Optimising canopy function to increase yield while maintaining wine quality with efficient use of resources



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Optimising canopy function to increase yield while maintaining wine quality with efficient use of resources

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Abstract

Concern existed that extended use of irrigation strategies such as regulated deficit irrigation (RDI) could reduce vine capacity until the vine becomes uneconomic. Using an existing irrigation trial this project has examined the long-term impact of RDI and a prolonged deficit strategy (PD) in comparison with a well-watered control. Intra-seasonal effects of the PD deficit and inter-seasonal effects of both treatments resulted in reduced canopy size and yield, with PD vines being the smallest and having the lightest crop. However, neither treatment resulted in year-on-year reductions in vine capacity, with reduced yields remaining stable for the duration of the project.

Executive summary

The increasing use of irrigation strategies such as regulated deficit irrigation (RDI) over the last 15 years has led to concern that a progressive decline in vine 'capacity' could occur, reducing the economic viability of a given planting. This project utilised an existing irrigation trial in Sunraysia, an area that produces a significant proportion of Australia's winegrape crop, to examine the long-term impact of such irrigation strategies and the mitigation of any long-term effects.

The trial site consisted of a 12 year old planting of Cabernet Sauvignon vines on own-roots irrigated using an RDI type strategy, with prolonged deficit (PD) and well-watered control treatments imposed in a replicated block design. The PD treatment extended RDI in both duration and severity by suspending irrigation and allowing soil moisture to be drawn down to a low level for two to three weeks after the milder site standard RDI deficit period. RDI had been used at this site since the vines were planted. The PD treatment had been in place from the 2002/3 season and the well-watered control was altered from additional water during the RDI deficit period alone (from 2002/3) to additional water throughout the season in 2005/6. Use of the well-watered control allowed the speed of recovery of RDI vines from any reduction in 'capacity' to be examined as well as acting as a non-water limited comparison for the longer-term impacts of RDI and PD.

Both the RDI and PD treatments reduced canopy size and yield relative to the well-watered control, but there was no evidence that these reductions were increasing year-on-year. In fact the relative differences were remarkably stable, showing little seasonal variation. These results suggest that not only is the current use of RDI sustainable and unlikely to result in a progressive decline in vine productivity, but further, that RDI strategies can be extended without any risk of such a decline.

Wines from RDI fruit had significantly improved colour compared with those from the well-watered control and sensory analysis indicated there were beneficial effects on aroma and flavour as well. However, although the PD treatment generated a further improvement in colour initially, this reduced in time and there was no significant difference by the 2008 vintage. Sensory difference testing also failed to separate the PD wines from RDI, and although the full sensory analysis demonstrated differences in aroma and by mouth attributes, these were minor. Consequently, the change in wine quality, relative to fully irrigated vines, resulting from using an extended RDI strategy (e.g. PD) is unlikely to be higher than impact from using RDI alone. On the other hand, the PD treatment did not have a negative effect on wine quality and reduced water consumption by approximately 20%. Therefore, an extended RDI strategy may offer an effective means to reduce irrigation where required, albeit with a commensurate reduction in yield.

Little difference was seen in leaf function between the well-watered control and RDI vines, with stomatal opening being slightly greater in the control vines when soil moisture was limiting under the RDI treatment. The observed effects of the control treatment, relative to RDI, were therefore driven primarily by the faster rate and longer duration of canopy growth, generating a larger canopy, greater whole vine water use and consequently a larger yield. On the other hand the PD treatment not only reduced stomatal opening, and consequently photosynthesis, during the deficit period itself, but there was an intra-seasonal carry-over effect, leading to a further reduction in carbon gain by the PD vines from veraison to harvest. This effect was also seen in field-grown grafted Cabernet

Sauvignon and Shiraz vines. Furthermore, the PD vines had a slower rate of canopy growth than the other treatments, which was necessarily an inter-seasonal effect as it occurred prior to the RDI or PD deficit periods. In fact, within a given season, canopy growth in the PD vines was complete prior to the PD deficit being applied and no loss of canopy during the deficit period was observed. It is likely that the growth effects were the result of changes in the carbohydrate storage pool. Only slight differences in the *concentration* of carbohydrate reserves between treatments were observed, but there was evidence, particularly below ground, that the *pool size* was affected.

The extent of the intra-seasonal effect on leaf function varied between seasons, but was not governed by the degree of water stress during the PD deficit period and it did not appear to be related to atmospheric conditions following the deficit. It is possible that interaction with phenological stage, i.e. proximity to veraison, may have been involved. This suggests that by applying the same 20% reduction in seasonal irrigation more evenly over the combined RDI-PD deficit period, but ensuring soil moisture is non-limiting prior to the start of veraison, may enable the same saving in irrigation volume with a more limited impact on canopy growth and yield. However, this is yet to be tested.

One risk of using deficit irrigation is that a high-temperature event occurs whilst the vines are water stressed. The impact of this was tested using two glasshouse experiments funded by the CSIRO Plant Industry Summer Scholarship program. These experiments confirmed anecdotal reports that well irrigated grapevines are able to withstand significant heat stress, e.g. two consecutive days at over 45°C, with few or no negative effects on the foliage. Measurements of leaf function found that stomatal opening remained high in well watered vines reducing leaf temperature relative to deficit irrigated vines. However, this came at the cost of a large increase in water use, with the well-irrigated vines using almost four times as much water at 45°C than at 28°C, illustrating the scale of additional irrigation required to minimise heat damage. These experiments also demonstrated that water-stressed vines can take several days for stomatal opening to recover, suggesting that unless re-irrigation of deficit irrigated vines is started well before a heat event, additional irrigation may not be of benefit.

The second phase of the project examined the inter-seasonal carry-over effects of the field based irrigation treatments by reverting all vines to the site-standard RDI strategy. As was the case whilst the treatments were in place, there was no evidence of an inter-seasonal effect on leaf function. Consequently, carry-over effects on canopy growth and yield were also driven by vine size, not physiology. Even in the second season after reverting to standard irrigation the yield of the previously well-watered control vines was higher than RDI and the canopy size of PD vines smaller. Interestingly, the treatment effects on berry size were maintained for at least the first 'recovery' season (berry size was not measured in the second 'recovery' season) with control berries being larger and PD berries smaller than under RDI, but compositional effects were not. During the three seasons of irrigation treatment sugar per berry was higher in control fruit and lower in PD. Juice titratable acidity (TA) was higher in control and pH slightly lower than under RDI. However, despite the difference in berry size in the 'recovery' season, TA was only slightly affected and pH not at all. On the other hand colour density of control wines remained lower in both 'recovery' seasons. This implies that some characteristics were driven by the deficit irrigation within a season (juice parameters), whereas others were driven by berry size or canopy effects (e.g. wine colour despite the fact that total anthocyanins was unaffected by the irrigation treatment).

Chapter 1

Project background

To remain internationally competitive in targeted export markets, the Australian industry recognised a need to improve vineyard productivity while maintaining quality and reducing inputs. Existing management practices were not necessarily consistent with this requirement. The Riverlink Wine Industry Research Forum, 2004, identified a requirement for "Increasing yield while maintaining quality with decreased inputs for Fast Moving Consumer Products" as a very high priority for further research to maintain sustainable, economic viability for growers within the Murray Darling Basin. These growers were the major suppliers of fruit targeted for export wine styles at commercially acceptable price points.

There was considerable industry concern that the combination of deficit irrigation (applied via drip irrigation systems) and/or severe pruning to enhance fruit colour and wine quality might not be economically sustainable in the longer term due to compounding losses in vine productivity associated with reduced 'vine capacity' linked to development of smaller vines. Recent research also supported this concern as production losses may be associated with development of smaller root systems, reduced canopy size and photosynthetic function, reduced levels of storage carbohydrates and impacts of stress (e.g. water, temperature, light and their interactions) at critical periods on crop development. The latter, i.e. impact of stress at critical periods on crop development, identified in recent CSIRO research (Ashley *et al.* 2006, Cooley *et al.* 2005, Cooley *et al.* 2008, Glenn *et al*. 2010, Pellegrino *et al*. 2008, Petrie *et al*. 2004) required further investigation to elucidate the impact of such stresses and to identify significant interactions. This included potential effects of post-set stress on bud fruitfulness, stress at budburst on inflorescence branching and flower number, effects of low carbohydrate reserves associated with stress in the previous season on budburst, shoot and inflorescence growth and in the current season, effects of stress on fruit set and berry development.

GWRDC implemented a 'Soil and Water Initiative' with considerable input from industry personnel. This addressed key sustainability issues associated with soil and water management across a number of well developed, experimental sites. It was recognised that canopy form and function and their impact on crop development, maturation and fruit quality was critical to understanding the effects of soil and water management on vine performance across the various sites.

The project formed one of two Sunraysia GWRDC Soil and Water Iinitiative experimental sites at Wingara and built on Cooperative Research Centre for Viticulture (CRCV) research that was aimed at development of integrated strategies to manage seasonal variation in wine grape maturation (Cooley *et* al. 2005, Cooley *et al.* 2008, Glenn *et al*. 2010). In addition CSIRO Food Futures Flagship research to enhance wine flavour and aroma (Boss *et al*. 2010, Bramley *et al.* 2011), and CRCV research on partial rootzone drying/canopy management impacts on Shiraz wine quality (Ashley *et al.* 2006) were also conducted on the Wingara property and included treatments that had been imposed for a number of seasons. The Cabernet Sauvignon site (Clingleffer *et al*. 2006, Cooley *et al.* 2005, Cooley *et al*. 2008) was established to assess the effects of water stress treatments and seasonal temperature conditions on crop development, fruit maturation and long-term

productivity (ie. vine capacity). It showed significant effects of water stress on canopy size and function (including photosynthesis and leaf temperature), crop development (including sugar accumulation) and wine aroma, flavour and colour. Significant seasonal (temperature, in particular) impacts were also demonstrated. Because treatments had been established for a number of seasons, the site was ideal for examining long-term impacts on productivity and could be modified to address the specific question of whether current deficit irrigation practices were economically sustainable.

The irrigation regimes at the site were focussed around studying the impact of regulated deficit irrigation (RDI), a term introduced to describe irrigating at less than ET_{crop} for a specific phenological period. Originally, RDI was trialled by reducing irrigation during phases I & II of stone fruit growth, which are periods when cell division is occurring in the fruit and when fruit carbon demand is greatly outstripped by that of the actively growing canopy (Chalmers *et al.* 1981). The result being that canopy growth was reduced without a negative impact on yield. Furthermore, total water use was reduced and water use efficiency, in terms of yield per unit of water transpired, was improved (Mitchell and Chalmers 1982). During the 1990s these techniques were adapted for winegrapes (Hardie and Martin 1990, Goodwin and Jerie 1992, McCarthy 1997) with the result that not only were irrigation and shoot growth reduced, but an increase in colour of red wines was often observed. The use of RDI is now widespread amongst red winegrape growers in the warm climate regions of Australia.

To date, the uptake of RDI has been driven by the need to control vine vigour and maximise fruit quality rather than the need to improve vineyard water use efficiency. However, globally, irrigated agriculture is the single largest user of fresh water, accounting for 70% of diverted water and 90% of consumed water globally (Shiklamanov 1997, UNESCO 2000) and locally, concern over recent low water allocations to irrigators and the cuts now proposed in the Murray-Darling Basin Plan (Murray-Darling Basin Authority 2010) are likely to result in more growers considering RDI as a means of reducing water use. As 88% of Australia's 173,000 ha of vineyard are irrigated (Australian Bureau of Statistics 2008), this still has significant potential to improve water use efficiency.

Due to the relatively recent introduction of RDI and the necessarily limited duration of most studies examining the technique, there was concern about the possibility of undesirable longer-term effects on vine productivity, as noted above. Indeed, the early studies of RDI use in stone fruit observed that effects on canopy growth were small in a given season but had a significant cumulative effect over three seasons (Chalmers *et al.* 1981). Furthermore, inter-seasonal differences in growing conditions can easily mask such cumulative effects in 2-3 year studies.

By utilizing an established irrigation trial, this project has presented a rare opportunity to determine if RDI usage results in cumulative effects that may jeopardise production in the long-term. The trial took two approaches, firstly it compared established vines that had been irrigated using RDI for over ten years with vines that were irrigated in excess of ET_{crop}, and secondly it compared these treatments with vines that had been irrigated with an extended RDI strategy (prolonged deficit: PD) for four to six seasons.

Chapter 2

Experimental site: description, history, irrigation regimes and environmental monitoring

Background

Prior to the start of the 2002/3 growing season an irrigation trial was established on 0.95 ha of a larger 13 ha block of mature Cabernet Sauvignon vines as part of a CRCV project. The block was part of the commercially managed Deakin Estate vineyard, owned by the Wingara Wine Group, sited approximately 30 km south-east of Mildura in the Sunraysia region of Victoria. The vines were planted in 1994 at 2.44 m intervals along rows with a 3 m spacing, on own roots, trained to a two wire vertical trellis with wires at 1.1 & 1.6 m height and mechanical hedged to a width of approximately 0.4 m.

The soil was a calcarosol (Isbell 1996) consisting of a sand to loamy sand increasing to a sandy loam (to approximately 80 cm) above a carbonate layer (containing < 30% calcrete fragments) leading to a restrictive clay base below 170 cm.

Irrigation treatments and experimental design

All vines were drip irrigated with dripper spacings of 0.75 m and dripper outputs of 3.5 L h^{-1}. The irrigation management used by the grower on this block was a regulated deficit irrigation (RDI) strategy, implementing a post-fruit set period of reduced irrigation. The extent of the deficit was determined empirically by the vineyard manager, based on monitoring soil moisture content at 60 cm depth. During the designated deficit period soil moisture was allowed to decrease further before irrigating than during the rest of the season.

Starting in November 2002, three seasons prior to this project, three irrigation treatments were established (Cooley et al. 2005, Cooley et al. 2008): (1) continuation of the RDI strategy: a deficit period typically running from mid-November until mid- to late-December, with the precise timing being dependent on the weather conditions within a given season; (2) a well-watered control: where double the standard irrigation was applied during the RDI deficit period with irrigation for the rest of the season being identical to that of the RDI treatment; (3) prolonged deficit (PD): irrigation of this treatment was the same as RDI except that an additional irrigation deficit was initiated at the end of the RDI deficit period, consisting of no irrigation for approximately three weeks, the timing again being dependant on the weather within a given season. The PD period generally ended prior to the onset of veraison and always before more than a few per cent of berries had coloured. The aim of the PD treatment was to extend the RDI irrigation strategy in both time and severity.

For the current project the 'control' irrigation treatment was altered to provide double irrigation throughout the season from the beginning of the 2005/6 season, with the aim of providing a treatment that was never limited by soil water. Thus at the start of the current project, the irrigation treatments were: the first season of a double irrigation **control**, the fourth season of **PD** and a continued **RDI** strategy. These treatments were continued for three years. From the beginning of the 2008/9 season irrigation in all treatments was returned to the site standard RDI. Table 1 presents seasonal and pre-harvest irrigation, rainfall and reference evapo-transpiration (ET_o) for each year of the study.

Table 1. Irrigation applied, rainfall and ET_o from 1st of September to harvest (pre-harvest) and annually for each season of field work at the Wingara vineyard. *Estimated.

Year	Irrigation Treatment	Irrigation		Rainfall		Total Water		ET_o	
		Pre-harvest (mm)	Season (mm)	Pre-harvest (mm)	Annual (mm)	Pre-harvest (mm)	Annual (mm)	Pre-harvest (mm)	Annual (mm)
2005/6									
	Control	1096	1157			1246	1404		
	RDI	548	578	150	247	698	825	1044	1473
	PD	397*	428*			150	247		
2006/7									
	Control	787	1061			867	1244		
	RDI	394	530	80	183	474	713	1081	1548
	PD	296	432			376	615		
2007/8									
	Control	969	1131			1048	1303		
	RDI	485	565	79	172	564	737	1081	1525
	PD	387	489			466	661		
2008/9									
	RDI	588	613	85	195	752	980	1066	1503
2009/10									
	RDI	606	643	167	323	937	1333	1080	1487

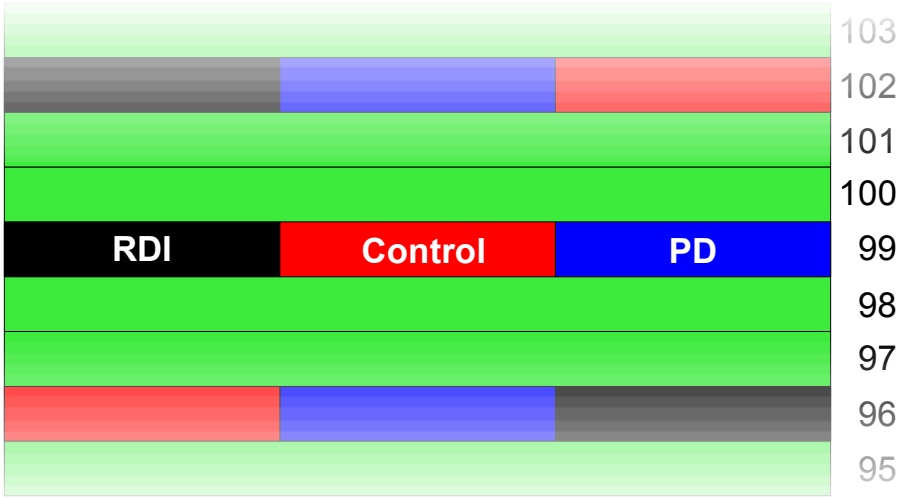


Figure 1. Layout of a typical replicate 'block' in the Cabernet Sauvignon experimental site at the Wingara vineyard. The measurement row is marked and the treatments are colour coded.

The treatments were laid out in balanced 'randomized' block design, with twelve blocks each consisting of three rows of vines. The blocks were adjacent to one another and each contained one replicate of each of the three irrigation treatments, assigned randomly to one of three positions, but constrained so that across the twelve blocks the 'column' formed by each position contained four replicates of each treatment (Figure 1).

Consequently, an individual replicate consisted of 36 vines arranged in three adjacent rows of 12, all receiving an identical irrigation treatment. All measurements were made only on the central eight vines of the central row, with the remaining rows/vines being treated as 'guards'.

Environmental measurements

Weather data for the experimental site, consisting of air temperature, relative humidity, wind speed, solar radiation and rainfall was obtained from two sources: a weather station maintained by the site owners, situated in a block of vines approximately 2 km from the experimental site and operated throughout the project, a weather station (MEA, Magill, SA, Australia) sited in the centre of the experimental area in December 2007 and operated until harvest 2010. Occasional gaps in the data set were filled using Bureau of Meteorology data obtained from their weather station at Mildura Airport (Bureau of Meteorology, 2010), approximately 28 km from the experimental site. Data was collected at 15 minute intervals. In addition vapour pressure deficit and reference crop evapo-transpiration (ET_o) were calculated from the above data, the latter according to FAO 56 (Allen *et al.* 1998).

Soil moisture measurements

Regular measurements of volumetric soil water content (θ) were made for all 36 replicates during the 2006/7 and 2007/8 growing seasons using a TRASE System 1 TDR (ICT International, Armidale, NSW, Australia) with 30 cm waveguides, inserted vertically, giving an integrated measure of soil moisture in the top 30 cm of the soil profile. These measurements were made directly under the drip line in the centre of every replicate. Occasional measurements were made in the same way during the 2008/9 and 2009/10 seasons using 30 cm and 60 cm waveguides.

Hourly readings of soil moisture throughout the growing season were made from 2007/8 to 2009/10 using eight 30 cm long CS615 soil moisture sensors attached to a CR10X data logger (all Campbell Scientific, Cairns, QLD), with two sensors in a PD replicate, two sensors in one RDI replicate, a single sensor in a second RDI replicate, two sensors in a control replicate and a single sensor in a second control replicate. These data were intended to compliment the TDR data by providing continual monitoring of soil moisture, albeit with less statistical power due to the small number of sensors.

Occasional neutron probe measurements were made down to 1 m at 10-20 cm intervals using a CPN 503DR Hydroprobe (ICT International, Armidale, NSW, Australia). There were two neutron probe access tubes per treatment, in separate replicates. For the 2006-2007 season the tubes were positioned approximately 10-15 cm from the drip line, but prior to the start of the 2007-2008 season they were moved to directly under the drip lines. These data provided information on soil moisture to a greater depth, albeit with less regularity.

Finally, the water table was monitored during the 2007/8 season onwards with two 3 m deep test wells in centrally positioned RDI replicates, one in the upper half of the experimental area, one in the lower, installed directly under the vine row and midway between two vines.

Where required, soil matric potential (Ψ_{sm}) as kPa was estimated from 0-30 cm TDR measurements using:

$$\Psi_{sm} = [(Te^{-26.6}-1)^{0.2052}]/3.76$$

and 0-60 cm TDR measurements using:

$$\Psi_{sm} = [(Te^{-28.43}-1)^{0.1959}]/2.675$$

where $Te = (\theta-0.02)/0.383$ and assuming a clay content of 12% in each case (Lanyon, pers. comm.).

Chapter 3

Development of a rapid and simple assay to quantify non-structural carbohydrates of grapevine tissues*

Introduction

Determination of total non-structural carbohydrates (TNC) was an important part of this project. There are many published methods for quantification of carbohydrates, ranging from various colourimetric analyses, to HPLC and GCMS. Although more advanced methods such as GCMS allow great detail in carbohydrate composition to be determined, they were generally too expensive and time consuming to be used for quantification of TNC in large numbers of samples. For our purposes the concentrations of individual sugars were not required and a method giving an overall concentration of carbohydrates was adequate. One commonly used method is based on the anthrone reagent (Dreywood, 1946), which reacts with a wide range of soluble carbohydrates (Yemm & Willis 1954). We adapted this method for use in a microplate together with a simple method for the extraction of soluble carbohydrates from grapevine tissues. The same sample was then used for starch determination. The combined method was validated using tissue samples 'spiked' with appropriate carbohydates in a range of tissue types. It offers a fast, high-throughput technique for determining TNC and was used for all carbohydrate concentrations presented in this report.

Materials and Methods

Grapevine tissue from leaf, cane, cordon, trunk and root was collected from the Cabernet Sauvignon vines described in Chapter 2 and freeze-dried before being ground to a fine powder. Approximately 5 mg of ground material was weighed into a microcentrifuge tube, with 0.5 mL of 80% aqueous ethanol or deionised water then added. The extract was thoroughly mixed with a vortex mixer and heated to 60°C for 5 minutes in a dry block heater. After heating the extract was centrifuged at 12,000 g in a microfuge and the supernatant removed. The extraction was repeated twice more with the supernatants being combined in a second microcentrifuge tube. The pellet was dried in a heat block to remove any excess supernatant and retained for starch analysis, whereas the combined supernatant was dried using a vacuum centrifuge and used for soluble carbohydrate analysis.

Soluble carbohydrate analysis: The dried supernatant was resuspended in 0.5-1.0 mL of deionised water. A 50 µL aliquot was transferred to a new microcentrifuge tube, put on ice and 1 mL of anthrone reagent added. The mixture was vortexed then left on ice for 10 minutes, before being heated to 85°C for 10 minutes using a dry block heater. The anthrone mix was then vortexed thoroughly to ensure a uniform color and 300 µL was transferred to a microplate well. Absorbance was read at 600 nm on a microplate reader. A fresh fructose standard curve was prepared each time the anthrone procedure was performed in the same manner as the extracts, typically using 0.016 to 0.5 mg mL^{-1} of fructose in deionised water.

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* This chapter is an extended summary of a manuscript accepted for publication in the *American Journal of Enology and Viticulture*. Permission for a summary was provided and the complete published paper is available at: http://www.ajevonline.org/cgi/content/abstract/ajev.2010.10051v1

Insoluble starch analysis: The method was adapted from Association of Analytical Communities International method 996.11 (AOAC 2007). The pellet was resuspended using 150 µL of thermostable α-amylase, thoroughly mixed using a vortex mixer and then heated to 90°C in a dry-block heater for 6 minutes, with mixing every 2 minutes. The starch extract was cooled to 50°C and 200 µL of sodium acetate buffer, followed by 5 µL of amyloglucosidase. The extract was then vortexed and heated at 50°C for 30 minutes. After cooling to room temperature the extract was used directly for analysis, either using the microplate anthrone method described above or "GOPOD" reagent (Megazyme International, Bray, Ireland).

Validation of analysis: 1) The linearity and degree of response of the anthrone reagent for fructose, glucose and sucrose standards was determined, 2) the effectiveness of aqueous ethanol or water as an extraction medium was examined, 3) the presence of resistant starch in trunk wood was tested for, and 4) the "GOPOD" and anthrone methods were compared for use in starch determination.

Results and Discussion

Due to the differing degrees of anthrone colour reaction, choice of standard will affect the estimate of total soluble carbohydrate. Comparison of serial dilutions of glucose, fructose and sucrose in our study demonstrated good linearity of response for all three, but a significant difference in the slope of each. Fructose was used in order not to overestimate TNC.

Aqueous ethanol is commonly used as an extraction medium (e.g. Reed *et al.* 2004). However, when extracts were spiked with fructose the recovery was variable and poor. Ethanol extracts cannot be measured directly using anthrone as alcohols interfere with the colour development (Clegg 1956). Consequently, extraction with water was examined as an alternative. When the reliability of using water as an extraction solvent was tested, the mean recovery was 91.4%, with no difference between the tissue types. Furthermore, recovery was determined on four separate occasions for trunk samples, with no significant difference between those tests. No 'resistant' starch, that is tightly packed amylose chains not easily accessible to enzymatic hydrolysis, was found in grapevine trunk material.

The possibility of using the anthrone method to measure the glucose produced during the insoluble carbohydrate assay offered a means of measuring TNC as one entity, without splitting it into soluble and insoluble fractions. When the GOPOD-based method and the anthrone method were carried out on the same extracts the results were well correlated (R^2=0.966, n=34), but the anthrone method overestimated the glucose by 24.7%. Consequently, whilst it is possible to use anthrone for the insoluble carbohydrate part of the assay, the accuracy is seriously impaired in comparison to the GOPOD-based method.

In conclusion, we have successfully adapted both the anthrone soluble carbohydrate assay and a GOPOD based commercially available starch assay kit to low volume extractions that can be read in a microplate reader, significantly reducing the cost and time involved in both assays as well as allowing small samples to be analyzed. We have tested these assays on a range of grapevine tissues and recommend that, when using the anthrone method, water rather than aqueous ethanol is used to extract soluble carbohydrates since the necessary ethanol removal step led to an underestimate of total carbohydrates in grapevine wood tissues.

Chapter 4

The effects of long-term use of regulated deficit irrigation strategies on Cabernet Sauvignon growth, yield and water use

Introduction

The potential long-term impacts of utilizing regulated deficit irrigation (RDI) strategies on vine vigour and capacity, discussed in the opening chapter, were investigated over three growing seasons using the existing Cabernet Sauvignon irrigation trial at the Wingara vineyard described in Chapter 2. The trial took two approaches, firstly it compared established vines that have been irrigated using RDI for over ten years with vines that were irrigated in excess of ET_{crop} (control) and secondly it compared these treatments with vines that had been irrigated with an extended RDI strategy (PD: prolonged deficit). The period described here covered the fourth to sixth seasons of the PD treatment and first to third seasons of the control treatment.

Materials and Methods

Experimental set-up and data collection

The long-term impacts of deficit irrigation were assessed during three seasons, 2005/6 to 2007/8 with RDI, PD and control irrigations treatments implemented as described in Chapter 2. Environmental and soil moisture measurements were also made as described in Chapter 2.

Canopy growth

Canopy growth was estimated as leaf area index (LAI - the ratio of leaf area to the ground area covered by the canopy), using a LiCor Li-2000 Plant Canopy Analyser (John Morris Scientific, Willoughby, NSW, Australia). It was not possible to make measurements as a transect across rows due to measurements within a replicate being restricted to a single row, therefore it was not possible to measure true LAI of the vineyard. However, by restricting the 'view' of the instrument, estimates were made of individual rows. Two measurements of each replicate were made, one with a 15° view and one with a 90° view. The 15° measurement represented the central area of the canopy, that is, the LAI of the thickest part of the canopy, but was more strongly influenced by the light interception due to the canes and wood of the cordon. The view of the second measurement encompassed the entire canopy, thus providing an LAI value for the whole vine, but potentially including the edge of the guard row canopy. In both cases the view along the row encompassed approximately two entire vines. Each measurement consisted of two reference readings made above the canopy and three measurement readings made from midway between the soil surface and the lower cordon. The raw reading was then adjusted in the FV2000 software, provided for use with the instrument, to account for the canopy height and the distance between the instrument and the canopy. LAI was not measured in the 2005/6 season, but readings were taken at two to eight week intervals whilst the vines were in leaf for each subsequent season, with more frequent readings taken during the canopy expansion phase of the 2007/8 season.

In each season the pruning weight of a single vine in each replicate was measured after

leaf fall was complete. Vines were pruned from the mid-point along the cordon on one side of the chosen vine to the mid-point along the cordon on the opposite side (2.44 m). The fresh weight of the collected prunings was recorded. The site as a whole was mechanically pruned, typically to a width of 0.4 m, and the hand pruning attempted to replicate this.

Trunk growth

During the 2007/8 season Verdtech 'Plantsens' dendrometers (Verdtech, Huelva, Spain) were used to record changes in trunk diameter. A total of six dendrometers were used, two for each treatment, but cable length limitations meant that both dendrometers for a given treatment had to be placed on vines within the same replicate, with the replicate for each treatment being in a separate block. The output from the sensors was logged at 15 minute intervals using a Unidata Prologger (MEA, Magill, SA, Australia).

Root biomass and soil properties

In August 2007, post-pruning, but prior to budburst, root biomass was estimated by taking a nine 0.75 m deep cores from two replicates for each treatment. Cores were taken in a grid at intersections 0, 50 & 120 cm from the vine trunk in the x axis (along the row) and 20, 70 & 150 cm from the row in the y axis, thus covering half the distance from the vine to it's neighbour in each direction. Cores were split into three 25 cm lengths, with roots being extracted from each by mixing the sample with water, removing the visible roots then sieving the water to extract any remaining roots. Extracted roots were then split into live and dead fractions based on colour and pliability. Each set of extracted roots was oven dried at 60°C and then weighed. Root biomass was estimated on an area basis by assigning a soil surface area to each core extending from the midpoint between the given core and an adjacent core, the midrow or the cordon. The root biomass in each core was then multiplied by the assigned soil surface area / the area of the core cross section.

A second set of 1 m deep cores, one from each of the 36 replicates, were taken to examine soil properties. Each core was taken 20 cm from the trunk towards the mid-row and split into four 25 cm lengths. Each core section was thoroughly mixed and a 50 g sub-sample taken which was air dried. Soil conductivity and pH were measured in 5:1 slurries made from each sample, using an Activon Model 301 Conductivity Meter (and a Beckman Φ50 pH meter respectively (both Activon Scientific Products, Thornleigh, NSW, Australia).

'Merbein bunch count'

At the beginning of the 2007/8 season a Merbein bunch count (Antcliff *et al.* 1972) was performed to estimate the bud number, shoot number flower number and proportion of budburst. A 20 cm wide transect was used through a single vine for each of the 36 replicates, with the transect being at one of 12 equally spaced positions along the cordon, six on either side of the trunk, and each position being used once per treatment, but assigned to a random replicate.

Harvest yield

Yield was estimated using a single vine from each replicate shortly before the block was harvested mechanically by the grower. Harvest dates for the three seasons were

6/3/2006, 1/3/2007 and 3/3/2008. In 2006 a randomly positioned 1 m transect was used, whereas in 2007 and 2008 whole vines were harvested (with a 'vine' defined in the same way as for pruning weights). In each case bunches were harvested by hand and the total fresh mass for a vine recorded, without removing the berries from the rachis. In years 2006 and 2008 harvested bunch number per vine was also recorded.

Vine water use

Whole vine water use was estimated from budburst to harvest using 12 Greenspan Sapflow loggers, each with two SF100 sensors attached (Greenspan Technology, Warwick, Queensland, Australia) in the 2006/7 and 2007/8 seasons. Four loggers were assigned to each treatment, with two blocks having a logger in every replicate and the other six loggers being spread out across the experimental block. In the 2006/7 season, both sensors with each logger were inserted in the same vine, approximately 1/3rd and 2/3rds of the distance between the cordon and soil surface, and in 2007/8 two sensors were inserted in adjacent vines at approximately the mid-point between the cordon and the soil surface. The Greenspan sensors use a heat pulse velocity system (HPV) and the data recorded was the 't2' time, that is the time after a heat pulse that the upstream and downstream temperatures match. Each sensor recorded a 't2' datum for each of two depths in the trunk at 15 minute intervals.

Errors were found when using the supplied software so the 't2' data was converted to a heat pulse velocity and then scaled to a whole vine flux using a spreadsheet. The effect of the wound width was calculated using Table 1 in Swanson & Whitfield (1981). The wound width itself was measured by taking trunk cores encompassing the sensor insertion hole from 12 vines at the end of the 2006/7 season, sectioning the cores with a razor blade and measuring the diameter of the wound under a dissecting microscope as the distance between conducting (clean, white) xylem vessels either side of the hole. An average value was used for all calculations. The heat pulse velocity was then adjusted for the wood fraction using equation 7 from Edwards & Warrick (1984), with the wood fraction being calculated from a further 12 trunk cores.

One hour running means were calculated for the resulting data and each was then weighted for the area of the trunk cross-section it represented according to equation 3 in Hatton *et al.* (1990). Data below the minimum resolvable in the field by the logger was then removed. This was determined for a given data set as the average night-time reading for the night with the lowest sapflow result plus two standard deviations of that average (typically n was between 16 & 32). Finally, data were summed on a 24 hour timestep to give daily sap flux as L per vine.

Statistical analysis

The additional measurements of LAI during the canopy growth period of the 2007-2008 season allowed aspects of that growth to be examined statistically. For each replicate the first 10 measurements (from 21/9/07 to 29/1/08) were used to fit a logistic growth curve of the form:

$$LAI = a + c / (1 + EXP(-b * (X-m)))$$

where a was the initial measurement, c was the extent of growth (the difference between

the initial LAI measurement and that once growth was complete), X was the date of the given LAI measurement, *m* was the date where half of total growth had been reached (the midpoint), and *b* was the shape parameter. In addition, the maximum rate of growth was estimated for each curve using:

$$LAI_{mgr} = (b * c) / 4$$

where LAI_{mgr} is the maximum rate of growth and *b* and *c* are as equation 1. The entire procedure was used for both the LAI values obtained using the 90° view and those using the 15° view. The values of the midpoint, the extent of growth and LAI_{mgr} were then analysed using ANOVA to test for treatment effects.

The sapflow results from the 2007/8 season were modelled using a multi-variate regression fitted using an artificial neural network with JNNS software (University of Tuebingen, Tuebingen, Germany) with ET_o, LAI and soil moisture as input variables. ET_o for the site was taken from the BoM weather data, LAI was calculated on a daily basis for each replicate using the growth curves described above and average daily soil moisture was estimated for each treatment using the results from the CS615 data (see chapter 2).

Differences in slopes were determined using ANCOVA, with a significant interaction between factor and co-variate being taken as a significant difference in slope. Other data were analysed as appropriate using ANOVA or Student's t-tests. All statistical analyses, including the logistic curve fits, were completed using Genstat v10 (VSNi, Helensburgh, NSW, Australia) and a probability below 0.05 was taken as significant unless otherwise stated.

Results

Soil Moisture & Properties

The water table ranged from 1.23 m to 1.55 m, both the maximum and minimum occurring in the summer months. The mean depth was 1.39 m with a standard deviation of 0.11 m. Soil moisture was measured in two of the three seasons and was directly related to irrigation treatment, with the double irrigation control treatment leading to a higher volumetric soil moisture content throughout the season and the PD treatment reducing soil moisture significantly only during the deficit period itself (Table 1). Estimated Ψ_{sm} indicated that, although there were differences between the RDI and control treatments, the intra-seasonal differences were much greater (Table 1). Post-PD soil moisture measurements were not made in the 2006-2007 season, but in the 2007-2008 season TDR measurements indicated that soil moisture in the PD treatment had recovered to 86% of that in RDI within three days of re-watering and neutron probe measurements indicated a 96% recovery after six days. There was no significant difference between TDR measurements in PD and RDI plots during the rest of the season. During the PD period the Ψ_{sm} of the PD treatment was over 10 MPa and consequently it was unlikely that any soil water was available to those vines in the top 0.3 m in which measurements were made. The neutron probe measurements indicated that the PD treatment reduced soil moisture to a depth of 0.8 m, but that this effect decreased with increasing depth (data not shown).

