Cooperative Research Centre for Viticulture

Australian Government
Grape and Wine Research and Development Corporation

Integrated strategies to manage seasonal variation in winegrape maturation



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The Cooperative Research Centre for Viticulture is a joint venture between the following core participants, working with a wide range of supporting participants.

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Cover Picture

Typical bunches from the three irrigation treatments applied in the detailed research component of this project. Control (left), Regulated deficit irrigation (middle) and Prolonged deficit irrigation (right).

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1. Abstract

The project aimed to develop strategies to minimise impaired or delayed fruit maturation. It investigated the effects of water deficits imposed through irrigation management on performance of own-rooted Cabernet Sauvignon vines. Linkages between treatment effects on vine physiology, carbohydrate status, yield components, berry development, fruit and wine composition were explored. Existing data sets from previous research and commercial sources were also analysed focusing on seasonal, regional and varietal effects on grape berry maturation. The study found that production may not been sustainable in the longer term with sustained deficit irrigation treatments, due to the reduced photosynthetic capacity and impacts on vine carbohydrate status. Management strategies to assist growers to improve the consistency of grape quality and meet set specifications were identified. These were (1) extended pre-veraison water deficits to enhance berry colour in seasons with low potential colour caused by high pre-veraison temperatures, applied with the caution that this treatment results in yield reductions and research on sustainability of this treatment beyond three seasons is yet to be conducted; (2) the application of particle film treatment (PFT) to reduce leaf temperature and minimise stresses associated with hot weather and/or water deficits.

2. Executive summary

The project had two main components. The first investigated the effects of water deficits imposed through irrigation management on vine performance, in particular berry development and fruit and wine composition of own-rooted Cabernet Sauvignon. Linkages between treatment effects on vine physiology, carbohydrate status, yield components, fruit maturation and fruit and wine composition were explored. In the second component of the project, existing data sets from previous research and commercial sources were analysed focusing on seasonal, regional and varietal effects on grape berry maturation.

In the Cabernet Sauvignon study the irrigation treatments imposed over three seasons were a control (Con) irrigation treatment, scheduled by the operators of the vineyard, a Regulated Deficit Irrigation (RDI) where a soil water deficit was applied in the post-fruit set to pre-veraison period and a Prolonged Deficit (PD) treatment which was similar to the RDI but had an extended period of extreme deficit (no water) after the RDI stress period until veraison. The effect of superimposing particle film treatment (PFT) on the above irrigation treatments was also assessed in some seasons with the aim to reduce canopy leaf temperature. In general for the parameters measured, seasonal effects were greater than treatment effects. Large differences between seasons were measured in yield (36%), berry characteristics (fresh and dry berry weight, the ratio of fresh to dry weight, skin, flesh and seed dry weight and sugar per berry), fruit composition (berry phenolics, total anthocyanins (1.3-1.9 mg/g), non-degraded anthocyanins and degraded anthocyanins) and wine spectral properties (colour density, hue, total anthocyanins, ionised anthocyanins and total phenolics).

Water deficits affected vine physiology, leading to reduced photosynthesis, leaf water potential and turgor, transpiration and stomatal conductance. They also increased leaf temperature for up to 3 weeks after the end of the stress period and had a long term impact on shaded leaf water potential for up to 2 months after return to normal irrigation during fruit ripening. The lower photosynthesis impacted on berry development, maturation, carbohydrate partitioning and storage. Application of PFT lowered leaf temperature and reduced the impact of soil moisture deficits on leaf physiology and may increase the sustainability of deficit irrigation treatments.

Compared to the control, deficit treatments (RDI and PD) had lower yields, smaller bunches, reduced berry size, but had no significant effect on flesh weight, seed weight, or the skin to flesh ratio or the fresh to dry weight ratio of berries. Deficit treatments had minimal effect on fruit composition (titratable acidity, pH, phenolics, total anthocyanins and non-degraded anthocyanins) but had a significant positive effect on wine quality attributes, particularly with PD (ie. colour density, hue, total anthocyanins, ionised anthocyanins and total phenolics).

With Cabernet Sauvignon maximum berry weight occurred about two weeks after 50% veraison and then remained relatively constant regardless of season until harvest one month later. Note, this is in contrast to the analysis of Shiraz data where the increase in sugar concentration at the later stages of ripening was associated with a significant berry weight loss attributed to moisture loss and dehydration. With Cabernet Sauvignon, differences between seasons and irrigation treatments were obvious at maximum berry weight. Total soluble solids concentration increased rapidly for two weeks after 50% veraison reaching 20 ^{0}Brix followed by a much slower rate of increase in concentration until harvest. Reductions in total sugar accumulation attributed to water deficits, particularly PD were most likely due to the limited supply of carbohydrates around veraison. The pattern of berry anthocyanins accumulation differed with different levels of water deficit. With the control and RDI treatment, anthocyanin concentration reached a maximum about 20 days after veraison whereas with PD anthocyanin concentration tended to increase for an extended period up to harvest. The PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons. Deficit irrigation treatment reduced malic acid levels and enhanced the tartrate to malate ratio during the ripening period.

Analysis of the commercial data sets showed that average ripening rates were relatively constant across most varieties and regions, except in the very cool regions. In most cases differences in harvest date between regions, varieties and growers within a region could largely be attributed to differences in the date of veraison rather than the rate of ripening. There were however differences between growers in a region which could not be accounted for by crop load, rootstock or pruning system.

The project has identified potential problems of production sustainability when deficit irrigation treatments are adopted with the aim to enhance wine quality associated with reduced photosynthetic capacity and impacts on vine carbohydrate status. It has shown that water deficits can impact on vine physiology for up to 2 months after irrigation treatments are returned to normal. The project has identified two rather contradictory vineyard management practices which, if proven under larger commercial conditions, should provide growers with further tools to improve the consistency of grape quality and assist in meeting set specifications, at least for red wine production. Firstly, the results with PD show that extended pre-veraison water deficits enhance berry colour, particularly in seasons with low potential colour caused by high temperatures in late December and early January. However, this benefit is potentially offset by yield reductions and the impact of this treatment on vine performance for more than three seasons is yet to be determined. Secondly, the application of PFT can potentially reduce leaf temperature and minimise stresses associated with hot weather and/or water deficits.

3. Background

3.1 Background

Growers in most winegrape producing regions of Australia have at some stage, experienced problems in getting grapes of various varieties mature within reasonable time frames. Season 2000 was a worst case scenario, with grapes maturing normally or ahead of schedule in the early part of the season, followed by a slow-down or halt in sugar accumulation, then either no or partial recovery later in the season. Many growers and corporate vineyard managers had no choice but to delay harvest until late in the season in the hope that total soluble solids (TSS) would increase, but this did not always happen and many crops had to be picked short of target sugar levels. Fruit was often low in acid with high pH. Winery intake schedules were severely affected with consequent inefficiencies and poor juice quality.

Not all varieties suffered the problem and in those that did, the effect was linked to a period of heat followed by periods of heavy rainfall. The effect highlights significant gaps in knowledge of grapevine source-sink relationships and their interaction with environmental factors. The problem was also evident in the considerable difference in maturation times for a single variety within a region that cannot always be explained by crop load effects. For example, in Sunraysia, the Shiraz crop at some sites matured in mid-February, while at other sites, the crop did not mature until April. There is a lack of understanding of the underlying management and associated physiological factors that lead to such variability. The increasing use of Regulated Deficit Irrigation (RDI) to achieve higher quality in combination with periods of heat stress may be a factor, but the reality is that little is known of the causes of the 'arrested maturation' problem. RDI has little margin for error and a combination of RDI and sudden, intense heat may negatively impact on vine physiology.

To address this question, a project was initiated to investigate the viticultural and physiological variables including water stress and its interaction with crop load to identify possible causes leading to fruit maturity differences. Although difficult to investigate in a field situation, the interaction between water stress and heat stress was monitored whenever possible. Grape colour was also monitored along with sugar levels. The effects of the stresses on photosynthetic capacity and inter-relationships with the developing crop's requirement for imported carbohydrate was followed. The role of trunk (reserve) water soluble carbohydrate in supplying carbohydrate to developing fruit was investigated. The project aimed to identify *strategies* to minimise delayed fruit maturation. In addition, historical data sets from both CSIRO and major wine companies were analysed with respect to seasonal and climatic influences on fruit maturation.

The research aimed to increase our understanding of the maturation process and facilitate vine management strategies and recommendations to achieve greater seasonal consistency. It aimed to demonstrate the impact of various "stresses" on fruit maturation and thus to identify irrigation and canopy management strategies most likely to achieve good fruit quality.

