio of vegetative growth and fruit yield that is capable of fully maturing fruit before harvest. Moreover, vines that are deemed as unbalanced fall into a vegetative cycle where an excessive amount of canopy is developed along with very little fruit. If not corrected, unbalanced vines can contribute to excessive canopy shading and low light conditions within the canopy interior that will reduce fruitfulness in subsequent years (Howell 2001). It is important to note vine balance is dependent on a number of factors such as cultivar, vineyard location, trellis configuration, and vine spacing (Kliewer and Dokoozlian 1995) which can lead to varying recommendations of the most ideal canopy.

Yield efficiency is a successful method of assessing vine balance where the ratio of leaf area to fruit produced is calculated (Kliewer and Dokoozlian 2005, Howell 2001). Research performed in Davis and Oakville, CA by Kliewer and Dokoozlian (2005) recommended yield efficiency values of 0.8 – 1.2 m^2/kg for single-canopy type trellis systems such as the California Sprawl. A separate

recommendation was made for horizontally divided canopies of $0.5 - 0.8 \text{ m}^2/\text{kg}$. So far, these values have been the only documented recommendations for yield efficiency of grapevines. Vines that are grown in climates that retain functional exposed leaf area after harvest for a considerable time, such as the hot climate of Central California, require less leaf area to ripen a crop (Howell 2001). Therefore, it is feasible that yield efficiency recommendations for the central San Joaquin Valley can be lower.

California is currently within an extended period of drought that has put a serious constraint on water availability for irrigation. Today's enhanced pressure on available water resources has increased global demand to improve the 'water footprint' of modern agriculture. The water footprint of crops is a metric that is expressed as m^3 of applied water per ton of crop harvested (Williams 2014a). However, it is not solely based on applied irrigation but also includes additions from precipitation. Additions from surface or groundwater are known as blue water while water additions from precipitation are known as green water. The proportion of water from the soil from grapevine ET_c is also classified as green water (Williams 2014b). The current drought that is being experienced in California has generated a situation where winter rainfall (green water) is insufficient to meet vine water demand, therefore causing a heavier reliance upon blue water. It is more desirable to have a higher consumptive use of green water compared to blue water in order to mitigate the use of limited pools of irrigation water (Williams 2014a). Therefore, it is important to increase the understanding of water footprint dynamics within the hot climate of the central San Joaquin Valley to better utilize limited resources more efficiently.

MATERIALS AND METHODS

Plant Material and Vineyard Management

This study was conducted from 2013-2015 at a commercial vineyard planted with Merlot/Freedom grapevines at a 2.1 m x 3.4 m (vine x row) spacing. The vineyard research site was located in Madera, CA (lat. 37°N, long. 120°W) and was planted in 1995 on Atwater loamy sandy that is a coarse-loamy, mixed, thermic typic haploxeralf (USDA 2003) and Cometa sandy loam which is a fine, mixed, superactive, thermic typic palexeralf (USDA 2006). Before the trellis conversion was performed, the vineyard was trained to a 1 m cordon wire with two, 8-node canes trained at 1.1 m and an additional two, 8-node canes that were trained at 1.4 m, in opposing directions. The vineyard was drip-irrigated with pressure compensating emitters spaced at 1 m with two emitters per vine delivering 1.5 L.h⁻¹. Pests were managed using an intensive integrated pest management (IPM) program. All other cultural practices were standard for the area and conducted according to the University of California Cooperative Extension guidelines (UCIPM 2014).

The experiment was a three (canopy management) x two (regulated deficit irrigation) factorial with a randomized complete block design and three replicated blocks. Each replication contained 376 vines per plot of which 24 were sampled (72 total samples) based on a grid pattern of every 50 vines that were 107 m distant from each other. The same vines were used for all treatments in all years of the study.

Canopy Management Treatments

Three canopy management treatments were applied. Before the trellis conversion was applied, the vineyard was cane pruned (CP) to four, 8-node canes.

This canopy management treatment was the control. 1) The CP treatment vines were trained to a 1 m cordon height above vineyard floor with two, 8-node canes retained in opposing directions. An additional two, 8-node canes were trained at 1.4 m in opposing directions. The other two canopy management systems were applied as follows: 2) the trellis was converted to a California Sprawl system (HP) with a cordon wire at 1.4 m above vineyard floor and two parallel catch wires at 1.7 m above the vineyard floor and spur pruned to retain 22 spurs with two nodes each per vine. 3) The trellis was also converted to a single high-wire sprawling system (SHMP). The cordon wire was established 1.7 m above the vineyard floor and a bi-lateral cordon was established to generate a single high-wire system that was mechanically box-pruned at a 100 mm spur height.

Deficit Irrigation Treatments

Two deficit irrigation treatments were applied by imposing different irrigation practices to the respective drip lines. Precipitation in winter and spring was insufficient to fill the soil profile in all years of the study. Since precipitation was insufficient, the root zone was irrigated beginning in the third week of March in all years based on a crop coefficient (K_c) of 0.2 and 80% of reference crop (grass) evapotranspiration (ET_o) obtained from CIMIS station 188 in Madera, CA. Irrigation was interrupted before bloom to allow the soil to dry down until mid-day leaf water potential (Ψ_l) was <-1.0 MPa to control shoot growth. After fruit set was reached, the RDI treatments were imposed. The ET_o and a vine based K_c , developed for fully irrigated grapevines in the San Joaquin Valley was used to calculate irrigation amounts based on vine water status (Williams 2001). A control treatment of sustained deficit irrigation (SDI) where 80% of daily estimated ET_c was applied when Ψ_l was below -1.2 MPa started at fruit-set and continued

through harvest. One regulated deficit irrigation (RDI) treatment was applied where 80% of ET_c was applied from bloom to fruit set when Ψ_1 reached -1.2 MPa. When Ψ_1 reached -1.4 MPa, 50% of ET_c was applied from fruit set to veraison, but not thereafter. After veraison was reached, 80% of ET_c was applied all the way through harvest. These water potential thresholds were able to be achieved in 2013 for a total application of 2.31 and 1.76 ML/Ha in SDI and RDI respectively. Due to the current severe drought in California drastically reducing the availability of irrigation water for 2014 and 2015, the set irrigation thresholds were not reachable. The irrigation treatments for 2014 and 2015 were adjusted to fit this environmental constraint. Minimal weekly applications of irrigation water were applied for a total of 1.67 and 1.25 ML/Ha in SDI and RDI treatments respectively, for 2014. In 2015, availability of irrigation water was drastically reduced. Only 0.39 and 0.34 ML/Ha were applied to the SDI and RDI treatments respectively, for 2015.

Canopy Architecture and Leaf Area

One week after berry set total shoots per vine was counted by the addition of count shoots (borne from count positions greater than 5 mm distal to the base of the bearing surface) and non-count shoots (borne from non-count positions less than 5 mm distal to the base of the bearing surface and secondary shoots). Indicators of canopy architecture measurements such as leaf layers, cluster contacts, and canopy gap percentage were measured as described by Smart and Robinson (1991). A ceptometer (AccuPAR-80; Decagon Devices, Pullman, WA) was placed directly above the cordon, within the fruiting zone on the east side of the canopies parallel to the vine row at the head of each vine. Four measurements were taken with the ceptometer from 4 vines within each experimental unit.

Ambient readings were taken at a height of 50 mm above the canopy surface. The remaining three measurements were taken within the fruiting zone at the head of the vine. Measurements were taken at canopy closure, veraison, and harvest at mid-day with photosynthetically active radiation (PAR) values ranging approximately 1900-2200 $\mu\text{mol}\cdot\text{m}^2\cdot\text{s}^{-1}$. The three fruiting zone PAR measurements were combined and expressed as the percentage of total PAR measured at mid-day. Leaf area was determined as follows. Two shoots per vine were randomly selected and stored at 2.2°C at 98% humidity until measured. Each shoot was separated into main and lateral axes. The number of leaves per axes was counted, and leaf area was measured with a leaf area meter (LI-3000; LI-COR, Lincoln, NE.). The canopy leaf area per vine was then determined as described by Keller et al. (2008).

Yield, Fruit Composition, and Yield Efficiency Assessment

Fruit yield and cluster numbers for each treatment were measured by hand-harvesting 4 vines from each experimental unit when the fruit reached 24 Brix. Average cluster weight was calculated by dividing fruit yield per vine by the number of clusters harvested per vine. Leaf area to fruit ratio was calculated by dividing the leaf area at 20 Brix of each vine by the yield per vine and was expressed as m^2/kg . Fruit composition was measured only at harvest in 2013 and was measured at canopy closure, veraison, and harvest for both 2014 and 2015. On each date, a random 20-berry sample was collected from 4 vines from each experimental unit, placed into polyethylene bags, stored on ice and analyzed within 24 h. Before analysis, the 20-berry samples were weighed and average berry mass was determined. The samples were then crushed by hand and the juice

placed in 100 mL beakers. A 5 mL sample was used to determine the brix using a digital refractometer (PAL-1; Atago Co., Tokyo). Juice pH was determined using a glass electrode and a pH meter (model XL15; Fisher Scientific, Pittsburgh, PA). Titratable acidity (TA) of each sample was determined by titrating to a pH of 8.2 with 0.1 N sodium hydroxide using an endpoint titrator (model DL 15; Mettler-Toledo International, Columbus, OH), and expressed as milligrams per liter (Iland et al. 2004).