Table 1. Volumetric soil moisture and estimated soil matric potential (Ψ_{sm}) for the top 30 cm of soil under the dripline of control, RDI and PD irrigation treatments during the 2006/7 and 2008/9 growing seasons. Each value is a mean of 12 replicates ± standard error. Superscript letters indicate significant differences using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10.

Season	Irrigation Treatment	Volumetric Soil Moisture (m^3 m^{-3})						Soil Matric Potential (kPa)*		
		Pre-PD		PD Period		Post-PD		Pre-PD	PD Period	Post-PD
		Mean	SE	Mean	SE	Mean	SE			
2006/7										
	Control	0.162[a]	0.008	0.221[a]	0.007		†	-59	-9	†
	RDI	0.135[b]	0.007	0.191[b]	0.006			-189	-22	
	PD	0.133[b]	0.005	0.073[c]	0.006			-209	-12,864	
2007/8										
	Control	0.162[a]	0.007	0.202[a]	0.007	0.235[a]	0.009	-59	-15	-6
	RDI	0.140[b]	0.007	0.182[b]	0.006	0.211[b]	0.008	-151	-29	-12
	PD	0.136[b]	0.006	0.074[c]	0.004	0.197[b]	0.007	-184	-11,188	-18

* See Chapter 1 for conversion from volumetric soil moisture.

† TDR measurements not taken post-PD in 2007.

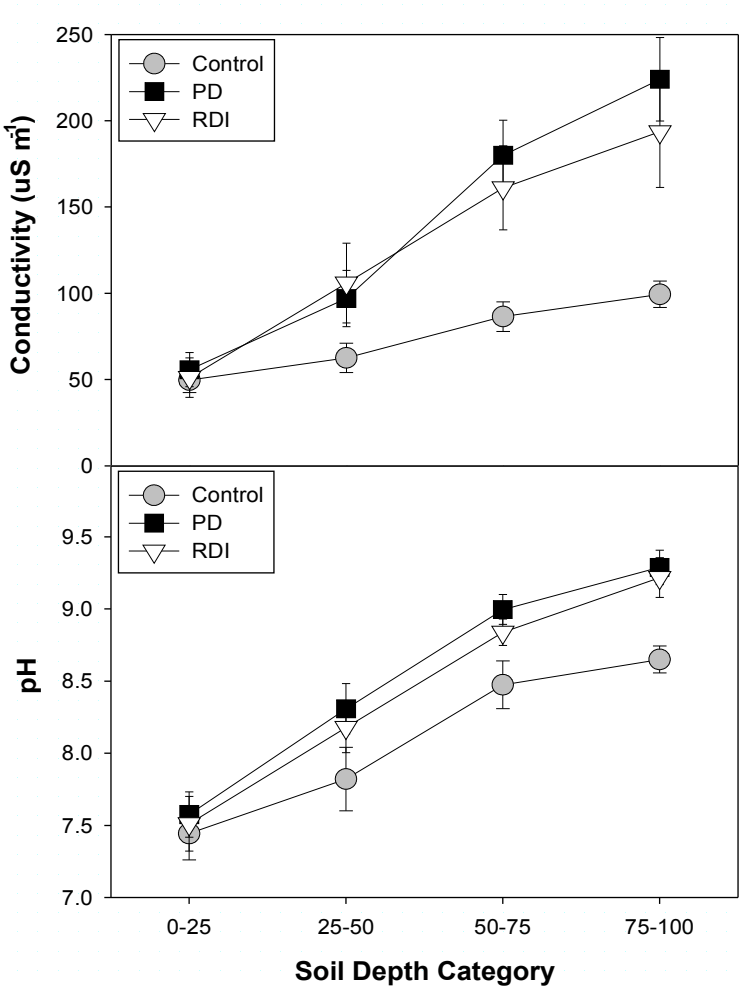


Figure 1. Conductivity (a) and pH (b) of 1:5 suspensions of soil samples taken from cores made adjacent to the dripline and split into four sections, representing four depths, in August 2007. Points are means of 12 replicates ± standard error.

The results from the soil samples taken prior to the 2007-2008 season suggested the irrigation treatments had a significant effect on soil properties (Figure 1a). In particular, conductivity of the double irrigation control samples was significantly lower than the other treatments at all but the shallowest of the depths; and was less than half the PD treatment at 0.75-1 m. Soil pH was also lower in control samples except at 0-0.25 m (Figure 1b), though the difference was not as great as with conductivity. Conductivity of soil from the PD treatment was slightly higher than RDI below 0.5 m. Both pH and conductivity increased with depth in all soils.

Canopy growth and size

Canopy size was estimated using LAI in two of the three seasons, 2006-2007 and 2007-2008, and in neither season was there any evidence that significant leaf loss occurred during the PD treatment period (Figure 2). However, there were clear effects of the irrigation treatments on the rate and extent of canopy growth. The 2006-2007 season whole vine (90° view) LAI data were analysed using repeated measures ANOVA in its entirety as well being split into three sections: canopy growth period, full canopy cover and post-harvest senescence. In all analyses the irrigation treatments had a significant effect,

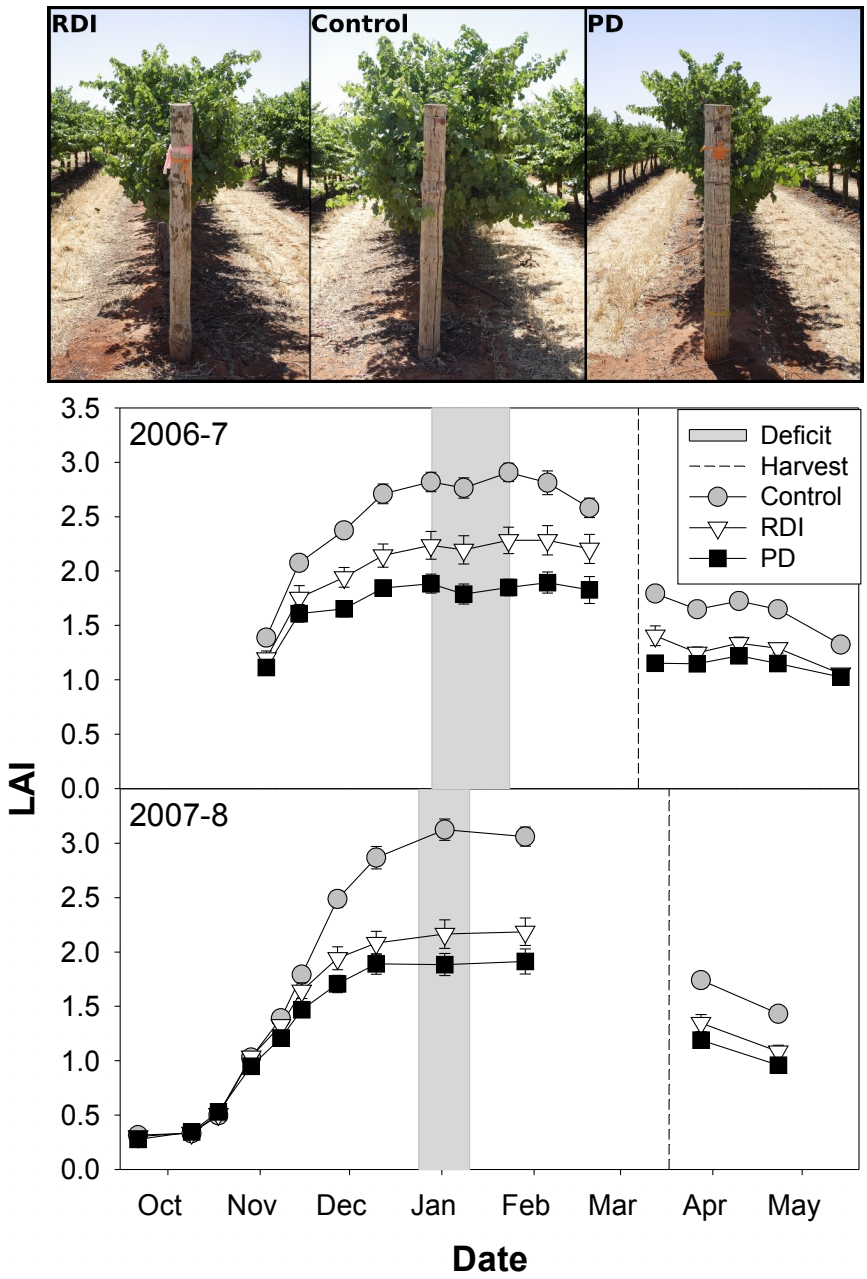


Figure 2. Canopy growth, a) photographs of three typical rows taken at flowering 2006, LAI during b) the 2006/7 and c) the 2007/8 growing seasons, estimated with a 90º view of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. The grey area denotes the duration of the PD deficit period in each season. Each point is a mean of 12 replicates ± standard error.

with both the RDI and PD treatments resulting in a smaller canopy than the well-watered control (Figure 2b). Furthermore, the canopy area of the PD treated vines was significantly smaller than that of the RDI vines through most of the season in both seasons. During the canopy growth period the interaction between time and treatment was also significant, indicating that the growth rate of the vines under the different treatments was different.

The greater number of measurement times during the canopy growth period of the 2007-2008 season (Figure 2c) allowed a more detailed analysis, with fitted curves being used to

estimate the maximum growth rate for each replicate along with the length of the growth period (Table 2). Growth rate, growth period and LAI at the end of canopy growth were all significantly affected by the irrigation treatments, with the control vines increasing LAI at a faster rate and for a longer period than the other treatments, whereas the PD vines had a slower growth rate than the RDI vines, but grew for a similar length of time.

Table 2. Calculated parameters from the 2007/8 season canopy growth period measured as whole vine LAI. The growth rate represents the maximum rate of change of the growth curve. The growth period mid-point is the time days from 21/9/07 to achieve 50% of total LAI, and the range is the difference between the peak LAI and the initial LAI value. Values are means of 12 replicates.

	Parameter	**Treatment**			**F probability**
		Control	**PD**	**RDI**	
90º View	Midpoint	43.2	33.5	35.4	<.001
	LAI Range	2.89	1.69	2.03	<.001
	Growth Rate	0.062	0.038	0.044	<.001
15º View	Midpoint	28.4	25.2	27.8	0.022
	LAI Range	2.85	2.48	2.51	0.001
	Growth Rate	0.093	0.080	0.09	0.181

The centre of vine LAI (15° view) responded in a similar way to the whole vine LAI in both seasons, but reached a higher peak LAI (approximately 3.7 in control, 3.4 in RDI and 3.3 in PD), and reached that peak sooner with smaller differences evident between the treatments. In 2006-2007 the interaction between time and treatment was not significant and in 2007-2008 there was no significant effect on the growth rate. However in neither season was there a difference in the starting LAI (i.e. the estimate of permanent wood), and in both seasons there was a significant treatment effect on the centre of vine LAI overall.

The differences in LAI between the RDI and the PD vines were seen from early after budburst. For instance, in 2006 budburst occurred in the last week of September, and the first measurement of whole vine LAI on 3/11/06 showed a difference between RDI and PD vines. Similarly, in 2007 budburst occurred in the first week of October and again, by the first week of November, LAI of PD vines was less than that of RDI vines. In both seasons canopy growth was complete prior to the onset of the PD deficit treatment itself.

Pruning weights were estimated at the end of each season and there was a significant effect of irrigation treatment, both across all three seasons together and within each of the three seasons (Figure 3). Comparison of means comparisons utilizing the Bonferonni adjustment, demonstrated a significant increase in pruning weight of the control vines in all three years, but not PD. However, the pruning weights of the PD treatment were lower than RDI in all three seasons and repeated measures analysis across all years did demonstrate that the PD treatment resulted in a marginally significant reduction in pruning weight ($p=0.067$).

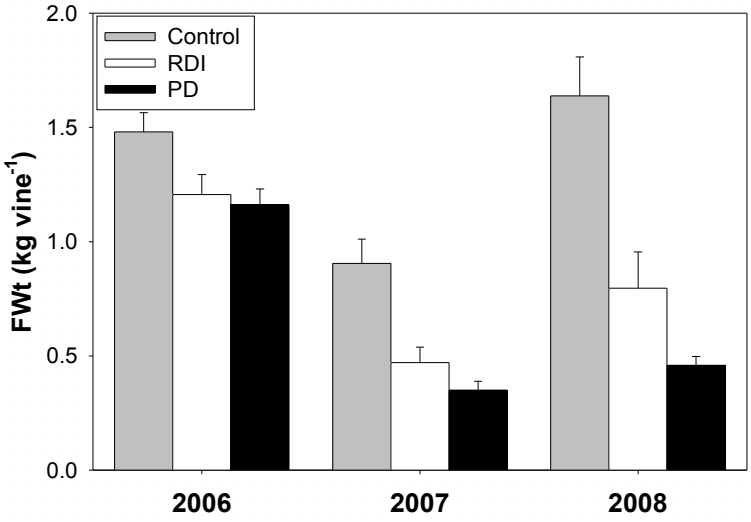


Figure 3. Pruning weights of Cabernet Sauvigon vines receiving control, RDI or PD irrigation after leaf fall in the 2005/6, 2006/7 and 2007/8 growing seasons. Each bar is a mean of 12 replicates + standard error.

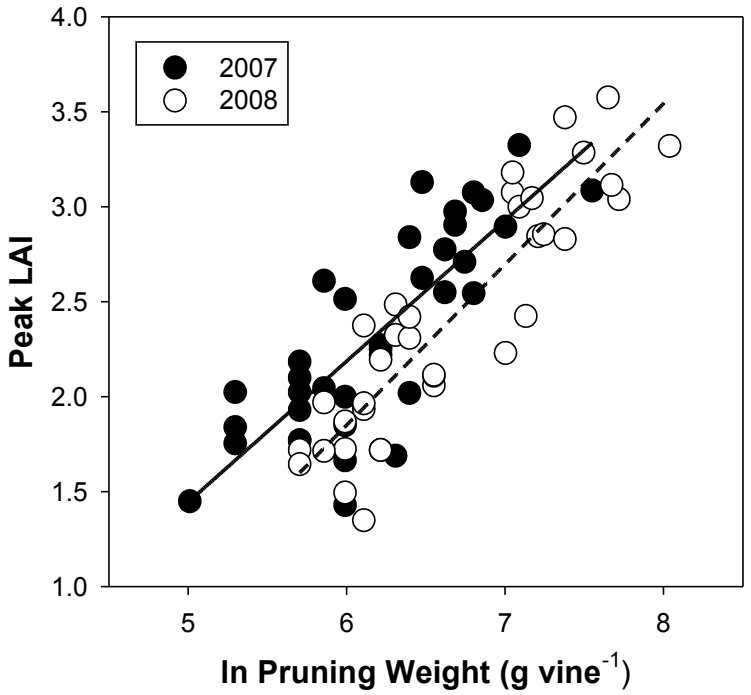


Figure 4. Regressions of pruning weights from 2007 and 2008 against maximum LAI for the preceding season. The natural log of the pruning weight was used to linearise the data. For each year *n*=36.

Matching LAI and pruning weight data were available for each replicate in two of the three seasons allowing a possible relationship between the two to be examined. A mean 'peak' whole vine LAI value was calculated for each replicate (measurements from 28/12/06 &23/1/07 were used for the 2006-2007 season and from 2/1/08 & 29/1/08 for the 2007-2008 season) and then plotted against the pruning weight for the given season (Figure 4). ANCOVA analysis indicated that there was no significant difference between the slopes of the regressions for the two seasons and the regression across the whole data set gave LAI = 0.721 * ln(Pruning weight in g) -2.266, with an adjusted R^2 of 0.68.

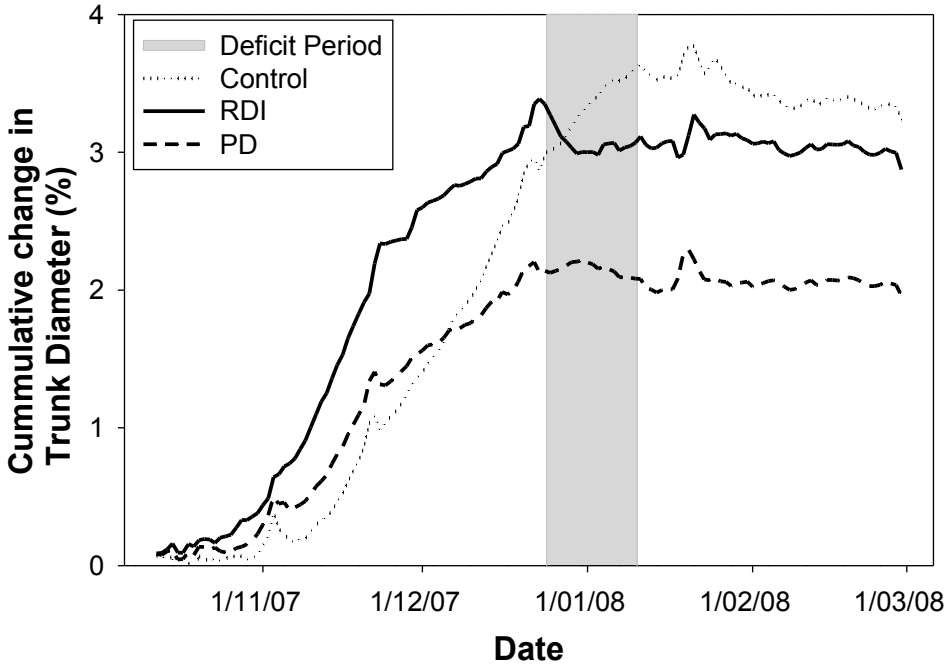


Figure 5. Cumulative change in daily minimum trunk diameter of Cabernet Sauvignon vines irrigated with control, RDI or PD strategies. The grey area denotes the duration of the PD deficit period. Each line is a mean of two replicates.

Vine trunk growth

The data from the trunk dendrometers exhibited a strong diurnal variation, with peak measurements also being affected by strong winds causing flexing of the trunk. The presented data is calculated using the daily minimum trunk diameter. Trunk expansion began soon after budburst, but appeared to be slightly delayed in the control vines (Figure 5). The rate of expansion was somewhat less in PD vines than in RDI, whereas the rate of expansion in control vines was similar to that of RDI. In both PD and RDI vines, expansion stopped before veraison and the trunk diameter was then maintained for the rest of the season. In control vines, trunk expansion continued until early veraison before also stopping. In this respect, trunk expansion closely matched the changes in LAI, although the point marking the end of growth was slightly later in the trunk than the canopy. There was also no further change in trunk diameter from harvest to leaf fall (data not shown).

Root biomass

The extracted roots could be split into a number of size classes visually and it was noticeable that almost all the extracted roots in the smallest classes, those < 0.3 mm in diameter, appeared to be dead. The fraction of total root mass to 0.75 m represented by the dead fine roots ranged from 0.11 in control, to 0.18 in RDI and 0.21 in PD. The total root dry mass was highest in control and lowest in PD (Figure 6a), with the treatment differences occurring in the root biomass at <0.5 m depth. Furthermore, treatment differences were primarily in the cores close to the drip line, with fewer roots in the midrow and no discernible effect of irrigation treatment (Figure 6b).

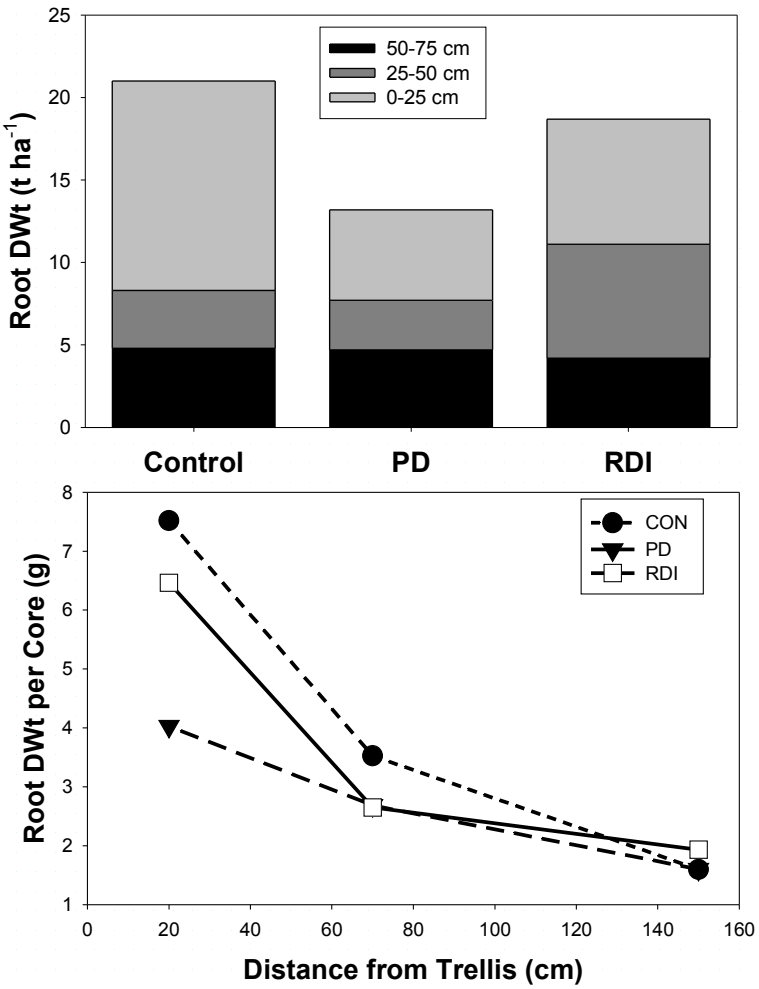


Figure 6. Root dry mass to 75 cm soil depth a) per unit area estimated from a grid of nine soil cores and b) change in mass with distance from trellis of Cabernet Sauvigon vines irrigated with control, RDI or PD strategies. In each case *n*=2, but both replicates are a mean of three cores taken along the row for b).

Table 3. Results of Merbein bunch count of Cabernet Sauvignon vines irrigated with control, RDI or PD strategies made shortly after budburst in 2007.

Irrigation Treatment	Bud No.		No. Burst Buds		Shoot No.		Bunch No.		% Budburst	% Fruitful Shoots
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Mean
Control	506	63	333	36	493	37	321	46	68	61
RDI	476	50	326	35	476	42	323	45	69	64
PD	543	68	365	35	533	48	376	47	70	67
Overall Mean	509		342		501		340		69	64
p	ns		ns		ns		ns		ns	ns

Merbein bunch count

There was no significant effect of the irrigation treatments on any of the parameters calculated from the Merbein bunch count (Table 3), including bud number per vine, number of buds burst per vine, number of shoots per vine, number of inflorescences per vine, the fraction of buds burst and the fraction of shoots with bunches. Overall the average bud number per vine was 509, with 69% burst and 64% of shoots being fruitful.

Harvest Yield

Across the three seasons the irrigation treatments had a significant effect on harvest yield, with PD treated vines having the lowest yield and no significant difference between seasons (Table 4). The interaction between season and treatment was marginally significant (p=0.051), but due entirely to the lack of any effect of the control treatment in its first year. Similarly, there was a significant effect of treatment and season on yield per unit of pruning weight. With a significant interaction due to the control treatment having an effect on pruning weight but not yield in 2006. In both 2007 and 2008, the control vines produced less fruit mass per unit of pruning weight than the two deficit treatments (Table 4). However, similar effects were not seen on the yield produced per unit area of canopy. Neither bunch number nor bunch weight (data not shown) at harvest were significantly affected by irrigation treatment in either of the years they were measured.

Table 4. Harvest yield, bunch number, yield / pruning weight ratio and yield / canopy area ratio of Cabernet Sauvignon vines irrigated with control, RDI or PD strategies. Each value is a mean of 12 replicates ± standard error. Superscript letters indicate significant differences using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10.

Year	Irrigation Treatment	Yield (t ha^{-1})		Bunch No. (vine^{-1})		Yield / Pruning Weight	Yield / Canopy Area (kg m^{-2})
		Mean	SE	Mean	SE	Mean	Mean
2006							
	Control	31.5^{a}	1.9	292	14	16.4	*
	RDI	31.9^{a}	1.5	271	16	20.6	
	PD	27.0^{b}	1.0	307	12	18.1	
2007							
	Control	34.8^{a}	1.3	*		31.6^{a}	1.24
	RDI	27.2^{b}	1.9			48.8^{ab}	1.21
	PD	23.0^{b}	1.5			58.5^{b}	1.27
2008							
	Control	31.6^{a}	1.8	351	22	15.4^{a}	1.03
	RDI	27.5^{ab}	1.4	341	20	41.3^{b}	1.19
	PD	24.5^{b}	1.6	318	27	35.0^{b}	1.33

* Data not collected in this season

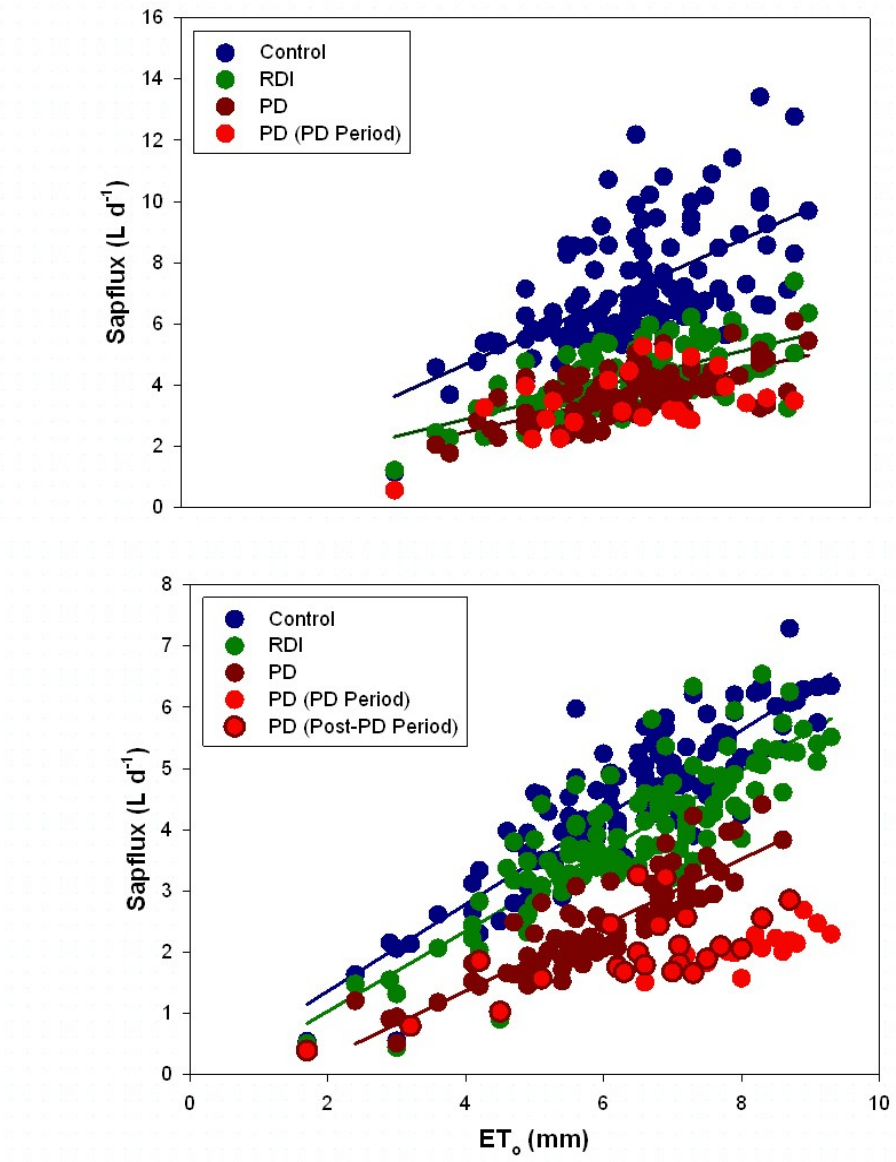


Figure 7. Daily sap flux of Cabernet Sauvignon vines irrigated with control, RDI or PD strategies during a) the 2006/7 and b) the 2007/8 growing seasons against ET_o from Bureau of Meteorology data. Each point is a mean of between two and four replicates, typically four.

Vine water use

Measured as sapflow, vine water use rose from zero at budburst to between 4 and 8 l day^{-1} vine^{-1} prior to veraison, with control vines having the highest and PD vines the lowest water use. Sapflow then reduced somewhat from veraison to harvest. Consequently, seasonal water use was greatest in the control treatment and lowest in the PD treatment (Table 5). Variation from day to day was very high, but strongly correlated with ET_o (Figure 7), which accounted for 38-46% of the variation in 2006/7 and 58-75% of the variation in 2007/8 (Table 4). The regression slopes represent the amount of water transpired by a vine in a day per mm of evaporation. For the 2007/8 season the slopes were significantly different between treatments and the slope of the PD period data was significantly different to that

of data collected prior to the PD period.

Table 5. Seasonal sapflow from budburst to harvest, slope of daily sapflow: daily ET_o regression for the period from an LAI of one to harvest, water use efficiency of yield (WUE_y = yield / irrigation applied) and crop water use index (CWUI = yield / water used). Regression results for the PD deficit period are also given for the PD treatment.

Season	Irrigation Treatment	Sapflow to Harvest	Sapflow : ET_o Regression Parameters				Water Use Efficiency	
		(Ml ha^{-1})	Season (LAI > 1)		PD Period Only		WUE_y	CWUI
			Slope (l mm^{-1} d^{-1})	R^2	Slope (l mm^{-1} d^{-1})	R^2	(t ML^{-1})	(t ML^{-1})
2006/7								
	Control	1.37	1.02	0.38			3.8	25.4
	RDI	0.76	0.56	0.45			6.0	35.9
	PD	0.64	0.50	0.46	0.39	0.26	6.2	34.3
2007/8								
	Control	0.81	0.71	0.75			3.3	38.8
	RDI	0.72	0.65	0.74			5.7	38.2
	PD	0.45	0.43	0.58	0.15	0.31	6.3	54.0

A major factor that is likely to affect these slopes, and the water use of the vines, is canopy size. Including canopy size into a multivariate-regression model using the 2007/8 season data together with soil moisture and ET_o greatly increased the predictive power of the regressions and allowed a single regression model to be used with all the treatments (Figure 8). The R^2 of the regression model across the whole data set was 0.87, indicating that 87% of the variation in the sapflow data was explained using only ET_o, canopy size and soil moisture.

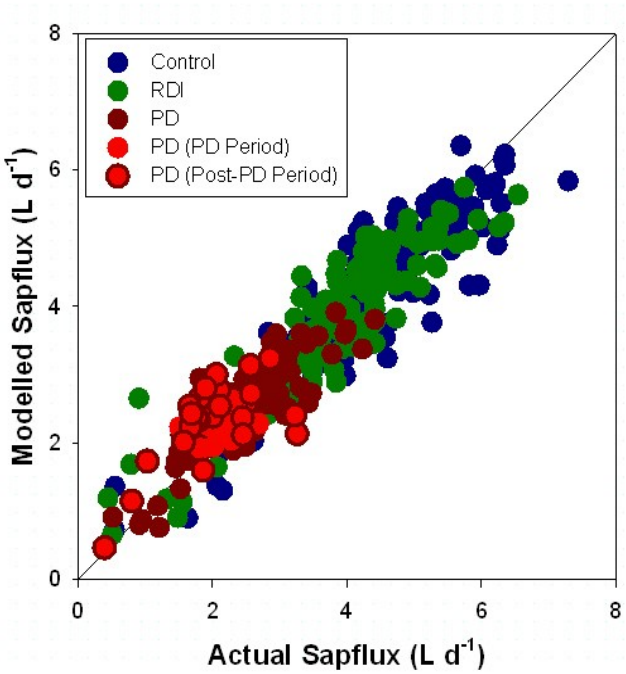


Figure 8. Daily sap flux modelled using a multi-variate regression with LAI, ET_o and soil moisture to 30 cm depth plotted against measured daily sap flux. The diagonal line represents 1:1.

Discussion

The intended effects of the irrigation treatments on available soil moisture were seen in both seasons where soil moisture was monitored, namely that the soil moisture content in the control treatment was unlikely to have been limiting to vine growth and that the PD treatment caused a reduction in soil water only during the PD deficit period. The estimated Ψ_{sm} of the top 30 cm of soil dropped to below -10 MPa during the PD deficit, well below the point at which the vine could potentially extract water considering the observed leaf water potentials (see Chapter 5). However, it is unlikely that Ψ_{sm} was as low at greater depth and as the water table was at a depth of less than 1.5 m it is likely that deep roots would have access to that water. Indeed during the soil coring roots were observed at a 1 m depth, albeit at a much lower density than in the top 50 cm.

Throughout both growing seasons volumetric soil moisture in the control treatment was greater than in the RDI treatment, which suggested that the RDI vines might be water-limited to some extent outside, as well as during, the RDI deficit period. This was the result of watering the control treatment in excess of ET_{crop} and consequently it was not possible to determine definitively whether differences between the control and RDI vines were entirely due to the RDI strategy or partially due to differences in total water applied. However, following the RDI period the estimated Ψ_{sm} of both treatments were high (> -30 kPa) so it is unlikely that the RDI vines were experiencing any water stress at that time or that the differences in soil moisture were having an effect on vine function. On the other hand it is possible that the soil moisture content under the RDI and PD vines prior to fruit set may have affected growth (Ψ_{sm} < -150 kPa cf. > 60 kPa for control). However, this value does not reflect the moisture content of the entire rooting zone. The deeper soil water measurements made 2008-10 (see Chapter 6) demonstrated that even early in the season Ψ_{sm} below 30 cm was also > -30 kPa in all treatments. Considering the differences in root distribution between the treatments (Figure 6) and assuming that the distribution of the overwintering roots was representative of the distribution in the following growing season, only about a quarter of the root system in the RDI and PD vines would have been experiencing a greater degree of water stress than the control vines prior to fruit set.

The soil samples taken in 2007 demonstrated that the irrigation treatments, two seasons of control and five seasons of PD, had altered soil chemical properties relative to RDI. Reduced conductivity and pH in the control treatment suggested that excess water had caused some leaching of salt. Greater conductivity below 50 cm depth in the PD treatment suggested a slight increase as a result of the reduced water application. However, even the highest conductivity readings at 1 m depth in the PD treatment were equivalent to a saturated soil paste value of only 2.2 dS m^{-1}, below the 2.3 dS m^{-1} threshold for detectable effects found for own-rooted grapevines in a similar soil by Zhang *et al.* (2002) and so did not suggest any level of salt stress likely to affect the interpretation of the experiment.

A primary objective of this phase of the project was to assess any long-term effect of deficit irrigation on vine growth, particularly canopy size. Canopy growth of all three treatments separated out in both seasons. Firstly, the difference between control and RDI vines indicated that the RDI strategy had reduced canopy size, a result in line with the expectations of growers implementing RDI and the published literature describing its effects (e.g. Mitchell & Chalmers, 1982; Goodwin & Jerrie, 1992; McCarthy, 1997). There are a number of mechanisms by which an RDI approach could potentially influence shoot

growth, ranging from direct effects of low water potential on growth (Keller *et al.* 2008), to changes regulated by hormonal signals (Davies *et al.* 2005, Sieburth & Lee 2010) to effects on vine carbon balance as a result of reduced stomatal conductance altering photosynthetic rates. These mechanisms all assume that the effect of RDI on growth occurs directly from the applied deficit period within a season, an idea that is supported by the literature and in this study by the reduced duration of the canopy growth period in the RDI and PD treatments, compared to control irrigation.

However, the detailed measurements of canopy growth in the 2007/8 season show that the canopy of the RDI vines was not only growing for a shorter period than the controls, but also at a slower rate and that this difference was present from budburst, suggesting a second effect of RDI on canopy growth that is due to the prior seasons of using RDI. It is also possible that this was because the RDI vines were somewhat water limited compared with the controls early in the season (see above).

The growth period of the PD vines was not significantly different to that of the RDI vines. The PD and RDI vines received identical irrigation within a season until canopy growth was complete, with the PD deficit period occurring after this. Furthermore, there was no reduction in canopy area, nor leaf loss during the PD deficit period, so any effect of the PD treatment on canopy growth could not be due to direct effects on canopy size within that season. However, the PD irrigated vines had both a smaller canopy and lower canopy growth rate than the RDI vines, so the additional effect of the PD deficit could only occur via an indirect mechanism, resulting from prior seasons of using the PD strategy. The reduced canopy growth rate of PD vines relative to RDI whilst having the same soil moisture also supports the suggestion that the early difference in canopy growth rate between control and RDI vines may be due to irrigation differences in previous seasons.