3.2 Preliminary and Previous Research

Several researchers have documented heat stress effects on fruit maturation. Kliewer (1977), using Emperor grapes, showed that extremes in temperature (37/32° C day/night) arrested anthocyanins accumulation and slowed sugar accumulation which did not increase above 12.9° Brix. It appears that anthocyanin synthesis is impaired at much lower temperatures than sugar accumulation (Buttrose et al. 1971; Kliewer and Lider 1970). In the field it is likely that periods of high day and night temperatures in combination with low levels of available soil moisture will lead to plant water deficits resulting in adverse effects on photosynthesis as well as colour and sugar accumulation. Numerous experiments conducted in the field and in pots show that imposition of water stress in grapevines may lead to a rapid decline in the rate of photosynthesis and sugar accumulation (Quick et al. 1992, Smart and Coombe 1983, Schultz 1996). Sommer et al (1993) in a field experiment conducted in Coonawarra with Cabernet Sauvignon vines found that leaf photosynthesis and accumulation of TSS in berries may be

completely arrested under soil water deficits. The problem seems to be further exacerbated in years with a high crop (Poni et al. 1994), although crop load does not appear to be the primary cause of differences in maturation across Sunraysia growers (Clingeleffer et al. 2001). In a field experiment conducted at Merbein, maturation of well-watered Cabernet Franc vines was severely impaired during the 1998/99 growing season beginning shortly after veraison (Sommer 2000). Preliminary evaluation of the 1998/99 season using the VineLOGIC model suggested that the average day and night temperature during veraison and harvest was several degrees above the long-term average. Crop load of Cabernet Franc vines in the 1998/99 season of the Merbein experiment also was above average. It is conceivable that heat induced transient water stress combined with a heavier than average crop contributed to the temporary arrest of colour and sugar accumulation in Cabernet Franc vines.

The problem of arrest of sugar accumulation in grape berries may have some similarities to other species, where import of assimilate to reproductive organs is influenced by heat shock, often in combination with water stress and inter-relationships between the sink's demand for assimilate and the inability of the stressed leaves to supply it together with the capacity of stem tissues to supply reserve water soluble carbohydrates when needed (van Herwaarden et al. 1999).

Grape berry total anthocyanin levels have also been shown to depend on the degree of bunch shading and resultant berry temperature (Haselgrove et al, 2000). The intensity of effects of heat shock and water stress may be different for anthocyanin formation and sugar accumulation.

There is a rising trend to impose Regulated Deficit Irrigation and Partial Root Zone Drying in vineyards of the irrigated inland growing districts. The aim of such management procedures is to reduce vigour and optimise fruit quality by reducing berry size. It is quite conceivable that such management procedures impose periods of transient water stress on the vine that, under extremely high temperatures and combined with a heavy crop, may be detrimental to sugar accumulation and colour formation.

3.3 Specific Objectives

- Examine environment triggers for the onset of ripening delay in major cultivars.
- Test the interaction of fruit load, water deficit, heat stress on berry development and composition.
- Examine cultivar site interactions on fruit ripening using historical data
- Develop strategies to minimize impaired or delayed fruit maturation

3.4 Relevant publications/references.

Buttrose, M.S., Hale, C.R. and Kliewer, W.M. (1971) Effect of temperature on the composition of 'Cabernet Sauvignon' berries. American Journal of Enology and Viticulture **22**, 71-75.

Clingeleffer, P.R., Krstic, M.P. and Sommer, K.J. (2001) Production efficiency and relationships among crop load, fruit composition and wine quality. American Journal of Enology and Viticulture (in press)

Iacono, F.; Sommer, K. J.; 1999: The measurement of chlorophyll fluorescence as a tool to evaluate the photosynthetic performance of grapevine leaves. In: E. H. Rühl, J. Schmid (Eds.): Proceedings of the First ISHS Workshop on Water Relations of Grapevines. Acta Horticulturae, **493,** 31-44. ISHS Leuven, Belgium.

Iland, P.G. (1987). Predicting red wine colour from grape analysis. Australian Grapegrower and Winemaker, 285,29.

Haselgrove, L., Botting, D. van Heeswijck, R., HØj, P.B., Dry P.R., Ford, C. and Iland, P.G. (2000). Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic

composition of V*itis vinifera* L. cv. Shiraz grape berries. Australian Journal of Grape and Wine Research **6,** 141-150.

Kliewer, W.M. and Lider, L.A. (1970) Effects of day temperature and light intensity on growth and composition of *Vitis vinifera* L. fruits. Journal of the American Society for Horticultural Science **95**, 766-769.

Kliewer, M.W. (1977) Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. American Journal of Enology and Viticulture **28**, 96-103.

Kliewer, W.M., Freeman, B.M. and Hossom, C. (1983) Effect of irrigation, crop level and potassium fertilization on Carignane vines. I. Degree of water stress and effect on growth and yield. American Journal of Enology and Viticulture **34**, 186-196.

Poni, S., Lakso, A.N., Turner, J.R. and Melious, R.I. (1994) Interactions of Crop Level and Late Season Water Stress on Growth and Physiology of Field-Grown Concord Grapevines. American Journal of Enology and Viticulture **45**, 252-258.

Quick, W.P., Chaves, M.M., Wendler, R., David, M., Rodrigues, M.L., Passaharinho, J.A., Pereira, J.S., Adcock, M.D., Leegood, R.C. and Stitt, M. (1992) The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. Plant, Cell and Environment **15**, 25-35.

Schultz, H.R. (1996) Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. In: S. Poni, E. Peterlunger, F. Iacono, C. Intrieri (Eds.): Proc. Workshop: Strategies to Optimize Wine Grape Quality. 251-266. ISHS, Acta Horticulturae 427.

Smart, R.E.; Coombe, B.G. (1983) Water relations of grapevines. In: T. T. Kozlowski (Ed.): Water deficits and plant growth. 137-196. Academic Press New York.

Sommer, K.J., Clingeleffer, P.R. and Ollat, N. (1993) Effects of minimal pruning on grapevine canopy development, physiology and cropping level in both cool and warm climates. Viticultural and Enological Sciences **48**, 135-139.

Sommer, K.J. (2000) Optimisation of quality wine grape production through modelling wine phenology, canopy architecture, light interception, water use and yield. Final Report Grape and Wine Research and Development Corporation. pp 54.

van Herwaarden, A.F., Richards, R.A., Farquhar,G.D. and Angus, J.F. (1999) 'Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser. 111 The influence of heat shock and water stress. Australian Journal of Agricultural research **49**, 1095-110

4. Project Aims and Performance Targets

4.1 Hypothesis:

Impairment of berry ripening appears to be caused by a combination of above average day and night temperatures post-veraison (the period of sugar loading in grapes) and a transient limitation in available soil water supply. If vines also carry an above average crop under such conditions it is likely that fruit maturation and ripening will be impaired and harvest delayed.

4.2 Overall Objectives:

The principal objectives were to test the interaction of fruit load, water deficit and if possible heat stress, on berry development and composition in the field; to identify the factors causing impairment of berry ripening; and to develop strategies to minimise impaired or delayed fruit maturation.

4.3 Performance targets:

Planned outputs and performance targets as described in the original application are provided below.

Outputs	Performance Target
1. Field experiments and experimental treatments established	1. Irrigation and crop load treatments imposed in each season (02,03,04,05)
2. Stress indicators assessed	2. Stress indicators (chlorophyll fluorescence, water potential) assessed fortnightly pre-veraison and weekly during maturation in each season (02,03,04,05)
3. Weekly characterisation of juice composition	3. Berries collected and analysed on a weekly basis for key maturity indicators (Berry volume and weight juice TSS, pH, titratable acidity, colour, organic acid composition) (02,03,04,05)
4. Sampling of perennial tissues and analysis of reserve carbohydrates	4. Carbohydrate measurements undertaken from sampled tissues of selected experimental treatments in each season (02,03,04,05)
5. Yield and fruit quality indicators assessed at harvest	5. Yield and juice quality indicators of treatments characterised by June each season (02,03,04,05)
6. Preliminary understanding of the conditions (environmental / physiological) that lead to the delayed maturation problem	6. Commercial and initial (02) experimental data sets obtained and analysed by December 2002
7. Strategies to minimise delayed and seasonal variation in grape berry maturation identified	7. Strategies identified by June 2004 and recommendations extended by harvest 2005

5. Methodology Overview

A key experimental site was established in a commercial vineyard (Wingara Wine Group) to assess effects of transient water stress on vine physiology, crop development, maturation and fruit and wine composition. For this purpose own-rooted Cabernet Sauvignon, planted in 1995 at a density of approximately 1366 vines per ha (2.44 m within row and 3 m between rows) on Nookamka sandy loam (Hubble and Crocker 1941) located in the Sunraysia region of Victoria, Australia were used. Vines were trained in east-west orientated rows, on a two wire vertical trellis and mechanically hedged. The study was conducted over three seasons (2002/2003 - 2004/2005). A control treatment (standard drip), which was irrigated to full point, was compared with a regulated deficit irrigation (RDI) treatment and a prolonged deficit irrigation (PD) treatment during the season. Treatments in 12 vine plots were arranged in a random design down the rows with a total of 12 replicates. In total the experimental site extended over 36 rows (ie. 2 guard rows and one measured row). The duration of the RDI was determined based on visual assessment of tendrils, canopy leaf area, vine canopy to crop ratio and soil water monitoring data. The RDI treatment was imposed post-veraison for a period of about three weeks depending on seasons. The PD treatment was identical to the RDI treatment but non-irrigation was extended for up to three weeks after the RDI period and presents an extreme deficit irrigation treatment. In addition in 2003/2004 and 2004/2005 a particle film treatment was superimposed on the study. This treatment involved application of a koalinite based, commercial product (ie. Surround) with the aim to reduce canopy leaf temperature and minimise the stress effects associated with the imposed irrigation treatments.