Berry Flavonoid Composition

The flavonoid composition of berries was determined using an exhaustive extraction method modified from Pastor del Rio and Kennedy (2006). At harvest, 20 random berry samples were collected, weighed and stored at -80°C until analyzed. Berry skins and seeds were manually separated and lyophilized (model: Triad Freeze Dry System; Labconco, Kansas City, MO). Skin and seed dry masses were recorded. Dry skin tissues were then extracted in 20 mL of 2:1 acetone:water for 24 h in darkness. Samples were filtered through a Whatman #1 90 mm filter under vacuum to exclude solid tissues. A 1-mL of the liquid sample was collected. The acetone from the 1 mL collected samples was then evaporated with a centrivap concentrator (model: 7810010, Labconco, Kansas City, MO) attached to a -103°C cold trap (model: 7385020, Labconco, Kansas City, MO). Following the evaporation of the acetone, the residue was brought to a volume of 5 mL with water. Samples were centrifuged for 15 minutes at 1400 g and the supernatant was subjected to HPLC-DAD analysis.

HPLC Analysis and Procedure

Flavonoid composition was measured using reversed-phase high performance liquid chromatography (HPLC) using an Agilent 1100 (Santa Clara,

CA) modular system. The system contained a G1313A injector, G1311A HPLC quaternary pump, on-line G1379A degasser, G1316A thermostatted column holder, G1315B photodiode array detector, and Agilent Chemstation software (version B03). A LiChrosphere 100 RP-18 reverse-phase column (5mm packing, 250 x 4mm) was used, protected with a guard column of the same material, and thermostatted at 40°C.

The mobile phase flow rate was kept at 0.5 mL/min and three mobile phases were used. The mobile phases used were (A) 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid, (B) 20% mobile A + 80% acetonitrile (v/v), and (C) 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH. Acetonitrile of HPLC-gradient grade, orthophosphoric acid of analytical grade, and ammonium phosphate of analytical grade were purchased from (Fischer Scientific, Waltham, MA). Eluting gallates, flavan-3-ols, flavonols, and anthocyanins were identified and quantified using Gallic acid, catechin, rutin, and oenin standards, respectively (Extrasynthese, Genay, France).

Statistical Analysis

Data for all measured parameters were tested to verify if the assumptions of analysis of variance (ANOVA) were met using Shapiro-Wilk's test. Data which failed to meet the assumptions of ANOVA were either log₁₀ or square root transformed and analyzed using the generalized linear model (GLM) procedure in SAS (version 9.3; SAS Institute Inc., Cary, NC). Significance level was set at $P \leq 0.05$ and means were separated using Tukey's honestly significant difference test. When the ANOVA showed significant differences, the mean separation test was conducted on the transformed data but the non-transformed means were presented for ease of discussion. Interactions between year and treatments were tested and

whenever these interactions were significant ($P < 0.05$) analysis was conducted separately for each year.

RESULTS

Climate and Experimental Site

Growing degree days (GDD) that were accumulated were calculated from 15 March through harvest. Degree day models normally start on April 1st but climate change has shifted bud break to earlier dates. The GDD accumulation was 1624 and 1813 in 2014 and 2015, respectively (refer to Appendix A, Table 1). The GDD accumulation was not calculated for 2013 due to the weather station being inactive for most of 2013. Precipitation was 27.5 mm and 30.5 mm between March 15th through harvest in 2014 and 2015, respectively. In 2014, the experimental site received 64 mm of precipitation from November to bud break, 27.5 mm from bud break to veraison, and 0 mm from veraison to harvest. Compared to the 10-year average the amount of precipitation received by the experimental site in 2014 was 51% of the annual sum, 53% of the dormant season and 48% of the growing season (refer to Appendix B, Figure 1). In 2015, the experimental site received 84.1 mm of precipitation from November to bud break, 30.3 mm from bud break to veraison, and 0.2 mm from veraison to harvest. Compared to the 10-year average the amount of precipitation received by the experimental site in 2015 was 38% of the annual sum, 38% of the dormant season and 42% of the growing season. Due to limitations on irrigation water availability the SDI and RDI treatments only received 1.67 ML/Ha and 1.25 ML/Ha of water in 2014, respectively. In 2015 available irrigation water was reduced drastically. Because of this, the SDI and RDI treatments only received 0.39 ML/Ha and 0.34 ML/Ha, respectively.

Both of the post trellis- conversion years of 2014 and 2015 exhibited similar extreme weather temperatures. In 2014, the number of days that exceeded

32°C was 71 and 73 in 2014 and 2015, respectively (data not shown). The crop coefficient was estimated during the 2014 and 2015 seasons. Based on the estimated values, the K_c varied from year to year (data not shown). In 2014, the K_c reached a maximum value of 0.51 when growing degree days reached 659. However, in 2015, the K_c reached a maximum value of 0.4 when growing degree days reached 942. These estimated values affected estimated grapevine water use each year based on a percentage of the ET_c calculated throughout the season. Despite having estimated K_c values to fine tune irrigation applications, the low availability of irrigation water at the research site caused a drastic reduction in amount of water applied.

Effect of Pruning Systems and Irrigation on Canopy Architecture

Canopy architecture was measured at two phenological periods, pre-veraison (PV) and pre-harvest (PH). There was an effect of experimental year on leaf layer numbers, cluster contacts, and percent canopy gaps for both the PV and PH periods (refer to Appendix A, Table 2). However, year only affected *PAR* transmittance during the PV period. Leaf layers increased from 2014 to 2015 for both the PV and PH collection periods. Both cluster contacts and percent canopy gaps decreased from 2014 to 2015 in both years. *PAR* transmittance increased from 2014 to 2015 only during the PV period. No effect of year was seen for leaf area measurements. In 2014, there was an interaction between year and pruning systems where the CP treatment had the lowest leaf layer number during the PV period. In 2014 and 2015, the SHMP treatment had the greatest number of cluster contacts during the PH period. The SHMP treatment only retained a higher cluster contact value during the PV period in 2015. In 2014, the CP treatment had the greatest canopy gap percentage value during the PV period. However, the same

result was not evident in the PH period or either of the periods in 2015. Leaf area was consistently greater in the SHMP treatment for both years and both the PV and PH periods. The SHMP treatment increased total vine leaf area by 28% and 33% compared to CP in 2014 and 2015 during the PV period, respectively. The SHMP treatment increased total vine leaf area by 26% and 22% over that of CP in 2014 and 2015 during the PH period, respectively. There were no significant effects from irrigation treatments in any of the measured variables throughout the entire study.

Effect of Pruning System and irrigation on Yield Components

Experimental year had an effect on berry weight, clusters per vine, cluster weight, yield, skin weight, and seed number per berry (refer to Appendix A, Table 3). Berry weight, clusters per vine, cluster weight, and yield decreased in subsequent years while skin weight and seeds per berry increased. The SHMP treatments displayed the least decrease in yield per year followed by the HP and CP treatment (data not shown). Pruning systems affect berry weights in all years. The CP treatment had lower berry weights during the 2013 conversion year compared to 2014 and 2015. The SHMP treatment reduced berry weights by 7% and 5% compared to other pruning systems in 2014 and 2015, respectively. Clusters number per vine was affected by pruning systems in all years. The HP treatment had greater cluster numbers in 2013 compared to CP and SHMP. Once the pruning systems were established, the SHMP treatment had greater clusters per vine by 21% and 24% in 2014 and 2015, respectively. Cluster weight was affected by pruning systems only in 2013. The CP treatment had the greatest cluster weight while SHMP had the lowest. Yield per vine was affected by pruning systems in all years of the study. The SHMP treatment had the lowest yield in 2013. However,

the SHMP treatment increased yield by 14% and 30% in 2014 and 2015, respectively. Seed weight and seeds per berry were only affected by pruning systems in 2013. The HP had a greater seed weight and seed count per berry compared to other treatments in 2013. There was a year by pruning system interaction seen in berry weights, clusters per vine, cluster weight, and yield. This interaction in berry weights shows the CP treatment having the smallest berry weight in 2013 but smaller in subsequent years. The HP treatment had a greater amount of clusters per vine in 2013, however the SHMP treatment had greater clusters per vine in subsequent years.

Effect of Pruning System and Irrigation on Berry Composition

The greatest Brix was seen in 2015 across all treatments when compared to 2013 and 2014. In 2013 during the conversion year, there was an effect of pruning system on Brix where CP treatment increased it by 4% compared to the HP treatment (refer to Appendix A, Table 4). However, the same result was not evident in 2014 and 2015. In 2014, there was an effect of irrigation on Brix where the SDI treatment increased it by 3% compared to the HP treatment. The same result was not evident in 2013 or 2015. Year had an effect on juice pH where values in 2014 were lower than in 2013 and 2015. Juice pH was affected by pruning systems in 2013. The SHMP treatment reduced juice pH by 3% compared to the CP and HP treatments. However, the same results were not evident in 2014 and 2015. In 2015, irrigation affected juice pH where the SDI treatment reduced values by 3% compared to RDI. These results were not seen in 2013 and 2014. Experimental year had an effect on TA where values increased from 2013 all through 2015. In 2013, pruning treatments affected TA where values were greatest for the SHMP treatment compared to the CP and HP treatments. However, the

same result was not evident in 2014 and 2015. There were no effects of irrigation seen on TA in all years of the study.

Effect of Pruning System and Irrigation on Anthocyanins

There was a year effect on all anthocyanin glucoside constituents (refer to Appendix A, Table 5). Cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside had their highest concentrations in 2014. Peonidin-3-glucoside decreased from 2013 to 2015. Conversely, malvidin-3-glucoside increased from 2013-2015. In 2014, there was a pruning system and irrigation interaction where a combination of the CP and SDI treatment produced the lowest concentration of malvidin-3-glucoside. However, this interaction was not apparent in 2015.