Pruning weight is commonly used as an index of vine growth and significant effects of the irrigation treatments on pruning weights were seen in all three seasons. Pruning weight was well correlated with peak LAI for a given season, so the 2006 pruning weights are likely to also be representative of peak LAI in the 2005/6 season where LAI was not measured. Interestingly, in 2006, the first season of double irrigation in the control treatment, the relative difference in pruning weight between control and RDI was much less than subsequent seasons, providing further evidence of the multi-seasonal nature of the control of canopy size.

Two potential mechanisms for this multi-seasonal effect of short-term deficit irrigation on canopy growth are a change in the extent/timing of budburst or a change in the carbohydrate reserves that provide the majority of carbon for growth until flowering (Zapata *et al.* 2004). Carbohydrate storage will be examined later (see Chapter 5), but Smith and Holzapfel (2009) observed that leaf removal at harvest reduced shoot expansion in the subsequent season and suggested that this was due to reduction in carbohydrate reserves. In contrast, it is clear from the Merbein bunch count that bud numbers were not driving the reduced canopy growth under either the RDI or PD deficit irrigation treatments. Timing of budburst was not accurately assessed, but regular visual observation did not indicate any difference between the treatments, with budburst occurring in late September in all treatments. Similarly, no effect of RDI on budburst timing was seen in Cabernet Franc (Matthews *et al.* 1987) or Shiraz vines (McCarthy 1997).

There was no evidence that productivity of the RDI vines was decreasing year-on-year

during the experiment. In fact, foliage area and, to a lesser extent, yield were remarkably stable across the five seasons of the project. A study of three RDI strategies in a temperate climate by Keller *et al.* (2008), whilst not having a well-watered control, also did not indicate any progressive drop in productivity over five seasons. The limitation of the control treatment in our study was in the uncertainty over how many seasons were required to remove the longer-term effects of the RDI strategy that had been in place previously so that a valid comparison could be made. Certainly, the control vines in the 2005/6 season were influenced by past irrigation, but comparing the yield, foliage area and relationship between those parameters for the 2006/7 and 2008/9 seasons did not indicate that significant changes in vine vigour and productivity were still occurring.

In general the relationship between foliage area and yield was constrained, with approximately 0.75-0.85 m^2 kg^{-1} of fruit in any given treatment or year, which is at the lower end of the 'ideal' range of 0.8-1.2 m^2 kg^{-1} suggested by Kliewer and Dokoozlian (2005). However, in 2006, where the irrigation was changed for the control vines this relationship was lost, with no yield difference between control and RDI treatments, but based on the LAI:pruning weight ratio in the subsequent seasons, the 2006 pruning weights indicated a significant difference in canopy size.

Although yield / pruning weight is often used as a surrogate of harvest index it doesn't take account of possible changes in carbon allocation to other parts of the vine. For example, a shift in carbon allocation from shoot to root as a result of deficit irrigation could potentially result in no change to yield / new biomass, but a significant change in yield / pruning weight. Prior to the start of the 2007/8 season root coring was used to estimate irrigation effects on overwintering root biomass and dendrometers were installed to examine effects on trunk growth over the following season. The two sets of results indicated that growth of the whole vine was indeed altered by the deficit irrigation strategies, with both trunk growth and root biomass reduced in the PD vines relative to RDI, but both parameters being greater in the control vines.

Understanding how much soil water is being used, and when, is the basis of irrigation management, but using short-term, regulated, deficit irrigation may have effects on yield and/or fruit quality that are not easily predicted from the changes in the total volume of water applied. Furthermore, altering irrigation volume may have effects on the proportion of soil water that is transpired, leached or lost through evaporation. Monitoring sap flux allows the water use efficiency of yield to be calculated as fruit mass per unit of water transpired (CWUI: crop water use index) as well as the more common fruit mass per unit of water applied. Table 4 provides both calculations for the 2006/7 and 2007/8 seasons. Sap flux measurements commonly underestimate actual transpiration (Steppe *et al.* 2010), and in this study conservative estimates were used for some of the scaling parameters, so comparing the actual values between the two calculations is difficult. However, it is clear that not withstanding this, the vines are not transpiring all of the applied water. More significantly, expressing the yield as CWUI suggests that in 2007/8 the reduced yield of the RDI vines when compared to the control was in direct proportion to the reduction in water use, whereas the PD vines had a higher CWUI and thus higher transpired water use efficiency. Simply examining the yield in terms of water applied leads to the opposite conclusion. In contrast, the 2006/7 CWUI results have the same pattern as the WUE_y, however, instrument problems reduced the reliability of the 2006/7 sapflow data (thus the much higher R^2 values in 2007/8), with the control data in particular likely to be over-estimated relative to the other treatments.

The difference between the CWUI and WUE_y calculations is due to the limited proportion of available soil water that is actually used by the vine. It was clear from the 2007/8 sap flux data that the primary driver of day to day variation in vine water use was the atmospheric conditions (i.e. ET_o), which were the same for all treatments, but that the irrigation treatments influenced this relationship. These treatment effects on daily and seasonal sap flux could be driven by a range of factors, such as differences in stomatal response to VPD, differences in available soil water or differences in canopy size. However, as the slope of the regressions between ET_o and sap flux, excluding the PD deficit period, were similar for all treatments, any differences in stomatal responses must have been minor (see also Chapter 5). The relationship between sap flux and canopy size across the entire data set (all three treatments) was nearly as strong as that with ET_o (R^2=0.29 vs R^2=0.35), whereas the relationship with soil moisture was negligible (R^2<0.03), so foliage area was probably the main factor in the different seasonal sap flux values of the vines.

On the other hand, as foliage area was not affected by the PD treatment during the deficit period itself, the reduction in water use by the PD vines during and immediately after the PD period in 2007/8 was almost certainly due to reduced stomatal conductance (see Chapter 5).

In conclusion, we found that using RDI over several seasons did not result in a progressive reduction in vine capacity and further that extending RDI using our PD irrigation strategy also did not result in decreasing yields or canopy size year-on-year. Secondly, although yields were stable, long-term use of RDI did reduce yield relative to a well-watered control and the PD treatment reduced yield still further.

Chapter 5

The effects of long-term use of regulated deficit irrigation strategies on Cabernet Sauvignon leaf physiology and carbohydrate reserves

Introduction

The multi-season effects of the RDI strategies, PD in particular, on vine growth described in Chapter 4 must have been driven by within-season effects of irrigation on vine physiology. Furthermore, although the primary driver of irrigation treatment differences in whole vine water use was canopy size, it is likely that stomatal conductance was also having an effect, at least during the PD deficit period (Chapter 4, Figure 7b).

Leaf level physiology was examined during the second and third seasons of the project, representing the fifth to sixth seasons of the PD treatment and the second to third seasons of the control treatment. In addition vine carbohydrate status was examined, with an emphasis on the third season, as changes in storage reserves represent a mechanism whereby short-term changes in leaf physiology can have an effect on vine growth in subsequent seasons.

Materials and Methods

Leaf water potential

Leaf water potential was measured using the balance pressure method with a Soil Moisture Equipment Corp. Model 3000 Plant Water Status Console (ICT International, Armidale, NSW, Australia) fitted with a digital peak hold meter (MEA, Magill, SA, Australia). Measurements were made on inner, shaded (Ψ_{sl}), and outer, sunlit (Ψ_{l}), canopy leaves. Young, fully expanded leaves from the northern side of the trellis, typically the eighth leaf from a cane tip were used and always placed under pressure within 20 seconds of being detached. Midday measurements were generally completed within ± 45 minutes of local solar noon and pre-dawn measurements were timed to be finished by civil twilight (30 minutes before dawn).

During the 2006/7 season midday Ψ_{l} was measured pre- and post-harvest (22/2/07 & 9/4/07), with Ψ_{sl} being measured only on 22/2/07. During the 2007/8 season midday Ψ_{l} was measured on nine occasions from mid-November to mid April, midday Ψ_{sl} on eight occasions, with pre-dawn measurements of both Ψ_{l} and Ψ_{sl} being made on four occasions (19/12/07, 9/1/08, 13/2/08 and 3/4/08). No leaf water potential measurements were made in the 2005/6 season. Typically a set of measurements included a single leaf from at least seven replicates for each irrigation treatment.

Stomatal conductance

Stomatal conductance (g_s) was measured on the abaxial side of leaves chosen as for the leaf water potential measurements (above), using a Delta-T AP4 Porometer (MEA, Magill, SA, Australia). Measurements were made in the late morning, normally between 9:30 and 11:30 AM on sunlit outer canopy leaves that were expected to be in direct sunlight for most of the day and on leaves from the inner canopy that were never fully illuminated by direct

solar radiation. Calibration of the porometer was always performed immediately before a set of measurements in the ambient conditions, and generally a recalibration was performed half way through the measurements.

Fifteen sets of porometer measurements were made during the 2006/7 season, from mid-October to late April and 11 sets of measurements during the 2007/8 season, from early November to mid-April. The timing of these measurements represented a period from before cap fall to post-harvest in each case. No porometer measurements were made in the 2005/6 season. A standard set of measurements included a single leaf from each of the twelve replicates per irrigation treatment.

Leaf gas exchange

Gas exchange measurements were made with a LiCor 6400 Portable Photosynthesis System (John Morris Scientific, Adelaide, SA, Australia) on a young but fully expanded leaf as above. Measurements consisted of either a) six to eight readings of assimilation (A) at different cuvette CO_2 concentrations under saturating light (2000 μmol m^{-2} s^{-1}), giving a photosynthetic CO_2 response curve (AC_i curve) or b) light saturated assimilation (A_{sat}) measured using a reference air CO_2 concentration of 400 ppm, providing a cuvette CO_2 concentration approximately the same as the ambient air. The AC_i curve CO_2 concentrations included a step at 400 ppm, which was considered equivalent to a measurement of A_{sat}. Cuvette air temperature was maintained at the expected typical ambient air temperature for the measurement period and humidity was not controlled. For each step change of CO_2, measurements were only made once photosynthesis and conductance readings were steady.

Transpiration (E), stomatal conductance (g_s) and leaf temperature were also recorded with each A_{sat} measurement. The C_3 model (Farquhar *et al.*, 1980) was fitted to each AC_i curve, allowing *in vivo* maximum carboxylation activity of Rubisco (V_{cmax}) and electron transport capacity (J_{max}) to be estimated. Where AC_i curves were not determined V_{cmax} was calculated from single A_{sat} readings using the C_3 photosynthesis model of Farquhar *et al.* (1980) as described in Harrison *et al.* (2009).

Leaf water use efficiency was calculated for 'instantaneous water use efficiency' (WUE_i) as A_{sat} / E and for 'intrinsic water use efficiency' (WUE_t) as A_{sat} / g_s.

A_{sat} measurements of outer canopy leaves were made on five occasions during the 2006/7 season, from the middle of the PD deficit period (9/1/07) to post-harvest (27/3/07). During the 2007/8 season A_{sat} measurements of outer canopy leaves were made on eight occasions from mid-November to mid-April and of inner canopy leaves on seven occasions. AC_i curves of outer canopy leaves were determined on five occasions and of inner canopy leaves on four occasions. Gas-exchange measurements were not made in the 2005/6 season. A measurement set typically included a single leaf from at least six replicates for each irrigation treatment.

Leaf total nitrogen analysis

Where leaves used for photosynthesis measurements were to be harvested for total nitrogen analysis (typically those used for AC_i curves) the position of the cuvette on the leaf was marked in indelible ink on the leaf blade after each measurement. Such leaves

were harvested when all measurements were completed and returned to the laboratory sealed in plastic bags on ice. The 6 cm^2 area that was used in the photosynthesis measurement was then cut out and fresh weight (FWt) determined, followed by oven drying at 60°C and the dry weight (DWt) determination. Specific leaf area (SLA) was calculated as 6 cm^2 / leaf segment DWt and 'leaf thickness' as FWt / 6 cm^2. Total leaf nitrogen was determined in the oven dried samples by the CSIRO Plant Industry Analytical Chemistry Group using Isotope-Ratio Mass Spectrometry.

Carbohydrate analysis

Vine carbohydrates were analysed using the methods described in Chapter 2, with water soluble carbohydrates (WSC) determined using the anthrone reagent and insoluble starch using the 'GOPOD' based analysis. In the 2006/7 season trunk samples were taken at veraison, harvest and leaf fall from single vines in at least six replicates from each irrigation treatment. In the 2007/8 season samples were taken of trunk, cordon, primary lateral root, leaf and cane at various times during the season (Table 1).

Table 1. Date of sample collection from various plant tissues for carbohydrate analysis at different phenological periods (season 2007/8).

Phenology	Sample Date	Trunk	Cordon	Root	Leaf	Cane
Budburst	02/10/07	X				
Flowering	13/11/07	X			X	
Canopy Closure	18/12/07	X	X	X	X	
Pre-veraison	06/01/08	X	X	X	X	
Pre-harvest	25/02/08	X	X	X	X	
Post-harvest	31/03/08	X		X	X	
Leaf fall	16/06/08	X	X	X		X

Trunk samples were taken by using a 5.15 mm diameter Haglöf increment borer (Forestry Tools, Willoughby, NSW, Australia) to obtain a core through the entire diameter of the trunk. Cordon samples were obtained by cutting a 90° 'wedge' from the top to the centre of the cordon. Primary lateral root samples were obtained by removing soil to expose a root with a minimum of 20 mm (but typically greater) diameter, then using the increment borer to take a core through the entire diameter of the root. Leaf samples consisted of two outer canopy leaf blades per vine and cane samples consisted of a two node/inter-node section of cane cut to provide the 4^{th} & 5^{th} internodes. All samples were immediately frozen in liquid nitrogen or dry ice and transported back to the laboratory where they were freeze-dried using a Telstar LyoQuest freeze-drier (AVT Services Beverley, SA, Australia), then ground to a fine powder (< 5 µm) using a Rocklabs Benchtop Ring Mill (AXT Pty Ltd, Terrey Hills, NSW, Australia).

Results

Leaf water potential

Pre-harvest midday Ψ_l in 2006, measured four weeks after the PD deficit period, was not significantly different between the RDI and PD treatments (-1.66±0.04 & -1.53±0.09 MPa respectively), but significantly higher in the control vines (-1.10±0.24 MPa). Midday Ψ_{sl} was higher than midday Ψ_l but exhibited the same differences, with the control vines

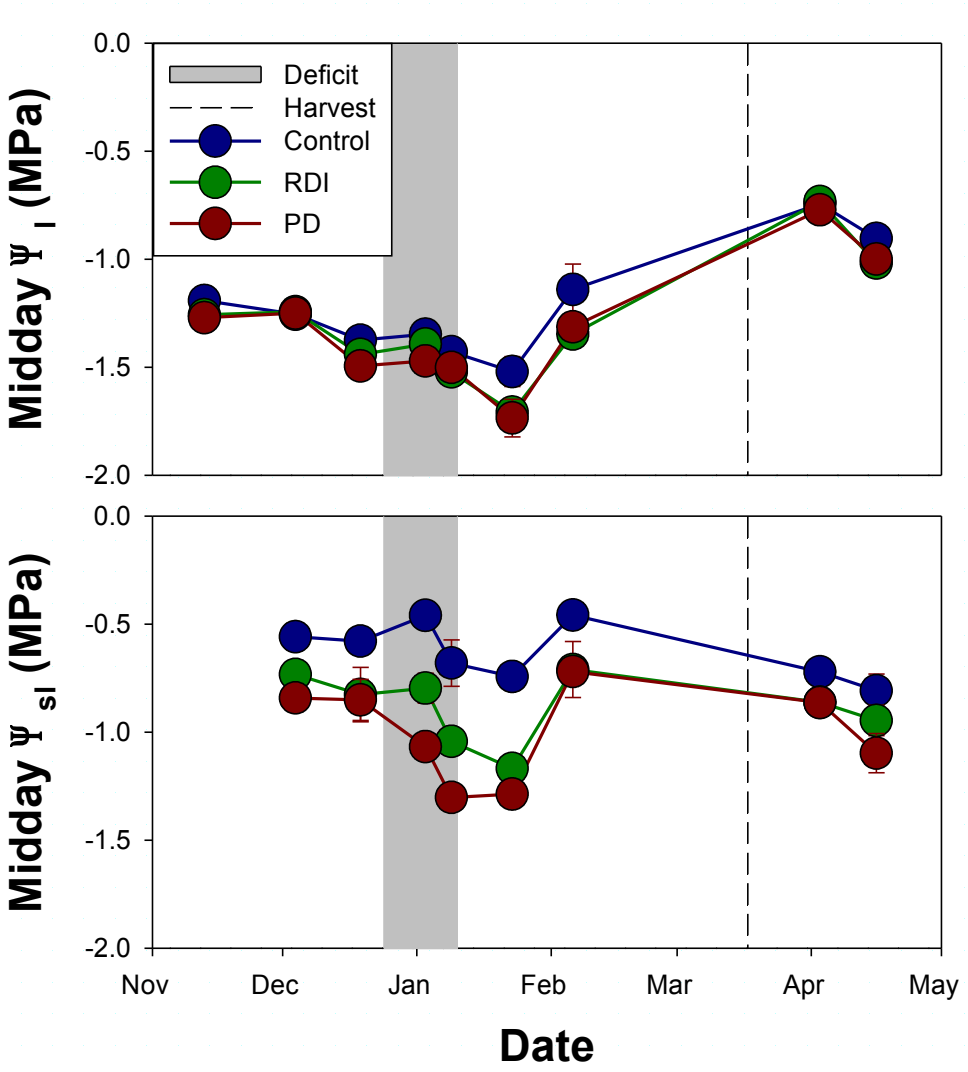


Figure 1. Midday leaf water potential during the 2007/8 growing season of a) outer canopy leaves (Ψ_l) and b) inner canopy leaves (Ψ_{sl}) of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. The grey area denotes the duration of the PD deficit period and the dotted line the date of harvest. Points are means of at least 6 replicates ± standard error.

having the highest midday Ψ_{sl}, (-0.78±0.13 MPa) and the deficit treatments significantly lower (-1.10±0.05 & -1.04±0.09 MPa for RDI and PD respectively). Following harvest there was no difference in midday Ψ_l between any of the treatments, with an overall mean of -1.45 MPa.

There were no significant effects of irrigation treatment on midday Ψ_l at any point of the 2007/8 season (Figure 1a), even during the PD deficit period. In contrast, midday Ψ_{sl} of control vines, which was invariably higher than midday Ψ_l in all treatments, was significantly higher than the deficit irrigated treatments until after harvest (Figure 1b). Prior to the PD deficit period there was no difference in midday Ψ_{sl} between RDI and PD vines, but during the PD deficit period midday Ψ_{sl} of the PD vines dropped progressively and was significantly lower than in the RDI vines for both measurements. The PD vines were still slightly, but significantly, lower than RDI 13 days after their return to standard irrigation, but this difference had completely disappeared at the next measurement (Figure 1b).

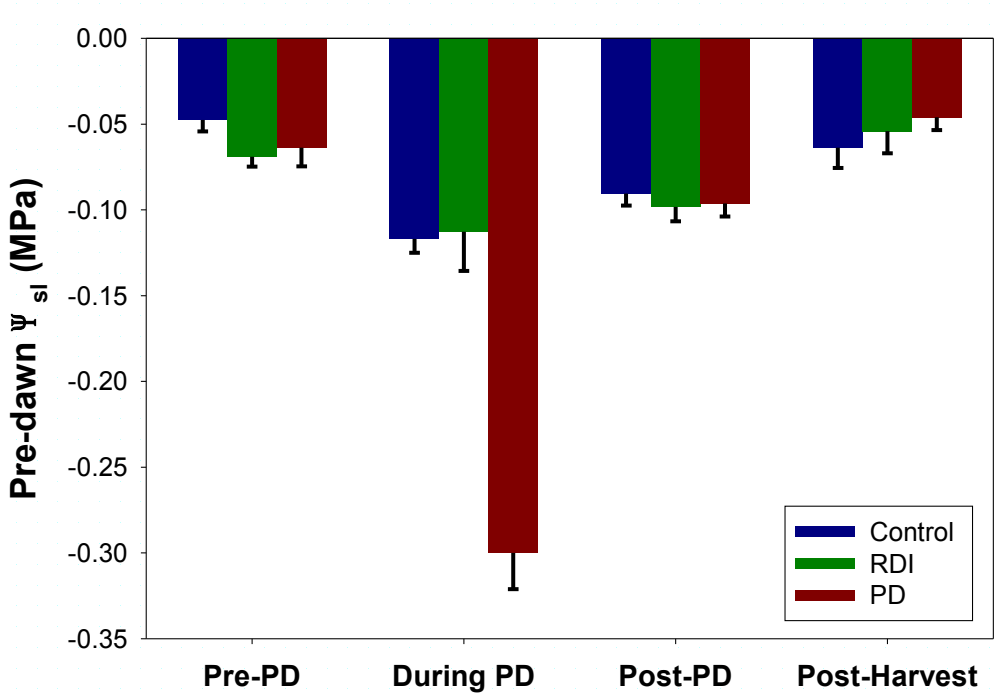


Figure 2. Pre-dawn leaf water potential during the 2007/8 growing season of inner canopy leaves (Ψ_{sl}) of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. Points are means of at least 6 replicates - standard error.

There was no difference in pre-dawn Ψ_{sl} between any of the irrigation treatments except during the PD deficit period, where Ψ_{sl} of the PD vines was almost three times lower than the other treatments (Figure 2). Pre-dawn Ψ_l was always slightly lower than pre-dawn Ψ_{sl} (data not shown) and although the PD vines had a lower pre-dawn Ψ_l during the PD deficit period, at the post-PD deficit period measurement the PD vines had a significantly *higher* pre-dawn Ψ_l (-0.14 MPa, cf. -0.21 & 0.26 MPa for RDI and control treatments respectively). This was due to the control and RDI vines having a much lower Ψ_l than at other times of the season, rather than an increase Ψ_l of the PD vines.

Stomatal conductance

In both the 2006/7 and 2007/8 seasons there was no significant effect of irrigation treatment on g_s of the outer canopy leaves early in the growing season or post-harvest (Figure 3). However, during the warmer part of the season g_s of the control vines was consistently and significantly higher than the deficit treatments. Irrespective of the difference between the control and deficit treatments there was no difference in g_s of outer canopy leaves between the RDI and PD vines prior to the PD deficit period. However, during that deficit period the g_s of outer canopy leaves of PD vines was significantly lower than either of the other treatments.

Following the return of the PD vines to standard watering, the g_s of outer canopy leaves of the PD vines remained lower than the other treatments, even though soil moisture and leaf water potential had recovered to be no different to RDI. This effect was greater in 2006/7 (Figure 3a) than 2007/8 (Figure 3b), despite the effect of the deficit period on outer canopy g_s of the PD vines being much greater in 2007/8, with the lowest values of g_s of outer canopy leaves being 107 mmoles m^{-2} s^{-1} cf. 253 mmoles m^{-2} s^{-1} in 2006/7.

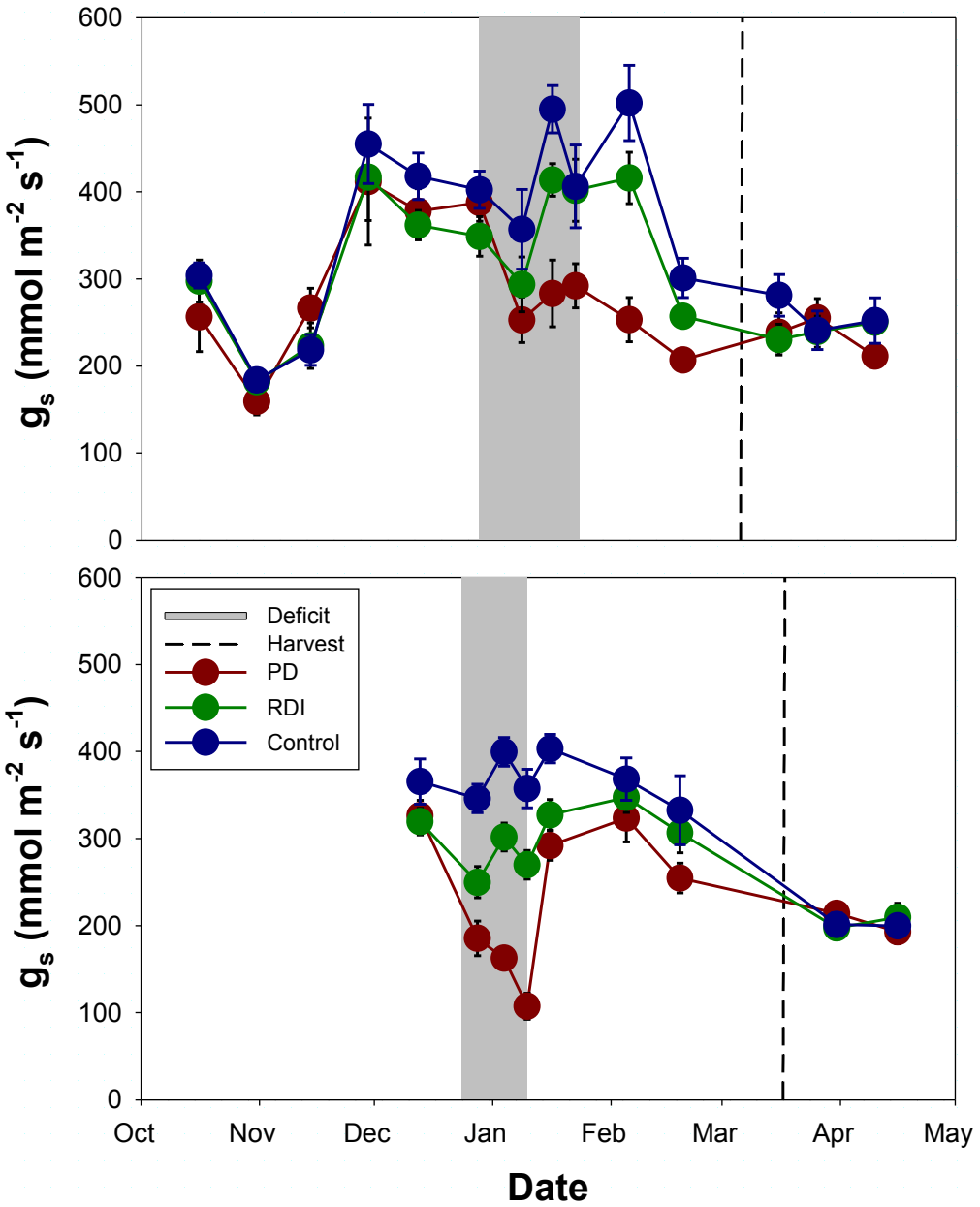


Figure 3. Stomatal conductance (g_s) of outer canopy leaves during a) the 2006/7 and b) the 2007/8 growing seasons of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. The grey area denotes the duration of the PD deficit period and the dotted line the date of harvest. Points are means of at 12 replicates ± standard error.

The g_s of the inner canopy leaves was much lower than the outer canopy leaves, typically below 100 mmoles m^{-2} s^{-1} with generally no difference between the treatments. However, in both seasons there was still a decrease in g_s of the inner canopy leaves of the PD vines during the PD deficit, but no difference between PD and RDI within a few days of irrigation being returned (data not shown). Furthermore, g_s of the inner canopy leaves of the control vines was never higher than the other treatments and occasionally significantly lower in both seasons. Incident radiation was not accurately monitored, but the light readings recorded by the porometer during those measurements indicated that within the inner canopy of the control vines there was less light than the other treatments (data not shown).

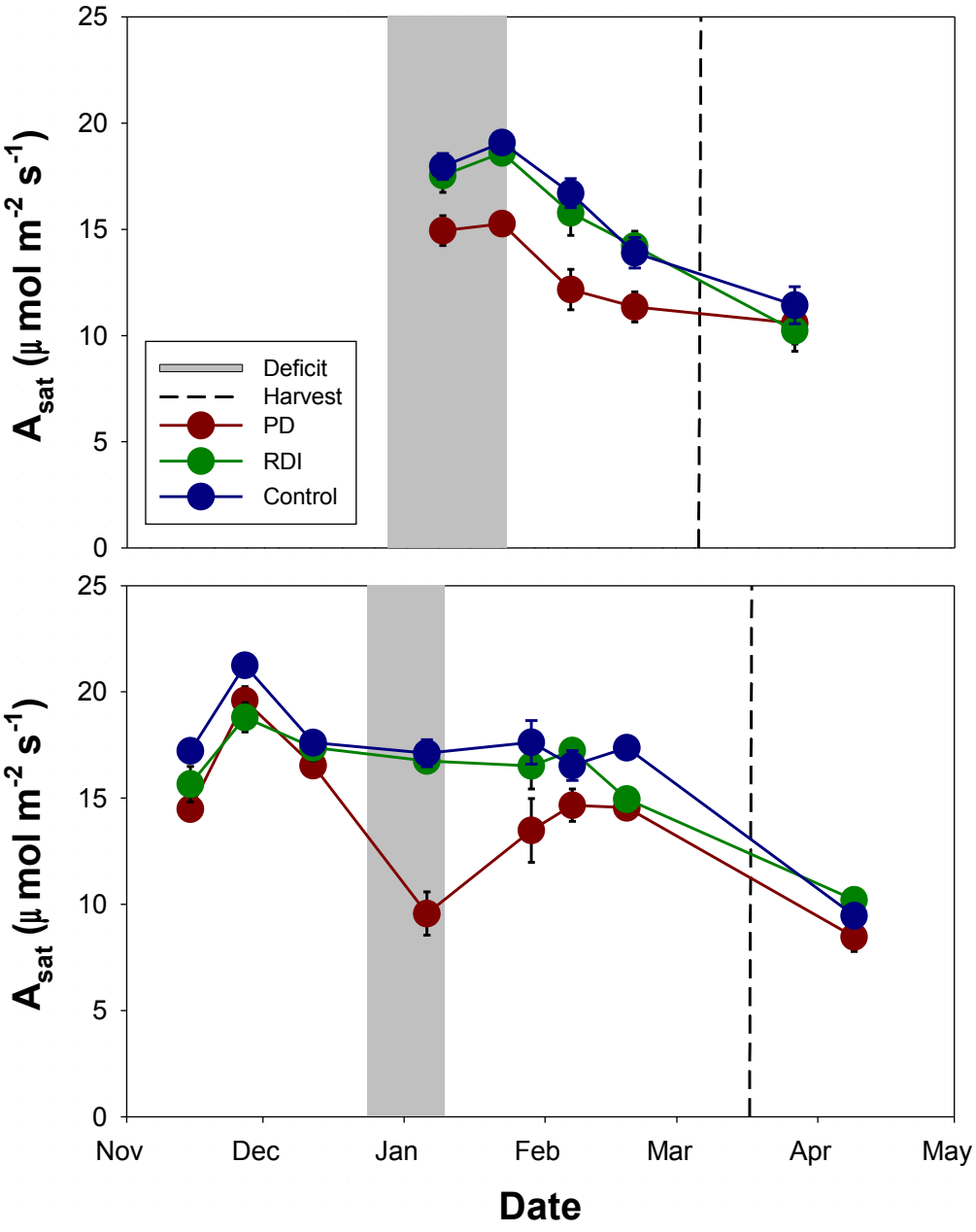


Figure 4. Assimilation under saturating light (A_{sat}) of outer canopy leaves during a) the 2006/7 and b) the 2007/8 growing seasons of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. The grey area denotes the duration of the PD deficit period and the dotted line the date of harvest. Points are means of at least eight replicates ± standard error.

Gas exchange

Stomatal conductance measured during the gas exchange measurements was broadly similar to the results above, despite being measured under controlled conditions, with the same effects of irrigation treatment and the same carry over in the PD vines following the return to standard irrigation (data not shown).

A_{sat} was not measured prior to the PD deficit period during the 2006/7 season, but during the PD period A_{sat} of the outer canopy leaves of the PD vines was significantly lower than the other treatments, consistent with the observed reduction in g_s (Figure 4a). Similarly, A_{sat} did not recover following the return of irrigation and only after harvest, when rates were almost half of their seasonal peak, was a carry-over effect of the PD deficit not seen. However, there was no difference in A_{sat} between the control and RDI treatments at any point in the 2006/7 season.

During the 2007/8 season A_{sat} of outer canopy leaves was slightly, but significantly, higher in the control vines than the other treatments early in the season, but not after fruit-set (Figure 4b). There was also no difference between the RDI and PD treatments prior to the PD deficit period. Whereas, during the deficit period A_{sat} of outer canopy leaves of PD vines was almost half that of the other treatments and as in 2006/7 there was a delay in recovery even after soil moisture and leaf water potential had recovered. However, as with g_s the difference between PD and the other treatments was much less than in 2006/7, despite the reduction during the PD deficit itself being much greater than the previous season. Again, matching the 2006/7 season results, there was no difference in A_{sat} of outer canopy leaves between any of the treatments following harvest and again rates were almost half the peak A_{sat} earlier in the season.

A_{sat} of inner canopy leaves was also measured during 2007/8, with no effect of irrigation early in the season, but once full canopy growth was complete A_{sat} of control vine inner canopy leaves was significantly less than the other treatments, and the PD vines were only affected whilst the PD deficit occurred (data not shown). There was also a difference between inner and outer canopy leaves in SLA, but no effect of treatments, with across treatment means of 10.9±0.2 & 26.8±0.8 m^2 kg^{-1} at harvest in 2007 and 12.4±0.4 & 28.5±0.7 m^2 kg^{-1} at harvest in 2008 for outer & inner canopy leaves respectively.

E was primarily driven by conductance, albeit modified by vapour pressure deficit (VPD), and therefore in outer canopy leaves was higher in controls early in the season and lower in PD vines during the PD deficit period (Table 2). The PD carry over effect was also apparent. Using *E* it was possible to calculate WUE_i, but although there was a slight gain in PD vines during the PD deficit in both seasons, generally WUE_i of all the treatments was similar on any given day (Table 2). In contrast, WUE_t was affected to a much greater extent during the PD deficit period and the carry over following the return of PD vines to standard irrigation was also apparent (Table 2).

Using the AC_i measurements an estimate of $V_{c\ max}$ was calculated and these results were supplemented with $V_{c\ max}$ estimates using the single point from the A_{sat} measurements. Although $V_{c\ max}$ was affected by leaf temperature and phenology, there was no significant effect of any irrigation treatment in either season, even during the PD deficit period itself (Figure 5). This suggested that the irrigation treatments had no effect on Rubisco content or activity and was supported by a lack of any irrigation effect on leaf N content (Table 2).

Measuring AC_i curves (but not A_{sat}) also allowed J_{max} to be estimated. Unlike $V_{c\ max}$ the values of J_{max} were reduced in the outer canopy leaves of PD vines during the PD deficit period and, although there was no difference early in the season, J_{max} was higher in the control vines (Table 2).

Table 2. Transpiration (E), instantaneous water use efficiency (WUE_i), intrinsic water use efficiency (WUE_t), electron transport capacity (J_{max}), ratio of J_{max} to maximum carboxylation rate (J/V_c) and leaf blade nitrogen content (leaf N). Each value is a mean of at least six replicates ± standard error. No measurements were made prior to the PD deficit period in 2006/7. Superscript letters indicate significant differences within a measurement set using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10 and are only added where a significant effect was present.