The effects of the deficit irrigation treatments on aspects of plant physiology involved monitoring canopy growth using a canopy analyser (LAI- 2000, Li-Cor, Nebraska), studies of vine water relations including pre-dawn or mid-day water potentials, monitoring leaf gas exchange, leaf chlorophyll fluorescence and C^{13} discrimination and analysis of carbohydrate status of perennial tissues.

Monitoring of fruit development on a weekly basis from veraison to harvest involved measurement of total soluble solids, berry weight, berry skin, flesh and seed weights (fresh and dry), pH and titratable acidity, organic acids, berry anthocyanins and phenolics. Yield, bunch numbers and bunch weights were assessed at harvest. Wine was made from small lots of fruit from all treatments and various attributes assessed.

The study also examined recent experimental data on grape berry maturation from GWRDC-funded projects, eg. the ‘crop development and prediction’ project and the ‘crop control for quality wine grape production’ project. These projects covered a range of sites and regions, and involved the major wine grape varieties. In addition a major commercial data set on grape berry maturation, covering a range of varieties, sites and regions was assessed.

When deemed appropriate, further methods and techniques are included in the results section.

6. Results

6.1. Vine physiology

6.1.1. Main experiment

Irrigation treatments

The application of irrigation water was similar for all treatments from budburst until after flowering (ie. up to the commencement of RDI) and from the completion of PD until leaf fall. The timing, duration and volume of water applied for each treatment during managed stress periods and in total are presented in Table 6.1.1.1. While the RDI treatment commenced at almost the same time in each season (21st – 25th November) the duration varied considerably (9th December - 2nd January), a result of commercial decisions taking into account seasonal factors, soil moisture and vine growth. Hence the water applied in the RDI period varied across the seasons from 34 -134 mm for the control and 18 - 67 mm for the RDI and PD treatment. The timing and duration of the PD period also varied between seasons with commencement from 20th December - 2nd January and cessation from 31st December - 18th January. The water applied to the control and RDI treatment in the PD period ranged from 25 - 50mm. Total application of water applied as irrigation in each season ranged from 4.5-6.5 ML/ha.

Irrigation treatment	RDI period Irrigation applied				PD period irrigation applied				Season total water applied (ML per ha)
	ET[1] (%)	ON	OFF	Water applied (mm)	ET[1] (%)	ON	OFF	Water applied (mm)	
Control	100	21-Nov-02	13-Dec-02	50	100	24-Dec-02	31-Dec-02	25	4.5
RDI	50	21-Nov-02	13-Dec-02	25	100	24-Dec-02	31-Dec-02	25	4.3
PD	50	21-Nov-02	13-Dec-02	25	0	24-Dec-02	31-Dec-02	0	4.0
Control	100	25-Nov-03	2-Jan-04	134	100	2-Jan-04	18-Jan-04	42	6.5
RDI	50	25-Nov-03	2-Jan-04	67	100	2-Jan-04	18-Jan-04	42	5.1
PD	50	25-Nov-03	2-Jan-04	67	0	2-Jan-04	18-Jan-04	0	4.9
Control	100	22-Nov-04	09-Dec-04	34	100	20-Dec-04	03-Jan-05	50	4.5
RDI	50	22-Nov-04	09-Dec-04	18	100	20-Dec-04	03-Jan-05	50	4.4
PD	50	22-Nov-04	09-Dec-04	18	0	20-Dec-04	03-Jan-05	0	3.8

Abbreviations: **RDI** regulated deficit irrigation, **PD** prolonged deficit irrigation and **ET** evaporative transpiration. [1] **ET** was based on soil water.

Table 6.1.1.1. Irrigation treatment durations and volumes applied during managed stress periods along with total season irrigation applied.

Soil moisture

Soil moisture within the rootzone (0-70cms) is shown for each treatment in each season (Figure 6.1.1.1). Compared to the control, both deficit irrigation treatments had lower soil moisture during the RDI period to varying degrees, the difference being largest in season 2002-03 while in 2003-04 differences were only achieved after an extended RDI period. Compared to the control and RDI treatments low soil moisture content was achieved with the PD treatment in each season, the difference being largest in 2002-03. In season 2003-04 there was some carryover of the RDI treatment into the PD period. Higher soil moisture levels with deficit irrigations in the ripening period when all treatments received similar quantities of water in 2004-05 and with PD in 2003-04 are indicative of reduced water use by vines which have been subject to water deficits. This adaptive change is most likely due to development of smaller canopies and long term impacts on uptake of water by roots and canopy function (see below).

Seasonal changes in leaf physiology

Leaf water potential and gas exchange measurements of fully exposed leaves were undertaken at different stages of canopy development over each growing season to determine the impact of deficit irrigation strategies on canopy function. During the RDI period, days 60-80 after budburst in season 2002-03, the PD treatment (and the identical RDI treatment) had lower assimilation rates, transpiration and stomatal conductance although dawn leaf water potentials were only slightly reduced (Figure 6.1.1.2). Leaf function of the RDI treatment was identical to the control during the ripening period indicating a complete recovery of gas exchange. By contrast, the PD treatment produced much lower assimilation, transpiration and stomatal conductance during the PD period. This effect was maintained for about three weeks after the irrigation was returned to the same level as the other treatments. Leaf water potential of PD was also lower when measured after the PD period. In season 2003-04 there were no apparent effects of irrigation treatment on leaf water potential (Figure 6.1.1.3). Compared to the control both deficit treatments had lower assimilation, transpiration and stomatal conductance at the end of the extended RDI period. These differences were maintained with both deficit treatments during the PD period, indicating a severe long term impact on leaf function from the extended period of RDI. In season 2004-05 there were some but not consistent reductions in assimilation, stomatal conductance, transpiration and leaf water potential with deficit irrigation treatments, particularly RDI (Figure 6.1.1.4).

Diurnal measurement of leaf physiology

Daily time course measurements of leaf water status and leaf gas exchange were also undertaken at key stages of vine development taking into account the duration of irrigation treatments. In general deficit irrigation treatments which produced a significant difference in soil moisture contents reduced canopy function during and in some cases, after the duration of the imposed stress period. Comparisons of the control and PD treatment measured on 23rd January 2003, 3 weeks after the end of PD stress period, are provided as an example. The results show a 50% reduction in assimilation (Fig. 6.1.1.5), a 2-fold decrease in stomatal conductance (Fig. 6.1.1.6) and a 2-fold reduction in transpiration (Fig. 6.1.1.7) which were maintained throughout the day. Leaf turgor (Fig. 6.1.1.8) and leaf water potential (Fig. 6.1.1.9), were reduced with PD compared to the control at all times during the day, except at dawn and late in the evening. Leaf temperature was increased by up to 5 ^{0}C with PD, a result of the reduced transpiration.