There was a year effect on all anthocyanin acetate constituents. Cyanidin-3-glucoside-acetate decreased from 2014 to 2015. Peonidin-3-glucoside-acetate decreased from 2013 to 2015. Petunidin-3-glucoside-acetate decreased from 2013 to 2014. Conversely, malvidin-3-glucoside-acetate increased from 2013 to 2015. In 2014 there was a pruning system and irrigation interaction where the combination of the CP and SDI treatment displayed the lowest concentration of malvidin-3-glucoside-acetate. However, this interaction was not visible in 2015.

There was a significant year effect on both of the 3-coumaryl-glucosides. Petunidin-3-glucoside-coumarate increased from 2013 to 2015. Malvidin-3-glucoside-coumarate displayed its highest concentration in 2013 and decreased from 2013 to 2014 with a minimal increase in 2015. In 2014 there was a pruning system and irrigation interaction where the combination of the CP and SDI treatments had the lowest concentrations of petunidin-3-glucoside-coumarate and malvidin-3-glucoside-coumarate. However, both of these interactions were not

seen in the following year. A significant year effect was seen in total soluble anthocyanins where concentrations were greatest in 2013. Furthermore, a pruning system by irrigation interaction was seen where the combination of the CP and SDI treatments had the lowest total soluble anthocyanin concentrations. This interaction was not apparent in 2015. No pruning system or irrigation treatment effects were seen across all anthocyanin constituents in both years.

Effect of Pruning System and Irrigation on Yield Efficiency, and Water Footprint

In both years, leaf area to fruit weight ratio was not affected by crop load management or irrigation treatments (table 6). However, there was a significant effect of year where the ratio increased from 2014 to 2015 across all treatments indicating less leaf area developed per unit of crop weight in the subsequent year due to the reduction in amount of water applied.

Experimental year had an effect on the water footprint of Merlot grapevines where values decreased from 2014 to 2015 across all treatments. There was a crop load management effect in both years where the SHMP treatment had a water footprint that was 25% fewer than that of HP in 2014, and 30% lower than that of CP in 2015. There was no significant effect of irrigation methods or interactions on the water footprint in both years of the study.

Effects of Pruning Systems and Irrigation on Gallates, Flavonols, and Flavan-3-ol Monomers of Skin Tissue

There was a significant effect of year on gallates where gallic acid increased from 2013 to 2015 across all treatments (refer to Appendix A, Table 7). Experimental year also had an effect on total flavan-3-ol monomers where they decreased from 2013 to 2015. Experimental year effected total flavonols where flavonols increased from 2013 to 2014 then decreased from 2014 to 2015. In 2014,

pruning systems and irrigation method interacted with total flavan-3-ol monomers where the combination of the SHMP system and RDI treatments had the lowest concentrations. However, this interaction was not evident in 2015.

DISCUSSION

Weather at Experimental Site

During this experiment, California has been in a period of extended drought where average temperatures have increased and average rainfall decreased. The first year of the study after the trellis conversion, only received 51% of the 10-year precipitation average while the second year received 38%. Furthermore, most of the precipitation received by the experimental site was concentrated during the winter period and earlier in the season. This type of environmental constraint experienced in the central SJV gives credence to the importance of applied irrigation throughout the season. However, the availability of irrigation water has become an issue for the area. Therefore, it is of the utmost importance to utilize techniques that applies irrigation in the most efficient manner possible.

One such technique is the use of K_c to calculate vineyard evapotranspiration (ET_c) (Williams and Ayars 2005) where the estimation of canopy shade on the vineyard floor can be used to estimate K_c values. These then can be used to calculate relatively accurate vine water requirements throughout the season (Allen et al. 1998). The highest values of K_c that were estimated for this experiment were 0.51 and 0.40 for 2014 and 2015, respectively (data not shown). Recommendations for K_c values within the literature have been variable. Allen and colleagues (1998) have concluded that a maximum K_c value of 0.70 in winegrapes is most desirable when row spacing is 3.35 m, which was similar to the spacing of this experiment. Conversely, Williams and Ayars (2005) recommended a K_c maximum of 0.98 for the same row spacing in California's hot climate as most favorable. However, the estimated values calculated in this study are much lower than the previous recommendations with the same row spacing and similar climate. Lower K_c values below those of the previous recommendations have also

been found in similar studies in region V and region IV of California (Nelson 2015, Cook 2015). These findings indicate that recommended values for specific vineyard configurations are unreliable as K_c is dependent on a number of factors such as canopy height, row spacing, and pruning system that interact and can be highly variable from one vineyard to another as well as the addition of seasonal variability.

As previously mentioned, it was quite apparent that rainfall in the study area has been severely limited with only 91 mm and 115 mm of precipitation in 2014 and 2015, respectively. Less than half of the precipitation fell during the winter period for both years. Mendez-Costabel and colleagues (2014) concluded that the exclusion of winter precipitation reduced canopy development, regardless of applied water amounts during the growing season. The minimal rainfall seen during the winter of this study combined with reduced applied water amount exacerbated the issue of canopies filling their allotted space. In 2015, the maximum K_c value was achieved later in the season at 942 GDD (data not shown) compared to 2014 where it reached its maximum value at 659 GDD at a higher value. However, similar canopy architecture values were measured for both years of the study. This can be attributed to the limited winter rainfall and less water applied for 2015. This reduction in canopy development in the current research setting had a measurable effect on the other measured parameters, as year effects were ubiquitous throughout the study. Projected scenarios of warming across the area have implications of earlier occurrence of phenological events and the compression of the grape berry growth period (Webb et al. 2007), which can have a significant effect on berry chemistry.

Canopy Architecture

Achieving optimum canopy architecture levels in hot climates like the SJV can be difficult as canopies tend to have a precocious and rapid development, particularly when resources are not limited leading to dense and mutually shaded canopies. This precocious and rapid canopy development was observed whether dormant season pruning was applied mechanically or by hand (Wessner and Kurtural 2013). However, during extended drought such as that experienced by this project's research site, grapevine canopy development can be severely restricted. The restriction of canopy development can be exacerbated further when winter precipitation is minimal (Mendez-Costabel et al 2014) as experienced during this experiment, which led to an effect of year where canopy architecture values decreased in subsequent years.

The trellis conversion from conventional systems to a mechanically pruned system performed in 2013 was successful in creating distinct canopy architectures. The cluster contacts for this experiment were the most affected architecture variable where pruning system was the primary contributor. Cluster contacts were consistently higher in the SHMP system indicating a higher cluster density within this canopy configuration. These results are in agreement with other mechanical pruning trials where the lower severity by the application of mechanized winter pruning had a tendency to leave a higher number of retained buds, therefore leading to more shoots and clusters per vine (Gatti et al. 2011, Geller and Kurtural 2013, Kurtural et al. 2013, De Toda and Sancha 1999, Reynolds 1988). More strikingly, the leaf layers measured in this study were found to range from 1.5 – 2 which are well below those of recommended values for the area. Reynolds and Vanden Heuvel (2009) recommended three leaf layers for the most efficient canopy while Terry and Kurtural (2011) recommended between three to four leaf

layers. These low values may indicate a greater amount of fruit exposure to direct sunlight, which has been shown to adversely affect berry phenolic composition (Bergqvist et al. 2001) particularly in hot climates such as the SJV.

In this study, there were few discernable differences seen between canopy architecture variables throughout all treatments. This indicated that canopies were fully developed relatively early in the season rather than continuing to develop as would be expected in cooler climates (Howell 2001). This plays a large role into one of the major advantages of mechanically managed systems. The data presented shows a precocious and greater development of total leaf area per vine by the SHMP system where it produced 19% more leaf area than what was generated by the traditional systems at pre-veraison. There is agreement within the literature that systems that maintain higher amounts of buds, such as mechanically pruned systems, generate and maintain larger leaf areas when compared to traditional pruning methods (Wessner and Kurtural 2013, De Toda and Sancha 1999, Poni et al. 2004, Wolf et al. 2003). The greater capacity of the SHMP system for both growth and production was attributed to greater canopy development and the length of time during which the leaves maybe photosynthetically active. The CP treatment reduced the total active leaf area compared to the other treatments. It has been reported that cane-pruned vines when supplied with sufficient irrigation to meet vine water demands can produce greater active canopies that may support greater yields (Wessner and Kurtural 2013). However, in drought situations where resources are limited, divided canopies tend to be heavily mitigated.

Yield Components

The economic feasibility of winegrape production in the central SJV has been called into question due to a group of factors such as lack of labor, seasonal drought, and limited availability of irrigation water due to dwindling sources. The current climatic situation of extremely low rainfall and high temperature events exacerbates the issue. However, it is essential for growers to maintain optimize yields to remain profitable. In response, a number of growers are converting trellises to a mechanized single high-wire system managed with reduced irrigation inputs in an attempt to maintain yield. By converting from already established systems, growers save the cost from replanting as well as lost yield revenue from the first few years of vine training. The current study shows that reducing the severity of winter pruning by mechanical box pruning is effective at maintaining yields over that of traditional systems in the current drought climate.