Season	Period	Irrigation Treatment	E (mol m^{-2} s^{-1})		WUE_i (µmol mol^{-1})		WUE_t (µmol mmol^{-1})		J_{max} (µmol m^{-2} s^{-1})		J / V_c	Leaf N (mmol g^{-1})	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	SE
2006/7													
	PD Deficit	Control	5.45	0.35	3.34	0.24	59.4	4.2	*		*	*	
	(9/1/07)	RDI	5.86	0.44	3.02	0.18	59.4	3.3					
		PD	3.94	0.26	3.81	0.13	93.0	3.9					
	Post-PD	Control	6.65	0.53	2.16	0.08	69.1	3.9				1.37	0.04
	(20/2/07)	RDI	7.19	0.50	2.02	0.07	68.6	3.7				1.31	0.01
		PD	5.58	0.44	2.08	0.06	81.9	4.2				1.28	0.04
2007/8													
	Pre-PD	Control	6.56	0.23	2.69	1.22	62.6	2.6	162.4	5.0	1.22	1.92	0.05
	(12/12/07)	RDI	6.57	0.21	2.65	0.08	61.2	3.7	154.4	4.2	1.18	1.84	0.06
		PD	6.15	0.23	2.70	0.09	69.1	5.5	157.0	6.2	1.17	1.80	0.06
	PD Deficit	Control	7.27	0.63	2.45	0.18	66.3	5.9	148.3	4.0	1.12	1.82	0.02
	(6/1/08)	RDI	7.36	0.30	2.29	0.06	61.9	4.4	137.7	5.6	1.06	1.78	0.08
		PD	3.60	0.36	2.66	0.06	102.3	7.5	121.3	5.6	0.93	1.78	0.04
	Post-PD	Control	6.11	0.91	3.16	0.45	47.3	6.3	153.3	5.2	1.42	1.78	0.12
	(19/2/08)	RDI	5.59	0.77	3.05	0.42	47.6	3.3	134.1	4.3	1.31	1.53	0.03
		PD	5.59	0.72	3.03	0.48	47.0	2.2	129.0	2.5	1.29	1.54	0.04

* Data not collected.

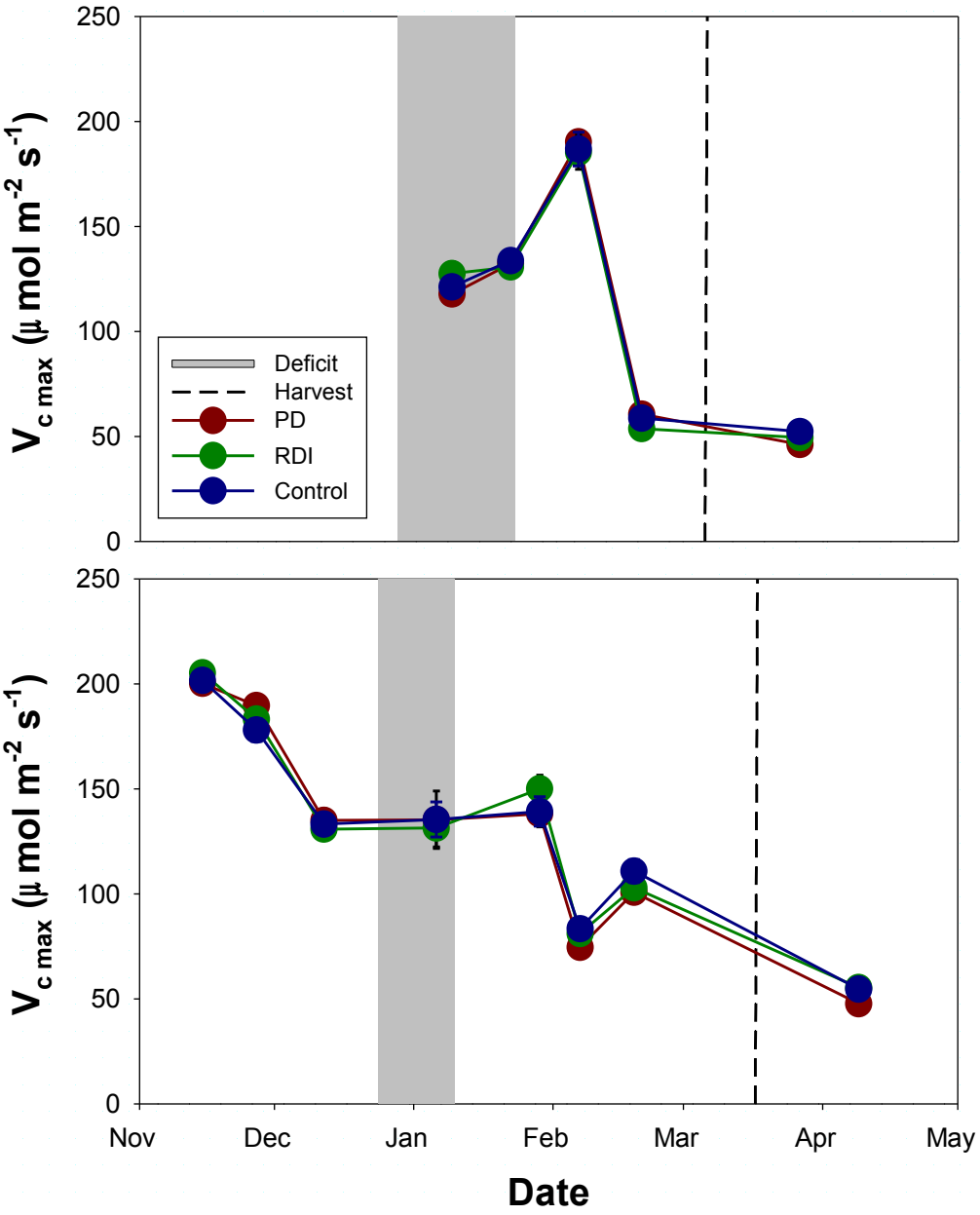


Figure 5. Maximum carboxylation activity ($V_{c\,max}$) of outer canopy leaves during a) the 2006/7 and b) the 2007/8 growing seasons of Cabernet Sauvignon vines receiving control, RDI or PD irrigation. The grey area denotes the duration of the PD deficit period and the dotted line the date of harvest. Points are means of at least eight replicates ± standard error.

Carbohydrates

There was no significant effect of irrigation treatment or phenology on TNC concentrations in the trunk samples taken during the 2006/7 season (Figure 6), with TNC accounting for about 15% of the wood dry mass in all samples. However, during the 2007/8 season, both treatment and phenology elicited responses in tissue carbohydrate concentrations.

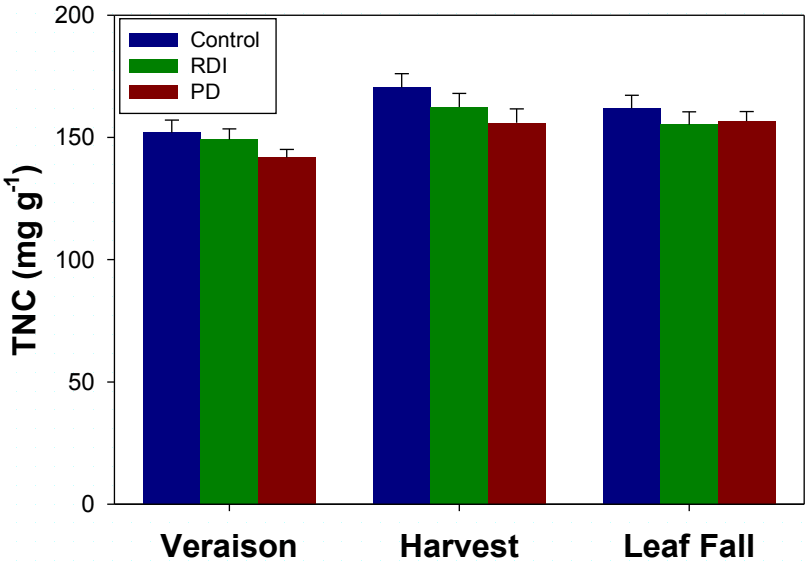


Figure 6. Total non-structural carbohydrate (TNC) concentration of trunk wood samples, taken at veraison, harvest and leaf fall of the 2006/7 growing season, from Cabernet Sauvignon vines receiving control, RDI or PD irrigation. Bars are means of 8-12 replicates + standard error.

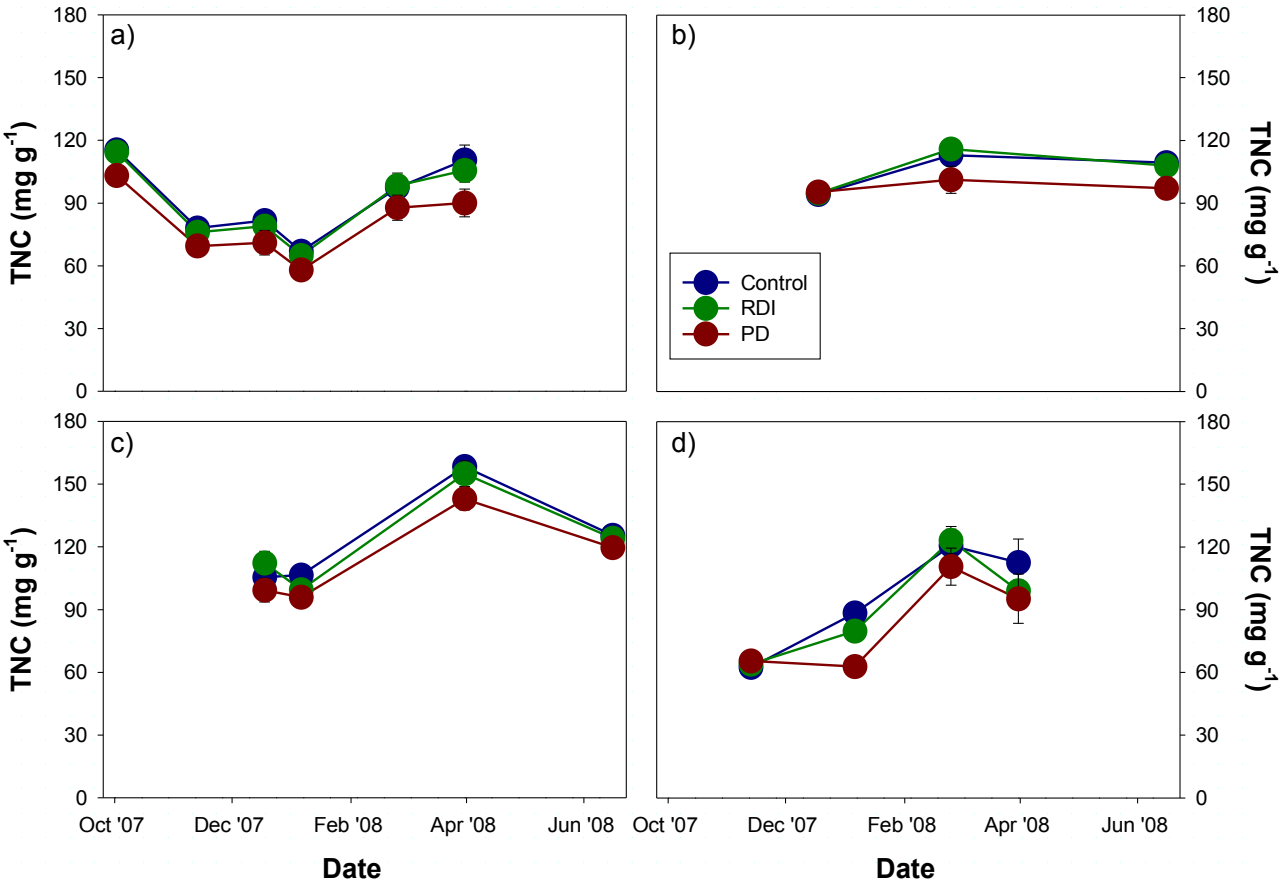


Figure 7. Total non-structural carbohydrate (TNC) concentration of a) trunk wood, b) cordon wood, c) primary lateral roots, and d) outer-canopy leaves, taken at various times during the 2007/8 growing season from Cabernet Sauvignon vines receiving control, RDI or PD irrigation. Bars are means of 12 replicates + standard error.

Table 3. Water-soluble carbohydrate and insoluble starch concentrations of vine tissues taken at various times during the 2007/8 growing season from Cabernet Sauvignon vines receiving control, RDI or PD irrigation. Data are means of 12 replicates and super-script letters denote significant differences.

Tissue type	Irrigation Treatment	Budburst		Flowering		Canopy closure		Pre-veraison		Pre-harvest		Post-harvest		Leaf fall	
		WSC	Starch	WSC	Starch	WSC	Starch	WSC	Starch	WSC	Starch	WSC	Starch	WSC	Starch
Trunk wood	Control	33.7	81.9[a]	25.7	52.4[a]	18.3	63.4	19.5[a]	47.5[a]	24.4	73.0	31.0	79.5[a]		*
	RDI	33.5	81.0[a]	26.4	49.6[a]	17.9	61.0	20.9[b]	44.0[ab]	26.4	71.9	31.9	73.7[ab]		
	PD	35.0	68.2[b]	26.7	42.7[b]	18.1	52.9	20.8[b]	37.1[b]	26.5	61.3	30.7	59.4[b]		
Cordon wood	Control			†		40.3	54.0		*	46.7	66.2		†	61.2	48.2[a]
	RDI					42.6	52.1			46.0	70.0			66.2	41.7[ab]
	PD					42.3	53.1			47.3	54.0			62.4	34.7[b]
Outer canopy leaves	Control		†	31.4	31.1		*	39.0	49.4[a]	66.9	53.7[a]	52.6	59.9		†
	RDI			32.3	31.7			36.8	43.0[a]	68.7	54.5[a]	45.4	53.6		
	PD			33.9	31.5			35.8	27.0[b]	67.8	42.7[b]	45.2	50.1		
Primary lateral roots	Control			†		45.6	59.9	50.8	55.7	*	67.1	52.8	105.6	47.7	77.7
	RDI					47.1	65.1	49.7	49.8		62.2	57.0	98.0	49.4	74.7
	PD					43.9	55.4	45.0	51.0		43.2	53.8	89.1	48.9	70.7
Cane	Control							†						56.2	64.1
	RDI													57.1	62.1
	PD													59.4	64.9

* Data not available.
† No sample taken.

Repeated measures analysis of all available data determined that phenology had a significant effect on TNC concentration in all the measured tissues; trunk, cordon, root and leaf (Figure 7). Generally, the highest TNC concentrations were observed at budburst and after harvest, with the lowest concentrations at veraison. Irrigation treatment effects on TNC were significant for trunk wood and marginally significant for leaves (p = 0.054), in each case it was the PD treatment that had lower TNC concentrations than the other two irrigation regimes. When the WSC and starch data were examined separately it was found that in general there were no effects of irrigation on WSC, although phenology did alter WSC concentration, but that starch concentration was significantly affected by irrigation in both trunk and leaf material (Table 3).

Whilst irrigation treatment did not significantly affect carbohydrate concentrations in cordon and root material across the entire season, there were effects for parts of the year in the cordons, with a marginally significant reduction in starch by PD shortly before harvest (p = 0.064) and a significant effect at leaf fall (Table 3). There were also differences between tissues. For instance, trunk wood invariably had lower WSC than the other tissues and the leaves were often lower in starch than the woody tissues.

Discussion

Leaf water potential can be a sensitive measure of vine water stress (e.g. Williams and Araujo, 2002), yet little difference was seen in midday Ψ_l between the irrigation treatments. Even when soil moisture content was extremely low in the PD treatment (Chapter 4 – Table1) there was no significant difference between midday Ψ_l of control and PD vines. In fact the lowest midday Ψ_l values occurred when soil moisture was high in all three treatments (on 6/2/08), but at a time where air temperatures were very high and relative humidity very low. Pre-dawn Ψ_l was better correlated with soil moisture, but still exhibited discrepancies, notably in the post-PD measurement where control vines had a pre-dawn Ψ_l similar to the PD vines exhibited during the PD deficit (data not shown). This may have been due to incomplete re-filling of the large control vine canopies, either as a result of nocturnal transpiration (Wheeler *et al.*, 1999; Rogiers *et al.*, 2009) or simply because the duration of the warm summer nights was too short.

In contrast, the midday Ψ_{sl} more closely reflected differences in soil moisture during the 2007/8 season and exhibited effects of the irrigation treatments. Furthermore, pre-dawn Ψ_{sl} was only affected by irrigation during the PD deficit period itself. While, Ψ_{sl} is effectively a less rigorous version of stem water potential, it is still influenced to some extent by atmospheric conditions, and stem water potential has been shown as a more effective measure of vine water status than midday Ψ_l (Choné *et al*., 2001). The use of pre-dawn or midday leaf water potential as an indicator of stress is discussed further in Chapter 7, but the data presented here particularly highlights the limitations of using midday Ψ_l as an indicator of vine stress in warm-climate Australian viticulture regions.

All four measures of leaf water potential, Ψ_l and Ψ_{sl} measured pre-dawn and at midday, showed no difference or indicated a rapid recovery of leaf water status in the PD vines following irrigation being returned. Only midday Ψ_{sl} was significantly lower in PD vines than RDI after the PD deficit period was over, with a difference of less than 0.12 MPa, but no difference 6/2/08. However, carry-over effects of the PD deficit on leaf gas exchange were observed beyond this date.

Both g_s and A_{sat} remained lower in PD vines than RDI following the end of the PD deficit in both seasons, despite the recovery of soil moisture and leaf water potential. With the exception of A_{sat} in 2007/8, these differences continued until harvest, where both parameters were almost half of their seasonal peak, at veraison. It is not possible to conclude directly from the A_{sat} and g_s data whether either the carry-over effect or the direct irrigation effects on A_{sat} were due to the changes in g_s alone or whether other factors, such as photodamage to the photosynthetic apparatus (Guan *et al.*, 2004) or changes in mesophyll conductance (Flexas *et al.*, 2002), were involved. Estimating $V_{c\,max}$ from CO_2 response curves and from the A_{sat} measurements demonstrated that there was no difference in maximal carboxylation rates ('photosynthetic capacity') between the treatments, either before, during or after the PD deficit period. Therefore, irrigation treatment differences in A_{sat} were primarily due to differences in inter-cellular CO_2 concentration caused by differences in g_s and not by direct effects of water stress on the photosynthetic apparatus.

Consequently, the carry over effect of the irrigation treatments on A_{sat} were also the result of changes in g_s and this was also supported by the lack of any direct or carry-over effect of irrigation treatment on leaf N concentration, often correlated with photosynthetic capacity (Wright *et al*. 2004), or $V_{c\,max}$ expressed per unit of N (data not shown).

The reduction in g_s during and after the PD deficit period necessarily resulted in a change in E as well as A_{sat}, with E being halved in both seasons during the deficit itself, similar to the effect of the PD deficit on sapflow in the 2007/8 season (Chapter 4 – Figure 7b). The carry-over effect on g_s also explained the observed carry-over effect on total daily sap flux in the PD vines for the 2007/8 season. Despite this and an improvement in both instantaneous and intrinsic water use efficiency during the PD deficit period, neither measure was higher in the PD vines after the PD deficit period was over.

Although there was no effect of irrigation on $V_{c\,max}$ there was an effect on J_{max}, 'electron transport capacity', which was significantly reduced in the PD vines during the PD deficit period, but recovered following re-watering. Consequently, there was also an effect on J_{max}/V_{cmax}. Whereas $V_{c\,max}$ is driven by the amount of Rubisco in the leaf and it's activation state, the control of J_{max} is more complex because there are many steps in the electron transport chain. Interestingly, an increase in J_{max}/V_{cmax} of woody deciduous species as the growing season progresses has previously been observed (Wilson *et al.*, 2000; Onoda *et al.*, 2004), but more pertinently to this study a decline in J_{max}/V_{cmax} has been associated with acclimation to higher temperatures (Hirosaka *et al.*, 1999) a process that may have been occurring here as leaf temperatures were higher in leaves of the PD vines due to lower stomatal conductance (data not shown). Irrespective of the cause, J_{max} was never low enough to limit photosynthesis in the field.

Seasonality was also observed in $V_{c\,max}$, with a decrease following veraison (late January). This was more evident when $V_{c\,max}$ was adjusted to a common temperature and well correlated with leaf nitrogen concentration, which also decreased following veraison. However, $V_{c\,max}$ expressed on a nitrogen basis (μmol CO_2 mmol N^{-1}) still declined with time, so translocation of nitrogen from the leaf could not fully explain this. The increase in J_{max}/V_{cmax} noted above suggests that a change in allocation of leaf nitrogen from Rubisco to the electron transport chain was occurring, again this has been previously observed in deciduous woody species (Wilson *et al.*, 2000). There are various potential explanations, but as the proportional increase in leaf TNC content, post-veraison, was greater than in

any other tissue measured, regulatory feedback may have reduced V_{cmax} in response, as has been observed in other species (e.g. Urban *et al.*, 2004). Feedback effects of increasing leaf carbohydrate on A_{sat} in grapevines have also been observed (e.g. Quereix *et al.*, 2001)

The reductions in A_{sat}, both during and after the PD deficit, represent a reduction in carbon available for the vine at a time where sink demand for fruit ripening and building storage reserves is high (Sommer and Clingeleffer 1996, Clingeleffer and Pellegrino 2006). The fruit yield per unit of canopy area (Chapter 4 – Table 4) was no lower in PD than the other treatments, consequently other sinks were likely to be impacted to the same extent as yield was, relative to the other treatments. This was true of various growth parameters (see Chapter 4), but large differences in the carbohydrate concentrations of storage tissues were not seen in either season, although small reductions in the PD vines were observed in 2007/8. Therefore, it is likely that the main effect of reduced carbon input was a reduction in the storage pool size. These are difficult to estimate, but a large difference was seen in the root biomass, the whole-vine effect of which may be compounded by the carbohydrate concentrations being higher in the roots than in any other tissue measured.

A link between TNC reserves and shoot growth during the following season has been observed in vines defoliated at harvest for two consecutive seasons (Smith and Holzapfel 2009), in a study that found very slight effects on wood TNC concentration, but up to a 50% reduction in root TNC concentration. However, TNC pool sizes were not estimated in either this study or our own so it is difficult to compare the effects. Even without a difference in TNC concentration a difference in the TNC pool size, e.g. trunk diameter or root volume, could result in large differences in the carbon available for early season growth.

In conclusion, we found that the effect of the PD deficit period on leaf physiology was compounded by a carry-over effect that reduced photosynthesis for a significant period after both plant and soil water status had recovered. This was due to a change in stomatal conductance, possibly a change in stomatal responsiveness to diurnal variation in humidity. The combined effect of the PD deficit and carry-over on photosynthesis did not have a major effect on tissue carbohydrate concentration, but may explain differences observed in growth parameters.

Chapter 6

Carry-over effects of long-term deficit irrigation on vine growth and physiology

Introduction

Chapter 4 described a range of effects on vine growth due to multi-seasonal impacts of irrigation strategy. Some of these could only have been due to 'carry-over' effects from one season to the next. Chapter 5 described effects of irrigation strategy on canopy physiology. These appeared to largely be driven by the irrigation received within the season, but in the case of the well watered control it was not possible to positively rule out multi-seasonal carry-over effects.

By returning all the experimental vines to the site standard RDI strategy a number of aims could be fullfilled. Firstly, any carry-over effects of converting well-watered vines to RDI could be examined using the previously control irrigated vines. Secondly, the impact of PD irrigation on vine physiology, growth and yield in subsequent seasons, the period required for 'recovery' of PD vines, could be examined. Finally, any carry-over effect on the leaf physiology of the control vines could be ascertained.

Materials and Methods

Experimental set-up and data collection

Irrigation of all vines was returned to the site standard RDI strategy prior to the first irrigation of the 2008/9 season and the impacts of this assessed over two seasons, 2008/9 and 2009/10 as described in Chapter 2. Environmental and soil moisture measurements were also made as described in Chapter 2.

Vine growth and physiology measurements

Canopy growth was estimated in both seasons using LAI measurements and pruning weights as described in Chapter 4. A 'Merbein bunch count' was also performed after budburst in the 2009/10 season. Harvest yield was estimated using a 'whole' vine as in 2007 and 2008 Harvest dates were 31/3/2009 and 2/3/2010.

Leaf water potential, stomatal conductance and leaf gas exchange were all measured in both seasons as described in Chapter 5. Midday Ψ_l and Ψ_{sl} were assessed at flowering, canopy closure and harvest in the 2008/9 season and at flowering and veraison in the 2009/10 season. Pre-dawn Ψ_l and Ψ_{sl} were assessed at flowering in the 2008/9 season only. Stomatal conductance of inner and outer canopy leaves was measured five times in 2008/9, from flowering to harvest, and three times in 2009/10, from flowering to veraison. Assimilation under saturating light was determined four times in 2008/9, from flowering to harvest and CO_2 response curves were measured once at canopy closure.

Table 1. Volumetric soil moisture and estimated soil matric potential (Ψ_{sm}) for 0-30 and 30-60 cm depths under the dripline of RDI irrigated Cabernet Sauvignon vines previously irrigated with control, RDI and PD strategies until June 2008. Each value is a mean of 12 replicates ± standard error, with each replicate value being a mean of two or more measurements made on different dates. Superscript letters indicate significant differences using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10.

Season	Depth (cm)	Past Irrigation treatment*	Volumetric Soil Moisture (m^3 m^{-3})				Soil Matric Potential (kPa)†	
			Pre-Veraison		Post-Veraison		Pre-Veraison	Post-Veraison
			Mean	SE	Mean	SE		
2008/9								
	0-30	Control	0.159[a]	0.008	0.213[a]	0.009	-68	-11
		RDI	0.143[b]	0.007	0.200[ab]	0.010	-129	-16
		PD	0.133[b]	0.008	0.189[b]	0.009	-205	-24
	30-60	Control	0.219	0.013	0.243	0.009	-9	-5
		RDI	0.221	0.010	0.250	0.007	-9	-4
		PD	0.221	0.014	0.240	0.007	-9	-5
2009/10								
	0-30	Control	0.178	0.012		‡	-33	‡
		RDI	0.160	0.008			-64	
		PD	0.153	0.009			-86	
	30-60	Control	0.221	0.008			10	
		RDI	0.216	0.009			11	
		PD	0.218	0.009			11	

* All vines were irrigated as RDI for 2008/9 and 2009/10.
† See Chapter 1 for conversion from volumetric soil moisture.
‡ TDR measurements not taken post-veraison in 2010.

Results and Discussion

Soil moisture

The final irrigation with the control treatment in place was in April 2008 and there was no difference in irrigation applied to the PD and RDI treatments from January 2008. No irrigations were applied between April and September 2008, with the only additions to soil moisture coming from winter rainfall (88.6 mm, with ET_o for the same period being 270.1 mm). As uptake of soil water in this period by the vines would be low it was possible that residual effects of the control treatment, in place from 2005/6 to 2007/8, on soil moisture could occur.

Indeed, an effect was seen in the TDR data for the top 30 cm of soil prior to veraison in the 2008/9 season (Table 1). This equated to a matric potential about 1/3rd of the other treatments and very similar to the same part of the season when treatments were in place (see Chapter 4, Table 1). The effect was only marginal later in the season, when matric potentials of all treatments were very high and there was no significant effect of prior irrigation during the 2009/10 season. Furthermore, there was no indication of an effect below 30 cm at any time in either season. Consequently, any difference in soil water available to the vine root system as a result of prior irrigation in the control treatment relative to RDI was likely to be small, temporary and affecting only part of the root system.

The water table ranged from 1.51 to 1.86 m over the two seasons, with the lowest depths occurring post-harvest and the highest between budburst and flowering. The mean depth was 1.66 m with a standard deviation of 0.12 m. This was lower than whilst the treatments were in place, but due to additional deep drainage installed by the vineyard owner rather than being an effect of ending the well-watered control treatment.

Vine growth and production

Differences in growth and productivity between vines previously receiving the PD treatment and RDI vines would be further evidence of the carry over effect described in Chapter 4 and, assuming the limited effects on soil moisture had a negligible effect on vine growth, any differences between RDI and previously control irrigated vines would be indicative of the impacts of moving from full irrigation (complete replacement of ET_{crop}) to RDI.

Such differences were indeed seen, with significant effects of prior irrigation strategy on canopy size apparent in both seasons (Figure 1). During 2008/9 LAI_{90} of all three treatments separated out, with control having a significantly larger canopy than the other treatments and the PD canopy significantly smaller. The greater canopy size of the previously control irrigated vines compared with the RDI vines suggested that RDI was indeed having a multi-seasonal effect on canopy size, above and beyond that within a given season. However, that difference was lost in the following season. Consequently, it may be that, i) any long-term impact of RDI on canopy size requires only two seasons to reach it's peak, ii) given the vine history of receiving RDI prior to the introduction of the well-watered control treatment, it may be that the multi-seasonal effect of that RDI was not completely removed from those vines prior to their return to RDI, or iii) the difference in soil moisture in the upper part of the profile between RDI and control vines was enough to affect canopy growth in 2008/9 and there was no long-term effect on canopy size.

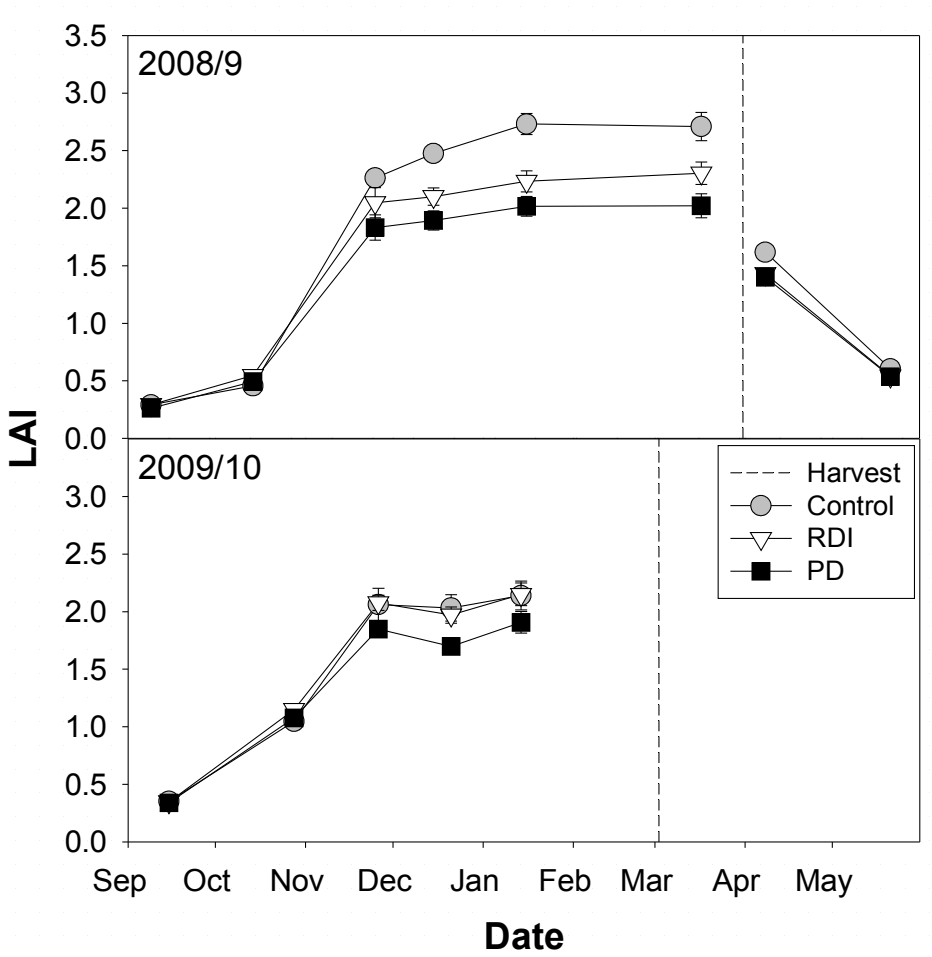


Figure 1. LAI during a) the 2008/9 and b) the 2009/10 growing seasons, estimated with a 90° view, of RDI irrigated Cabernet Sauvignon vines, having previously received control, RDI or PD irrigation until June 2008. Each point is a mean of 12 replicates ± standard error.

In contrast, the difference in canopy size between the RDI vines and those previously PD irrigated remained in the second season (Figure 1), despite receiving identical irrigation and no difference in soil moisture being observed. Furthermore, the size of the difference was similar in both seasons to that when the PD treatment was in place. Clearly, not only did this extended RDI strategy have a multi-seasonal impact on canopy growth, but two seasons without the treatment in place were not adequate for vine vigour to recover.

As in the previous two seasons, the centre of vine canopy measure, LAI_{15}, during 2008/9 exhibited the same separation of treatments as the 90° view, but with a higher peak. In fact the absolute values were also very similar to the previous season (approximately 3.6 in control, 3.4 in RDI and 3.3 in PD). In contrast, during the 2009/10 season there were no significant differences between any of the treatments with an overall average peak LAI_{15} 3.2.

Given the previously observed close correlation between LAI and pruning weight, it was not surprising that the carry over effects on canopy size were also seen in the pruning weights for 2008/9. In fact, pruning weights of previously control irrigated vines were significantly higher in both seasons, despite the lack of any effect on LAI in 2009/10,

although the difference was less in 2009 than 2010 (Figure 2). Conversely, and similar to when the irrigation treatments were in place, the differences in pruning weight between the RDI and PD treatments were not significant in either given year.

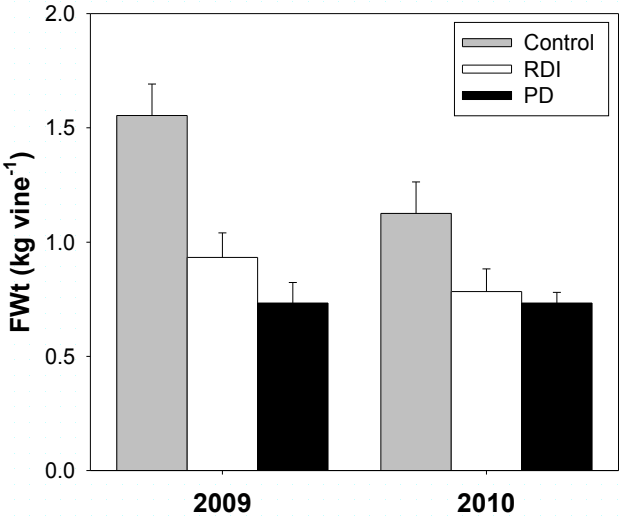


Figure 2. Pruning weights of RDI irrigated Cabernet Sauvignon vines, having previously received control, RDI or PD irrigation until June 2008, after leaf fall in the 2008/9 and 2009/10 growing seasons. Each bar is a mean of 12 repliactes + standard error.

As described in Chapter 4, 'peak' LAI values were calculated for each treatment, using measurements on 16/1/09 & 17/3/09 for the 2008/9 season and on 21/12/09 & 14/1/10 for 2009/10. Peak LAI and pruning weight from 2009 were significantly correlated with an adjusted R^2 of 0.54 (Figure 3) and the ANCOVA result demonstrated that the relationship was not significantly different to that for the previous two seasons (see Chapter 4). On the other hand there was no significant correlation between LAI and pruning weight in 2010, the only season during the project in which this was the case. The lack of a relationship was driven by there being an effect of prior irrigation on pruning weight, but not LAI_{90} in the control vines and on LAI_{90} but not pruning weight in the PD vines.

One explanation for this might be a change in canopy structure, with a greater cane mass per unit leaf area in the previously control irrigated vines for instance. Visual observation did suggest that there was little growth from the lower cordon in the control vines in 2009/10, with almost all of the leaf area being borne on canes originating from the upper cordon. However, no measurements were attempted to confirm this impression.

Canopy growth differences did not appear to be driven by budburst, as none of the parameters calculated from the Merbein bunch count were significantly affected by past irrigation regime (Table 2), including bud number per vine, number of burst buds per vine, number of shoots per vine, number of inflorescences per vine, the fraction of buds burst and the fraction of shoots with bunches. Overall the average bud number per vine was 672, with 40% burst and 49% of shoots being fruitful. With the exception of number of burst buds, all the parameters were significantly different in 2009 to those of 2007 (see Chapter 4); bud number being significantly higher and the other parameters significantly lower. These results suggest that pruning by the vineyard owner may have been lighter, leading to the retention of higher bud numbers with subsequent compensatory effects on budburst, shoot numbers and fruitfulness (Clingeleffer 1993, Clingeleffer 2009).