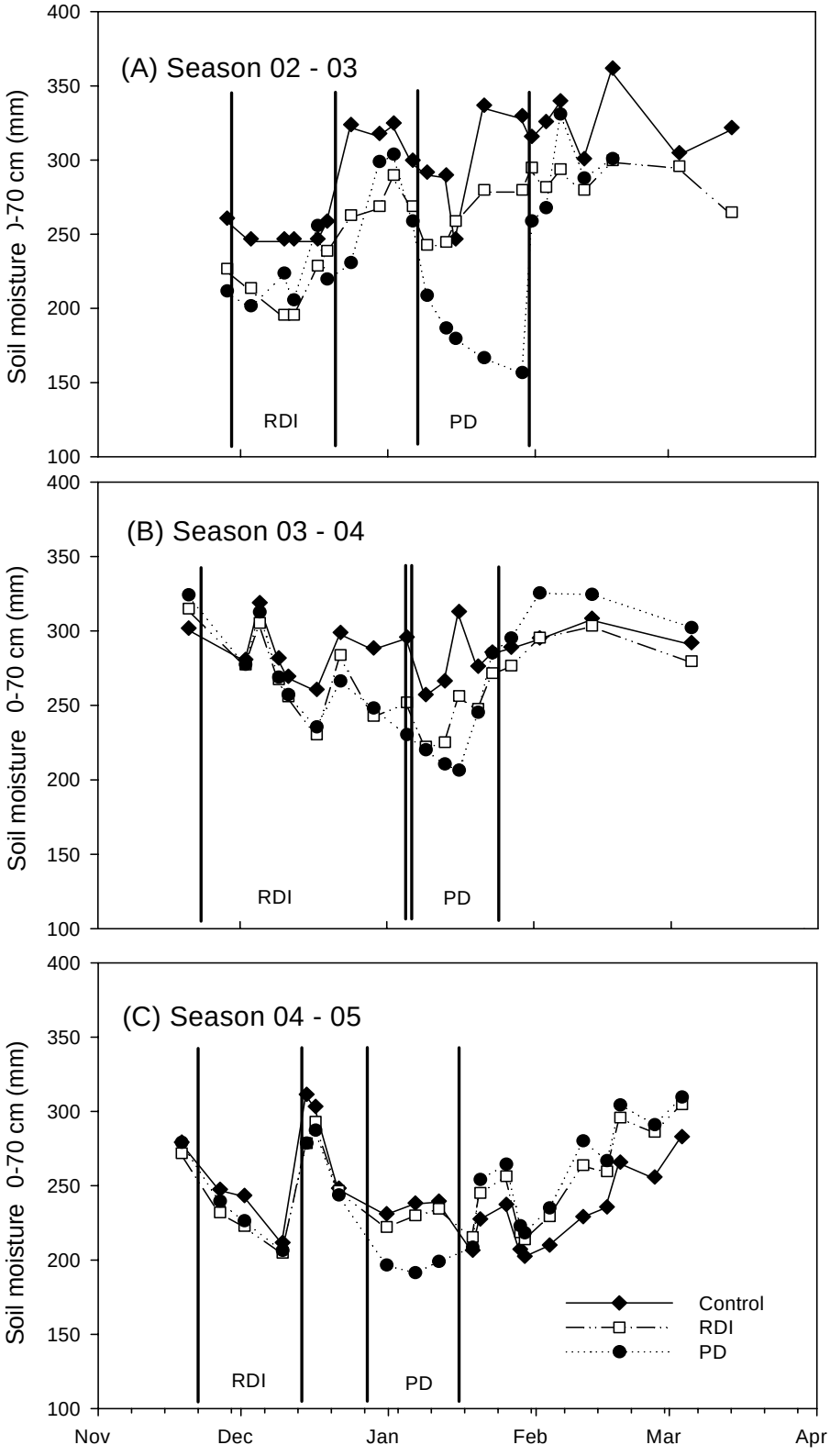


Figure 6.1.1.1. Soil moisture 0-70 cm throughout the season for (A) season 2002-2003, (B) season 2003-2004 and (C) season 2004-2005. Readings measured using neutron probes at 10 cm intervals to a depth of 1.2 meters. Readings were acquired on a bi-weekly basis between 6.00 and 11.00 am.

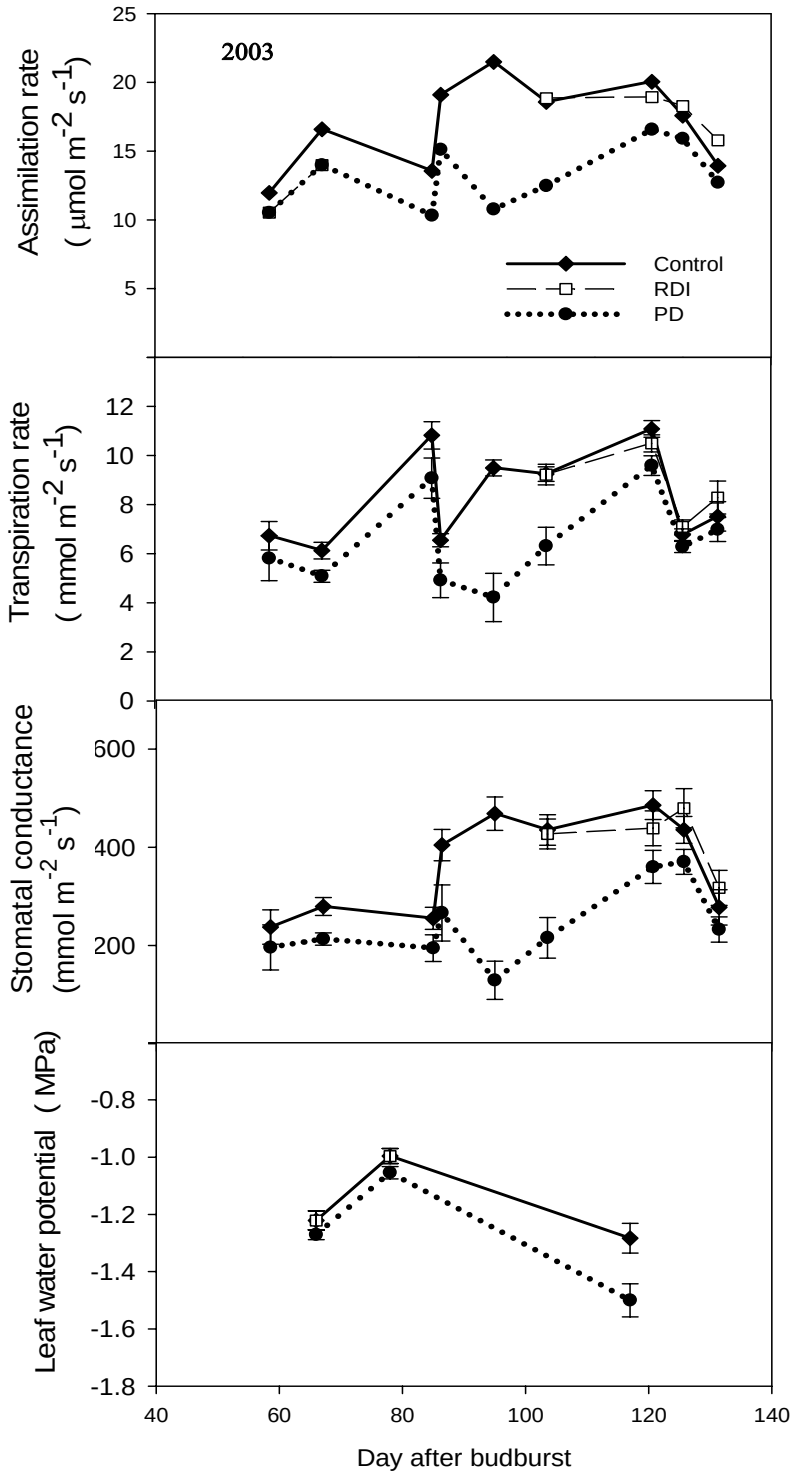


Figure 6.1.1.2 . Changes in assimilation, transpiration, stomatal conductance and leaf water potential of the three irrigation treatments (ie. Control, RDI, PD) during season 2002-03 (n=8).

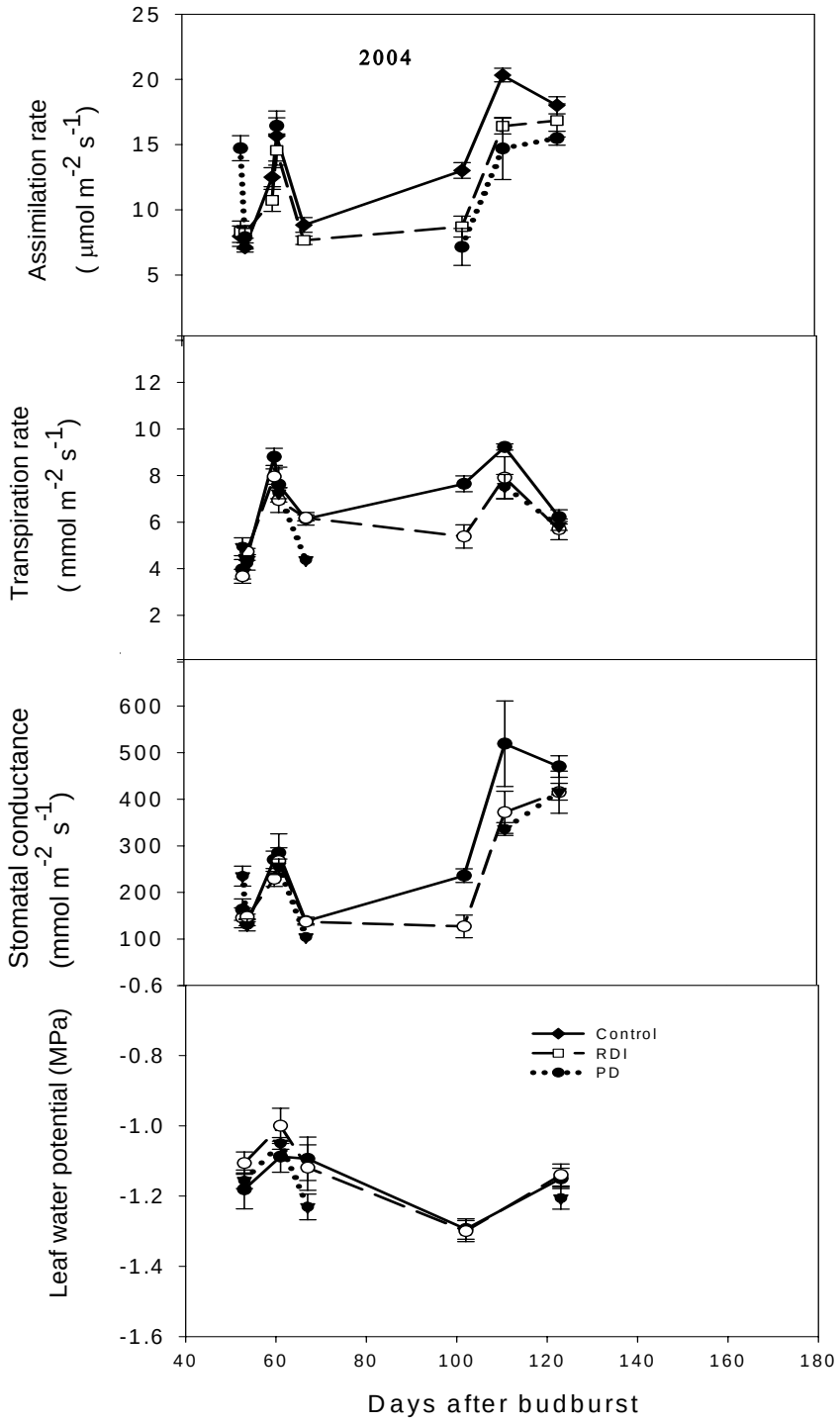


Figure 6.1.1.3 . Changes in assimilation, transpiration, stomatal conductance and leaf water potential of the three irrigation treatments (ie. Control, RDI, PD) during season 2003-04 (n=8).