There was a strong effect of year on the majority of yield components along with a number of effects attributed to pruning systems. Pruning system effects were widespread throughout the trellis conversion year in 2013 where the CP treatment was already established and the HP and SHMP treatments were in transition. The CP treatment displayed the smallest berry weight and largest clusters during this transition. However, after the completion of the canopy conversion, pruning system effects on yield components were more favorable for the SHMP treatment. The data indicates that the mechanically box-pruned system maintained the smallest berry weights once the conversion was complete. Smaller berry sizes linked to mechanical pruning have been documented through a number of studies across many cultivars (Reynolds 1988, Terry and Kurtural 2011, Zabadal et al. 2002). However, this was likely more attributed to the compensating response of the grapevine that has a higher number of retained buds (Bates 2008,

Reynolds et al. 1996). In addition, arid winters have also been shown to reduce berry weights in subsequent years (Mendez-Costabel et al. 2014). This relatively rapid reproductive compensation response by the mechanized system displays the grapevines ability to manage the partitioning of photoassimilates to the reproductive organs of the plant without affectively starving itself of resources that are needed for other plant systems (Keller 2015). This is a very important compensation response when deciding to implement mechanization of winter pruning because when this response is coupled with a high level of canopy efficiency as shown with the SHMP system, the system is capable of maintaining higher yields with similar quality when compared to traditional systems (Keller et al. 2004, Zabadal et al. 2002) as found within this experiment.

The increased retention of higher node counts in mechanized systems leads to a greater distribution of shoots along cordons with a concomitant increase in cluster numbers per vine (Gatti et al. 2011, Geller and Kurtural 2013, De Toda and Sancha 1999), which is similar to the findings of this study. The results of this study indicated that increases in yield were strongly associated with clusters per vine in agreement with previous studies (Nelson 2015, Petrie et al. 2004). Furthermore, the fewest decrease in yield per vine in spite of the extended drought was found within the mechanized system.

The nature of the significant year by pruning system interactions seen throughout the yield components lies in a number of environmental factors the vineyard was exposed to during the experiment. The goal of the experiment was to implement a regulated deficit irrigation strategy where 80% of ET_c would be applied from bloom to fruit set and then 50% of ET_c would be applied from fruit set to veraison and 80% thereafter. The second treatment would be 80% of ET_c throughout the whole season. However, the extended California drought

drastically reduced the water table and affected the availability of water for irrigation applications as noted previously. Severe reductions in irrigation applications along with reduced precipitation are known to reduce yield (Romero et al. 2010). Previous research by Mendez-Costabel et al. (2014) showed that arid winters, such as that experienced during this experiment, reduced yield regardless of applied water amounts during a given year. Furthermore, deficit irrigation carry-over effects have been shown to effect yield in subsequent years (Lakso et al. 1999, Petrie et al. 2004, Cook et al. 2015) regardless if in season irrigation. The lack of irrigation treatment effects within this experiment are likely due to the quite extreme reduction in irrigation applications coupled with drought.

Skin tissue weights were not affected by pruning system or irrigation treatments in any of the post conversion years. However, skin weights were reduced in 2014 and 2015 due to the drought conditions with the lowest values being found in 2014. The reductions in skin weights seen in this experiment were directly linked to the reduction in berry size and therefore were likely to be associated with the environmental and climatic stresses experienced during the experiment.

Fruit Composition

Effects on berry composition were generally inconsistent during the trellis conversion year. However, juice TA increased in subsequent years while juice pH displayed lowest values in 2014. Most important to recognize from results is that the SHMP treatment did not alter fruit composition when compared to the traditional systems in the current drought climate. Previous mechanization studies in the warm climate of California shared similar findings where mechanization of winter pruning had minimal effects on fruit composition (Geller and Kurtural

2013, Terry and Kurtural 2011). Similarly, systems that retain more buds by mechanically hedging are able to minimize effects on berry composition (Poni et al. 2004, Reynolds 1988). Therefore, it seems that mechanization of winter pruning does not negatively affect fruit composition compared to traditional hand pruned systems when managed under irrigation deficits.

Anthocyanins

There is agreement within the literature that increased sunlight exposure into the fruit zone of grapevine canopies increases production of anthocyanins (Bergqvist et al. 2001, Downey et al. 2006, Spayd et al. 2002). However, previous research by Downey et al. (2004) concluded that anthocyanins retained the ability to accumulate in the absence of light indicating that light is not an absolute requirement for its biosynthesis. More importantly, it was concluded that light had a major effect on anthocyanin composition where shaded clusters contributed to a decrease in the glucosides of malvidin, delphinidin, and petunidin and an increase in the glucosides of cyanidin and peonidin (Cortell and Kennedy 2006, Downey et al. 2004). These findings indicated a response of light exposure to the distribution of anthocyanins down two different pathways within the flavonoid pathway.

Although there were no pruning system or irrigation treatment effects seen within the current study, there was a strong effect of year indicating the effects of drought and severe water deficits. The most apparent differences were seen within experimental years where there was an increase in the contribution from tri-hydroxylated anthocyanins (glucosides of delphinidin, petunidin, and malvidin) and a reduced contribution from di-hydroxylated anthocyanins (glucosides of cyanidin and peonidin) (table 5). The data also indicated a shift in phenolic composition based on the methoxylation pattern of the B ring. Light exposure was

not a limiting factor within this experiment as *PAR* values in the fruit zone were as high as 35% of the ambient (table 2). This greater level of exposure within the fruit zone led to an increase in the proportion of anthocyanins with two methoxyl substituents on the b-ring (malvidin-3-glucoside) and a decrease in the proportion of those with a single methoxyl group on the b-ring (delphinidin and petunidin-3-glucoside). Similar results were reported in Syrah (Downey et al. 2004) and Pinot noir (Cortell and Kennedy 2006). These results indicate that high levels of *PAR* exposures, due to reduced canopy development as a result of the current drought, can up-regulate the expression of *F3'5'H* genes and possibly contribute to the down-regulation of *F3'H* genes leading to a higher proportion to delphinidin based anthocyanins.

As mentioned previously, applied water amounts in 2015 were drastically reduced due to the current California drought. Despite seeing no differences between the irrigation treatments, similar results were seen within each year where there was a higher contribution from tri-hydroxylated and methoxylated anthocyanins. Furthermore, the concentration of total soluble anthocyanins increased from 2014 to 2015. Previous research by Castellarin and colleagues (2007b) concluded that biosynthesis of anthocyanins is strongly up-regulated when grown under drought conditions. Grapevines under severe water deficits displayed higher transcription of flavonoid 3'5'-hydroxylase (*F3'5'H*) and *O*-methyl-transferase (*OMT*). The up-regulation of these genes that encode specific enzymes within the flavonoid pathway led to anthocyanin compositions that were more enriched in more hydroxylated and methoxylated derivatives. Castellarin et al. (2007b) also concluded a much higher proportion of di-methoxylated malvidin compared to the non-methoxylated precursor (delphinidin-3-glucoside) the more intense the water deficit became, similar to what was experienced by this

experiment. Therefore, it can be concluded that increased sunlight exposure can have a synergistic effect with more severe water deficits in transcriptionally regulating anthocyanin biosynthesis where the pathway shifts more towards delphinidin based constituents. These induced changes occurred regardless of berry size as concluded in previous studies (Castellarin et al. 2007a, Nelson 2015). This shift towards more 3'4'5'-hydroxylated anthocyanins in the hot climate can have a profound effect on wine quality as this leads to higher contributions of purple/blue pigments in the must (Castellarin et al. 2007a).

In 2014, there was an interaction between irrigation and pruning system treatments where the combination of the CP and RDI treatments reduced a number of anthocyanin constituents. However this interaction was not apparent in the following year. In 2014, the CP treatment under more severe water deficits appeared to be under a heavier crop level due to having similar yield to the mechanized treatment but a lower active leaf area with similar microclimate values. High crop levels are known to reduce the production of anthocyanins (Jackson and Lombard 1993). In a companion study where whole vine carbon assimilation and transpiration rates were measured throughout the growing season, the CP treatment under the more severe RDI treatment had reduced assimilation rates and significantly higher transpiration rates at the onset of anthocyanin biosynthesis (data not show) indicating a source limitation.

In general, the acetate derivatives of petunidin and malvidin increased from 2014 and 2015 while cyanidin and peonidin constituents were reduced. The coumarate derivatives of petunidin and malvidin displayed similar results. Previous research has shown that increasing temperatures led to increases in acetate and coumarate derivatives, although minimal possibly showing these particular compounds being relatively more stable under heat stress (Spayd et al.

2002). Mori et al. (2007) concluded that malvidin derivatives were relatively unaffected by incidences of heat stress while a reduction was seen in other components. Higher proportions of methoxylated, acylated, and glycosylated forms of anthocyanins are known to increase the thermal stability of anthocyanins (Jackman and Smith 1996). The results of this experiment do indeed indicate a higher stability of tri-hydroxylated and methylated forms of anthocyanins in the hot and dry climate of the central San Joaquin Valley due to the up-regulation of *F3'5'H*, *UFGT*, and *OMT* genes under drought stress.

Gallates, Flavonols, Flavan-3-ols of Skin Tissue

As discussed previously, the interactive effects of light and temperature can have a significant effect on skin anthocyanins. There is agreement that these same environmental factors affect flavonol and flavan-3-ol composition in skin tissues (Castellarin et al. 2007a, Cortell and Kennedy 2006, Downey et al. 2004, Ristic et al. 2007). In hot climates such as the central San Joaquin Valley, the addition of increased temperature can have a negative effect on the accumulation of flavonoids (Tarara et al. 2008) in contrast to production in cooler climates.