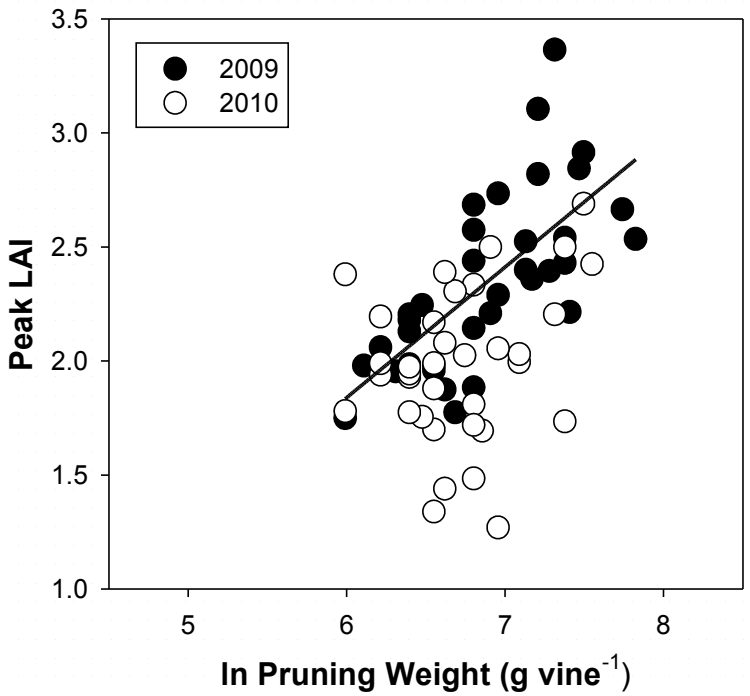


Figure 3. Regressions of pruning weights from 2009 and 2010 against maximum LAI_{90} for the preceding season. The natural log of the pruning weight was used to linearize the data. For each year n=36

Table 2. Results of Merbein bunch count, approximately four weeks after budburst in 2009, of Cabernet Sauvignon vines previously irrigated with control, RDI or PD strategies until June 2008, then irrigated with RDI.

Past Irrigation Treatment	Bud No.		No. Burst Buds		Shoot No.		Bunch No.		% Budburst	% Fruitful Shoots
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Mean
Control	612	35	344	21	390	24	250	30	56	47
RDI	671	61	374	40	440	41	251	27	55	51
PD	734	65	418	36	465	34	282	32	57	49
Overall Mean	672		379		431		261		40	49
p	ns		ns		ns		ns		ns	ns

Also as in 2007, the lack of any difference in the number of inflorescences did not prevent treatment effects, or in this case effects of prior treatment, on yield at harvest. Carry over effects were observed on fruit yield in both seasons, with control and PD both having a significant effect in 2009, but only control having a significant effect in 2010 (Table 3). In fact the relative difference between RDI and control in 2009 was greater than in any of the years were the control vines actually received more water than RDI (see Chapter 4). There was no difference in bunch number at harvest (Table 3), so this effect was driven primarily by bunch weight, with average bunch weight of the previously control irrigated vines being significant higher than those of the continuously RDI irrigated vines (59.8 and 47.5 g respectively) in 2010. Berry weights weren't measured in 2010, but in 2009 berries of the control treatment had a higher fresh mass than those of RDI (see Chapter 8).

Table 3. Harvest yield, bunch number, yield / pruning weight ratio and yield / canopy area ratio RDI irrigated Cabernet Sauvignon vines, having previously received control, RDI or PD irrigation until June 2008. Each value is a mean of 12 replicates ± standard error. Superscript letters indicate significant differences using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10.

Year	Irrigation Treatment	Yield (t ha^{-1})		Bunch No. (vine^{-1})		Yield / Pruning Weight	Yield / Canopy Area (kg m^{-2})
		Mean	SE	Mean	SE	Mean	Mean
2009							
	Control	41.8^{a}	1.2		*	21.0	1.55^{a}
	RDI	31.2^{b}	1.8			27.8	1.37^{b}
	PD	26.4^{c}	1.0			28.7	1.32^{b}
2010							
	Control	25.0^{a}	1.3	307	12	19.3	1.25
	RDI	21.3^{b}	1.4	330	18	22.6	1.05
	PD	21.3^{b}	1.3	304	13	22.0	1.19

Yield / pruning weight of control vines was marginally lower in 2009 (p = 0.079) than that of RDI vines, but not affected by any treatment in 2010 (Table 3). On the other hand, although there was no significant difference in canopy size between control and RDI in 2009/10 the significant difference in yield led to a significant effect on yield / canopy area. Comparing these data with those for seasons 2006-8 (Chapter 4, Table 4) it is clear that neither seasonal nor irrigation effects in yield have been driven by bunch number and that although irrigation treatments perturbed relationships between yield and pruning weight, inter-seasonal differences were equally large. Furthermore, the only significant effect in the four seasons of measurements on yield / canopy area (2009) was the result of a 'carry over' effect on growth rather than a direct effect of irrigation applied.

Leaf physiology

If the observed difference in soil moisture to 30 cm between the previously control irrigated vines and other treatments early in the 2008/9 season represented a limitation in available water, a difference in pre-dawn Ψ_l might be expected (Ritchie and Hinckley, 1975). However, neither pre-dawn nor midday Ψ_l was significantly different between any of the former irrigation treatments at flowering in 2008/9 or on any measurement date in either 2008/9 or 2009/10 (Figure 4). This was also the case for pre-dawn and midday Ψ_{sl} (Figure5). In fact, pre-dawn Ψ_l was higher than the estimated soil matric potential for the top 30 cm for the previously PD irrigated treatment, indicating that vine water status was not limited by having part of the root system in soil with a higher tension and suggesting that the difference in soil moisture between the vines previously control irrigated and the previously RDI and PD vines was unlikely to be determining differences in canopy growth. Past irrigation treatment also had no effect on g_s of outer or inner (data not shown) canopy leaves in either 2008/9 or 2009/10 (Figure 6).

* Data not collected in this season

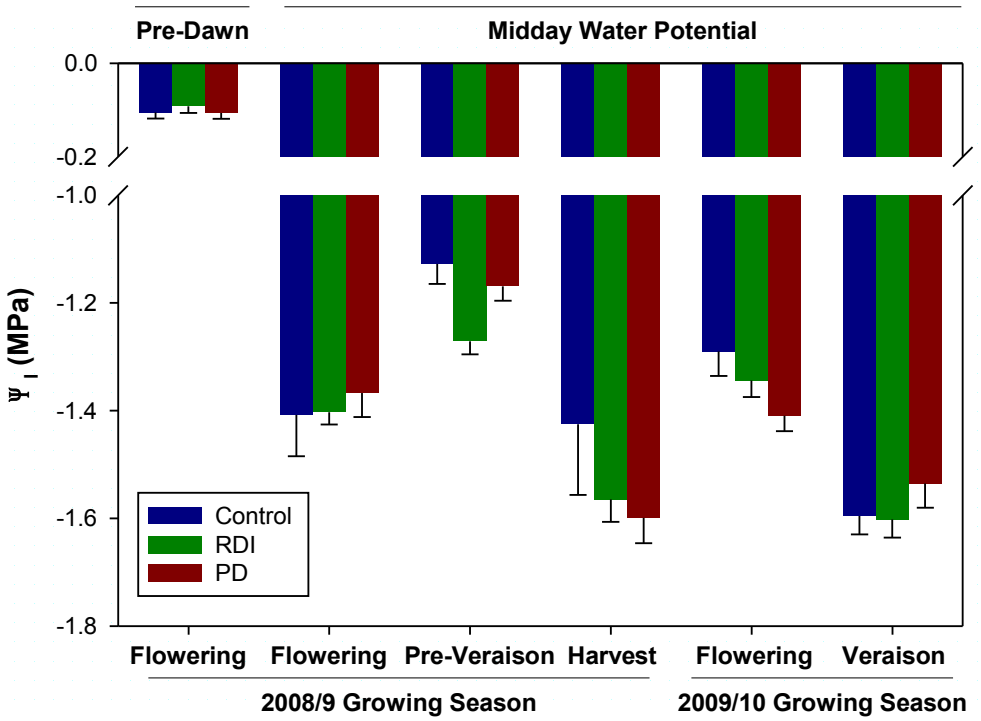


Figure 4. Pre-dawn and midday leaf water potential during the 2008/9 and 2009/10 growing seasons of outer canopy leaves (Ψ_l) of RDI irrigated Cabernet Sauvignon vines having previously received control, RDI or PD irrigation until June 2008. Each bar is a mean of at least six replicates – standard error.

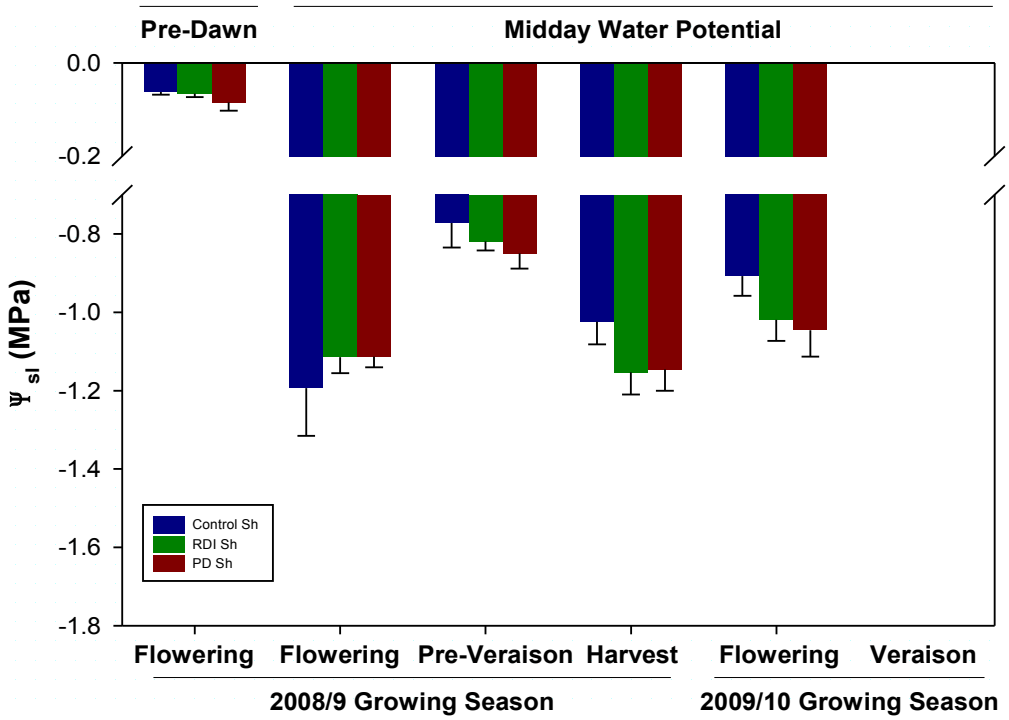


Figure 5. Pre-dawn and midday leaf water potential during the 2008/9 and 2009/10 growing seasons of inner canopy leaves (Ψ_{sl}) of RDI irrigated Cabernet Sauvignon vines having previously received control, RDI or PD irrigation until June 2008. Each bar is a mean of at least six replicates – standard error.

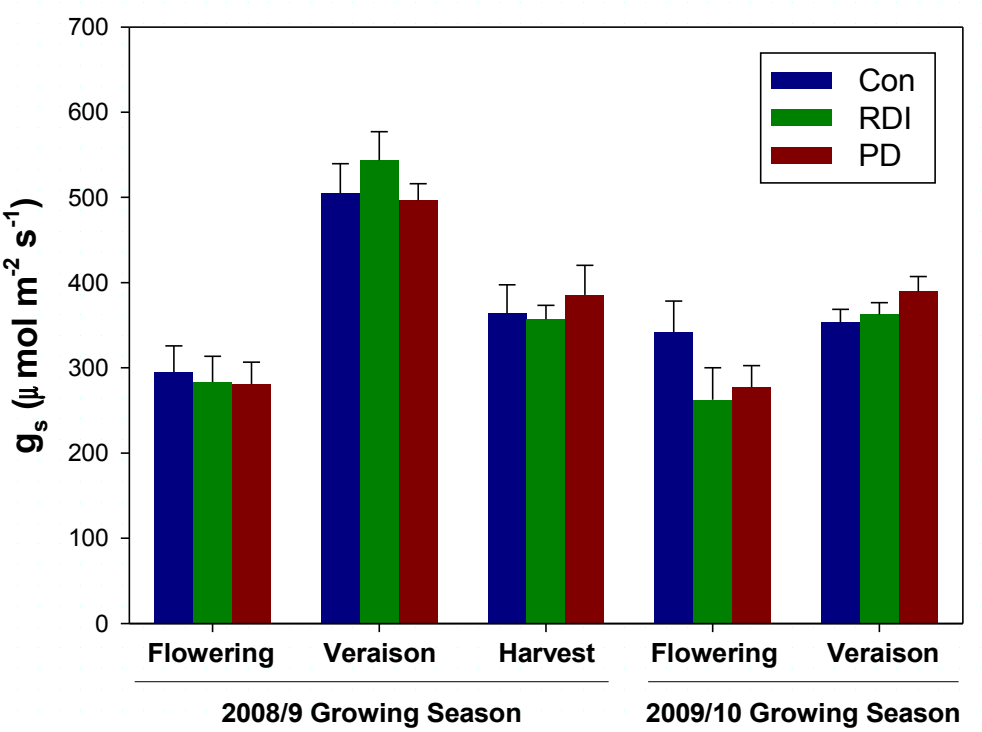


Figure 6. Stomatal conductance g_s of outer canopy leaves during the 2008/9 and 2009/10 growing seasons of RDI irrigated Cabernet Sauvignon vines having previously received control, RDI or PD irrigation until June 2008. Each bar is a mean of 12 replicates – standard error.

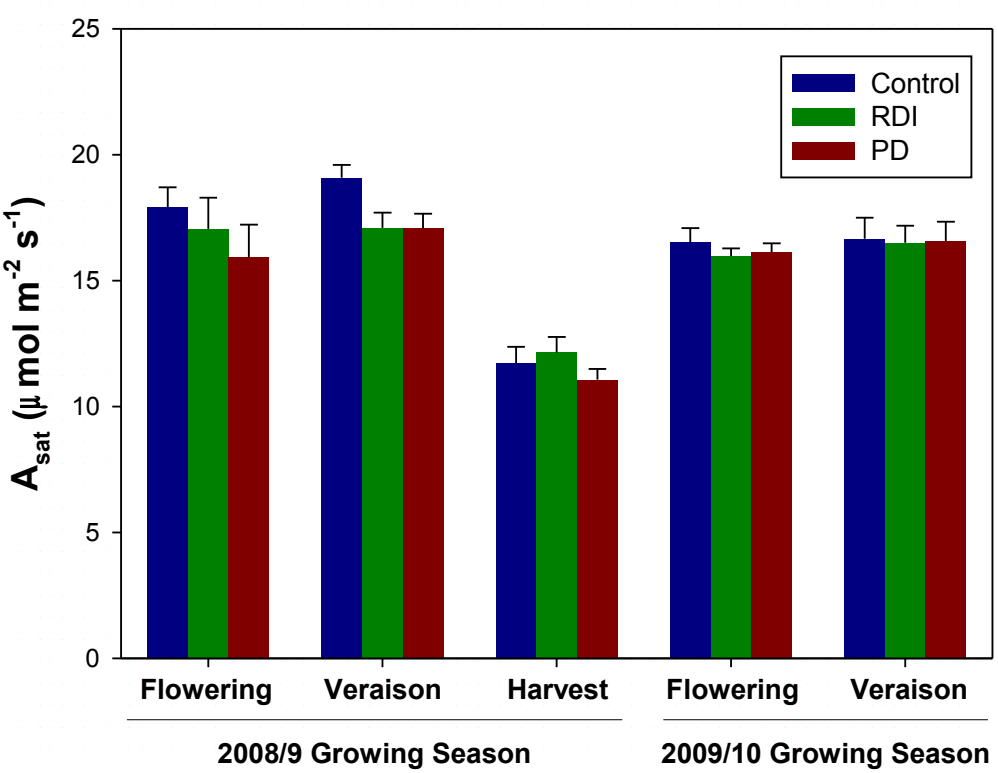


Figure 7. Assimilation under saturating light (A_{sat}) of outer canopy leaves during the 2008/9 and 2009/10 growing seasons of outer canopy leaves of RDI irrigated Cabernet Sauvignon vines having previously received control, RDI or PD irrigation until June 2008. Each bar is a mean of at least six replicates – standard error.

Both Ψ_l and g_s varied with time of season, with g_s being lowest when Ψ_l was lowest and vice-versa, as would be expected for typical leaf responses to soil moisture and VPD. Both parameters were lowest when soil moisture was lowest and highest when soil moisture was highest. Consequently, leaf water relations in all vines appeared to be reacting in the same way to their environment and there was no evidence that prior irrigation, or any carry-over effects of prior irrigation on soil moisture, were having an effect at the leaf level.

A_{sat} of outer canopy leaves was slightly higher in the previously control irrigated vines than the continuously RDI irrigated vines in three of four measurement dates. The exception was at harvest, when rates were significantly lower than earlier in the season in all treatments, but the difference was only significant at veraison (Figure 7). Other photosynthetic parameters were also unaffected by prior irrigation treatment, except for a small difference in *E* at veraison (Table 4).

Conclusions

There were no carry-over effects of past irrigation regime on the aspects of leaf function examined (except A_{sat} on a single occasion), these being similar in all treatments. There was also no interaction between intra-seasonal responses and past irrigation. However, both vine growth and fruit yield were affected by inter-seasonal carry-over from prior irrigation regime in both the 'recovery' seasons examined. Furthermore, the links seen throughout the project between canopy size and yield were broken by the change in irrigation regime.

Table 4. Transpiration (E), instantaneous water use efficiency (WUE_i), intrinsic water use efficiency (WUE_t), and carboxylation activity ($V_{c\ max}$),of outer canopy leaves of RDI irrigated Cabernet Sauvignon vines having previously received control, RDI or PD irrigation until June 2008. Each value is a mean of at least six replicates ± standard error. Superscript letters indicate significant differences within a measurement set using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10 and are only added where a significant effect was present.

Season	Period	Past Irrigation Treatment	E (mol m^{-2} s^{-1})		WUE_i (µmol mol^{-1})		WUE_t (µmol $mmol^{-1}$)		$V_{c\ max}$ (µmol m^{-2} s^{-1})	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
2008/9										
	Flowering	Control	4.63	0.15	3.87	0.15	88.9	3.2	140.4	2.9
	(10/11/08)	RDI	4.64	0.45	3.73	0.20	90.3	7.5	138.7	1.0
		PD	4.22	0.39	3.80	0.13	92.9	10.3	129.8	6.4
	Veraison	Control	8.78[a]	0.31	2.20	0.11	49.9	2.1	135.6	2.8
	(22/1/09)	RDI	7.56[b]	0.22	2.27	0.09	59.6	4.2	133.9	3.4
		PD	7.54[b]	0.32	2.29	0.11	58.0	4.1	129.0	3.3
	Harvest	Control	5.24	0.41	2.28	0.14	49.7	3.7	70.6	3.6
	(19/3/09)	RDI	5.13	0.53	2.52	0.27	58.9	6.5	78.8	2.5
		PD	5.43	0.22	2.04	0.05	52.7	3.4	71.3	3.0
2009/10										
	Flowering	Control	6.62	0.21	2.51	0.09	55.5	2.6	114.2	11.3
	(24/11/98)	RDI	6.36	0.22	2.53	0.12	55.8	2.3	106.5	7.3
		PD	6.51	0.12	2.48	0.05	51.2	1.6	105.4	5.2
	Veraison	Control	8.39	0.48	2.00	0.06	58.6	4.2	144.6	2.9
	(26/1/09)	RDI	8.40	0.35	1.98	0.07	56.7	2.3	140.7	4.6
		PD	8.31	0.16	2.00	0.06	59.2	3.6	144.9	3.8

Chapter 7

Grapevine canopy response to a high temperature event during deficit irrigation*

Introduction

A potential risk of using a deficit irrigation strategy in grapevines is that a high temperature event may occur whilst the crop is water stressed. For instance, an extreme heat event occurred during the PD deficit period in January 2008, affecting the experimental plot used in this project and resulting in greater stress at the leaf level than seen in previous seasons (see preceding chapter). There is relatively little known about the combined effect of heat and water stresses on C_3 plants, as most studies have examined the stresses individually. In wheat, water potential, osmotic potential and relative water content have all been found to be affected to a greater extent when high temperature and drought stresses are combined than when experienced separately (Machado and Paulsen 2001) as have photosynthesis and grain filling (Shah and Paulsen 2003). Similar effects have also been observed on grain filling in barley (Savin and Nicolas 1996) and on photosynthesis in the C3 grass Leymus chinensis (Xu and Zhou 2006). Metabolic profiling of Arabidopsis has indicated that applying heat and drought stresses together alters the expression of several hundred transcripts not affected by either stress applied individually (Rizhsky *et al.* 2004). Consequently it is unlikely that plant responses to the combined stress can be predicted from investigations of the individual stresses alone.

Little published data exist on grapevines exposed to an experimentally manipulated combination of water deficit and high temperature stress. This is despite numerous studies on grapevine water relations, a range of publications describing effects of high seasonal temperatures on grapevine phenology (Webb *et al.* 2007, Petrie and Sadras 2008) and anecdotal reports of yield reductions in response to high temperature events (Webb *et al.* 2009). However, 68% of the 2007 and 61% of the 2008 Australian winegrape harvests were produced in areas classified as 'warm climate' (Australian Wine and Brandy Corporation 2008), all of which are subject to mean January temperatures in excess of 23°C and daily maximum temperatures of over 40°C for an average of 4-5 days per season.

Although the likely increase in variability of Australia's air temperature due to climate change is small, the mean air temperature is expected to be between 2.8°C and 3.5°C higher than present by the end of the century and 0.4°C to 2°C higher by 2030, leading to an increase in the number of days that would currently be considered 'extreme' heat events (Christensen *et al.* 2007). For example, in the Sunraysia region, where this project was based, daily maximum temperatures have exceeded 45°C on six occasions since the year 2000, but only reached that maximum four times in the previous thirty years (Bureau of Meteorology, 2010). This warming is expected to be unequally distributed over the year, with greater warming in summer than winter (Suppiah *et al.* 2004), leading to significantly more days over a given temperature during the grapevine growing season than at present.

Consequently, it is vital that not only is the effect of higher growth temperatures on grapevines understood, but also any interaction with irrigation strategies that reduce the

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water status of the vine, potentially putting it at greater risk of damage during a high temperature event. This study used glasshouse based experiments with potted grapevines to examine the role of plant water status in the physiological response of the vine to a simulated high temperature event.

Materials and Methods

Plant Material

The experiments were carried out during the summer months of 2007/8 and 2008/9. In September of each year, cuttings of Cabernet Sauvignon grapevines (*Vitis vinifera* L.), clone G9V3 were obtained commercially (Victorian and Murray Valley Vine Improvement Association, Irymple, VIC, Australia), trimmed back to the topmost two buds and rooted in tubes of No. 1 Potting Mix (Envirogreen, Gepps Cross, SA, Australia) in a glasshouse. All flowers were removed as soon as they were distinguishable from new leaf growth in order to maximise vegetative production. The rooted cuttings were transplanted into 140 mm diameter pots and transferred to a shadehouse. In early December of each year they were transplanted again, into 250 mm diameter, 10.5 L, pots with a small amount of Osmocote All Purpose fertiliser (Scott's Australia, Baulkham Hills, NSW, Australia) and moved into a glasshouse, allowing direct solar illumination.

Experiment A

The potted cuttings were arranged on a steel mesh bench in a series of 18 blocks, each containing eight vines. The vines were individually watered using a 'trickler' adjustable dripper (Water Dynamics, Mildura, VIC, Australia), allowing the amount of water applied to each vine to be individually controlled. The vines were watered twice per day at 8 AM and 5 PM for 15 minutes, with the timing controlled by an automatic watering system. The watering fully saturated the soil each day. Three T-Tec 6-1 air temperature and %RH loggers (T-Tec, Henley Beach, SA, Australia) were placed in open-based boxes covered in aluminium foil, one at each end and one in the centre of the bench. The glasshouse was fitted with evaporative air conditioning, allowing the daytime temperature to be limited to 28°C, with night-time temperatures being uncontrolled. Excluding the heat stress event, the daily maximum glasshouse air temperature averaged 26.7°C with a standard deviation of 1.9°C and the daily minimum was 18.0 ± 2.2°C.

On 22 December 2008, four vines from each block were assigned at random to one of two irrigation treatments: 'full', approximately 400 ml per pot per irrigation, intended to saturate the soil, or 'deficit', approximately 30 ml per pot per irrigation. Irrigation events were reduced to once per day for both treatments to facilitate a rapid reduction in soil moisture in the deficit irrigation treatment. Volumetric soil moisture was measured on at least 16 pots per treatment using a TRASE TDR system (ICT International, Armidale, NSW, Australia) with 15 cm waveguides and demonstrated that the differing irrigation levels did lead to differing soil moisture in the pots, being 0.29 ± 0.01 and 0.12 ± 0.01 m^3 m^{-3} in the full and deficit irrigation treatments respectively. Volumetric soil moisture was also monitored in a limited number of pots (eight) on an hourly basis using 30 cm CS615 water content reflectometer sensors connected to a CR10X logger (Campbell Scientific, Townsville, QLD, Australia). These were assigned randomly to four pots for each irrigation treatment.

On 7th January 2009, during a period of sunny weather, a heat stress event was generated by adjusting the glasshouse temperature control to give a maximum daytime temperature over 40°C and minimum night-time temperature of 30°C. This was maintained for two days and returned to the previous setting of 28°C daytime and no minimum night-time temperature on the morning of 9 January 2009. Actual glasshouse temperatures reached a daily maximum of 41.4 and a minimum of 21.8 during the two days of heat stress. Half of the pots, two per treatment per block, were moved to an adjacent glasshouse chamber, maintained at a daily maximum of 28°C, for the duration of the heat event to act as ambient temperature controls. Air temperature on each day of the heat stress event exceeded that of the non-heated glasshouse by approximately 8:30 AM and reached a difference of over 10°C by 12:00 PM. Air temperature did not drop to that of the control glasshouse until the heat event ended, but the difference fell below 10°C at 7:00 PM.

All pots were watered up to field capacity and restored to 'full' irrigation on the evening of 16 January 2009. They were returned to two irrigations per day on 20 January 2009 and continued to be fully irrigated from this point on.

Experiment B

The potted cuttings were arranged on a steel mesh bench in a series of 24 blocks, each containing four vines. The vines were individually watered once per day at 8 AM as described above. Air temperature, %RH and soil moisture were also controlled and/or monitored as described for Experiment A. Prior to the heat stress event the daily maximum glasshouse air temperature averaged 30.2°C with a standard deviation of 2.0°C and the daily minimum was 21.9 ± 3.0°C.

Each vine within a block was assigned to one of four irrigation treatments: 'full', approximately 400 ml per pot, which was intended to saturate the soil, and 50%, 25% or 10% of full irrigation. Prior to irrigation treatments being applied on the 21 December 2007, all pots were given 'full' irrigation. Soil moisture was measured as described above by TRASE TDR in the afternoon prior to the heat treatment and was 0.15, 0.14, 0.11 and 0.09 m_3 m_{-3} in the full, 50%, 25% & 10% treatments respectively (SE of measurements was < 0.01 in each case).

On 3 January 2008, during a period of sunny weather, a heat stress event was generated by adjusting the glasshouse temperature control to give a maximum daytime temperature of 45°C and minimum night-time temperature of 30°C. This was maintained for two days and returned to the previous setting of 30°C daytime and no minimum night-time temperature on the morning of 5 January 2008. Air temperature on each day of the heat stress event exceeded that of the day before heating by approximately 9:30 AM and reached a difference of over 8°C by 1:00 PM. Air temperatures did not drop below those prior to the heat event at the same time of day until the heat event ended, but the difference fell below 8°C at 5:00 PM. There was no ambient temperature control for this experiment as it was short-term only, so the measurements made immediately before the heat event were deemed to be sufficient controls for those made during the heat event.

All pots were watered up to field capacity and restored to 'full' irrigation on the evening of 7 January 2008. From this point on all vines were fully irrigated until being harvested after leaf fall.

Stomatal Conductance

Stomatal conductance (g_s) was measured on the abaxial side of sunlit, young, fully expanded leaves in the late morning on at least eight vines per treatment, chosen at random, using a Delta-T AP4 Porometer (MEA, Magill, SA, Australia). A full set of measurements took approximately 1.5 hours to complete and the mid-point of the measurement period was typically between 10:30 and 11:00 AM. For both experiments measurements were made prior to the heat event (4 January 2009 & 2 January 2008), during the heat event (8 January 2009 & 3 January 2008), shortly after the heat event, but prior to re-watering (13 January 2009 & 7 January 2008) and after re-watering (20 January 2009 & 14 January 2008). Calibration was always performed immediately before a set of measurements in the ambient conditions, and typically a recalibration was performed half way through the measurements.

Leaf Water Potential

Leaf water potential of detached leaves was estimated by the balance pressure method using a 3000 series Plant Water Status Console (ICT International, Armidale, NSW, Australia), fitted with a digital peak hold meter (MEA, Magill, SA, Australia). Leaves were always placed under pressure within 10 s of removal from the vine, and the same vines were never used for consecutive sets of measurements to minimise any effect of leaf removal on subsequent growth.

Midday leaf water potential (Ψ_m) was measured during Experiment A on 8-12 sunlit, young, fully expanded leaves from 4-6 blocks per treatment at local solar noon two days prior to the heat event and on both days of the heat event itself. Pre-dawn leaf water potential (Ψ_{pd}) was measured during Experiment A on 12-16 leaves (6-8 blocks), two days prior to the heat event (5 January 2009), during the heat event (8 January 2009) and seven days after re-watering (23 January 2009). Ψ_{pd} was measured during Experiment B prior to deficit irrigation being applied (20 December 2007), during the heat stress (4 January 2008) and 18 days after re-watering (25 January 2008).

Photosynthesis

Leaf photosynthesis was measured during Experiment A on young, fully expanded leaves on the day prior to the heat event (6/1/2009) and on the first day of the heat event (7/1/2009). Light saturated assimilation (A_{sat}), transpiration (E), stomatal conductance (g_s) and leaf temperature were measured on 12-16 vines per treatment using a LiCor 6400 Portable Photosynthesis System (John Morris Scientific, Balwin, VIC, Australia). The reference air CO2 concentration was maintained at 400 ppm and the air temperature within the LiCor 6400 measurement chamber was maintained at the same temperature as the air within the glasshouse. No attempt was made to alter the humidity of the air within the leaf chamber of the LiCor 6400. In addition, instantaneous water use efficiency (WUEi) was calculated as A_{sat}/E. The in vivo maximum carboxylation activity of Rubisco (V_{cmax}) was calculated from single Asat readings using the C_3 photosynthesis model of Farquhar, von Caemmerer and Berry (1980) as described in Harrison *et al.* (2009) then adjusted to a common temperature of 25°C using the temperature dependence functions of von Caemmerer (2000).

Leaf Damage & Biomass Estimates

An arbitrary scoring system was used to estimate the permanent leaf damage occuring during the heat stress treatment. In Experiment A, a random selection of eight vines per treatment were assessed, whereas in Experiment B every vine was assessed. Leaf damage was defined as dead/dry leaves or areas of leaf; vines were scored as having no evidence of leaf damage, up to 25% loss of leaf area, 25-50% loss, 50-75% loss or 75-100% loss of leaf area. Leaf area loss was estimated visually.

During Experiment A whole plant biomass and it's components (leaves, new canes, trunk and roots) were measured using a destructive harvest of eight vines per treatment immediately after the heat event (9/1/09). After being separated into their components the vine parts were oven dried at 60°C and weighed to an accuracy of 1 mg.

Statistical Analysis

Data in the text are presented as mean ± standard error. All statistical analysis was carried out using Genstat version 11 (VSNi Australia, Helensburgh, NSW, Australia), except the Fisher's exact tests, which were calculated using R (R Foundation, Vienna, Austria). Analysis of variance (ANOVA) was used for all measurements except the leaf damage scores and data were analysed either with a one-way ANOVA, using block as a random factor, for measurements made on a given day or with a repeated measures ANOVA across two or more sets of measurements. Blocking was not able to be used in some repeated measures ANOVAs due to measurements necessarily being made on plants from different blocks on different days (e.g. leaf water potential). Unpaired, two-tailed Student's t-tests were used to compare two specific groups of data, e.g. comparison of a measured variable in a single treatment on two different days only.

The leaf damage scores for Experiment B were analysed as a comparison of frequency distributions using Fishers exact test for count data, applied to a 5 x 4 table (score x treatment). Individual comparisons were then made using a 5 x 2 table for each pair of treatments.

A p value under 0.05 was taken as significant unless otherwise stated. Means and frequency distribution comparisons were made using a Bonferonni adjustment, and an experimentwise probability of 0.10, generally providing a comparisonwise probability <0.020.

Results and discussion

Leaf physiology

The 'deficit irrigation' used in this study was deficit in the broad sense that less water was applied than the plant would potentially use, it was not 'regulated' in that it did not track a specific fraction of potential crop evapotranspiration (ET_{crop}). This was used to produce plant material at a range of water deficits with the aim of determining how vines under differing degrees of water stress responded to an additional heat stress. Leaf level responses to the deficit irrigation alone in both experiments were typical of C_3 plants (e.g. Davies and Kozlowski 1977, Thomas and Easmus 2002), grapevines (Bota *et al.* 2001) and Cabernet Sauvignon in particular (Nikolaou *et al.* 2003), in that g_s (Figure 1) and Ψ_{pd}

(Figure 2) were reduced in deficit irrigated vines in both experiments (pre-heat stress data) and that in Experiment B the reduction was greater with decreasing soil moisture. The deficit irrigation treatment in Experiment A and the 10% irrigation treatment in Experiment B both resulted in g_s approximately one third of the well watered vines. It was, perhaps, surprising that in the latter experiment there was no significant difference in g_s between the 50% and full irrigation treatments. However, the continuous soil moisture monitoring (data not shown) indicated that, despite the difference in water applied, at the time of the heat event the difference in soil moisture between these two treatments was slight and only lasted for a short period after irrigating.

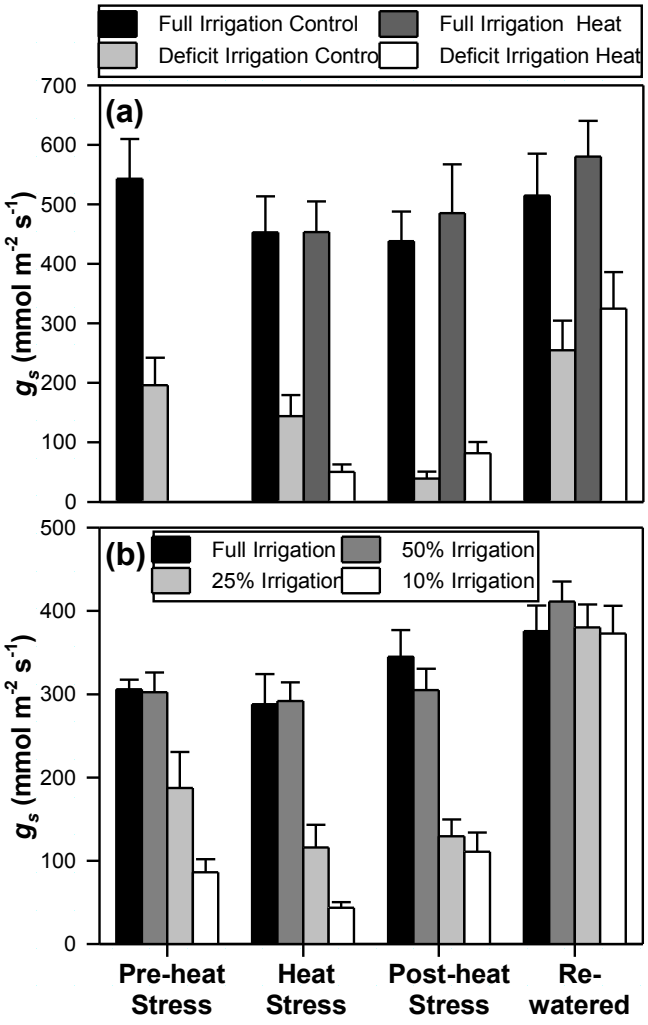


Figure 1. Stomatal conductance (g_s) of vines during irrigation treatments alone (pre-heat stress), two days of heat stress with irrigation treatment (heat stress), recovery from heat stress whilst irrigation treatments were maintained (post-heat stress) and recovery from irrigation treatment (re-watered) in a) Experiment A: full and deficit irrigation with a daily maximum temperature >41°C or <28°C and b) Experiment B: full, 50%, 25% and 10% irrigation with a daily maximum temperature >45°C. Each bar is a mean of at least eight measurements on separate plants + standard error.