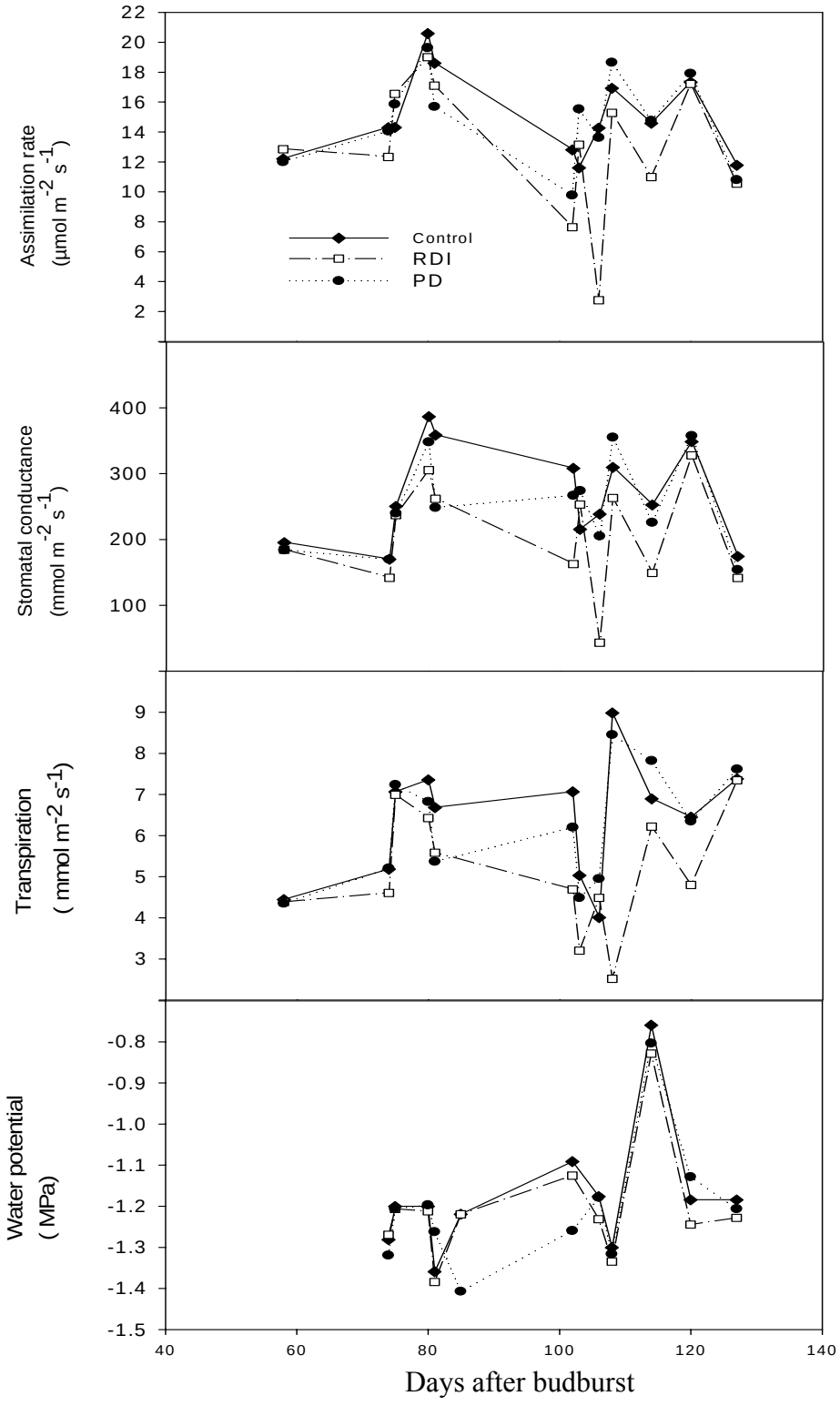


Figure 6.1.1.4 . Changes in assimilation, transpiration, stomatal conductance and leaf water potential of the three irrigation treatments (ie. Control, RDI, PD) during season 2004-05 (n=8).

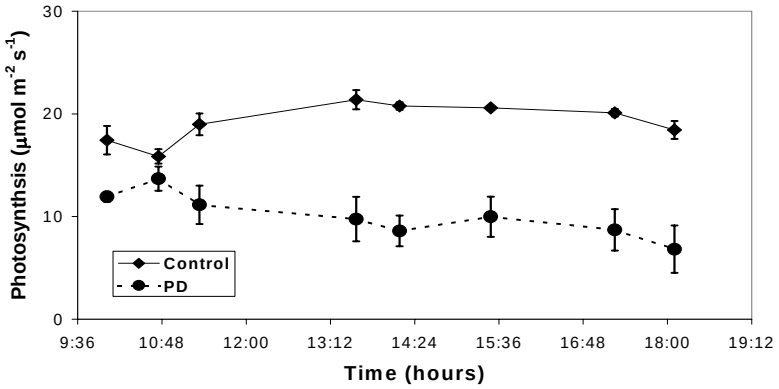


Figure 6.1.1.5. Daily time course of photosynthesis (assimilation) of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

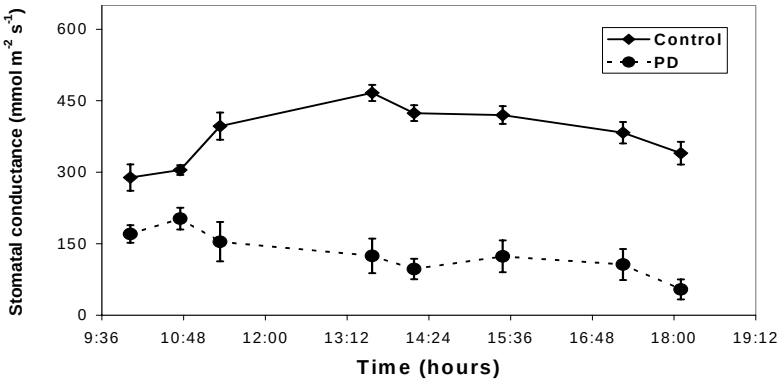


Figure 6.1.1.6. Daily time course of stomatal conductance of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

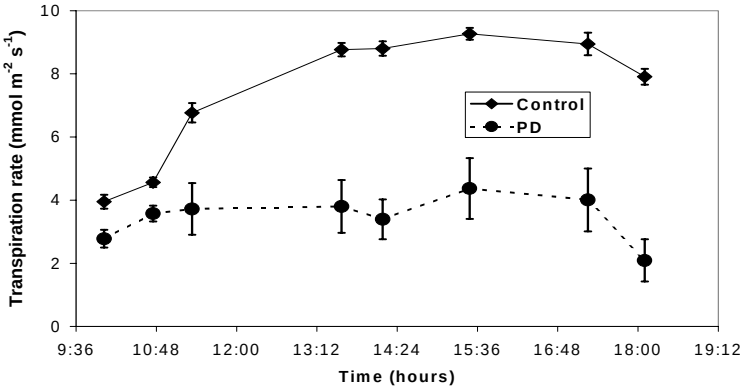


Figure 6.1.1.7. Daily time course of transpiration of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

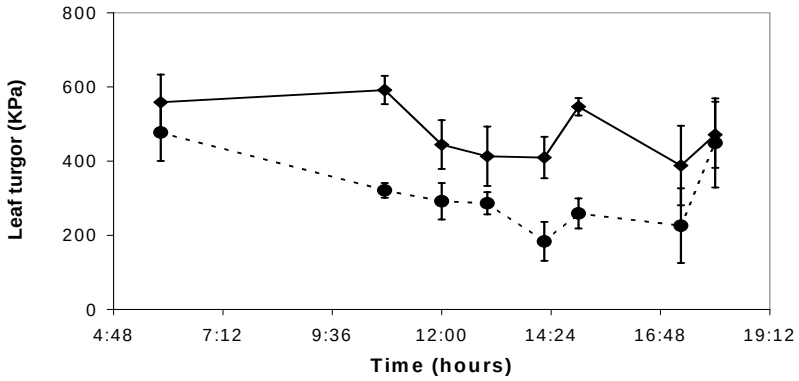


Figure 6.1.1.8. Daily time course of leaf turgor of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

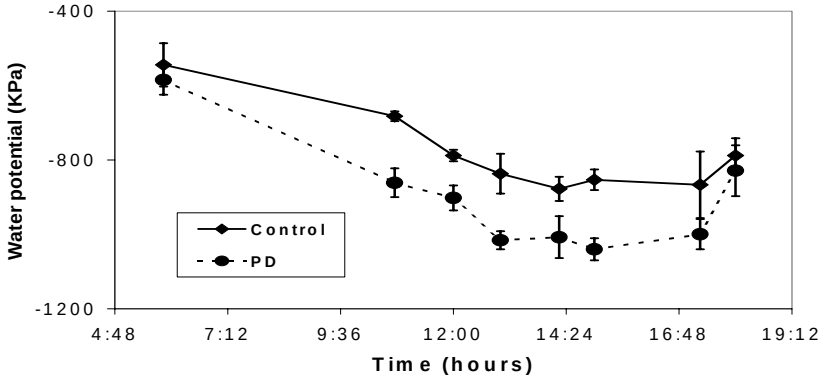


Figure 6.1.1.9. Daily time course leaf water potential of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

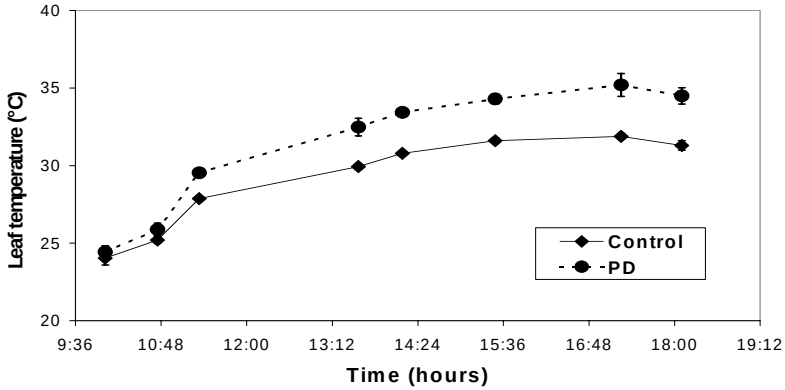


Figure 6.1.1.10. Daily time course of leaf temperature of control and PD treatments on 23rd February 2003, 23 days after the end of the PD period.