Flavonols are normally present in much smaller concentrations than anthocyanins and are strictly produced within the epidermis. Their primary purpose is to act as a protectant from UV-B radiation. Fruit that is more exposed to light generally has a much higher amount of flavonols than their shaded counterparts (Downey et al. 2004). Therefore, an expected response to increased incidence of light within the fruit zone would be a concomitant increase in flavonol content as a response to up-regulation of genes within the flavonoid pathway, such as *FLS1*, that stimulate their biosynthesis (Castellarin et al. 2007a, Downey et al. 2004). A decrease in canopy development was seen within this

study from 2014 to 2015 as indicated by a reduced K_c maximum in 2015 (data not shown). Furthermore, a reduction in a number of canopy microclimate variables was seen in 2015 (refer to Appendix A, Table 2). Cortell and Kennedy (2006) reported higher flavonol content as cluster exposure to light increased in a cool climate. In agreement, Cook (2015) attributed an increase in flavonol content due to higher *PAR* values as a result of pre and post-fruit set leaf removal in a hot climate. In contrast, there was a strong effect of year on total flavonols within this experiment where content decreased in 2015 where an increase would normally be expected as a response to higher exposure. However, exposure levels within this experiment displayed fruit zone *PAR* values around 35% during fruit maturation indicating the role increased temperatures may have on the inhibition or degradation of flavonol compounds. Inhibition of flavonols in red wine grapes has been reported as a consequence of exposure to higher temperatures (Goto-Yamamoto et al. 2010, Nelson 2015, Spayd et al. 2002). Previous research has supported the idea of improvements in the light microclimate of grapevine canopies as a method of improving flavonol content (Cook et al. 2015, Downey et al. 2004). However, due to the current California drought, canopy development was mitigated to undesirable levels that increased fruit exposure to high temperatures. It can be concluded that reductions in flavonols can be a result of exposure to increased temperatures, despite increase exposure to light in a hot climate.

A reduction of total flavan-3-ol monomers within skin tissues was also seen in 2015 within this experiment (refer to Appendix A, Table 7). Flavan-3-ols within the skin have been linked to maturation of fruit as content declines with increasing sugars within the berry (Cortell and Kennedy 2006). The higher values seen in 2014 can be attributed to the fruit being harvested well before commercial

maturity was reached (refer to Appendix A, Table 4). Light exposure has also been shown to effect flavan-3-ol content. Exposed fruit has been shown to have higher flavan-3-ol content than their shaded counterparts (Cortell and Kennedy 2006). Similar to the findings on flavonols within this experiment, increased exposure due to mitigation of canopy development led to a decrease in total flavan-3-ol monomers. Furthermore, it appears that water deficits within this study had a greater effect on anthocyanin composition than on flavonols and flavan-3-ols. These findings are consistent with results seen by Castellarin et al. (2007a) and Roby and Matthews (2004) who reported that water deficits significantly increased anthocyanins but had minimal effects on flavonols and skin tannins.

Yield Efficiency and Water Footprint

The data generated in this study indicates that adjustments to current recommended yield efficiency values can be adjusted for the hot climate of the central SJV. Although no treatment effects were apparent, there was a significant effect of year on yield efficiency. Previous research performed in Davis and Oakville, California concluded that the ideal leaf area/fruit weight ratio for single curtain canopies, such as the California Sprawl system, is 0.8-1.2 m²/kg while values for divided canopies were lower showing ideal values between 0.5-0.8 m²/kg (Kliewer and Dokoozlian 2005). In 2014 of the experiment, the CP treatment was the only one that made commercial maturity of 23 brix but displayed efficiency values below what was recommended showing 0.44 m²/kg. However, it must be noted that the fruit was harvested earlier in 2014 as indicated by the lower degree days accumulated at harvest (see Appendix A, Table 1). In 2015, all of the pruning systems reached commercial maturity where the SHMP and HP systems had lower values than those recommended. It would appear that

lower efficiency values would be viable in both single and divided canopies in the hot climate of the central SJV. The relatively shorter fruit maturation in the hot climate allows for an extended period after harvest where canopies stay functional and accumulate photoassimilates to store in the permanent structures of the vine. This activity can essentially reduce the amount of leaf area required to ripen a given crop size (Howell 2001). Similar research performed in the southern SJV had similar conclusions showing that lower efficiency values in both single and divided canopies were capable of reaching commercial maturity (Nelson 2015).

The looming threat of climate change has caused a higher demand to better utilize the application of limited water supplies. Due to current climatic stress and increases in water costs, it is inevitable that less water will be used particularly in those areas that have the highest plant water demand such as the San Joaquin Valley. The biggest fear is that deficit irrigated farming of wine grapes may be economically unfeasible, particularly when matched with arid winters (Mendez-Costabel 2014, Williams 2012). Furthermore, it was reported that irrigated production in hot and dry climates have very high water footprints (Morison et al. 2008) with values shown to be up to 608 m³ per ton (Williams 2014a). It should be noted that the previous value might not take into account the effect of applied water deficits. However, the findings of this study show a decrease in water footprint with decreasing water applications indicating an increase in water productivity. It was reported that vines under sustained water deficits have increased water productivity compared to fully irrigated vines (Chaves et al. 2007, Shellie 2014). The water footprint of the SHMP treatment was consistently lower from year to year when compared to the HP and CP pruning treatments. This is likely due to the mechanized systems yield buffering ability to yield reductions in response to applied deficits as previously discussed where both the CP and HP

treatments had significantly higher yield losses. Nelson (2015) found water footprint values that were much lower than those found in cooler northern California climates (Williams 2014a) with values ranging from 111-136 m³/ton of fruit harvested at commercial maturity. Data from this experiment generated water footprint values of 36-52 m³/ton of fruit. Water footprint values in this experiment indicate that mechanization of winter pruning under severe water deficits is able to improve the efficiency of water use by grapevines in the central San Joaquin Valley.

CONCLUSION

The results reported within this experiment indicate that conversion of traditional systems over to a single high wire mechanically box-pruned system can be effectively managed under severe water deficits while maintaining fruit quality in Merlot grapevines. The strong effect of year seen in subsequent years throughout the study was effective in showing the buffering capacity the SHMP treatment has in response to severe climate conditions. This was shown through the minimal reductions in yield in the SHMP system in subsequent years compared to the HP and CP treatments. There are a number of compensating mechanisms that grapevines have in response to a higher number of retained nodes. However, the predominant mechanism shown in this study was the production of significantly smaller berries within the SHMP system. The SHMP system also displayed a more precocious and earlier development of leaf area indicating a higher degree of canopy efficiency. Earlier development of canopy architecture when coupled with sufficient reproductive compensating responses allows for increased yields while maintaining quality such as that found within the current study. Not only was there extreme drought experienced throughout this study, but there was also minimal amounts of water applications that exacerbated the reductions in a number of growth parameters. However, the mechanized system was able to maintain higher yields without a loss in quality along with displaying the lowest water footprint. The lack of treatment effects between the irrigation treatments indicate an opportunity to use less water without any deleterious effects while saving money, considering the increased cost of water in times of drought. Furthermore, the combination of the SHMP and RDI treatments are recommended for growers within the hot climate of the central San Joaquin

Valley as a means to increase the sustainability of production during times of extended drought while not sacrificing fruit quality.

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APPENDICES

APPENDIX A: TABLES

Table 1. Phenological progression of 'Merlot 01/Freedom' in 2014 and 2015 in the central San Joaquin Valley of California.

<u>Phenological Stage</u>	<u>Modified Eichorn-Lorenz stage^z</u>	<u>Date in 2014</u>	<u>GDD accumulated in 2014</u>	<u>Date in 2015</u>	<u>GDD accumulated in 2015</u>
Bud Break	4	20-Mar	24	17-Mar	25
Bloom	25	5-May	309	30-Apr	310
Fruit Set	27	9-Jun	583	1-Jun	595
Veraison	35	14-Jul	1070	11-Jul	1193
Harvest	38	6-Aug	1624	17-Aug	1813
Dormant Pruning	-	15-Feb-15	-	22-Feb-16	-

^zModified Eichhorn-Lorenz stage = Modified E-L system for identifying major and intermediate grapevine growth stages (Coombe 1995). 2013 phenological progression was omitted due to weather station being unavailable during growing season.

Table 2. Effects of crop load management systems and applied water amounts on the phenological development of canopy architecture and microclimate of 'Merlot 01/Freedom' in the central San Joaquin Valley of California in 2014 and 2015 (n = 3).