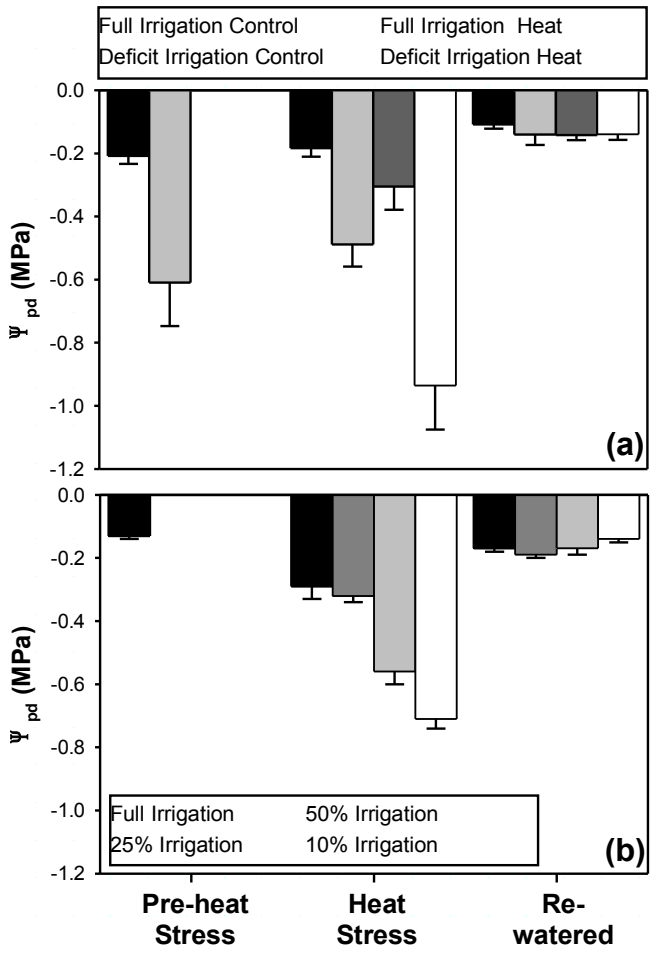


Figure 2. Midday leaf water potential (Ψ_m) during Experiment A with full or deficit irrigation; prior to heat stress and during one or two days of heating at > 41°C. Each bar is a mean of 12 measurements on separate plants + standard error.

In contrast to these results, Ψ_m of vines in Experiment A two days before the heat event, was not affected by the irrigation treatments, being approximately -1.2 MPa in both (Figure 3), despite the significant effects on stomatal conductance, soil moisture and Ψ_{pd} measured on the same day being three times lower in the deficit irrigated vines than in the fully irrigated vines (Figure 2a).

Repeated measures ANOVA of Ψ_m during the two days of the heat event demonstrated that Ψ_m was significantly lower in both full and deficit irrigated vines than the non-heated control vines and that Ψ_m was also more negative in deficit irrigated vines than fully irrigated vines, with no significant difference between the days (Figure 3). The relative differences in Ψ_{pd} between the deficit irrigated heated vines and fully irrigated heated vines during the heat event of Experiment A were much greater than seen in Ψ_m (Figure 2a), with the non-heated vines not being significantly different than before the heat event.

In Experiment B, Ψ_{pd} of vines subjected to irrigation treatment alone was not measured, although as with Experiment A there was no significant effect of irrigation on Ψ_m just prior to the heat event (data not shown). During the heat stress Ψ_{pd} of vines in all irrigation treatments were lower than Ψ_{pd} in fully irrigated vines measured before irrigation treatments were imposed and decreased with decreasing irrigation (Figure 2b).

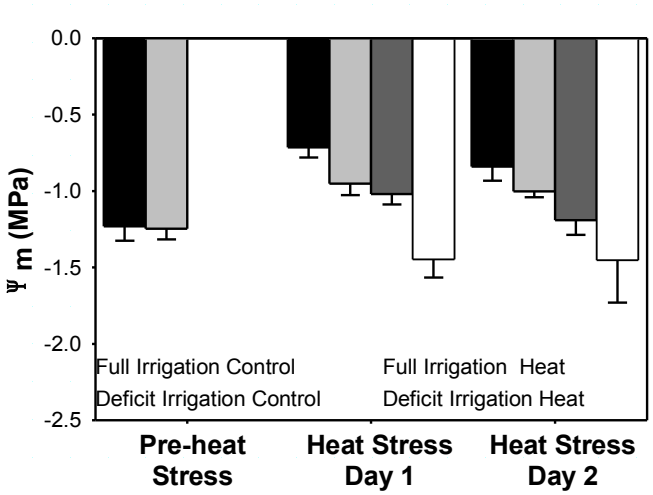


Figure 3. Pre-dawn leaf water potential (Ψ_{pd}) of vines during irrigation treatments alone (pre-heat stress), two days of heat stress with irrigation treatment (heat stress) and recovery from irrigation treatment (re-watered) in a) Experiment A: full and deficit irrigation with a daily maximum temperature >41°C or <28°C and b) Experiment B: full, 50%, 25% and 10% irrigation with a daily maximum temperature >45°C. Each bar is a mean of at least eight measurements on separate plants - standard error.

There is generally little evidence for any direct effect of heat on gs in C_3 plants (Warrit *et al.* 1980, Sepúlveda and Kliewer 1986), although positive responses to temperature may occur (Sheriff 1979, Lu *et al.*, 2000). However, in the field environment a decrease in g_s with increasing temperature is often seen due to changes in the vapour pressure deficit (VPD) of the air (Lange *et al.* 1971, Schulze and Hall 1982) although it is thought that changes in g_s are largely determined by transpirational flux rather than a direct effect of VPD (Mott and Parkhurst 1991).

In fact, despite air temperatures over 40°C in Experiment A and 45°C in Experiment B, there was no significant effect of the heat stress event on late morning g_s of well watered vines in either experiment (Figure 1). Field heating of well watered Shiraz grapevines to 42°C found that not only was there no decrease in g_s but that at certain times of the day there was actually an increase in g_s of heated vines (Soar *et al.* 2009), although Shiraz is a variety known for poor stomatal control (Soar *et al.* 2006).

In this study all heated vines experienced the same VPD and air temperature within each experiment, but only the deficit irrigated treatments exhibited a response in g_s. ANOVA analysis indicated that despite the low g_s of the non-heated controls in Experiment A the g_s of heated deficit irrigated vines was significantly reduced by more than 40%. Similarly in Experiment B, g_s of the 10% and 25% irrigated vines was 38-49% lower when heated than at the same time on the previous day, though this was only significant for the 10% irrigation treatment.

These data match previous observations in other species that have shown stomatal control of transpiration in response to VPD to be greater in droughted than well watered plants (e.g. Thomas and Eamus 1999, Turner *et al.* 1985). Indeed, in Experiment A

transpiration of deficit irrigated vines was 27% lower when heated than the non-heated deficit irrigated controls, whereas transpiration of fully irrigated vines when heated was over 300% of that of the controls due to the differential effects on g_s. The difference in maximum VPD was over 375%, from 1.5 to 5.7 kPa, indicating that the hydraulic system could maintain an adequate water flow to the leaves, even under very heavy evaporative demand, providing that adequate soil water is available.

Transpiration was not directly measured in Experiment B, but an approximation of transpiration can be calculated using VPD x g_s (after Aparicio-Tejo *et al.* 1980). Using this, the observed differences in g_s between the morning of the first day of heat stress and the morning of the previous day in Experiment B indicated that transpirational water loss during the heat stress was probably similar to the previous day (without heat stress) in the two drier treatments, but about 50% higher in the two wetter treatments (data not shown). Thus also indicating a much stronger stomatal response to VPD in the deficit irrigation treatments.

The mass flow of water from the leaf as transpiration is a primary determinant of Ψ_m and the observation that the transpiration rate of the deficit irrigated vines in Experiment A was lower than the fully irrigated vines probably explains the lack of any difference in Ψ_m between those vines prior to the heat treatment (also see below). There is little evidence of Ψ_m having any direct control over stomatal opening (Tardieu *et al.* 1996), although correlations between Ψ_m and g_s are commonly observed. In this study the effect of the heat event on Ψ_m appears to be independent of that on g_s, as heating lead to a decrease in Ψ_m irrespective of irrigation, but there was only a heat effect on g_s in the deficit irrigated vines. The large difference in transpiration may well explain the former observation, but there was no evidence for transpiration driving the latter. One explanation might be that greater draw-down of the limited soil moisture was occurring after the daily irrigations in the deficit irrigated vines. Temperature may also have been having a direct effect on components of Ψ_m other than leaf water content, such as the solute potential.

It might be expected that the large difference in transpiration rate between the fully and deficit irrigated vines during heating would have an effect on leaf temperature due to differences in evaporative cooling. Indeed the leaf temperature data collected with the photosynthetic measurements demonstrated that the deficit irrigated vines did have hotter leaves, being only 0.07±0.22°C cooler than the air in the leaf chamber compared with 2.65±0.36°C cooler for the fully irrigated leaves. This difference was somewhat larger than the 1-1.8°C difference seen between leaves of well watered and droughted field grown rice (Jones *et al.* 2009). Field grown Cabernet Sauvignon with high and low irrigation regimes and g_s of 524 ± 24 and 233 ± 16 mmol m^{-2} s^{-1}, similar to the rates in Experiment A here, exhibited a 4.69°C difference in whole of canopy temperature estimated using an infra-red thermometer (Loveys BR, 2009, unpublished data). Loveys *et al.* (2008) also observed a difference in canopy temperature of over 2°C in non-irrigated Shiraz vines compared with irrigated vines, the same vines had a decrease in g_s of over 50%. Clearly deficit irrigation results in higher leaf temperatures and deficit irrigated vines are therefore more likely to reach any threshold temperature for tissue or photosynthetic apparatus damage should an extreme high temperature event occur.

Photosynthesis (A_{sat}) of the vines was reduced by 30% during deficit irrigation and 22% during heating (Figure 4b), a similar magnitude to the effect seen in well watered Semillon grapevines heated to 40°C at flowering (Greer & Weston 2010). In contrast A_{sat} of deficit

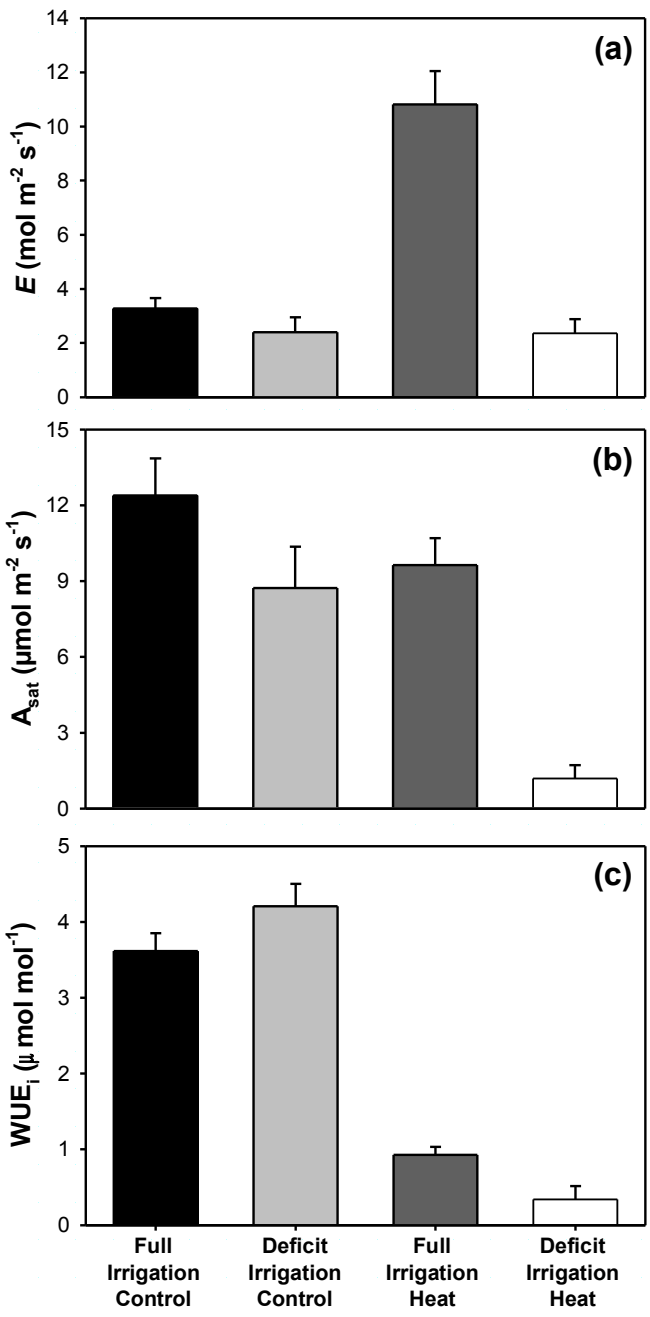


Figure 4. Leaf gas exchange during Experiment A of vines with full or deficit irrigation and exposed to a daily maximum temperature >41°C; a) assimilation under saturating light (A_{sat}), b) transpiration (E) and c) instantaneous water use efficiency (WUE_i). Each bar is a mean of 12 measurements on separate plants + standard error.

irrigated vines during the heat event was almost 85% less than the non-heated deficit irrigated vines and 90% less than the control vines. Despite this there was no difference in intercellular CO_2 concentrations (C_i) during heating, being 320 ± 20 and 291 ± 8 ppm for deficit and fully irrigated vines respectively. Consequently, although g_s of the deficit irrigated vines was lower when heated this did not account for the reduction in A_{sat}, which was entirely attributable to reduced carboxylation activity rather than being an effect of g_s.

Indeed, V_{cmax} adjusted to 25°C was only 10% of the non-heated deficit irrigated plants on the same day (3.81 ± 1.62 cf. 38.9 ± 6.03 µmol m^{-2} s^{-1}) indicating a more direct effect of heat on the photosynthetic apparatus, probably high temperature damage to photosystem II (Berry and Björkman 1980, Allakhverdiev *et al.* 2008). Furthermore, whilst the effect of heating on Asat of the fully irrigated vines was not significant, V_{cmax} at 25°C was significantly reduced (28.7 ± 3.0 and 52.5 ± 6.0 µmol m^{-2} s^{-1} for heated and non-heated vines respectively), indicating damage to the photosynthetic apparatus or down-regulation of Rubisco. Future examination of chlorophyll fluorescence in heated and droughted vines would provide useful data.

Instantaneous water use efficiency (WUEi = A_{sat} / E) of non-heated deficit irrigated vines was significantly higher than that of fully irrigated vines (Figure 4c). Such a response to water deficit is common in C_3 plants and has notably been observed in the drought adapted *Vitis* hybrid Richter-110 (Pou *et al.* 2008). Heating greatly reduced WUEi in fully irrigated vines, but due to the almost total loss of photosynthetic activity in the heated deficit irrigated vines WUEi was significantly lower again despite the lack of a heating effect on E.

Given the low Ψ_m and g_s measured during the first day of heat stress and the fact that irrigation was not applied until after dawn, it was notable that previously wilted leaves no longer appeared wilted (visual observation only) in all deficit treatments in both experiments by the time of pre-dawn measurements (approximately 4 AM) on the following day. However, Ψ_{pd} of heated vines was still lower than in non-heated vines or those measured prior to treatments being applied. Ψ_{pd} is generally assumed to be in equilibrium with soil moisture and thus more likely to vary with differences in irrigation. Soil moisture was not measured in non-heated pots so it is not possible to say whether the heat event resulted in greater losses of water from the soil, although it seems likely. Heated vines invariably had a lower Ψ_{pd} than non-heated vines which might reflect a difference in soil water availability during the heat event, be a direct effect of the heat stress on the ability of the vine to transport water into the leaf or a combination of the two.

In both experiments Ψ_{pd} of deficit treatments during the heat event was below -0.4 MPa and that of well watered treatments below -0.2 MPa, indicative of severe and moderate water stress respectively in grapevines (Carbonneau 1998), though any such categorisation is affected by many other factors. There is a significant volume of literature examining the relative merits of Ψ_m and Ψ_{pd} as indicators of stress in grapevines. For example, Sousa *et al.* (2006) concluded that Ψ_m was a poor measure of water stress after finding no significant relationship between irrigation regime and Ψ_m. A weak or no relationship between Ψ_m and a range of photosynthetic parameters has also been noted in a number of other studies (e.g. Medrano *et al.* 2003, Choné *et al.* 2001). In contrast, Ψ_m has been used to schedule differing irrigation regimes in grapevines (Girona *et al.* 2006). The isohydric like behaviour of these Cabernet Sauvignon vines in the data presented here, shown by the lack of any irrigation effect on Ψ_m before the heat stress treatment and the only slight response during it, indicates that Ψ_m is of limited use as an index of environmental risk. Whereas, the interactions between irrigation treatment and temperature on other measured parameters, A_{sat} and leaf damage for example, were well matched by Ψ_{pd}.

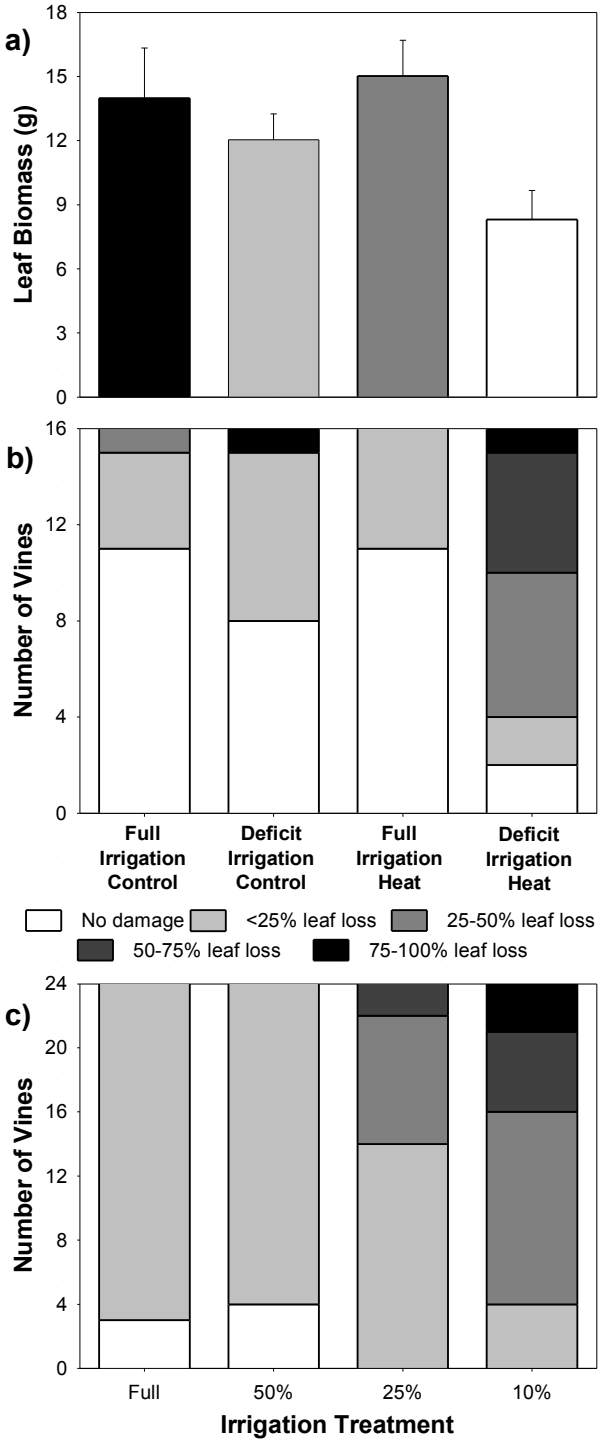


Figure 5. Effect of irrigation treatment on a) dry mass of live leaves with each bar being a mean of eight vines ± standard error, b) visual estimates of leaf area loss following two days with a daily maximum temperature >41°C during Experiment A and c) visual estimates of leaf area loss following two days with a daily maximum temperature >45°C during Experiment B. Each section within a bar represents the number of vines scored for no loss, 0-25%, 25-50%, 50-75% or 75-100% loss of leaf area.

Canopy damage

The effect of combination heat and water stress was most clearly seen in the loss of leaf area. The harvest of vines after the heat event in Experiment A found no significant difference in leaf biomass between the fully irrigated vines, heated or not, or the deficit irrigated vines that had not been heated. However, there was a significant 45% reduction in leaf biomass of the deficit irrigated vines that had been subjected to the heat event (Figure 5a), but no effect of any treatment on the other components of plant biomass (stem, cane and root).

The visual scoring of leaf area loss indicated minor damage in the non-heated vines, which was caused by handling of those vines and, to a lesser extent, a small amount of senescence of lower canopy leaves. Heating did not result in any additional leaf area loss to the fully irrigated vines, but the deficit irrigated vines were affected to a much greater extent, with three-quarters losing 25% or more of their leaf area (Figure 5b).

Prior to the heat stress treatment in Experiment B there was no visible leaf damage or wilting, even in the vines receiving only 10% of full irrigation. During the first day of heat treatment wilting of leaves and growing shoot tips occurred in all the irrigation treatments, but was most apparent in the 10% and 25% treatments. Furthermore, some shoot tips, expanding leaves and recently expanded leaves appeared to dry up and die in these treatments. Wilting occurred again during the second day of heat stress, but did not appear more severe than on the first day.

Visual estimates of the loss of leaf area after heating were similar in the full and 50% irrigation treatments and not significantly different, with all 24 vines in each treatment having between 0 and 25% loss of leaf area (Figure 5c). Damage was greatest in the vines receiving 25% irrigation, with 10 of 24 vines losing more than a quarter of their leaf area, and in vines receiving 10% irrigation, where 20 vines had more than 25% loss of leaf area and eight vines had more than 50% loss of leaf area. In both severe deficit treatments leaf loss was significantly more than in the two higher irrigation treatments. Furthermore, the difference in leaf loss between the 10% and 25% irrigation treatments was also significant.

The ability of the vines to withstand and recover from heat shock is at the upper end of the range observed during a survey of 54 warm climate Australian native sclerophylous species subjected to a similar heat shock to these experiments (>45°C for two days), which resulted in up to 73% of species losing up to 89% of their leaf area (Groom *et al.* 2004). The result of such leaf loss is a lower leaf area ratio (leaf area/plant dry mass), which, in terms of growth analysis and assuming that net rates of photosynthesis are unchanged, leads to a reduction in relative growth rate (dry matter accumulation per unit of dry matter per day). Consequently, if a heat event occurs during the period of active canopy growth, precisely the period where deficit irrigation is typically applied, the effect of such leaf loss on whole of season vine carbon accumulation will be proportionally greater than the loss of leaf area itself.

Post-treatment recovery

Sepúlveda and Kliewer (1986) reported the recovery of g_s in three grape cultivars following heat stress of 40°C for several days within two to five days, although these were all

maintained at full soil moisture throughout the heat period. As discussed above, in our study g_s of well watered vines was not affected by heat stress, but there was evidence that g_s of deficit irrigated vines could recover to pre-heat stress values in a similar time frame even whilst still deficit irrigated. In Experiment A there was no significant difference in g_s between heated and non-heated vines four days after the heat stress period, irrespective of irrigation treatment (Figure 1a). Furthermore, in Experiment B g_s of the 10% irrigated vines had recovered to pre-heat stress values three days after the heat stress (Figure 1b). This may have been assisted by the reduced leaf area in the heated, deficit irrigated vines, which should have resulted in improved water availability on a leaf area basis in those treatments, but there was certainly no evidence that the heat stress had resulted in any longer-term impairment or alteration of stomatal function. Indeed, although the difference was not quite significant ($p=0.076$) the heated, deficit irrigated vines in Experiment A had higher g_s than those that had not experienced any heat stress.

Once full irrigation was restored to the deficit treatments there was a relatively rapid recovery of g_s with partial recovery within 24 hours in Experiment A (data not shown). In Experiment B, g_s of the 25% irrigation vines had fully recovered in less than 48 hours and g_s of the 10% irrigated vines more than doubled to approximately 70% of the g_s of the fully irrigated treatment (data not shown). Full recovery of all treatments in Experiment B had occurred by seven days after re-watering (Figure 1b) and Ψ_{pd} measurements were no different to those prior to the irrigation treatments (Figure 2b). The latter measurements were not made until more than two weeks after re-watering, but demonstrated that neither the deficit irrigation, nor the combination with heat stress resulted in any longer-term effect on Ψ_{pd}. However, in Experiment A g_s of deficit irrigated vines was only about half of that in the fully irrigated vines four days after re-watering (Figure 1a). Düring (1988) found that time for recovery of g_s from stress after re-watering typically took three to five days, but was cultivar dependent. The slower recovery of the deficit irrigated vines in Experiment A and the 10% treatment in Experiment B could have been due to the greater damage caused by the heat stress period, or be due to the more severe water stress experienced. However, the heat event was more severe in Experiment B than Experiment A (45°C cf. 41°C), so if heat stress had been the cause the expectation would be that the Experiment A vines should recovery more quickly rather than the reverse. The time for recovery following re-watering may be dependant on the degree of the water deficit, as seen in Acacia spp exposed to water stress (Liang and Zhang 1999). However, soil moisture in the deficit irrigation treatment of Experiment A was similar to the 25% irrigation treatment in Experiment B. On the other hand the period of deficit was longer in Experiment A (25 cf. 17 days). Consequently, it may be that the length of time a deficit is applied prior to re-watering is important in determining the recovery period duration, but neither experiment addressed that directly.

Extrapolating from a pot experiment to the field is often difficult (Passioura 2006) due to many factors, particularly rooting volume. A further factor in this work is the lack of any fruit load on the vines, thus altering the source-sink balance. However, it might be expected that the short-term leaf level responses of fruited vines to a given combination of drought and heat stress would be similar to those reported here. Another difference is that for a given temperature VPD is likely to be higher in the field than a glasshouse, so VPD driven responses, such as effects on transpiration, may be larger.

Conclusions

The greater use of deficit irrigation strategies and the likely effects of climate change will increase the probability of vines being water stressed when a high temperature event occurs. Short-term leaf physiology responses to a high temperature event, including stomatal conductance, pre-dawn leaf water potential and net photosynthesis, were more severe in deficit-irrigated grapevines. Furthermore, the loss of leaf area due to heat stress was also higher in deficit irrigated vines. Consequently, growers need to carefully manage vine water status, using irrigation scheduling to ensure that root-systems have access to water and that vines are actively transpiring prior to a heat event occurring.

Chapter 8

Inter- and intra-seasonal effects of deficit irrigation and past irrigation strategy on grape maturation, yield components and wine quality

Introduction

Irrigation regime has the potential to affect fruit maturation and quality, as shown previously at this site (Cooley *et al*. 2005, Ashley *et al*. 2006). Furthermore, the impact of the intra-seasonal carry-over effect on leaf photosynthesis in the PD irrigated vines (Chapter 5) was likely to have reduced the amount of fixed carbon during the maturation period. This reduced vine growth and carbohydrate pools (Chapter 4), but could still have had an effect on sugar available for berry ripening.

For four of the five seasons that this project covered, 2006-2009, fruit maturation was examined from the point where berry soluble solid concentration reached approximately 18°Brix, typically the last week of January each year. This covered all three seasons where the control vines received double irrigation and the first season that all treatments were put back to the standard RDI strategy.

Changes in fruit quality attributes impact wine composition and quality attributes. Even minor effects can be magnified by the winemaking process leading to a larger impact on wine quality (Ashley *et al*. 2006, Clingeleffer 2009). Consequently, small lot wines were made for each treatment in each season, 2006-2010, including the Shiraz fruit resulting from the additional PD trial (Chapter 9).

Materials and Methods

Maturation analysis

For each vintage from 2006 to 2009 grape maturation series were conducted by taking 5-6 bunches from each of the 36 replicates at weekly intervals from late January. Initial sampling dates and total soluble solids concentration (TSS) for each treatment in each year is given in Table 1. At each sampling date bunches were taken from all upper and lower sections of a given vine on the North and South side of the row. One vine per replicate was sampled on a given date and vines were not sampled more than once in any given season. Sampling was repeated at approximately weekly intervals until harvest.

For each sampling date 100 berry sub-samples were taken at random, weighed, then crushed to extract juice without maceration in a plastic bag using a rubber pestle. The 'free run' juice was then centrifuged in 50 ml centrifuge tubes for 10 minutes at 3500 rpm using a Eppendorf 5810 R centrifuge. TSS of the centrifuged juice sample was determined using a Atago temperature compensating digital refractometer and pH and titratable acidity determined using a Metrohm Titrino auto-titrator. Sugar per berry was calculated as berry fresh weight * TSS.

In addition, bunch weight and number of berries per bunch was determined in 2006, 2007 and 2008 using five bunches per replicate. Berry dry weight was determined in 2007 and 2008 by taking a separate 50 berry sub-sample and oven drying at 60°C. The sample was weighed at weekly intervals to determine the point at which no further loss of mass was

detectable and this point defined as berry dry weight. Oven drying typically took six weeks. Berry water content was calculated using the fresh and dry weights and soluble solids per berry calculated as TSS * berry water volume. Berry dry mass was then apportioned to soluble solids and non-soluble solid fractions for these samples.

Winemaking and wine analysis

Each vintage three 35 kg replicate wines were made for each treatment from a combined crush of all the harvested fruit by Provisor at CSIRO Merbein. The exception was in 2008 where four replicate wines were made. The grapes were cooled overnight at 1.0°C prior to crushing and de-stemming. Tartaric acid was added to the must to adjust pH to 3.4. SO2 additions (1ml/L of 10% solution) were also made at that time. Fermentation was conducted at 18°C using EC118 yeast with the cap plunged twice daily. The wines were pressed when total soluble solids reached approximately 3°Brix and fermentation maintained until 'dry' (residual sugar <2.5 g/L). The wines were then racked, pH adjusted (if required), free sulphur adjusted to 30 ppm and CuSO4 added (1ml/L of 0.4% solution) prior to settling for 14 days at 18°C. The wines were racked with pH and free sulphur again adjusted. After cold stabilistation for a further 20 days at 1°C, pH and free sulphur were again adjusted, if required, prior to filtering and bottling.

After bottling an aliquot of each wine replicate was analysed spectrophotometrically for colour density, colour hue, ionised anthocyanins, and total phenolics in each year except 2010, for which the wines weren't available at the time of writing, according to the methods of Sommers and Evans (1977) using a GBC UV/VIS 918 spectrophotometer (GBC, Dandenong, VIC, Australia). A further aliquot of each wine was used to estimate total tannins in epicatechin equivalents according to Sarneckis *et al.* (2006).

Wine sensory analysis

Sensory analysis was conducted by Provisor on behalf of the CSIRO Food Futures Flagship. Difference testing was carried out to establish whether wines made from the different treatments showed perceivable differences. A panel of trained assessors performed duo-trio tests under standard conditions. In each comparison, the first sample presented was a labelled reference, followed by two other coded samples, one of which was identical to the reference. The instructions to panellists were to smell and taste the samples and identify the same sample as the reference.

Descriptive analysis was then conducted to identify and quantify the detailed sensory properties of wines. A trained panel developed a list of key attributes for the trial wines in a series of training exercises designed to familiarise assessors with the samples and to record the main product differences. For the final rating, each sample was assessed three times per person in a randomised order. Specific attributes were rated on a 0-15 scale, with anchors of 'none' and 'intense' placed at each end of the scale.

Results and Discussion

Maturation series

In 2006 the maturation series was started a week earlier than in subsequent years and consequently, TSS at the first sampling date was lowest in this season (Table 1).

However, at the second sampling date (02/02/2006) TSS was 17.8±0.4, 18.1±0.3 and 17.4±0.3°Brix for the control, RDI and PD treatments respectively. Consequently, the seasonal variation was actually very slight, with 2009 having the lowest TSS at the end of January.

Table 1. Initial sampling date and total soluble solids concentration of Cabernet Sauvignon grapes irrigated with control, RDI or PD irrigation strategies (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009). Each value is a mean of 12 replicates ± standard error. Superscript letters indicate significant differences using mean comparisons with the Bonferonni adjustment and an experimentwise significance level of 0.10.

Season	First sampling date	Number of sampling dates	Total soluble solids of first samples (°Brix)					
			Control		RDI		PD	
			Mean	SE	Mean	SE	Mean	SE
2005/6	23/01/06	6	14.4	0.5	15.0	0.2	14.5	0.3
2006/7	30/01/07	5	18.1	0.3	18.4	0.3	18.3	0.3
2007/8	30/01/08	7	18.4[a]	0.3	18.3[a]	0.3	17.2[b]	0.4
2008/9	02/02/09	7	16.2	0.3	19.9	0.3	16.7	0.3

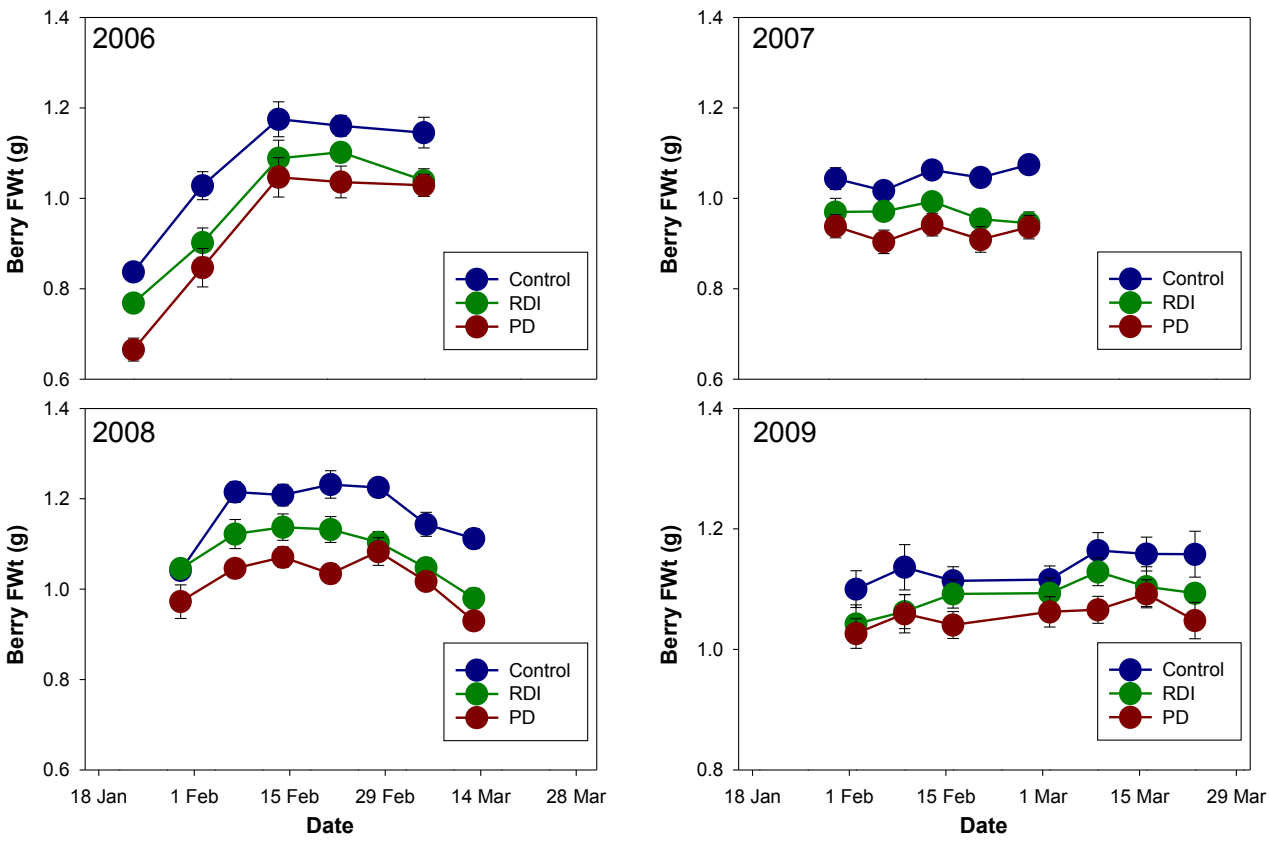


Figure 1. Berry fresh weight (FWt) of Cabernet Sauvignon fruit from vines receiving control, RDI or PD irrigation (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009). Each point is a mean of 12 replicates ± standard error.