6. 1. 2. Studies with particle film technology

In seasons 2003/04 and 2004/05 studies involved use of mineral reflectant, particle film treatments (PFT) which were superimposed on the main experiment to assess the effectiveness of PFT in reducing the severity of the water deficits and potentially increase the sustainability of deficit irrigation. These studies were undertaken in collaboration with Dr. Michael Glenn, a visiting scientist from the United States Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, WV, USA. In addition to the superimposed PFT treatments he was able to introduce techniques for continuous logging of shaded leaf water potential over the season which were applied to all treatments. These techniques have provided new insights into grapevine responses to water deficits. The following abstract from a paper in preparation (*Leaf water potential, canopy temperature and water use efficiency of grapevines under regulated deficit and prolonged deficit irrigation and with particle film treatments*, Glenn et al. 2006), summarises the key issues relating to vine physiology.

Abstract

Leaf-air temperature (ΔT), shaded leaf water potential (SLWP), water use efficiency (WUE), photosynthesis (A), transpiration (E), stomatal conductance (Gs) and responses of Cabernet Sauvignon (*Vitis vinifera* L.) vines were measured in control or standard irrigation (STD), regulated deficit (RDI) and prolonged deficit (PD) irrigation treatments, each with and without a particle film (PFT). The STD was maintained at close to field capacity throughout the entire growing season and was referred to as 100% of the estimated evapotranspiration (ET) based on soil water extraction. The RDI and PD treatments received the same irrigation as STD up to 25 November, after which RDI received 50% ET for 38 days followed by 100% ET for the remainder of the season. The PD treatment incorporated a RDI treatment followed by an 18 day period of no irrigation. After the no irrigation interval, 100% ET was applied to the PD for the remainder of the season. STD and RDI irrigation increased ΔT compared to PD for the non-PFT treatments. The PFT treatments increased ΔT and WUE (measured as isotopic carbon discrimination Δ) in all irrigation treatments. Pre-veraison SLWP indicated an irrigation by PFT treatment interaction in which the non-PFT RDI had significantly more negative SLWP compared to other treatments. After veraison, the STD non-PFT treatment maintained less negative SLWP compared to the PD and RDI non-PFT treatments. SLWP was less negative with plus-PFT for all irrigation treatments. During the period of PD stress A, E, and Gs were reduced compared to the STD. Moreover, A, E and Gs in the RDI treatment, when measured at a similar time, were also reduced, despite the vines having been returned to 100% ET. Four days after resumption of 100% ET in the PD treatment, the PD treatments had reduced A, E, and Gs compared to the STD, and the RDI was intermediate. Sixteen days after resumption of 100% ET in the PD treatment, there were no significant differences in gas exchange. The addition of PFT to the deficit treatments may increase the sustainability of deficit irrigation particularly in the case of the PD treatments.

Continuous monitoring of leaf water potential (leaf ψ)

Results of monitoring of the stem leaf water potential (SLWP) pre- and post veraison are shown together with the maximum daily temperature in Fig. 6.1.2.1. Analysis of variance (ANOVA) of pre-veraison SLWP indicated an irrigation by PFT treatment interaction in which the non-PFT RDI had significantly more negative SLWP compared to other treatments (Table 1). ANOVA of the post-veraison SLWP did not indicate any two or three way interactions. Averaged over PFT treatments, the STD irrigation treatment had significantly less negative SLWP than the RDI or PD treatments (-0.33, -0.61, and -0.55, respectively, *P=0.05*). The application of PFT to irrigation treatments resulted in less negative SLWP following veraison in all irrigation treatments (Table 1).

Irrigation Treatment	Pre-veraison period		Post-veraison period	
	plus-PFT	non-PFT	plus-PFT	Non-PFT
STD	-0.50 B	-0.54 B	-0.21 C	-0.45 B
RDI	-0.55 B	-0.62 A	-0.48 B	-0.74 A
PD	-0.46 C	-0.51 BC	-0.46 B	-0.63 A

Table 6.1.2.1 Mean shaded leaf water potential in the pre-veraison and post-veraison periods for Cabernet Sauvignon (*Vitis vinifera* L.) receiving irrigation treatments STD, RDI and PD each plus-PFT and non-PFT. Irrigation treatments: STD=Full irrigation; RDI=Regulated Deficit Irrigation; PD=Prolonged Deficit Irrigation. PFT=Particle Film Treatment. Mean separation within time periods by protected LSD, *P=0.05*

These results have major implications for sustainability of production for the use of irrigation stress strategies as it is clear that both the RDI and the PD treatments were clearly under stress during fruit ripening, a significant time after water application had been returned to normal for all treatments. PFT reduced this stress during ripening following a significant period of high temperature in early February. It is clear that further research is required into the mechanisms associated with the long term stress responses which may well be linked to effects on root development and/or function.

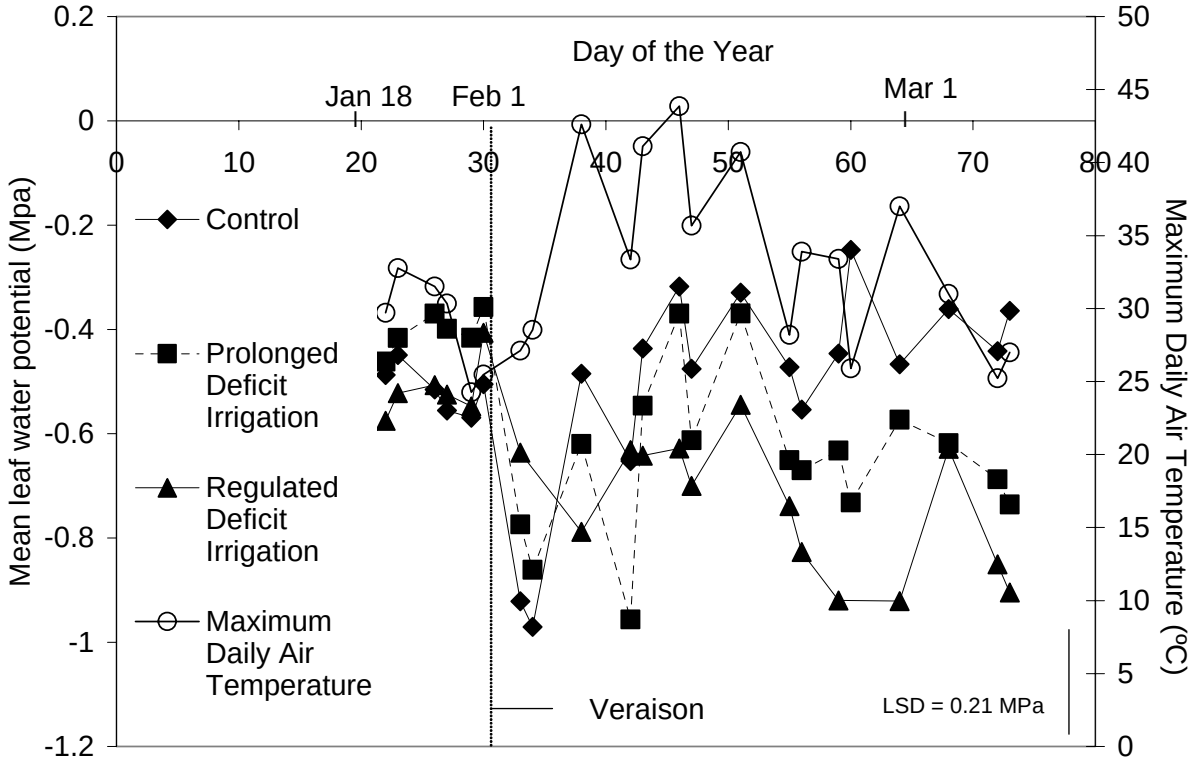


Figure 6.1.2.1 Mean leaf water potential (leaf ψ) for the control, regulated deficit and prolonged deficit (PD) irrigation treatments pre- and post-veraison in season 2004. The maximum daily air temperature is also included.

Insert:
6.1.2.1 Poster summary of vine physiology involving water stress and PFT treatments

An overview of the vine physiology in season 2004 is provided in a poster prepared for the ComBio 2005 conference in Adelaide in 2005 (*Plant physiological responses to water stress applied in warm climates Cooley et al. 2005*).