Systems/Methods	<u>Leaf Layers^x</u>		<u>Cluster Contacts^w</u>		<u>Canopy Gap %^v</u>		<u>PAR^u</u>		<u>Leaf Area (m²/vine)</u>	
	PV	PH	PV	PH	PV	PH	PV	PH	PV	PH
Pruning System^z	2014									
Cane-pruned (CP)	1.43	1.83	0.5	0.68b ^t	18.3a	8.3	16.9	35.6	2.99b	3.02b
Spur-pruned (HP)	1.72	2.05	0.6	0.69b	10.8ab	8.3	12.6	32.3	3.39ab	3.91a
Mechanical box-pruned (SHMP)	1.62	1.68	0.64	0.88a	9.2b	5.0	16.8	35.2	4.13a	4.10a
<i>Pr>F</i>	0.0830	0.0719	0.1039	0.0387	0.0294	0.4390	0.1535	0.8188	0.0313	0.0099
Irrigation method^y	2015									
Sustained deficit irrigation (SDI)	1.57	1.81	0.57	0.76	12.8	8.3	15.1	32.5	3.27	3.65
Regulated deficit irrigation (RDI)	1.61	1.89	0.59	0.74	12.0	6.1	15.7	36.2	3.73	3.74
<i>Pr>F</i>	0.6891	0.4996	0.7978	0.8135	0.7776	0.3646	0.6260	0.4284	0.1173	0.4399
Pruning system × irrigation (P)	0.7008	0.2217	0.1745	0.5948	0.0621	0.2400	0.7712	0.8925	0.197	0.4547
Pruning System	2015									
Cane-pruned (CP)	1.84	1.58	0.37b	0.67b	9.2	11.7	24.2	33.1	2.75b	3.39b
Spur-pruned (HP)	2.11	2.00	0.43ab	0.61b	6.7	6.7	25.1	30.5	3.11ab	3.59ab
Mechanical box-pruned (SHMP)	1.98	1.62	0.58a	0.88a	8.3	12.5	19.9	29.90	4.09a	4.33a
<i>Pr>F</i>	0.2390	0.1141	0.0294	0.0428	0.7469	0.2873	0.1623	0.5574	0.0086	0.0259
Irrigation method	2015									
Sustained deficit irrigation (SDI)	1.92	1.65	0.44	0.74	7.2	9.40	20.4	33.6	3.35	3.79
Regulated deficit irrigation (RDI)	2.03	1.81	0.48	0.69	8.9	11.1	25.6	28.8	3.32	3.76
<i>Pr>F</i>	0.3244	0.4820	0.5398	0.3677	0.5414	0.6077	0.0931	0.0806	0.9271	0.5684

Table 2 (cont.)

Systems/Methods	Leaf Layers ^x		Cluster Contacts ^w		Canopy Gap % ^v		PAR ^u		Leaf Area (m ² /vine)	
	PV	PH	PV	PH	PV	PH	PV	PH	PV	PH
Pruning system × irrigation (P)	0.6929	0.7689	0.7830	0.8260	0.3291	0.5406	0.3711	0.1637	0.6879	0.4733
Year (Pr>F)	<.0001	<.0001	0.0017	<.0001	0.0042	0.0436	0.0284	0.5377	0.4112	0.8303
Year x pruning system (Pr>F)	0.0492	0.7745	0.8446	0.2737	0.8066	0.8333	0.2781	0.9919	0.7223	0.2457
Year x irrigation (Pr>F)	0.1565	0.6949	0.8055	0.3906	0.7669	0.8244	0.6963	0.1206	0.2501	0.7008
Year x pruning sys. x irrigation (Pr>F)	0.8505	0.2078	0.6114	0.3862	0.2661	0.4612	0.1634	0.4091	0.7434	0.9967

^xHP = spur-pruned to retain 21 nodes m⁻¹; 15 February 2015, 22 February 2016. CP = Four 8-node canes tied on N-S direction. Two canes were trained to a 1m cordon wire in opposing directions.

Two canes were trained at a 1.4m cordon wire in opposing directions. SHMP = Mechanically box-pruned to a 10 cm spur height; 15 February 2015, 22 February 2016.

^ySDI = Sustained Deficit Irrigation (SDI) was initiated at bud-break and maintained till harvest. 1.67 megaliters/HA were applied in 2014 and 0.39 megaliters/HA were applied in 2015.

Regulated Deficit Irrigation (RDI) was initiated at bud-break. A third-less of irrigation water was applied from fruit-set to veraison compared to SDI treatment for an applied deficit.

1.25 megaliters/HA was applied in 2014 and 0.34 was applied in 2016.

^xLeaf layers = Total number of leaf contacts divided by the number of insertions measured using four point quadrat analysis.

^wCluster contacts = number of clusters in contact with the insertion needle, measured using four point quadrat analysis.

^vCanopy gaps (%) = Total number of gaps divided by number of insertions, measured using four point quadrat analysis.

^uPAR transmittance (%) = percentage of total ambient photosynthetically active radiation measured at mid-day in the fruit zone. Data failed to meet assumptions of analysis of variance and were log₁₀-transformed. Non-transformed values are presented for ease of discussion.

^yValues with different letter designations represent significant mean separation according to Tukey's significant difference test at $P \leq 0.05$.

Table 3. Effects of pruning systems and deficit irrigation methods on yield components of ‘Merlot 01/Freedom’ in the central San Joaquin Valley of California in 2013, 2014, and 2015 (n=3).

	Berry weight (g)	Cluster no.	Cluster weight (g)	Yield (kg/vine)	Skin wt (mg/berry)
Pruning System^z					
			2013		
Cane-pruned (CP)	1.09b ^u	93b	146.83a	13.66a	25.8
Spur-pruned (HP)	1.20a	110a	125.93b	13.71a	22.8
Mechanical box-pruned (SHMP)	1.23a	94b	111.40c	10.64b	25.4
<i>Pr>F</i>	0.0054	0.0057	<0.0001	0.0005	0.5364
Irrigation method^y					
Sustained deficit irrigation (SDI)	1.19	102	127.82	12.91	25.7
Regulated deficit irrigation (RDI)	1.17	97	128.28	12.42	23.7
<i>Pr>F</i>	0.5469	0.2780	0.9228	0.4850	0.4189
Pruning system × irrigation (P)	0.7115	0.6751	0.6315	0.4933	0.1981
Pruning System			2014		
Cane-pruned (CP)	0.78a	93b	85.90	8.10ab	27.4
Spur-pruned (HP)	0.76ab	91b	79.81	7.25b	23.1
Mechanical box-pruned (SHMP)	0.71b	117a	81.42	9.46a	23.1
<i>Pr>F</i>	0.0383	0.0016	0.3578	0.0304	0.1554
Irrigation method					
Sustained deficit irrigation (SDI)	0.77 a	104	80.43	8.23	25.7
Regulated deficit irrigation (RDI)	0.72 b	94	84.69	8.17	23.5
<i>Pr>F</i>	0.0176	0.2081	0.3909	0.4590	0.1555
Pruning system × irrigation (P)	0.4364	0.5883	0.8864	0.4359	0.0719
Pruning system			2015		
Cane-pruned (CP)	0.91ab	68b	82.40	5.71b	29.5
Spur-pruned (HP)	0.98a	75b	78.56	6.04ab	30.8
Mechanical box-pruned (SHMP)	0.86b	99a	86.43	8.60a	27.8
<i>Pr>F</i>	0.0119	0.0025	0.7659	0.0201	0.6069
Irrigation method					
Sustained deficit irrigation (SDI)	0.90	84	84.87	7.36	27.98
Regulated deficit irrigation (RDI)	0.93	77	80.06	6.21	30.73
<i>Pr>F</i>	0.4165	0.3537	0.3864	0.2358	0.2718
Pruning system × irrigation (P)	0.8919	0.2883	0.1461	0.0794	0.4937
Year (Pr>F)	<.0001	<.0001	<.0001	<.0001	0.0094
Year x pruning system (Pr>F)	0.0012	0.0001	0.0266	<.0001	0.5499
Year x irrigation (Pr>F)	0.0837	0.9270	0.4799	0.6298	0.1038
Year x pruning sys. x irrigation (Pr>F)	0.9391	0.6205	0.2669	0.2131	0.4692

^zHP = spur-pruned to retain 21 nodes m⁻¹; 15 February 2015, 22 February 2016. CP = Four 8-node canes tied on N-S direction. Two canes were trained to a 1m cordon wire in opposing directions. Two canes were trained at a 1.4m cordon wire in opposing directions. SHMP = Mechanically box-pruned to a 10 cm spur height; 15 February 2015, 22 February 2016.

^ySDI = Sustained Deficit Irrigation (SDI) was initiated at bud-break and maintained till harvest. 1.67 megaliters/HA were applied in 2014 and 0.39 megaliters/HA were applied in 2015. Regulated Deficit Irrigation (RDI) was initiated at bud-break. A third-less of irrigation water was applied from fruit-set to veraison compared to SDI treatment for an applied deficit. 1.25 megaliters/HA was applied in 2014 and 0.34 was applied in 2016.

^uValues with different letter designations represent significant mean separation according to Tukey's honestly significant difference test at $P \leq 0.05$.

Table 4. Effect of pruning system and deficit irrigation on chemical composition of 'Merlot 01/Freedom' in the central San Joaquin valley of California in 2013, 2014, and 2015 (n = 3).