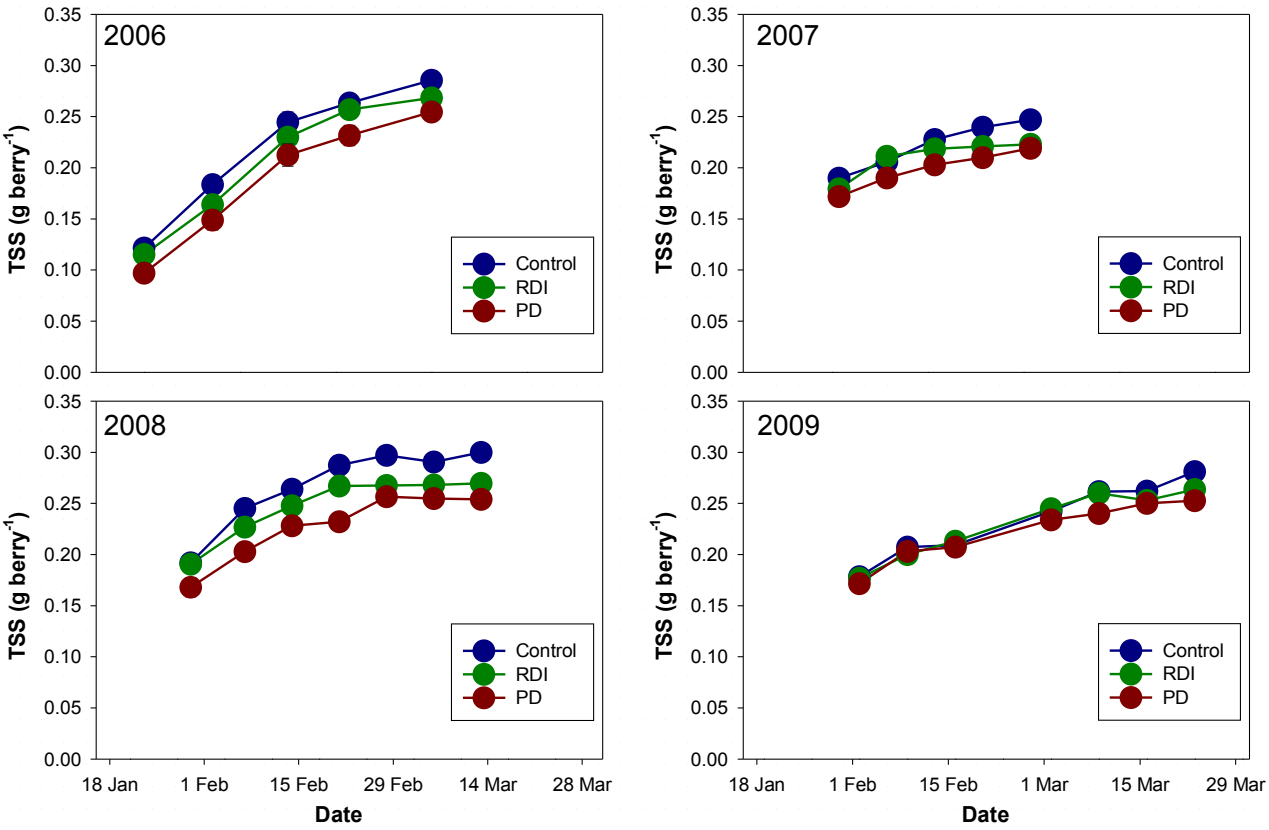


Figure 2. Sugar per berry (TSS per g FWt) of Cabernet Sauvignon fruit from vines receiving control, RDI or PD irrigation (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009). Each point is a mean of 12 replicates ± standard error.

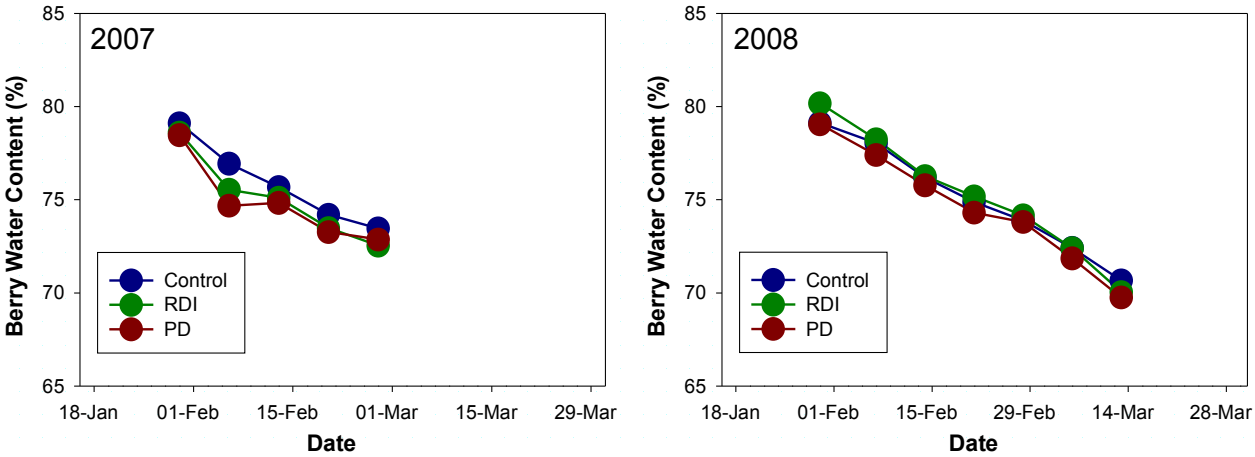


Figure 3. Berry % water content of Cabernet Sauvignon fruit from vines receiving control, RDI or PD irrigation. Each point is a mean of 12 replicates.

There was also only limited seasonal variation in bunch weight at the start of the series, with RDI vines having an average bunch weight at the end of January of 91.1±4.2, 92.5±5.2 and 84.1±3.5 g for 2006, 2007 and 2008 respectively. In general there was an increase in bunch weight during the maturation series of approximately 10%, irrespective of treatment with peak bunch weight being highest in the control vines and lowest in the PD vines in all three seasons in which it was measured. Berry fresh weight was also generally highest in control and lowest in PD (Figure 1), with some seasonal variation in maximum weight and the relative effect of the treatments. Notably, this effect on berry weight was continued in 2009, despite all vines having received identical irrigation. As berries per bunch was not affected by treatment in any season this effect was likely to account for some of the inter-seasonal carry-over effect of the irrigation treatments on yield (Chapter 6), particularly as bunch number per vine was not affected in the seasons of measurement (Chapters 4 & 6). In 2007 and 2009 berry growth, in terms of FWt, was complete by the start of the maturation series (end of January), with growth being complete in 2006 and 2008 one to two weeks later. Interestingly, the latter two years were also the years when berry FWt was highest.

In general TSS was not significantly different between the treatments in any year, with the start of the maturation series in 2008 being the only exception, where PD fruit had a slightly lower TSS than the other treatments. TSS increased throughout the series in every treatment in each year and did not vary between treatments in any season by harvest. However, when calculated on a per berry basis accumulation slowed in all years (Figure 2) reaching approximately 0.27 g berry^{-1} in RDI fruit except 2007, where TSS per berry only reached 0.22 g berry^{-1}. Although TSS was not affected by treatment, due to the difference in berry FWt, TSS per berry was higher in control fruit and lower in PD fruit when compared to RDI. Even in 2009, where irrigation treatments were no longer in place there was a slight effect of the prior irrigation strategy.

The difference in TSS accumulation when estimated on a juice (data not shown) or per berry basis implied that berry water content must be decreasing. Indeed, in the two years where berry dry mass was measured, this was the case, with berry water content as a proportion of FWt dropping throughout the maturation series in all three treatments, from 79% at the end of January to 73% at the end of February (Figure 3).As berry FWt didn't decrease the corollary of this was that berry DWt must have increased. Furthermore, TSS calculated per unit of berry water instead of per g berry FWt not only reduced in rate of accumulation during the maturation series, but stopped by late February in both 2007 and 2008, consequently the dry mass fraction that was not soluble solids continued to increase throughout the maturation series (Figure 4).

There was a tendency for juice pH to be lower in the control treatment than RDI. This was significant throughout the maturation series during 2006 and 2007, but not during 2008 nor during 2009, the first year of return to RDI. However, although significant, the effect was small, approximately 0.1 pH units. In all treatments and all years, pH rose throughout the maturation series, although there was a plateau in 2009 (data not shown) corresponding with slow sugar accumulation.

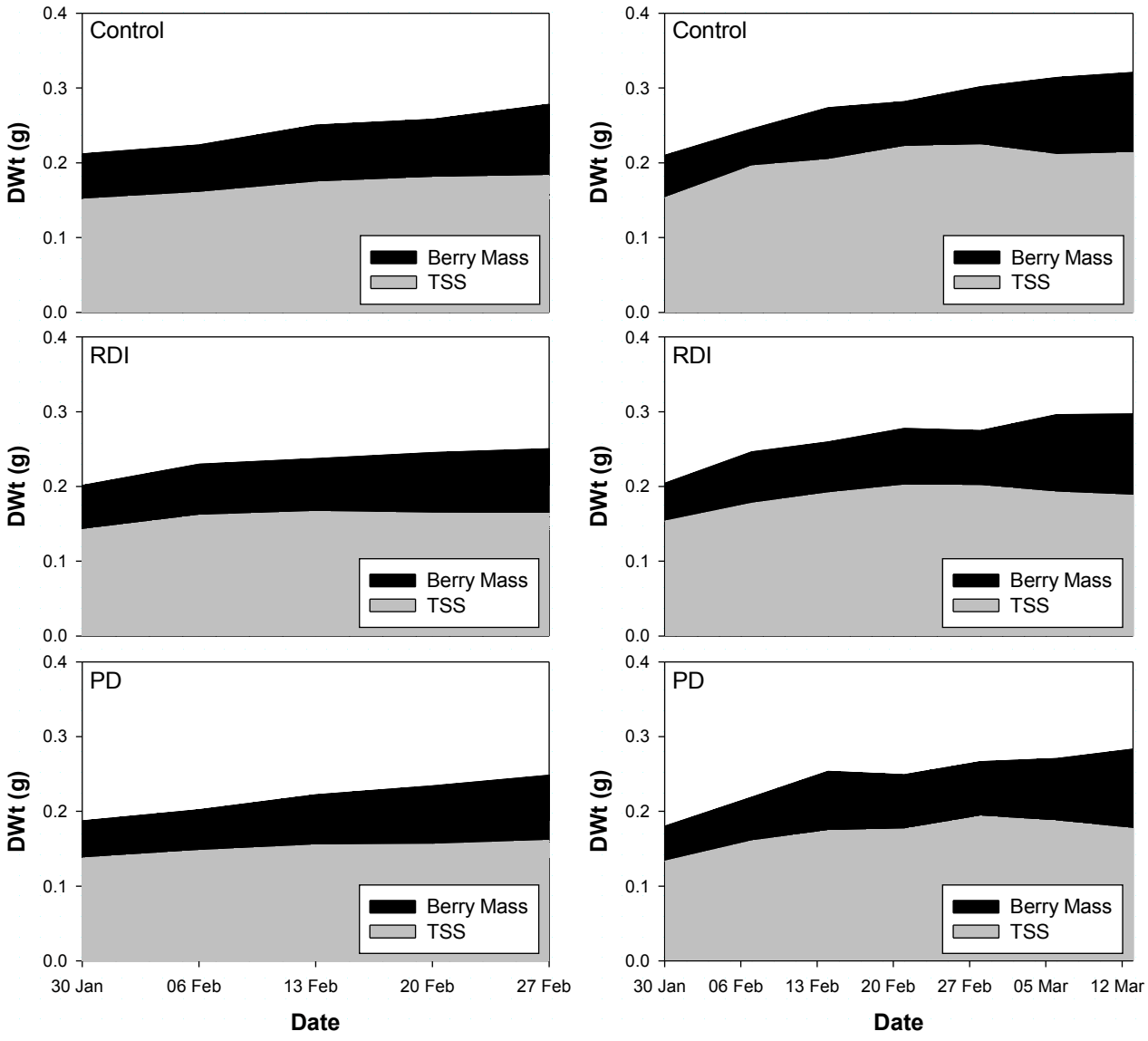


Figure 4. Total berry dry weight (DWt) and estimated dry weight of total soluble solids (TSS) of Cabernet Sauvignon fruit from vines receiving control, RDI or PD irrigation strategies. The chart areas represent the mean of 12 replicates.

For the three years where the double irrigated control was in place, TA was significantly higher in the control fruit than the other treatments (Figure 5). The absolute value of this effect declined during the maturation series, but the relative difference was maintained at approximately 15% throughout the series in all three seasons. In 2009 there was a much smaller increase in TA in the previously control irrigated fruit, averaging only 5%.

Comparing the results from 2009 with those from previous years demonstrates that whilst the irrigation treatments had an inter-seasonal carry-over effect on berry size and yield, they had a much smaller effect on berry composition. Irrigation effects on sugar per berry, pH and TA were all almost or completely absent in 2009, despite yield differences being larger than in any previous season (Chapter 6).

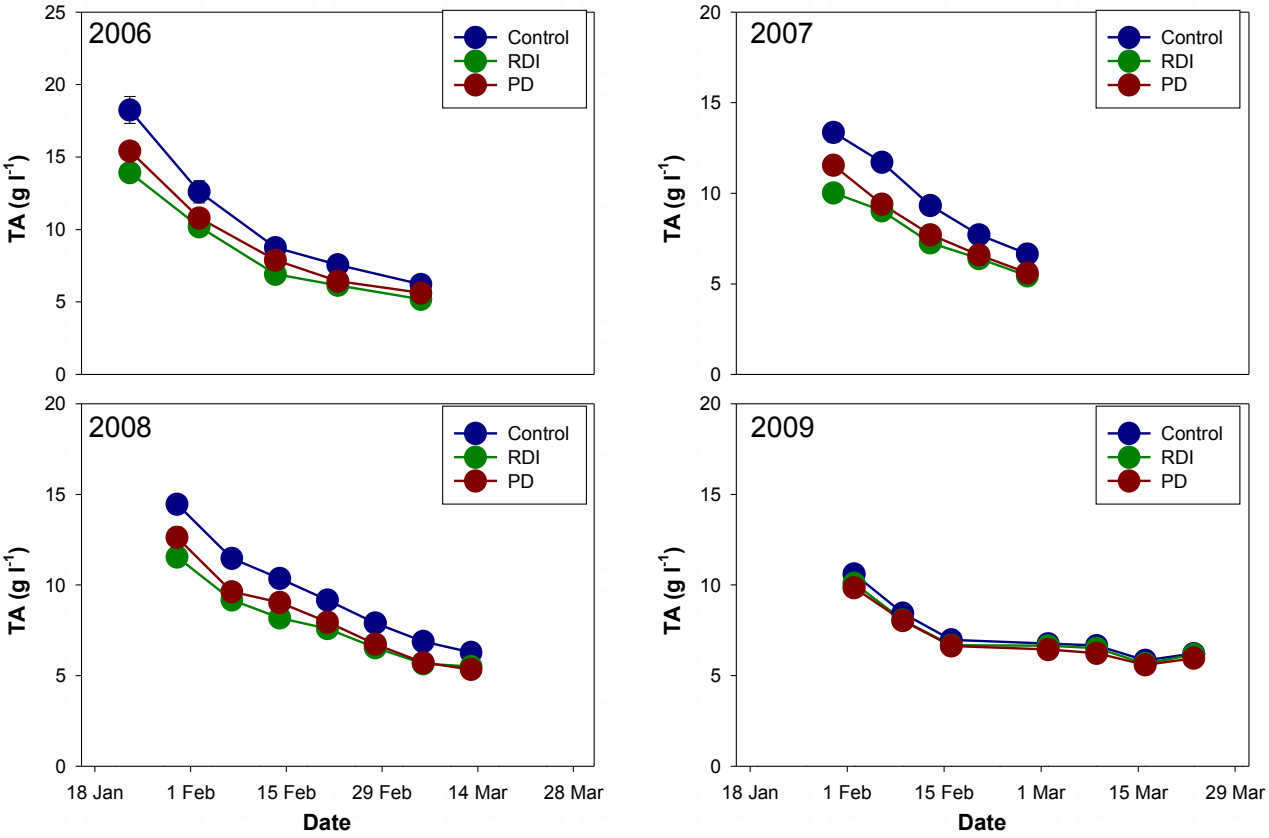


Figure 5. Titratable acidity of Cabernet Sauvignon fruit from vines receiving control, RDI or PD irrigation (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009). Each point is a mean of 12 replicates ± standard error.

Wine analysis

Spectrophotometric analysis of the Cabernet Sauvignon wines demonstrated a significant impact of the irrigation regimes on the wine, with colour density, colour hue and ionised anthocyanins all affected by both control and PD treatments when compared with RDI (Figure 6). Control irrigation significantly reduced colour density and ionised anthocyanins in each season and increased colour hue. This continued to be the case in 2009 and 2010, when irrigation was identical to RDI, but yield was greater. The PD treatment significantly increased colour density and ionised anthocyanins in 2006 and 2007, but no significant effect was seen in 2008, when the treatment was still in place, nor in the subsequent years where the vines were irrigated with the site standard regime. Colour density and ionised anthocyanins of RDI vines fell from 2006 to 2008 and the relative effect of PD fell as well, so it is possible that the positive response of colour to the PD treatment was dependent on other, possibly seasonal, factors driving colour across all treatments.

Total anthocyanins were generally unaffected by treatment, with a reduction in the control wines in 2007 and 2009 being the exceptions. Consequently, the treatment effects on colour were driven by the degree of ionisation, rather than the accumulation of anthocyanins themselves.

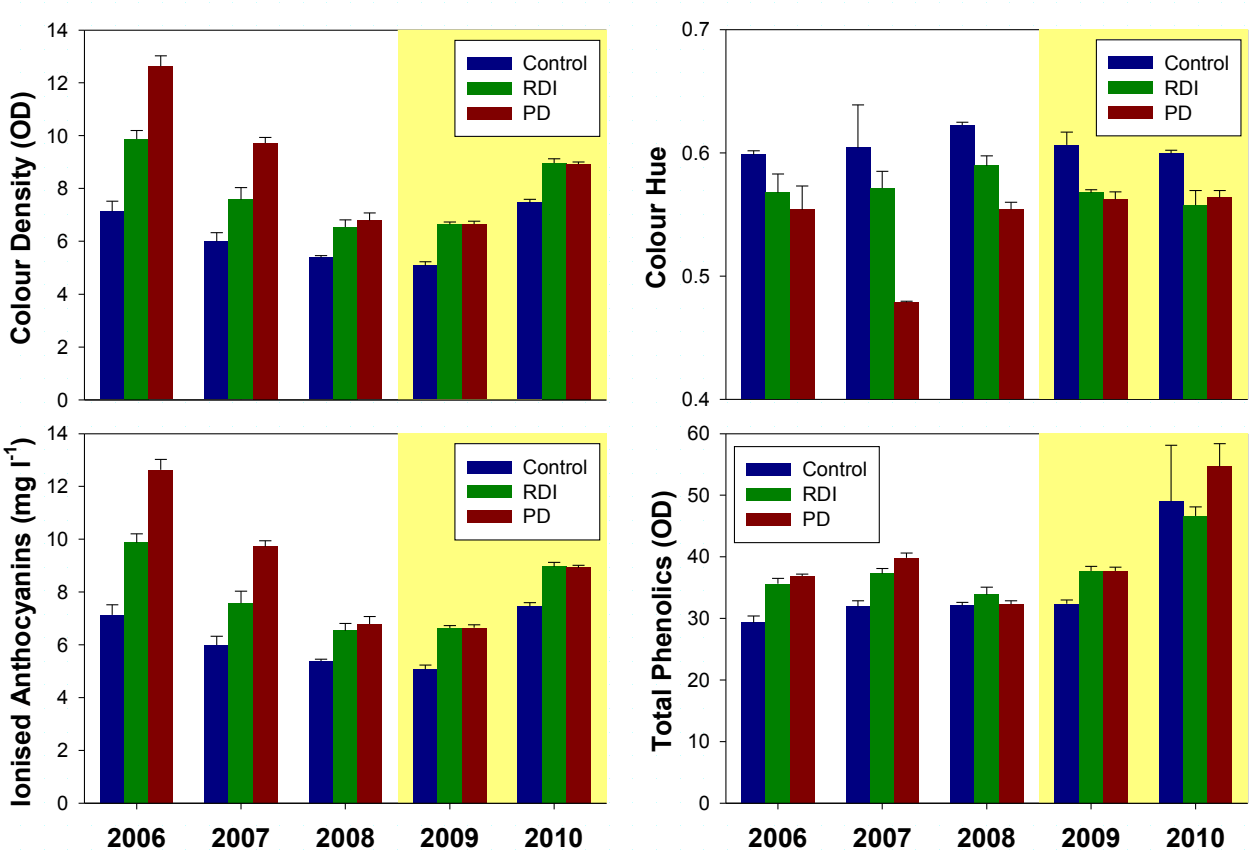


Figure 6. Colour density, colour hue, ionised anthocyanins and total phenolics of Cabernet Sauvignon wines made from vines receiving control, RDI or PD irrigation (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009-10, indicated with a yellow background). Each bar is a mean of 3-4 replicates + standard error.

Total phenolics estimated spectrophotometrically (Figure 6) and total tannins, estimated using a precipitation method (Figure 7) were similar in three out of the four years where both sets of measurements were made, with 2006 being the exception. However, tannins in the 2006 wine were measured three years after bottling, whereas all the other tannin and colour analyses were made within 12 months of bottling. The control treatment caused a significant reduction in phenolics in 2006, 2007 and 2009 relative to RDI, with the reduction in 2008 being non-significant, whereas control irrigation reduced tannins in 2007 to 2009 inclusive. The effect of PD relative to RDI was less predictable, with no significant response of total phenolics in any season, a significant increase in tannins in 2007, but a significant decrease in 2008.

In the Shiraz wines no significant effects of the PD treatment were observed for either season (2009 & 2010, data not shown). There were seasonal differences, with the 2009 wines having higher colour density and anthocyanin content than those from 2010. As well as the lack of an effect of the PD treatment on the Shiraz wines there was also no significant effect on yield (see Chapter 9), so the effects of PD seen in the Cabernet wines may be a consequence of the carry over effects observed after multiple seasons of treatment, or may represent varietal differences.

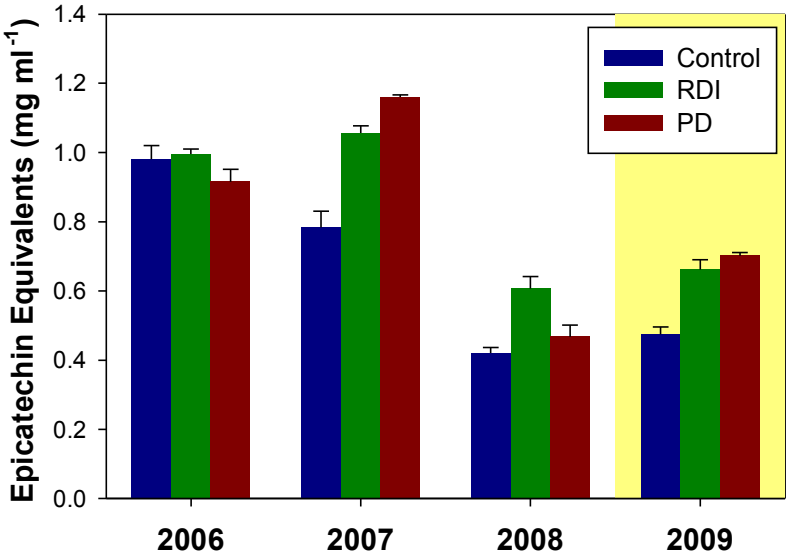


Figure 7. Total tannin content, expressed as epicatechin equivalents of Cabernet Sauvignon wines made from vines receiving control, RDI or PD irrigation (2006-8) or returned to site standard RDI strategy following at least three seasons of being irrigated with control, RDI or PD strategies (2009). Each bar is a mean of 3-4 replicates + standard error.

In summary, although the well watered control had predictable effects on wine and, to a lesser extent, total phenolics and tannins, the effects of the PD treatment were more variable, particularly on tannins. Inter-seasonal effects of the treatments were similar, with the previously control irrigated vines having reduced colour even in the second season on standard irrigation and PD having no carry-over effect on any measured wine parameter.

Wine sensory analysis

Difference testing was based on aroma and flavour and for vintages where this was performed a significant proportion of the testers correctly determined differences between control and RDI wines, but the difference between control and PD wines was only significant in 2008 (Table 2). The testers were not able to significantly determine a difference between PD and RDI wines in either year, despite the significant differences in analysis described above. Even where significant differences were determined testers reported that the differences were slight.

Table 2. Results of duo-trio difference tests for Cabernet Sauvignon wines irrigated with control, RDI or PD irrigation strategies. Winemaking replicates were combined for these tests, 27 tests were conducted for each comparison in 2007 and 30 tests in 2008.

Wine 1	Wine 2	2007		2008	
		% Correct	*p* value	% Correct	*p* value
Control	RDI	70	0.010	67	0.021
Control	PD	59	0.124	70	0.008
RDI	PD	52	0.351	57	0.181

The descriptive analysis found only one of 14 aroma attributes marginally different between the 2007 wines, with *red berry* being less intense in RDI than the other treatments (p = <0.100), whereas five aroma attributes were significantly different in the 2008 wines, with *leafy* and *vegetal* attributes being more intense in the control wine, *berry jam* higher in the PD wine and *red berry* highest in the RDI wine and lowest in the PD wine (Figure 8).

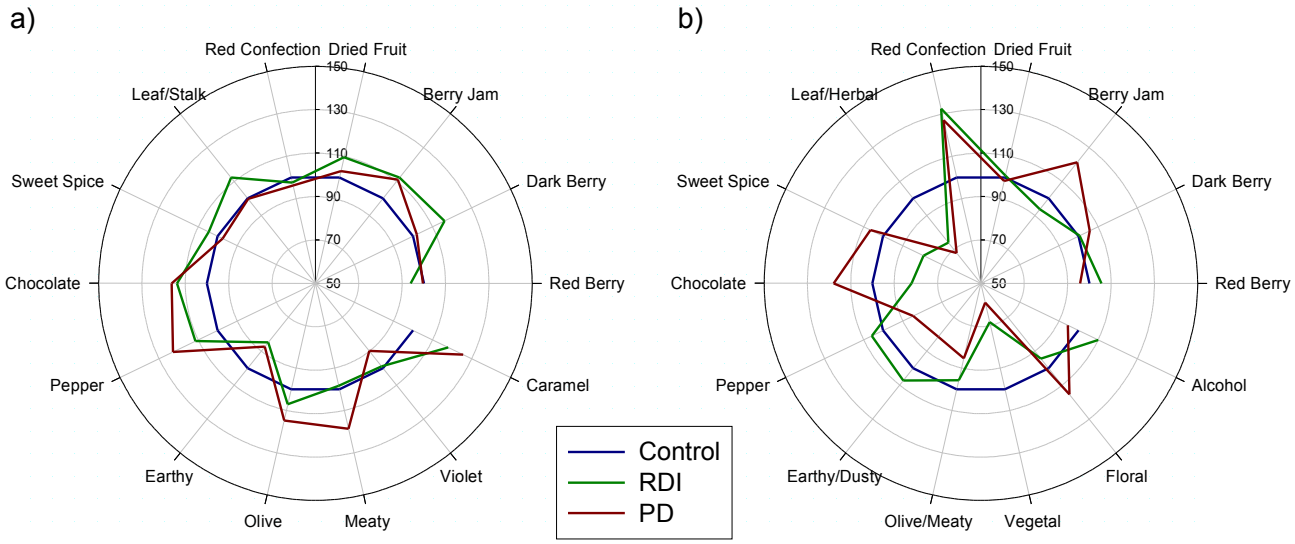


Figure 8. Aroma profiles of Cabernet Sauvignon wines from a) 2007 and b) 2008, made from vines receiving control, RDI or PD irrigation. Scores are relative to control wines.

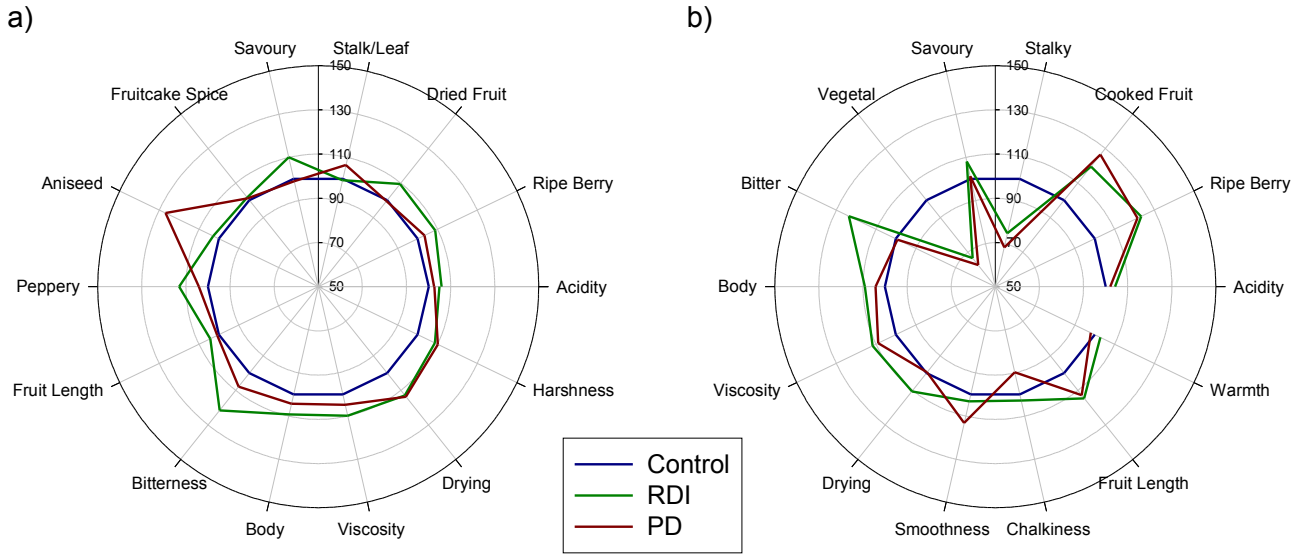


Figure 9. By mouth profiles of Cabernet Sauvignon wines from a) 2007 and b) 2008, made from vines receiving control, RDI or PD irrigation. Scores are relative to control wines.

There were larger differences in the by mouth attributes, with five of 14 attributes being significantly different between the 2007 wines and 13 of 15 attributes significantly different

in the 2008 wines (Figure 9). Most of these differences were between the control wine and the other treatments, but a number were more intense in the RDI wine than the other treatments, e.g. *acidity*, *savoury*, *drying* and *warmth* in the 2008 wines.

Conclusions

Whilst in place, up to the 2007/8 season, the irrigation treatments had effects not only on yield, but also on berry size and compositional attributes, though not TSS. However, these effects were not driven by berry size or yield alone as most of the effects on composition were not present in the 2009 maturation series, despite a larger effect of the prior irrigation on yield than in previous years. The decrease in berry water content during ripening is consistent with the reduction in commercial 'juice' yields where fruit is harvested at high maturities, although this may also be associated with berry weight loss and shrivel in some varieties (e.g. Shiraz) which was not the case in this study. The fact that the increase in berry dry weight during ripening cannot be accounted for totally by the increase in soluble solids is a significant observation which indicates potential changes in structural components or possibly non-soluble forms of carbohydrate, i.e. starch, which warrants further investigation. There was however, an inter-seasonal carry-over effect on the wines, with ionised anthocyanins and colour density continuing to be lower in the previously control irrigated wines. In the standard small-scale winemaking process used in this study, pH was adjusted, where required, through the addition of tartaric acid to give a pH around 3.45. Despite this adjustment, irrigation treatment effects on both the hue of the wine and wine colour density associated with the degree of ionisation of the anthocyanins occurred. Further research is required to understand the effects of deficit irrigation strategies on colour development during the winemaking process and potential linkages to berry anthocyanin profiles and their release, as total anthocyanins were unaffected despite the differences in berry size. Sensory analysis of the 2009 and 2010 wines had not been completed at the time of writing, but although differences between the wines were not large, each had a distinctive profile, with the control wines in particular standing out from the other two treatments. The reasons for these carryover effects warrant further investigation to investigate the underpinning causes which may be associated with impacts of the long term treatments on soil characteristics (physical or chemical) or vine growth characteristics (e.g. root architecture or canopy structure).

Chapter 9

Further examination of intra-seasonal carry-over effect of PD treatment on leaf physiology of grafted Shiraz and Cabernet Sauvignon vines

Introduction

The extent of the effect that the PD treatment had on vine growth (Chapter 4) was probably driven by the carry-over effect observed on leaf physiology (Chapter 5). The necessity of altering the irrigation regime of the main experimental plot for the second phase of the project prevented any further examination of the cause of this intra-seasonal carry-over effect. A variety of mechanisms could have caused the carry-over, with the most likely being a change in stomatal responsiveness to VPD, as seen in Shiraz under a partial-rootzone drying regime (Collins 2006), and Cabernet under sustained deficit irrigation (Collins and Loveys 2010). The collected weather data indicated that the post-PD period in 2006/7 was much hotter and drier than in 2007/8, so any change in stomatal response to VPD would have been seen to a greater extent in 2006/7 than 2007/8. Indeed, this is exactly what was observed (Chapter 5). In order to further examine this possibility two new field trials with PD and ongoing RDI treatments were put in place, both adjacent to the original trial, using Shiraz and Cabernet vines.

Materials and Methods

Two experimental blocks were established, one with Shiraz scions on Schwarzman rootstocks and one with Cabernet Sauvigon on a mixture of rootstocks, mainly Teleki 5A, but including some Freedom and K51-40. Both blocks were within 20 m of the original experimental plot described in Chapter 2, the vines were also the same age, on the same soil type and irrigated in the same manner as the original trial prior to a PD treatment being imposed. Each of the plots consisted of 24 rows by 24 vines and were split into eight blocks of three rows. Each block was split centrally into two replicates, one maintained with an RDI irrigation strategy and one with a PD strategy imposed. The replicates were assigned to a random position within a block, but constrained so that four replicates of each treatment occurred within each of the two columns created by the central split. The plots were maintained for two seasons, 2008/9 and 2009/10.

The PD deficit was applied to both plots from 22/12/2008 to 12/1/2009 and from 23/12/2009 to 14/1/2010. Table 1 presents the irrigation applied to each treatment in each plot, rainfall and ET_o data are given in Chapter 2, Table 1. Environmental measurements were used as described in Chapter 2 and soil moisture measurements were made using the TRASE System 1 TDR as described in Chapter 2 on four occasions during the 2008/9 season and on three occasions during the 2009/10 season, from mid December to mid-January, in each case.

Stomatal conductance and leaf water potential measurements of outer, sun exposed, leaves were made as described in Chapter 5 during and after the PD deficit in 2008/9 and before, during and after the PD deficit in 2009/10.

In addition, LAI was measured as described in Chapter 4 on two occasions in the 2009/10 season (21/12/09 and 14/1/2010) and a hand picked harvest yield estimate was made in both seasons. A whole vine was used for the Shiraz harvest in each season, with harvest

dates of 6/3/2009 and 5/2/2010, whereas a half-vine was used for the Cabernet Sauvigon estimate on 30/3/2009 and 2/3/2010. Two replicate wines were made from the Shiraz fruit in each season, with the winemaking as described in Chapter 8. Once bottled, 'colour' and tannin analysis was carried out, also as described in Chapter 8.

Results and Discussion

Soil moisture

The first PD deficit period applied began in December 2008. Prior to this any difference in soil moisture between the treatments in either plot would indicate a confounding effect caused by soil properties or vine growth. However, no such difference was found for either variety to a depth of 30 cm (Table 1, 2008/9 pre-PD). Soil moisture wasn't assessed during the PD deficit period in 2009/10, but during 2008/9 there was a large and significant decrease in both varieties and no reason to suspect the same wasn't true in the second season (Table1). Despite this, once irrigation was returned to normal in the PD treatments recovery in the top 30 cm of soil was rapid, with no difference observed four days after re-irrigation in 2008/9 for either variety. A marginally significant ($p=0.09$) difference for Shiraz two days after re-irrigation in 2009/10 was seen, but in this case it was the RDI treatment with the lower soil moisture content. Soil matric potentials were non-limiting in the Cabernet plot at all times except where the PD deficit was in place. In the Shiraz plots soil moisture content was always lower than Cabernet and consequently soil matric potential was higher, but no obvious effects of this difference were seen in the leaf physiology (see below), so it is possible that either the different rootstocks had different abilities to extract soil water or the water was extracted from a greater depth under the Shiraz vines.