Aims

To examine the impacts of water stress and application of particle film technology (PFT), which may reduce leaf temperature, on *Vitis vinifera* during season 2003-2004. The relationship between water potential and leaf temperature post water deficit along with vine physiology was investigated.

Methodology

- **Three Irrigation treatments were applied**
- A **control** treatment (137 mm/ha / RDI period, 100%ET).
- A **regulated deficit irrigation** (RDI) treatment where a water deficit is applied following fruit set (67 mm/ha / RDI period, 50%ET, 25-Nov-03 until 02-Jan-04).
- A **prolonged deficit** (PD), a more extreme water stress treatment, applied following the RDI deficit until veraison (0 mm/ha / PD period, 0% ET, 02-Jan-04 until 18-Jan-04). 1 All treatments were applied were as standard drip irrigation and water replenishment for the RDI treatment was based on neutron-probe data readings (data not shown).

- **Partial Film Technology (PFT) treatments**
  Particle film technology is a white kaolin based film. Four applications were applied (28-Nov-03, 12- Dec-03, 23-Dec-03 & 5-January-04). The treatments with PFT are termed **plus-PFT** while the treatments without the PFT spray are termed **non-PFT**.

Results

Physiology during and post water deficit

13 January 2004 (during PD water deficit period).
There was an irrigation treatment by PFT interaction for transpiration (E) and photosynthesis (A) (Table 1). The PD plus-PFT treatment had significantly lower E and A than the PD alone. The control treatment had greater stomatal conductance (Gs), E and A than the RDI and PD treatments.

22 January 2004 (post water deficit period).
There were no interactions when all treatments had resumed 100% ET replacement (18-Jan-04). The irrigation treatments plus-PFT treatments had significantly lower E and Gs compared to the non-PFT treatments, but there was no irrigation plus-PFT effect on A.

Treatment	Transpiration (mmol m^{-2} s^{-2})	Photosynthesis (µmol m^{-2} s^{-2})	Stomatal conductance (mmol m^{-2} s^{-2})
13 January 2004			
Control	7.8	13.3	244 A[y]
RDI	5.3	8.5	125 B
PD	3.4	5.5	77 B
Non-PFT	5.9	9.9	160
PFT	5.1 ns	8.2 ns	137 ns
Interaction [z]	**	**	ns
Control	7.6 A[z]	13.0 A	239
Control+PFT	8.0 A	13.5 A	281
RDI	5.4 B	8.7 B	127
RDI+PFT	5.2 B	8.3 B	124
PD	4.4 B	7.2 B	104
PD+PFT	2.4 C	3.8 C	50 ns
22 January 2004			
Control	9.1 A	20.1 A	494 A
RDI	7.4 AB	14.9 B	324 AB
PD	6.8 B (0.09)	14.1 B	286 B (0.07)
Non-PFT	8.2 A	17.1	409 A
PFT	7.3 B	15.6 ns	326 B
Interaction [z]	ns	ns	ns

Table 1 Physiological Responses

Post-deficit observations

The control mean leaf water potential (ψleaf) was less negative than the deficit irrigation treatments. PFT mean leaf water potential (ψleaf) was less negative than the non-PFT treatments (Table 2).

Irrigation	Plus-PFT	non-PFT
Control	-0.30 E	-0.50 B
RDI	-0.47 BC	-0.67 A
PD	-0.44 CD	-0.58 AB

Table 2 Leaf water potential (MPa)

No relationship was evident between ψleaf and maximum daily air temperature (MDAT) for all irrigation treatments pre-veraison (data not shown). Post-veraison, ψleaf is responsive to MDAT for all irrigation treatments (RDI only, Fig. 1). Application of PFT results in less negative ψleaf. This observation was found with all irrigation treatments.

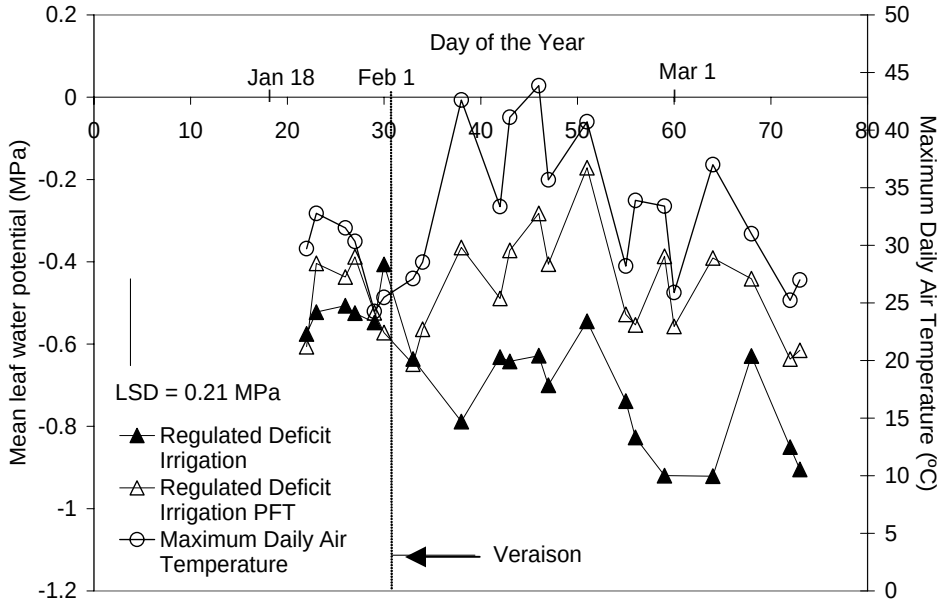


Figure 1. RDI plus-PFT and RDI non-PFT leaf ψ

As MDAT increases ψleaf becomes more negative when PFT is applied (Fig. 2). This observation was found with all irrigation treatments.

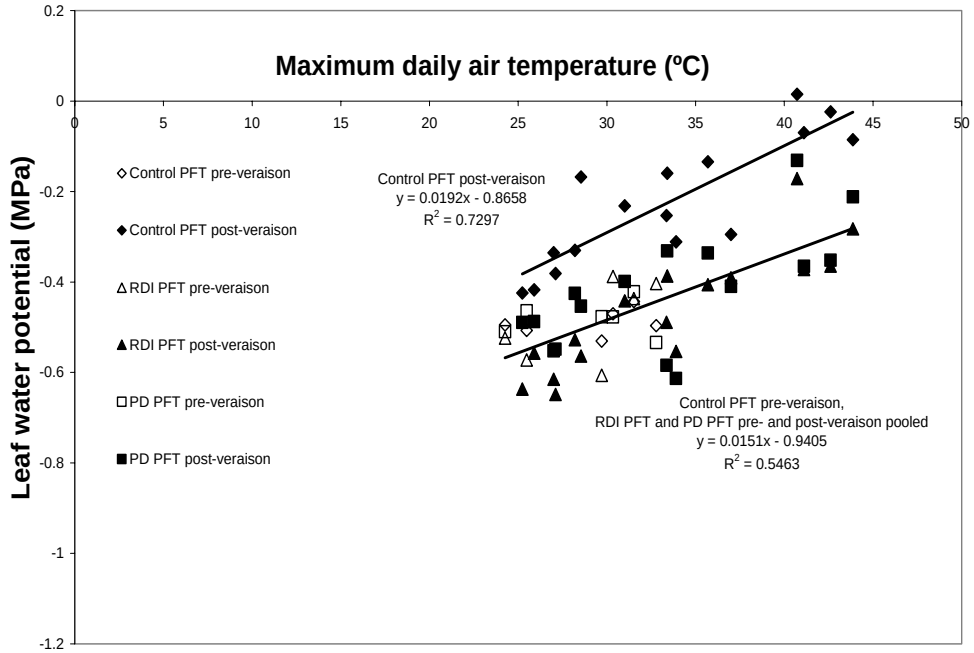


Figure 2. Relationship between leaf ψ MDAT pre and post-veraison control plus PFT

Overall, ΔT declined as the MDAT increased for all irrigation strategies (Fig. 3). The regression relationship of ΔT with MDAT during the pre-veraison period was not significantly different from the post-veraison period where irrigation strategies were different (Fig. 3). The regression relationship of ΔT with MDAT for all the irrigation treatments plus-PFT treatments indicated significant differences in comparison with all the non-PFT irrigation treatments.