	<u>Brix</u>	<u>Juice pH^x</u>	<u>TA (g/L)^w</u>
2013			
Pruning System^z			
Cane-pruned (CP)	23.1a ^y	3.53a	5.02b
Spur-pruned (HP)	22.1b	3.54a	5.20b
Mechanical box-pruned (SHMP)	22.5ab	3.48b	5.99a
<i>P_{Pr>F}</i>	0.0098	0.0045	<.0001
Deficit Irrigation^y			
Sustained deficit irrigation (SDI)	22.6	3.51	5.49
Regulated deficit irrigation (RDI)	22.6	3.53	5.33
<i>P_{Pr>F}</i>	0.9786	0.1307	0.2387
Pruning system × irrigation (Pr>F)	0.0604	0.5810	0.1803
2014			
Pruning system			
Cane-pruned (CP)	23.3	3.34	6.23
Spur-pruned (HP)	22.8	3.37	6.19
Mechanical box-pruned (SHMP)	22.4	3.33	6.39
<i>P_{Pr>F}</i>	0.1270	0.4069	0.8747
Deficit Irrigation			
Sustained deficit irrigation (SDI)	23.2a	3.34	6.29
Regulated deficit irrigation (RDI)	22.4b	3.37	6.25
<i>P_{Pr>F}</i>	0.0208	0.3917	0.9128
Pruning system × irrigation (Pr>F)	0.3768	0.6611	0.5238
2015			
Pruning system			
Cane-pruned (CP)	24.3	3.60	6.66
Spur-pruned (HP)	24.5	3.69	6.26
Mechanical box-pruned (SHMP)	23.7	3.59	6.61
<i>P_{Pr>F}</i>	0.1926	0.2342	0.4803
Deficit Irrigation			
Sustained deficit irrigation (SDI)	24.00	3.57b	6.54
Regulated deficit irrigation (RDI)	24.32	3.68a	6.49
<i>P_{Pr>F}</i>	0.4251	0.0038	0.9257
Pruning system × irrigation (Pr>F)	0.4208	0.4420	0.3466
Year (Pr>F)	<.0001	<.0001	<.0001
Year x pruning system (Pr>F)	0.2681	0.5672	0.1981
Year x irrigation (Pr>F)	0.0704	0.2488	0.9220
Year x pruning sys. x irrigation (Pr>F)	0.8392	0.6610	0.4060

^zHP = spur-pruned to retain 21 nodes/m-1; 15 February 2015, 22 February 2016. CP = Four 8-node canes tied on N-S direction. Two canes were trained to a 1 m cordon wire in opposing directions. Two canes were trained at a 1.4 m cordon wire in opposing directions.

SHMP = Mechanically box-pruned to a 10 cm spur height; 15 February 2015, 22 February 2016.

^ySDI = Sustained Deficit Irrigation (SDI) was initiated at bud-break and maintained till harvest. 1.67 megaliters/HA were applied in 2014 and 0.39 megaliters/HA were applied in 2015.

Regulated Deficit Irrigation (RDI) was initiated at bud-break. A third-less of irrigation water was applied from fruit-set to veraison compared to SDI treatment for an applied deficit.

1.25 megaliters/HA was applied in 2014 and 0.34 was applied in 2016.

^xJuice pH = Acidity of solution, expressed on a logarithmic scale.

^wTA (g L⁻¹) = Titratable acidity, total of all acids present in grape juice.

^yValues with different letter designations represent significant mean separation according to Tukey's honestly significant difference test at *P* ≤ 0.05.

Table 5. Table 5. Effects of pruning systems and deficit irrigation methods on exhaustively extracted anthocyanins (mg/kg) of 'Merlot 01/Freedom' from skin tissue in the central San Joaquin Valley of California in 2013, 2014, and 2015 (n = 3).

	<u>Di-hydroxylated</u>		<u>Tri-hydroxylated</u>			<u>3-Acetyl-glucosides</u>				<u>3-Coumaryl-glucosides</u>		<u>TSA^m</u>
	<u>c-3-g^x</u>	<u>po-3-g^w</u>	<u>d-3-g^v</u>	<u>pe-3-g^u</u>	<u>m-3-g^t</u>	<u>c-3-g-a^s</u>	<u>po-3-ga^r</u>	<u>pe-3-ga^q</u>	<u>m-3-ga^p</u>	<u>pe-3-gc^o</u>	<u>m-3-gcⁿ</u>	
Pruning system^z	<u>2013</u>											
Cane-pruned (CP)	77.4	135.9	77.4	103.0	916.5	25.8	92.7	537.1	39.2	10.8	538.1	2501.2
Spur-pruned (HP)	77.2	142.3	77.2	104.5	982.3	28.1	118.0	620.3	51.8	13.3	687.0	2861.4
Mechanical box-pruned (SHMP)	72.6	129.1	72.6	98.2	834.5	26.3	112.0	581.4	45.2	11.3	534.0	2464.8
<i>Pr>F</i>	0.9606	0.9488	0.9606	0.9635	0.6589	0.8983	0.4693	0.7344	0.5253	0.4626	0.2604	0.6098
Irrigation method^y												
Sustained deficit irrigation (SDI)	26.2	156.5	81.9	109.5	924.0	26.3	105.1	576.1	47.0	12.2	586.0	2661.8
Regulated deficit irrigation (RDI)	19.5	114.9	69.6	94.2	868.1	27.2	110.1	583.1	43.8	11.3	586.7	2556.5
<i>Pr>F</i>	0.9695	0.2392	0.4465	0.4515	0.8454	0.8353	0.7700	0.9357	0.7251	0.5048	0.9933	0.7705
Pruning system × irrigation (P)	0.5324	0.8386	0.5948	0.8178	0.7990	0.4285	0.1231	0.5365	0.6056	0.3919	0.3458	0.5926
Pruning system	<u>2014</u>											
Cane-pruned (CP)	37.6	108.0	157.1	151.4	752.9	29.9	6.5	38.3	361.7	16.1	224.4	1891.9
Spur-pruned (HP)	32.6	116.7	145.7	150.5	852.9	28.3	4.7	44.5	433.9	19.1	266.3	2132.4
Mechanical box-pruned (SHMP)	27.4	96.3	147.4	148.9	834.1	28.4	4.7	38.6	422.4	19.2	265.7	2036.1
<i>Pr>F</i>	0.0669	0.2450	0.7527	0.9854	0.4212	0.8346	0.3178	0.1573	0.1433	0.2440	0.2198	0.4511

Table 5 (cont.)

	<u>c-3-g^x</u>	<u>po-3-g^w</u>	<u>d-3-g^v</u>	<u>pe-3-g^u</u>	<u>m-3-g^t</u>	<u>c-3-g-a^s</u>	<u>po-3-ga^r</u>	<u>pe-3-ga^q</u>	<u>m-3-ga^p</u>	<u>pe-3-gc^o</u>	<u>m-3-gcⁿ</u>	<u>TSA^m</u>
Irrigation method^y												
Sustained deficit irrigation (SDI)	33.3	110.6	151.0	149.6	795.6	28.8	5.9	40.7	389.9	17.5	247.2	1978.7
Regulated deficit irrigation (RDI)	31.8	103.0	149.2	150.9	830.9	29.0	4.8	40.0	422.1	18.7	256.7	2061.6
<i>Pr>F</i>	0.7068	0.464	0.8908	0.9202	0.5922	0.9226	0.1573	0.3178	0.3127	0.5483	0.6477	0.5960
Pruning system × irrigation (P)	0.1667	0.2186	0.0894	0.0685	0.0491	0.1056	0.5733	0.5733	0.0398	0.0073	0.0239	0.0273
Pruning system							2015					
Cane-pruned (CP)	20.9	96.9	115.1	129.7	966.5	20.5	3.8	46.7	477.8	36.7	323.8	2238.6
Spur-pruned (HP)	19.6	97.7	120.2	136.6	1027.4	22.3	4.7	55.9	539.3	40.2	348.5	2431.7
Mechanical box-pruned (SHMP)	17.5	82.8	108.8	124.4	968.5	19.6	3.5	44.2	493.7	35.7	345.3	2308.6
<i>Pr>F</i>	0.5154	0.3737	0.7994	0.807	0.8729	0.6860	0.1833	0.1844	0.6451	0.6374	0.8199	0.8125
Irrigation method												
Sustained deficit irrigation (SDI)	18.7	86.3	111.3	125.2	940.8	20.4	3.9	45.0	486.2	35.6	331.9	2205.8
Regulated deficit irrigation (RDI)	20.0	98.6	118.1	135.2	1034.1	21.2	4.1	52.8	521.0	39.5	346.6	2450.7
<i>Pr>F</i>	0.4674	0.2021	0.6295	0.5153	0.3916	0.7663	0.7520	0.1867	0.5320	0.3352	0.6749	0.3360
Pruning system × irrigation (P)	0.5398	0.4223	0.6836	0.5383	0.3782	0.8120	0.8176	0.5845	0.5971	0.4562	0.5244	0.5728
Year (Pr>F)	<.0001	0.0156	0.0003	0.0437	0.0181	<.0001	0.0038	0.0394	0.0085	<.0001	0.0001	0.0558
Year x pruning system (Pr>F)	0.8848	0.9713	0.7616	0.9002	0.9027	0.7052	0.0771	0.7832	0.8538	0.8829	0.9443	0.7379
Year x irrigation (Pr>F)	0.2666	0.1030	0.6560	0.6567	0.6413	0.8804	0.2088	0.1953	0.9108	0.9685	0.9978	0.1847

Table 5 (cont.)

	<u>c-3-g^x</u>	<u>po-3-g^w</u>	<u>d-3-g^v</u>	<u>pe-3-g^u</u>	<u>m-3-g^t</u>	<u>c-3-g-a^s</u>	<u>po-3-ga^r</u>	<u>pe-3-ga^q</u>	<u>m-3-ga^p</u>	<u>pe-3-gc^o</u>	<u>m-3-gcⁿ</u>	<u>TSA^m</u>
Year x pruning sys. x irrigation (Pr>F)	0.1083	0.0876	0.1039	0.0712	0.0344	0.1892	0.5615	0.0836	0.0589	0.0270	0.0308	0.0633

^zHP = spur-pruned to retain 21 nodes`m-1; 15 February 2015, 22 February 2016. CP = Four 8-node canes tied on N-S direction. Two canes were trained to a 1m cordon wire in opposing directions. Two canes were trained at a 1.4m cordon wire in opposing directions.

SHMP = Mechanically box-pruned to a 10 cm spur height; 15 February 2015, 22 February 2016.