Leaf physiology

Prior to the PD deficit being applied, measurements of pre-dawn Ψ_l (Figure 1b & d) and midday Ψ_l (Figure 2b & d) in 2008/9, and g_s in both 2008/9 and 2009/10 (Figure 3 & 4) all indicated no difference in leaf function between the PD and RDI treatments. Although no difference would have been expected before the first PD deficit was applied in 2008/9 the lack of any effect on leaf physiology before the PD deficit period in 2009/10 supports the conclusions of Chapter 5 that found no inter-seasonal effect on physiology at the leaf level.

During the PD deficit period the same responses as previously observed in the main experiment occurred, with both varieties exhibiting similar large decreases in both pre-dawn Ψ_l (Figure 1) and g_s (Figures 3 & 4). Interestingly, although no effect of the PD treatment on midday Ψ_l was seen in Cabernet vines in the main trial (Chapter 5), there was a significant effect of the PD treatment on midday Ψ_l of Shiraz vines in both seasons and Cabernet vines in the second season. In both, the difference between treatments was much smaller than that seen in pre-dawn Ψ_l or g_s. This could be an effect of being grafted, rather than own-rooted or a difference in soil moisture, though comparing Table 1 in this chapter with that in Chapter 4 does not suggest any major difference in soil water availability during the PD deficit period between the two experiments. Shiraz is generally viewed as a more anisohydric like variety than Cabernet (Collins and Loveys 2010) and, consequently, might be expected to exhibit larger effects of water stress on Ψ_l. However, although both pre-dawn and midday Ψ_l were significantly lower in Shiraz and the effect of

Table 1. Volumetric soil moisture and estimated soil matric potential (ψ_{sm}) for the top 30 cm of soil under the dripline of Cabernet Sauvigon and Shiraz vines irrigated with RDI and PD strategies during the 2008/9 and 2009/10 growing seasons. Each value is a mean of 12 replicates ± standard error. Superscript letters indicate significant differences using Student's t-test and significance level of 0.05.

Variety/ Season	Irrigation Treatment	Volumetric Soil Moisture (m^3 m^{-3})						Soil Matric Potential (kPa)*		
		Pre-PD		PD Period		Post-PD		Pre-PD	PD Period	Post-PD
		Mean	SE	Mean	SE	Mean	SE			
Cabernet										
2008/9	RDI	0.212	0.012	0.228[a]	0.007	0.277	0.007	12	11	2
	PD	0.216	0.009	0.096[b]	0.009	0.270	0.008	10	2,414	3
2009/10	RDI	0.223	0.013	†		0.190	23	8	†	23
	PD	0.202	0.016			0.189	0.012	15		23
Shiraz										
2008/9	RDI	0.141	0.008	0.174[a]	0.009	0.213	0.011	142	46	11
	PD	0.137	0.008	0.067[b]	0.002	0.206	0.006	176	27,291	14
2009/10	RDI	0.124	0.008	†		0.089	0.005	321	†	2,984
	PD	0.122	0.006			0.108	0.008	365		834

* See Chapter 1 for conversion from volumetric soil moisture.

† TDR measurements not taken during PD deficit period in 2009/10 due to equipment failure.

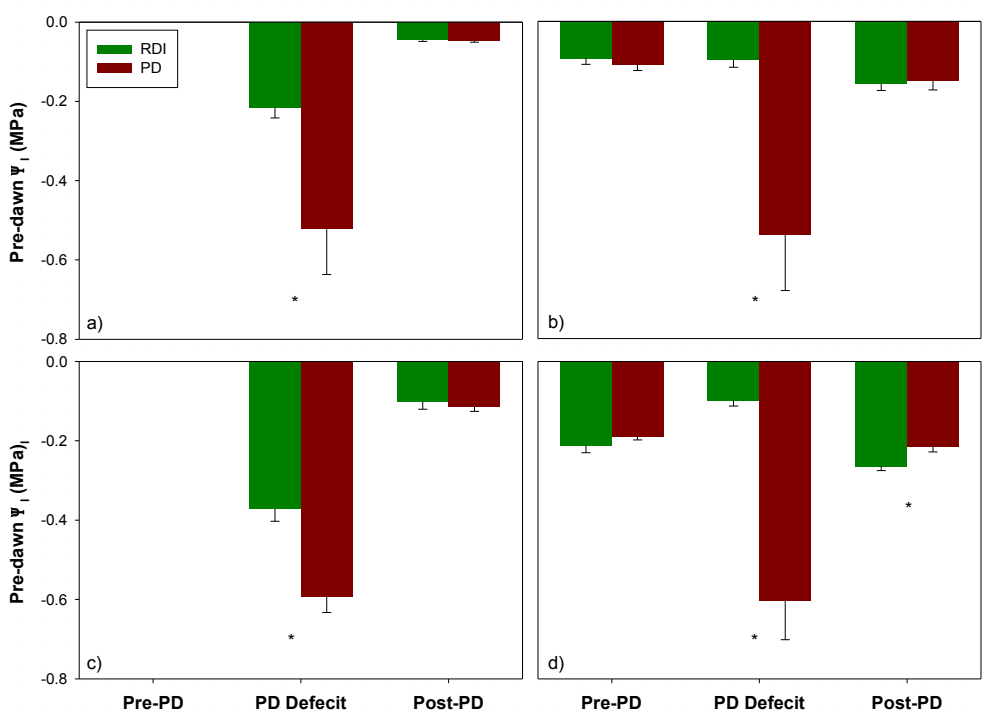


Figure 1. Pre-dawn leaf water potential (Ψ_l) of Cabernet Sauvigon (a-b) and Shiraz (c-d) vines during the 2008/9 (a & c) and 2009/10 (b & d) growing seasons receiving RDI or PD irrigation. Bars are means of eight replicates - standard error and asterisks denote significant differences.

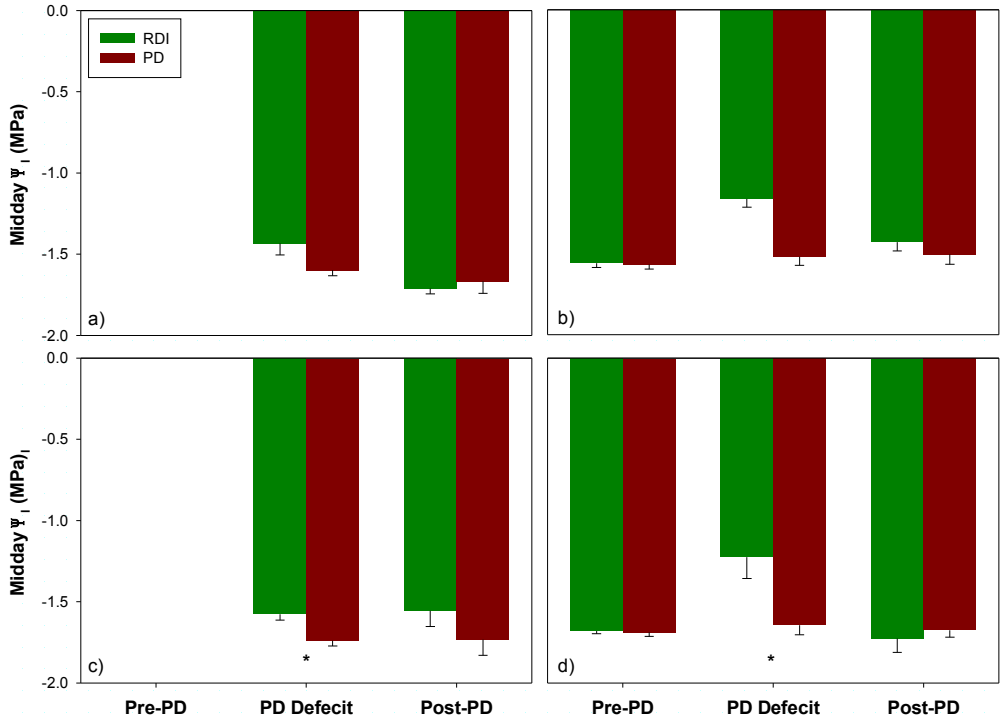


Figure 2. Midday leaf water potential (Ψ_l) of Cabernet Sauvigon (a-b) and Shiraz (c-d) vines during the 2008/9 (a & c) and 2009/10 (b & d) growing seasons receiving RDI or PD irrigation. Bars are means of eight replicates - standard error and asterisks denote significant differences.

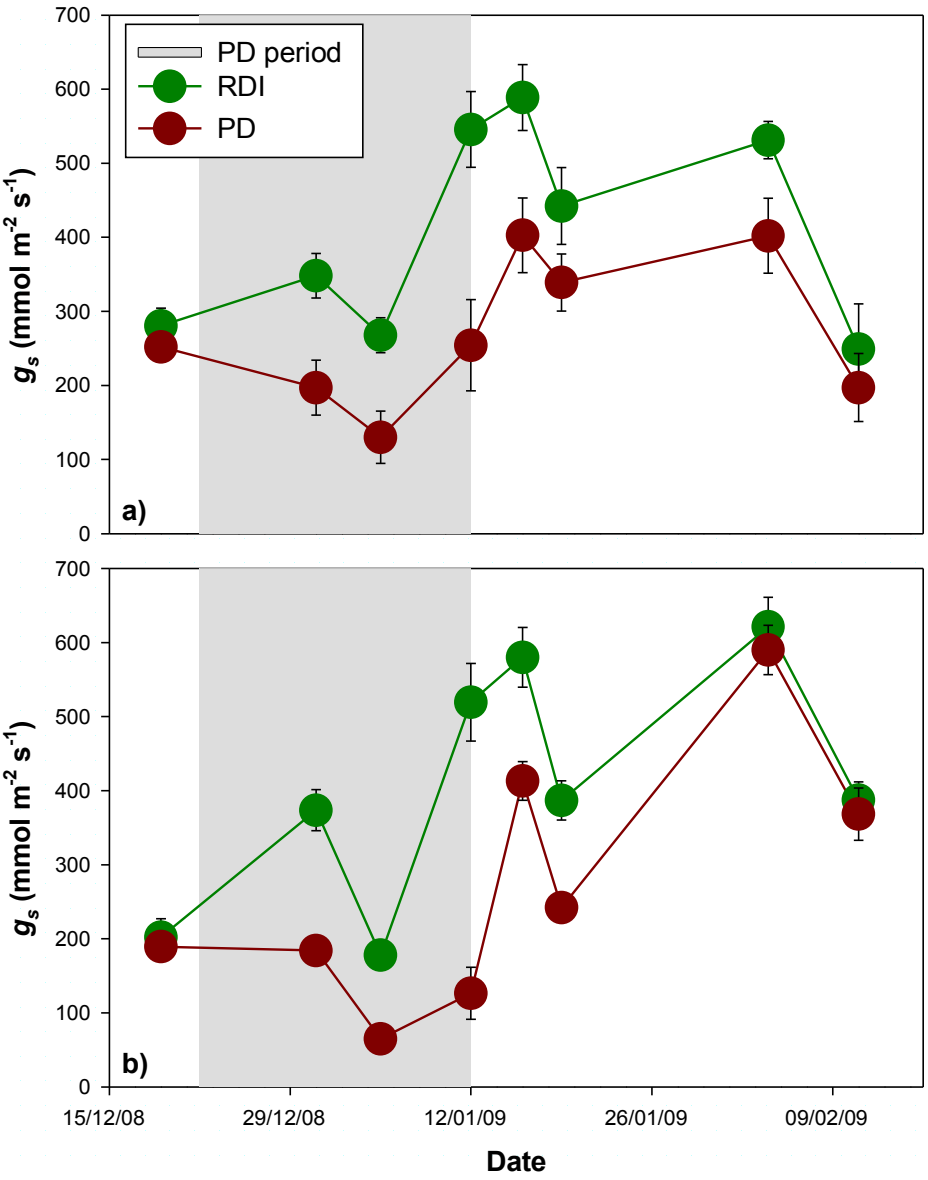


Figure 3. Stomatal conductance (g_s) of a) Cabernet Sauvigon vines and b) Shiraz vines during the 2008/9 growing season, receiving RDI or PD irrigation. Points are means of eight replicates ± standard error.

the PD treatment was significant in both seasons, soil moisture was much lower under the Shiraz vines and the effects of the PD deficit on midday Ψ_l was proportionally similar to that on Cabernet.

The effect of the PD deficit on g_s was also similar in both varieties and both seasons, though the minimum reached by the PD vines was lower in Shiraz (Figures 3 & 4). Following the return to standard irrigation at the end of the PD period in the 2008/9 season a carry over effect on g_s was seen in both varieties, with g_s being lower in the PD vines of each, despite no difference in soil moisture content to 30 cm (Table 1) and no difference in pre-dawn Ψ_l (Figure 1). The effect was similar to that seen in the main experiment (Chapter 5), but did not last for as long, full recovery having occurred by early February in Shiraz and soon afterwards in Cabernet. Also, no carry-over effect on g_s was seen in the 2009/10 season.

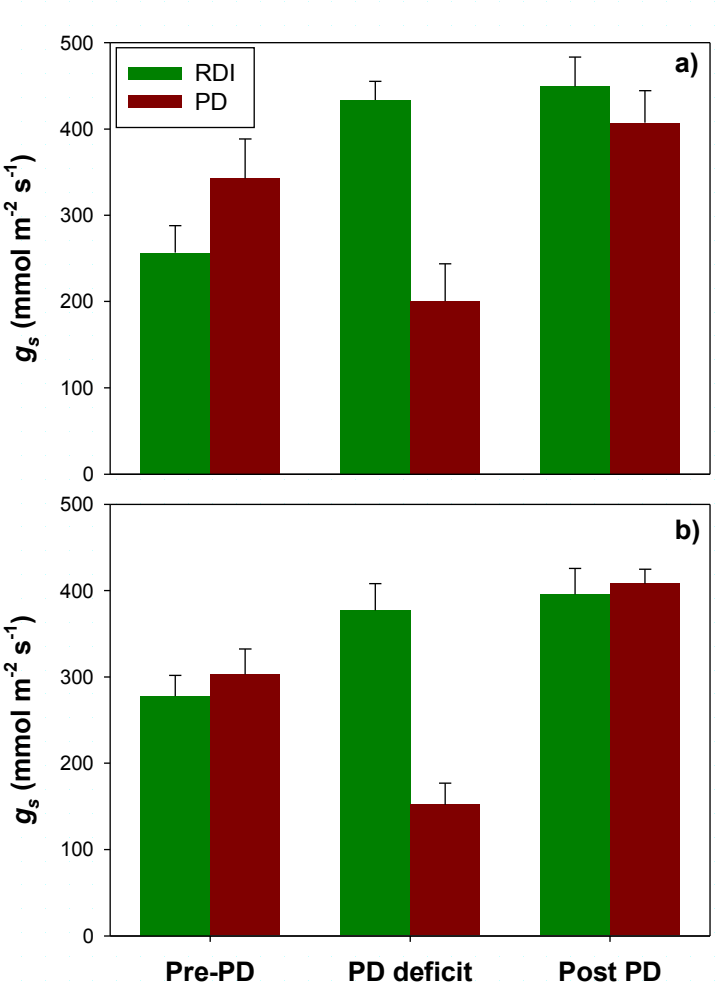


Figure 4. Stomatal conductance (g_s) of a) Cabernet Sauvigon vines and b) Shiraz vines during the 2009/10 growing season, receiving RDI or PD irrigation. Bars are means of eight replicates + standard error.

Weather conditions in the two seasons in the period following the PD deficit were similar. Comparative measurements of g_s made in the morning when VPD was low and the afternoon when VPD was high on three different days did not indicate any difference in VPD response between treatments (data not shown). Consequently, it does not appear that the intra-seasonal carry-over effect on leaf function observed after the PD deficit period in both the main experiment and this supplemental experiment was driven by changed stomatal responsiveness to atmospheric conditions.

The only estimate of potential PD effects on growth made during this part of the project was a measure of peak LAI during the second season. There was no significant effect with LAI of PD vines being 2.45±0.23 & 1.30±0.10, and that of RDI vines 2.43±0.13 & 1.50±0.14 for Cabernet and Shiraz vines respectively. However, no effect of one season of PD irrigation on LAI was observed in the original CRCV project on the primary Cabernet trial either (Cooley *et al.*, unpublished data), but the significant effects of further years of PD irrigation are clearly demonstrated in Chapter 4.

Table 2. Harvest yield and yield / canopy area ratio of RDI and PD irrigated Cabernet Sauvignon and Shiraz vines. Each value is a mean of 6-8 replicates ± standard error.

Year	Irrigation Treatment	Cabernet Yield (t ha^{-1})		Shiraz Yield (t ha^{-1})		Cabernet Yield / Canopy Area (kg m^{-2})		Shiraz Yield / Canopy Area (kg m^{-2})	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
2009									
	RDI	38.4	1.5	26.1	2.2			*	
	PD	35.4	2.1	24.8	2.0				
2010									
	RDI	32.5	1.9	26.2	2.1	1.58	0.18	2.12	0.26
	PD	24.7	3.5	22.4	1.8	1.08	0.15	1.59	0.20

Similarly, in the original CRCV study no significant effect of PD on yield was observed until the third season (Cooley *et al.* unpublished data) and no significant impact on yield in the current experiment was observed in either year for an individual variety. However, the lower yield in the Cabernet PD vines compared with that of RDI vines was marginally significant in 2010 ($p=0.085$) and when all the 2010 data was analysed together using ANOVA, the effect of PD on yield was significant, whereas the effect of variety was only marginally significant ($p=0.075$). On the other hand, the effect of variety was significant in 2009, but not treatment. Yield per unit area of canopy was significantly higher in Shiraz than Cabernet and in RDI vines than PD vines.



Figure 5. Photographs of Cabernet Sauvignon vines, fruit inset, irrigated with a) RDI and b) PD strategies following a natural heat event in January 2010.

* Canopy area not measured in 2008/9 season.

The potential effect of the PD treatment on the 2010 harvest was complicated by an extreme heat event that occurred in January 2010 during the PD deficit period. A number of the Cabernet vines on K51-40 and Schwarzmann rootstocks suffered significant leaf and berry damage as a result of the combined heat and water stress (Figure 5). This had a direct effect in reducing yield, whereas previous impacts of PD on yield were probably indirect, via the carry-over effect on leaf physiology. This may also explain part of the difference in yield per unit area of canopy seen between the PD and RDI, but the Shiraz PD vines were generally not as severely affected by the heat event and had a similar difference in yield per unit area of canopy as Cabernet.

Conclusions

The additional PD trials confirmed that the intra-seasonal carry-over effect of the PD deficit period on leaf physiology, was not limited to own-rooted Cabernet Sauvignon vines, finding a similar effect in Shiraz vines and grafted Cabernet vines. In both cases soil moisture and Ψ_l measurements made on the same day as g_s measurements indicated no difference in plant or soil water status whilst g_s continued to be negatively affected.

Chapter 10

Conclusions and recommendations

The project undertook, and completed, four years of field based research of the long-term impact of regulated deficit irrigation. Whilst it was delayed initially due to the period required to appoint staff, this enabled an additional season of irrigation treatments to be applied, enhancing the project in that the different effects of the control and RDI irrigation strategies could be determined with greater rigour. With the proviso that some non-essential chemical analyses are still ongoing, the project met all of its planned outcomes and performance targets.

The project's primary aim of identifying limitations imposed on canopy form and function by the use of RDI in relation to 'vine capacity' was met with the conclusion that, although an RDI strategy does limit canopy size and yield in comparison with unlimited 'luxury' soil moisture, this occurs in a sustainable manner. Furthermore, there was a clear enhancement of colour characteristics of the RDI wines and sensory analysis indicated that a number of favourable attributes were more intense compared with the control wines. The typical use of an RDI strategy in commercial viticulture of red winegrapes is to control vigour and in this respect the limitation imposed on canopy size by RDI is desirable. The industry concern, prior to this project being funded, was that there may be a long-term decline in the ability of the vines, managed under RDI or deficit strategies imposed by drought, to produce a crop of acceptable size and quality. However, throughout the four years of canopy size data and five years of yield data there was a remarkable consistency in both measures in the RDI irrigated vines, with no evidence of a progressive decline in growth or capacity.

The use of the PD irrigation treatment prior to and during this project, having been applied from 2002/3 to 2007/8, demonstrated that even a more extreme RDI type irrigation strategy was sustainable. The PD treatment reduced yield and canopy size still further than the RDI approach, but effectively this was a step change, albeit taking a number of years to complete. Relative to RDI, the effects of the PD treatment were also remarkably stable, with no progressive decline (Figure 1).

The second phase of the project was intended to meet the aim of 'optimising' canopy function, maximising yield in RDI irrigated vines without reducing wine colour and tannins, by adjusting pruning and/or irrigation regimes. As RDI irrigated vines had shown no progressive decline in yield but significant improvements in wine quality attributes over time a decision was made to return all treatments to the site standard RDI would provide the most industry relevant results, particularly in the context of drought recovery. Namely, i) whether vines subject to an extended RDI approach (the PD treatment) were able to recover their previous yield capacity and the duration required for this and ii) the duration of any carry-over effect of the control irrigation on yield and wine quality. In addition the change in irrigation of the control vines from and to RDI provided insights into the value of using rotational, multi-year, irrigation strategies.

Returning all vines to RDI demonstrated that both increasing and decreasing irrigation had effects lasting for at least two seasons (Figure 1). In the first 'recovery' season yield and canopy size differences were maintained despite all vines receiving identical irrigation, although the difference in canopy size between control and RDI vines was reduced. In the

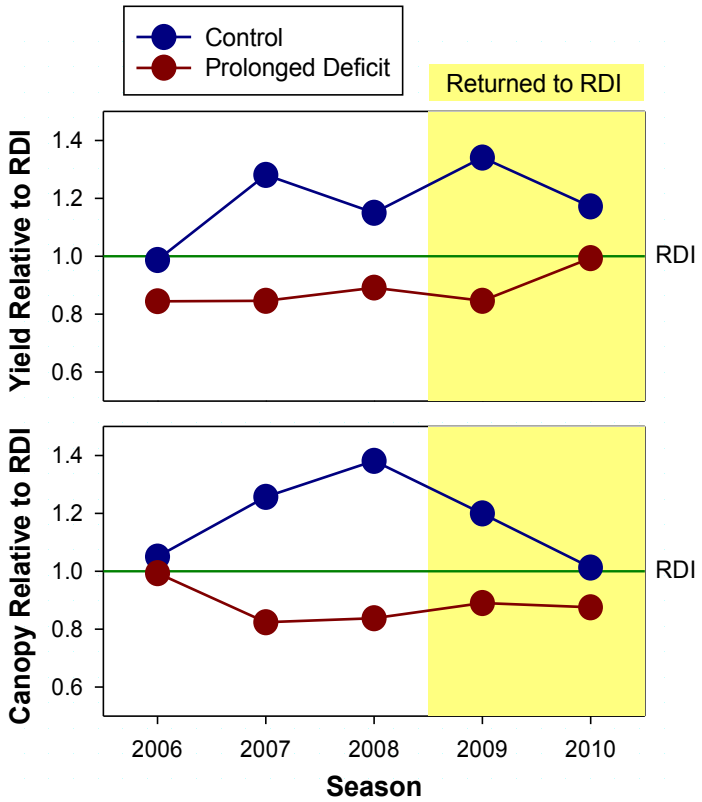


Figure 1. The effect of four to six seasons of PD irrigation and one to three seasons of control irrigation (2006-8) and two seasons after being returned to site standard RDI (2009-10) on a) yield and b) canopy size relative to RDI.

second 'recovery' season PD vines recovered to match the RDI yield, but canopy size remained affected and control vines maintained a higher yield, but not canopy size. These results show, not only that altering irrigation regime can take a number of seasons before the effects can be determined, but that the link between canopy size and yield ('vine balance') is broken in the period following a change in irrigation.

A significant effort was allocated to determining and understanding the short and long-term effects of the irrigation regimes and their alteration on canopy function. In general, no effect was seen of past irrigation regimes on leaf physiology, with gas exchange, water status etc. determined solely by the irrigation regime in the current season. This was true both whilst the irrigation treatments were in place and in the seasons following return to the site standard RDI. Consequently, whole vine effects such as alteration of storage reserve pools, vine water use etc. were determined by longer-term effects of irrigation on growth and canopy structure rather than effects at the leaf level. However, the additional stress imposed by the extended RDI approach of the PD treatment did result in short-term effects at the leaf level, with typical water stress responses occurring. The impact of the PD deficit period was almost certainly magnified by the intra-seasonal carry-over of those stress responses, with reduction in carbon gain continuing even once both soil and plant water status had been returned to match those prior to the imposed stress. This occurred in the original experiment with Cabernet vines on own-roots and in both Cabernet and Shiraz scions grafted onto rootstocks.

The mechanism by which this occurred was not determined, though it seems unlikely that it is via a simple adjustment in stomatal responsiveness to VPD. Other mechanisms, such

as an interaction with vine phenology, remain possible. It is likely, however, that by extending the RDI strategy with a greater than normal water stress over the entire RDI-PD duration, but less severely than imposed during the PD period the *intra*-seasonal effect on leaf physiology would be less and the *inter*-seasonal effect on vine growth and production of the PD strategy reduced.

The final aim of the project was to develop integrated approaches with the CSIRO Food Futures Flagship to assess treatment effects on wine aroma and flavour. Whilst it is outside the scope of this document to provide detailed results of the Flagship project, both fruit and wines from the treatments have been utilised by the Flagship in each year of the project for sensory and chemical analysis (with GCMS and LCMS) as part of their ongoing characterisation of environmental effects on wine aroma and flavour. The sensory data provided in Chapter 8 represent a small part of those studies and demonstrate the ability to determine statistically significant effects of changes in irrigation regime alone on resultant wines, particularly on mouth feel characteristics.

The project as a whole demonstrates to winegrape growers that RDI can safely be applied for many years without a progressive decline in vine capacity. Further, that irrigation water use can be reduced by extending the RDI approach in time and severity, also without having a progressive effect on vine capacity. However, whilst further extension of RDI generated improvements in wine attributes that could be determined in the laboratory setting, these were not detectable by sensory analysis. This work provides limits within which growers can reduce their current irrigation use to maximise fruit yield and wine quality without risking future vine productivity. It also highlights the limitation in our understanding of the interaction between vine water status, vine phenology and crop development. With little mechanistic knowledge current practices are based on very broad empirical results. Developing an understanding of *how* different degrees of water stress can have different effects at different points within the growing season could lead to much more efficient application of strategies such as RDI. Furthermore, the improved CWUI of the PD vines (yield per unit of water transpired), despite a reduction in yield proportional to the reduction in applied water suggests that the spatial provision of irrigation should be examined in conjunction with any further development of regulated deficit irrigation strategies.

In addition, the project has highlighted a significant risk of using severe water deficits, in that the negative impact of an extreme high-temperature weather event is far greater in water-stressed vines. Glasshouse experiments together with non-controlled field data demonstrated that well-watered vines are able to withstand multiple consecutive days of up to 45°C with no leaf loss, whereas water stressed vines suffer an increasing amount of damage with increasing water stress. However, the actual cause of this, be it higher leaf temperatures causing a threshold temperature to be reached more easily, a change in the threshold for irreparable damage to occur or some other mechanism is uncertain. Determining this could lead to more targeted advice for growers without unlimited access to water or with a limited capacity for irrigation application.

Appendix 1

Project aims and performance targets

OBJECTIVES:

- Optimisation of canopy function to increase yield while maintaining wine colour and tannins with efficient use of resources
- Identify limitations imposed by current management practices, particularly on canopy form and function and their relevance to 'vine capacity'
- Develop integrated approaches, eg. through CSIRO Food Futures Flagship wine aroma and flavour project, to provide targeted assessment of treatment effects on wine aroma and flavour.

Outputs and Performance Targets 2005-06

Outputs	Performance Targets
1. Phase 1 irrigation and pruning strategies implemented	Irrigation treatments and pruning treatments modified with the aim to improve yield and maintain quality
2. Canopy form determined	Canopy development and structure characterised for treatments over the growth period (ie. September to April)
3. Canopy function characterised	Targeted physiological measurements to characterise treatment effects on canopy function over the growth period (ie. September to April)
4. Crop development characterised	Crop development monitored to characterise treatment effects on yield components
5. Berry composition measured and wines evaluated	Berry composition monitored over the ripening period and small- scale wines produced at optimum harvest maturity and assessed by chemical techniques
6. Vine carbohydrate status assessed	Vine carbohydrate status determined for key phenological stages from dormancy to leaf fall

Outputs and Performance Targets 2006-07

Outputs	Performance Targets
1. Phase 1 irrigation and pruning strategies refined based on previous results	Irrigation treatments and pruning treatments modified with the aim to improve yield and maintain quality
2. Canopy form and function determined	Canopy development and structure characterised for treatments over the growth period (ie. September to April). Targeted physiological measurements undertaken over the growth period (ie. September to April)
3. Crop development characterised	Crop development monitored to characterise treatment effects on yield components
4. Berry composition measured and wines evaluated	Berry development and composition monitored over the ripening period and small-scale wines produced at optimum harvest maturity and assessed by chemical techniques
5. Vine carbohydrate status assessed	Vine carbohydrate status determined for key phenological stages from dormancy to leaf fall
6. Phase 1 results analysed, preliminary	All data collated and analysed as a basis for imposing modifications to irrigation treatments and pruning systems.

recommendations made	Preliminary recommendations prepared and communicated to industry.

Outputs and Performance Targets 2007-08

Outputs	Performance Targets
1. Phase 2 irrigation and pruning treatments imposed based on phase 1 recommendations	Irrigation treatments and pruning treatments modified to demonstrate recovery in productivity of low capacity vines
2. Canopy form and function determined	Canopy development and structure characterised for modified treatments over the growth period (ie. September to April). Targeted physiological measurements undertaken over the growth period (ie. September to April)
3. Crop development characterised	Crop development monitored to characterise modified treatment effects on crop development
4. Berry composition measured and wines evaluated	Berry development and composition monitored over the ripening period and small-scale wines produced. Targeted wine aroma and flavour compounds measured in fruit and wine via links to CSIRO Flagship project.
5. Vine carbohydrate status assessed	Vine carbohydrate status determined for key phenological stages from dormancy to leaf fall
6. Extension and communication	Presentation to industry forum and paper submitted to industry journal

Outputs and Performance Targets 2008-09

Outputs	Performance Targets
1. Phase 2 irrigation and pruning treatments further refined	Irrigation treatments and pruning treatments imposed for a final season to demonstrate ongoing recovery in productivity of low capacity vines
2. Canopy form and function determined	Canopy development and structure characterised for modified treatments over the growth period (ie. September to April). Targeted physiological measurements undertaken over the growth period (ie. September to April)
3. Crop development characterised	Crop development monitored to characterise treatment effects on crop components and recovery of low capacity vines.
4. Berry composition measured and wines evaluated	Berry development and composition monitored over the ripening period and small-scale wines. Targeted wine aroma and flavour compounds measured in fruit and wine via links to CSIRO Flagship project.
5. Vine carbohydrate status assessed	Vine carbohydrate status determined for key phenological stages from dormancy to leaf fall to demonstrate recovery of vine capacity
6. Report produced and recommendations to industry finalised	All data analysed and key linkages between vine capacity, canopy form and function, crop development and grape and wine quality summarised and communicated to industry by Final Report and presentation/paper
7. Recommendations for future research	Through industry consultation, future research needs considered and proposed to GWRDC

Variations:

The project commenced on June 1st, 2006 when Dr. Everard Edwards was appointed as the Research Fellow to the project, as a consequence the end date was adjusted until 31/3/2010. A further variation to extend the end date until 30/6/2010 was agreed to by GWRDC on 17th April 2009 to account for time spent on development and delivery of the GWRDC Drought Module 11 by Dr Edwards. The project end date has since been extended to 30/9/2010 to allow chemical analyses to be completed for the final report.

Appendix 3

Communication

Communication to industry and the scientific community has been an integral part of this project. Results have been presented at a range of industry and scientific meetings throughout the life of the project, starting less than 12 months after the appointment of the chief investigator. In addition to the presentations and publications listed below the results have been presented at annual GWRDC Soil and Water Initiative meetings, seminars within CSIRO and a number of external seminars at the Waite, elsewhere in Australia and in the UK. A video was produced of the module developed as part of the GWRDC Water and Vine program and made available on the GWRDC website. Components of the work have also been included as part of more general, vine management presentations by P. Clingeleffer at international symposia.

Refereed publications

2011 Edwards EJ, Downie AF & Clingeleffer PR. Development of a simple microplate assay to quantify non-structural carbohydrates of grapevine tissues. *American Journal of Enology and Viticulture*. 62, 133-137.

2011 Edwards EJ, Smithson L, Graham DC & Clingeleffer PR. Grapevine canopy response to a high temperature event during deficit irrigation. *Australian Journal of Grape and Wine Research*. In press.

2010 Clingerleffer PR. Plant management research-status, what it can offer to address the challenges and limitations. *Australian Journal of Grape and Wine Research* **16** supplement 1: 25-32.

Scientific Conferences

2010 Edwards EJ, Downie AF & Clingeleffer PR. Vine response to, and recovery from, multiple years of deficit irrigation. Oral presentation at the 14th Australian Wine Industry Technical Conference 3-8th July 2010.

2010 Edwards EJ, Smithson L, Graham DC & Clingeleffer PR. What if a high-temperature event occurs whilst vines are deficit irrigated? Poster presentation at the 14th Australian Wine Industry Technical Conference 3-8th July 2010.

2009 Clingeleffer P. Integrated vine management to minimise the impacts of seasonal variability on yield, fruit composition and wine quality. *Proceedings 16th International Symposium, GiESCO 2009.* pp259-264.

2008 Edwards EJ, Lanyon D & Clingeleffer PR. Above and below-ground effects of six years of deficit irrigation in Cabernet Sauvignon grapevines. *8th International Symposium on Grapevine Physiology & Biotechnology – Book of Abstracts*. p 88.

2008 Edwards EJ & Clingeleffer PR. Intra- and inter-seasonal effects of short-term water deficit on grapevine physiology. *8th International Symposium on Grapevine*

Physiology & Biotechnology – Book of Abstracts. p 189.

2008 Edwards EJ & Clingeleffer PR. Intra- and inter-seasonal effects of short-term water deficit on grapevine physiology. *Combio 2008 – Combined Conference Abstracts*. POS-TUE-42.

2007 Edwards EJ, Hanlin RL & Clingeleffer PR. Does deficit irrigation have a long term effect on canopy growth and function? *Conference Proceedings of the 13th Australian Wine Industry Technical conference* pp298-299.

Industry workshops and presentations

2010 Edwards EJ. How vines cope with water stress. GWRDC Innovators Network workshops at Franklin River, Margaret River and Swan Valley, 21-23 November 2010.

2010 Loveys BR & Edwards EJ. How to determine vine response to irrigation and the environment. Workshop W43 at the 14th Wine Industry Technical Conference 3-8th July 2010.

2010 Edwards EJ. Heat and drought tolerance. Presentation as part of workshop W03 at the 14th Wine Industry Technical Conference 3-8th July 2010.

2010 Edwards EJ & Loveys BR. Understanding how vines cope with periods of hot weather and extended drought conditions. GWRDC Innovators Network workshops at Bunbury 21st January 2010 and Swan Valley, 22nd January 2010.

2009 Edwards EJ & Loveys BR. Understanding how vines cope with periods of hot weather and extended drought conditions. GWRDC Innovators network workshop at Cowra 1st December 2009.

2009 Edwards EJ & Loveys BR. Understanding how vines cope with periods of hot weather and extended drought conditions. GWRDC Water and Vine Program at Cowra 30th January 2009 and Canberra 27th February 2009.

2008 Edwards EJ & Loveys BR. Understanding how vines cope with periods of hot weather and extended drought conditions. GWRDC Water and Vine Program at Lindenwarrah, Wangaratta, Mildura and Griffith, October-December 2008.

2007 Edwards EJ, Hanlin RL & Clingeleffer PR. Long-term effects of deficit irrigation. Riverlink Wine Grape Symposium, 14th June 2007.

2007 Edwards EJ, Hanlin RL & Clingeleffer PR. Does deficit irrigation have a long term effect on canopy growth and function? The 56th Mildura Horticultural Field Days, 29-30th May 2007.

2007 Loveys BR & Edwards EJ. The impact of drought on vine performance. ASVO 'Drought Response' workshops, May 2007.

Appendix 3

Intellectual property

The information generated by this project will enter the public domain and will be accessible for future reference.

Appendix 4

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Appendix 5

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Appendix 6

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