^ySDI = Sustained Deficit Irrigation (SDI) was initiated at bud-break and maintained till harvest. 1.67 megaliters/HA were applied in 2014 and 0.39 megaliters/HA were applied in 2015. Regulated Deficit Irrigation (RDI) was initiated at bud-break. A third-less of irrigation water was applied from fruit-set to veraison compared to SDI treatment for an applied deficit. 1.25 megaliters/HA was applied in 2014 and 0.34 was applied in 2016.

^xc-3-g = cyanidin-3-glucoside, ^wpeonidin-3-glucoside, ^vdelphinidin-3-glucoside, ^upetunidin-3-glucoside, ^tmalvidin-3-glucoside, ^scyanidin-3-glucoside-acetate, ^rpeonidin-3-glucoside-acetate, ^qpetunidin-3-glucoside-acetate, ^pmalvidin-3-glucoside-acetate,

^opetunidin-3-glucoside-coumarate, ⁿmalvidin-3-glucoside-coumarate, ^mTotal skin anthocyanins.

Data failed to meet assumptions of analysis of variance and were log10-transformed. Non-tranformed values are presented for ease of discussion.

Table 6. Effects of pruning systems and deficit irrigation methods on yield efficiency, and water productivity of 'Merlot 01/Freedom' in central San Joaquin Valley of California (N = 3).

Pruning system	Leaf area:fruit (m ² /kg)	Water footprint (m ³ /t) ^b
	2014	
Cane-pruned (CP)	0.43	132.81ab ^c
Spur-pruned (HP)	0.51	147.79a
Mechanical box-pruned (SHMP)	0.44	110.69b
<i>Pr>F</i>	0.1661	0.0304
Irrigation method^a		
Sustained deficit irrigation (SDI)	0.46	136.51
Regulated deficit irrigation (RDI)	0.46	126.06
<i>Pr>F</i>	0.7582	0.0262
Pruning system × irrigation (P)	0.2887	0.4359
Pruning system		
2015		
Cane-pruned (CP)	0.81	52.23a
Spur-pruned (HP)	0.72	47.3ab
Mechanical box-pruned (SHMP)	0.66	36.56b
<i>Pr>F</i>	0.6931	0.0201
Irrigation method		
Sustained deficit irrigation (SDI)	0.72	46.42
Regulated deficit irrigation (RDI)	0.74	44.31
<i>Pr>F</i>	0.3029	0.9915
Pruning system × irrigation (P)	0.3372	0.0794
Year (Pr>F)	0.0022	<.0001
Year x pruning system (Pr>F)	0.5892	0.5351
Year x irrigation (Pr>F)	0.2231	0.1803
Year x pruning sys. x irrigation (Pr>F)	0.2344	0.1354

^aSDI = Sustained deficit irrigation was initiated at budbreak and had a target of 80% of crop evapotranspiration (ET_c). Regulated deficit irrigation (RDI) was initiated at budbreak and had targets of 80%, 50%, and 80% of ET_c at budbreak to fruit set, fruit set to veraison and veraison to harvest, respectively.

^bWater footprint (m³/t) = Irrigation applied in cubic meters per hectare divided by metric tons per hectare harvested.

^cValues with different letter designations represent significant mean separation according to Tukey's honestly significant difference test at p≤0.05.

All factors measured in this table failed to meet assumptions of analysis of variance and were log₁₀-transformed. Non-transformed values are presented for ease in discussion.

Table 7. Effects of pruning systems and deficit irrigation methods on exhaustively extracted gallates, flavan-3-ols and flavonols (mg/kg) of 'Merlot 01/Freedom' skin tissue in the central San Joaquin Valley of California in 2013, 2014, and 2015 (n = 3).

	<u>Gallates^x</u>	<u>Total Flavan-3-ols^w</u>	<u>Total flavonols^y</u>
Pruning system^z		<u>2013</u>	
Cane-pruned (CP)	397.3	212.1	204.7
Spur-pruned (HP)	495.5	259.6	218.8
Mechanical box-pruned (SHMP)	362.1	223.3	190.8
<i>Pr>F</i>	0.6806	0.8470	0.6943
Irrigation method^y			
Sustained deficit irrigation (SDI)	453.8	225.5	215.0
Regulated deficit irrigation (RDI)	363.7	242.8	194.5
<i>Pr>F</i>	0.2291	0.6795	0.4530
Pruning system x irrigation (P)	0.8878	0.4127	0.8896
Pruning system		<u>2014</u>	
Cane-pruned (CP)	397.3	212.1	204.7
Spur-pruned (HP)	495.5	259.6	218.8
Mechanical box-pruned (SHMP)	362.1	223.3	190.8
<i>Pr>F</i>	0.6806	0.8470	0.6943
Irrigation method			
Sustained deficit irrigation (SDI)	453.8	225.5	215.0
Regulated deficit irrigation (RDI)	363.7	242.8	194.5
<i>Pr>F</i>	0.2291	0.6795	0.4530
Pruning system × irrigation (P)	0.8878	0.4127	0.8896
Pruning system			
Cane-pruned (CP)	501	109	671

Table 7 (cont.)

	<u>Gallates^x</u>	<u>Total Flavan-3-ols^w</u>	<u>Total flavonols^v</u>
Spur-pruned (HP)	672	112	679
Mechanical box-pruned (SHMP)	583	96	691
<i>Pr>F</i>	0.2924	0.5226	0.8972
Irrigation method			
Sustained deficit irrigation (SDI)	523	106	664
Regulated deficit irrigation (RDI)	648	105	696
<i>Pr>F</i>	0.2120	0.6839	0.8161
Pruning system × irrigation (P)	0.3253	0.0385	0.1136
Pruning system		<u>2015</u>	
Cane-pruned (CP)	1144	34	115
Spur-pruned (HP)	977	33	123
Mechanical box-pruned (SHMP)	1052	32	146
<i>Pr>F</i>	0.8579	0.8720	0.2230
Irrigation method			
Sustained deficit irrigation (SDI)	1100	35	141
Regulated deficit irrigation (RDI)	1017	31	115
<i>Pr>F</i>	0.2778	0.2109	0.0983
Pruning system × irrigation (P)	0.8047	0.8522	0.7906
Year (Pr>F)	0.0148	<.0001	<.0001
Year x pruning system (Pr>F)	0.4417	0.5622	0.9883
Year x irrigation (Pr>F)	0.1337	0.6404	0.4067
Year x pruning sys. x irrigation (Pr>F)	0.4027	0.2159	0.3291

^zHP = spur-pruned to retain 21 nodes.m-1; 15 February 2015, 22 February 2016. CP = Four 8-node canes tied on N-S direction. Two canes were trained to a 1m cordon wire in opposing directions. Two canes were trained at a 1.4m cordon wire in opposing directions. SHMP = Mechanically box-pruned to a 10 cm spur height; 15 February 2015, 22 February 2016.

^vSDI = Sustained Deficit Irrigation (SDI) was initiated at bud-break and maintained till harvest. 1.67 megaliters/HA were applied in 2014 and 0.39 megaliters/HA were applied in 2015. Regulated

Table 7 (cont.)

Deficit Irrigation (RDI) was initiated at bud-break. A third-less of irrigation water was applied from fruit-set to veraison compared to SDI treatment for an applied deficit. 1.25 megaliters/HA was applied in 2014 and 0.34 was applied in 2016.

^xGallic acid, ^wincludes (+)-catechin and (-)-epicatechin constituents, ^vincludes quercetin and myricetin constituents.

Data failed to meet assumptions of analysis of variance and were log₁₀-transformed. Non-transformed data are presented for ease of discussion.

APPENDIX B: FIGURE

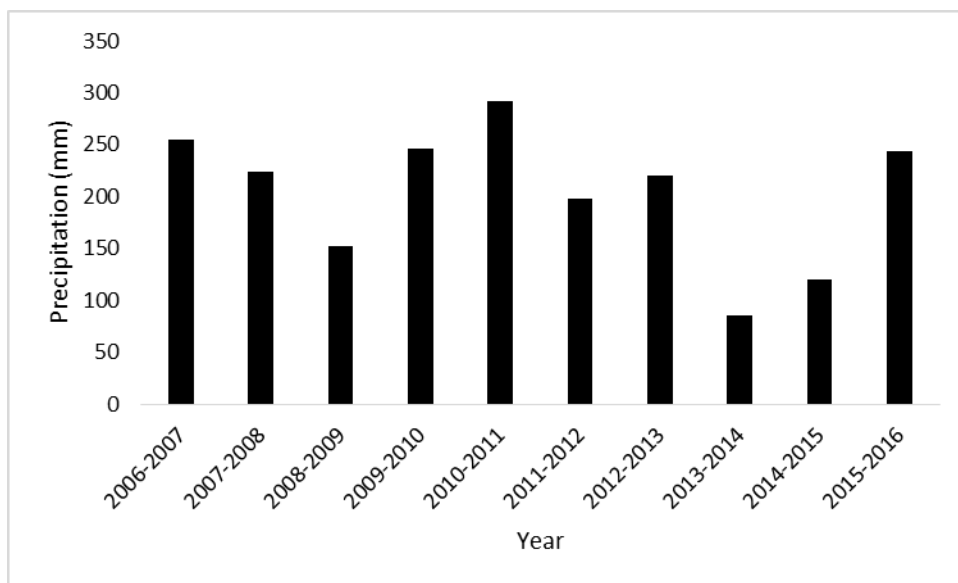


Figure 1. Seasonal summation (March-Feb.) of precipitation from California Irrigation Management Information System (CIMIS weather stations 188 and 145).

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