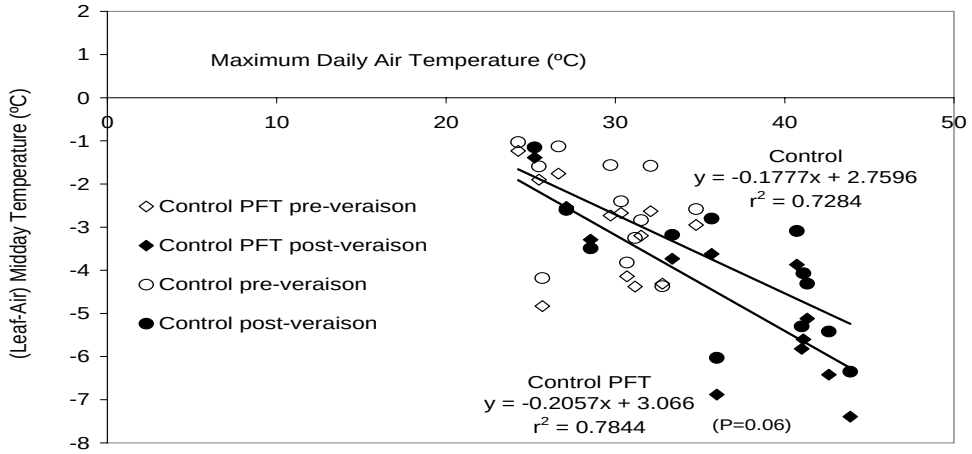


Figure 3. Leaf temperature minus air temperature (ΔT) for control plus-PFT & control non-PFT treatment

Conclusions

The absence of a correlation between ψ leaf and MDAT for the pre-veraison period indicates an ability of these vines to maintain stomatal opening even at high temperatures. In contrast, in the post veraison period, as MDAT increases leaf ψ becomes more negative for the PFT deficit treatments, indicating partial stomatal closure and a greater ability for water conservation.

In this study, high MDAT is most likely correlated with high VPD. The post-veraison RDI plus-PFT and PD plus-PFT treatment maintained less negative ψ leaf than the post-veraison RDI non-PFT or PD non-PFT treatment. This relationship continued after full irrigation was resumed (18th January 2004) indicating long-term effects of RDI and PD on plant water relations.

6.1.3. Conclusions from treatment effects on vine physiology

The imposed irrigation treatments (RDI and PD) produced significant soil moisture deficits which had affected vine physiology, leading to reduced photosynthesis during key stages of vine development. Due to climatic effects there were large differences between seasons in the ability to achieve moistures deficits and in the subsequent physiological responses. A reduction in photosynthesis resulting from severe water deficits is likely to impact on berry development, maturation, carbohydrate partitioning and storage and hence, the long term sustainability of production. Application of particle film PFT reduced the impact of soil moisture deficits on leaf physiology. Significant findings from the studies are listed below.

- There were large seasonal differences in the ability to both impose moisture deficits during targeted deficit periods and in recovery of soil moisture on return to normal irrigation. There were also large seasonal differences in water requirements (ie. 4.5-6.5 ML/ha).
- Post-stress measurements of soil moisture indicate that vines subjected to deficit treatments adapted to the conditions over time and had lower requirements for water during ripening and post-harvest periods in latter seasons. This may be a result of the development of vines with smaller canopies carrying smaller crops and possibly disrupted root function.
- Water deficits reduced leaf water potential and turgor, increased leaf temperature and lowered transpiration, assimilation and stomatal conductance.
- Effects of deficit treatments on leaf physiology (assimilation, stomatal conductance and transpiration) were measured up to 3 weeks after the end of the stress period.
- Water deficits had a long term impact on shaded leaf water potential (eg. for up to 2 months after the end of the RDI period). Both the RDI and the PD treatments were under stress during fruit ripening in season 2003-04, a significant time after water application had been returned to normal.
- Addition of PFT to the deficit treatments reduced stress leaf temperature and stress responses and may increase the sustainability of deficit irrigation treatments

6.2. Carbohydrate partitioning

6.2.1. Introduction

The effect of season and irrigation treatments on vine carbohydrate status was determined by measurement of trunk carbohydrates at significant phenological stages over three seasons. The results and linkages with other aspects of physiology presented in the previous chapter have been summarised in a paper prepared for the vine carbohydrate dynamics and source-sink relationships workshop held in Mildura in January 2006. The paper '*Effects of water stress on grapevine carbohydrate reserves, Cooley et al. 2006'* is presented below. Furthermore, an extract from a further paper presented at the workshop, '*Pruning, rootstocks and seasonal impacts on vine carbohydrate status', Clingeleffer and Pellegrino 2006* showing seasonal trends in trunk starch concentration from season 2004/2005 is included.

Insert:

6.2.2. Effects of water stress on grapevine carbohydrate reserves (Cooley et al. 2006)

Abstract :
The effects of deficit irrigation treatments on Cabernet Sauvignon grapevines were investigated in a commercial vineyard in the warm climate of the Sunraysia region of south-eastern Australia. The control was standard drip irrigation (STD) throughout the season as scheduled by the operators of the vineyard. This was compared with a Regulated Deficit Irrigation (RDI) treatment consisting of standard plus a soil water deficit in the post fruit set to pre-veraison period and a Prolonged Deficit (PD) treatment consisting of standard with RDI plus a period of extreme deficit (no water) applied after the RDI stress until veraison.

On a warm day during the extreme deficit period of the PD treatment water potential measurements were taken throughout the day after which leaves were collected for measurements of starch concentration. Leaf water potential became more negative throughout the day for both the standard and PD treatments. Water potential for the PD treatment by 1:00 pm was near -1.0 MPa and remained at this level until 5:00 pm when it became less negative. Water potential for the PD treatment was significantly more negative than the STD treatment. Significant reductions in rates of photosynthesis and stomatal conductance were found with the PD treatment compared to the STD treatment throughout the day. Leaf starch concentration of STD increased by 70% from pre-dawn measurements to early evening. The PD treatment vines had significantly lower leaf starch concentration at most time points throughout the day.

Trunk carbohydrates were measured at the time of water stress, post water stress and leaf fall over three seasons. Starch concentrations during season 2003 were lower in the trunks of RDI and PD treated vines at the time of water stress and post water stress, but were lower only in the PD treatment at leaf fall. Significant season to season variation was found with all carbohydrates measured (starch, sucrose, glucose and fructose). This work has shown that deficit irrigation practices have the potential to impact on both source and sink reserves in grapevines and that this response is subject to significant season to season variation.

Key words :
RDI, deficit irrigation, carbohydrates, starch, gas exchange

Introduction

Deficit irrigation strategies have been implemented in warm climate grape-growing regions to improve the quality of wine grapes. The most common is the use of regulated deficit irrigation (RDI) where the irrigation amount is reduced after berry set. Reductions to irrigation at this particular phenological stage slow down canopy development and result in reductions to berry size (Kriedemann and Goodwin 2004 and references therein). As seasonal variation can significantly alter the relationship between source characteristics (leaf area, net canopy photosynthesis) and sink carbohydrate partitioning into berries, stems, trunk and root the degree of soil water deficit can have a potentially significant impact on carbon assimilation and carbohydrate partitioning to the various sinks.

Reductions in leaf water potential occur when grapevines are subjected to water stress (Bota et al. 2004; Cifre et al. 2005 references therein). Such reductions often result in reductions to stomatal conductance (Gs) (Escalona et al. 1999), transpiration (T) and photosynthesis (A) (Rodrigues et al. 1993; Flexas et al. 1999; Iacono & Sommer 2000; Bota et al. 2004), but responses can vary depending on the level of reduction in leaf water potential, the cultivar (Gomez-Del-Campo et al. 2004; de Souza et al. 2005; Cifre et al. 2005) and other seasonal effects (Moutinho-Pereira et al. 2004). High temperatures have also been shown to inhibit Gs and A (Sinclair et al. 1984).

Reductions in leaf carbohydrate concentrations during water stress may have implications for whole plant carbohydrate reserves. Leaf starch concentration decreased in Savatiano grapevines during a drought cycle and this reduction was found regardless of leaf age (Patakas & Noitsakis 2001). Water stress in Alfonso Lavallé plants resulted in significant reductions to carbohydrate production and export from leaves (Bota et al. 2004). Data also suggested that assimilation rate (source activity) was more effected by water stress than phloem transport (Bota et al. 2004). Source carbohydrate and subsequent partitioning has been shown to be sensitive to other environmental impacts, for example fluctuation in temperature can result in alterations to carbohydrate levels (Koblet 1996).

The relationship between source assimilation activity and sink carbohydrate partitioning is particularly important in grapevine management especially in situations where water deficits are applied. Accordingly, source leaf physiological responses to water deficit were investigated in Cabernet Sauvignon grapevines in a warm climate region. In particular, the responses of source leaf carbohydrate concentrations and sink reserves (trunk and berry) to deficit irrigation strategies were examined.

Methods and Materials

Cabernet Sauvignon (*Vitis vinifera* L.) was planted on own roots in 1995 at a density of approximately 1390 vines per ha. Vines were spaced 2.4m within the row and 3m between rows and formed part of a 5 ha commercial vineyard located in the Sunraysia region of Victoria, Australia. The soil type was Nookamka sandy loam (Penman et al. 1939). Vines were trained on a two wire vertical trellis with

wires at 1.5 and 1.8m in east-west running rows. Vines retained approximately 150 buds by mechanically hedging.

Irrigation treatments

Drip irrigation was employed with dripper spacing at 0.6m intervals which delivered 1.56 ml per h. Soil moisture was monitored throughout the season in all treatments by nine neutron probes (Hydroprobe model 503DR, CPN, Corporation, 2830 Howe Rd, Martinez, CA, USA) (data not shown). Three irrigation treatments were initiated after fruit set each season, starting in November 2002 and continuing through to 2005 (Table 1): (1) The standard irrigation (Standard) treatment was set-up by employing two standard drip lines that delivered twice as much water during the stress period (drippers were evenly spaced to ensure an even spread of water around each vine). The standard was maintained at close to field capacity throughout the season by soil water replenishment and with set points determined from soil water use data of previous seasons; (2) A regulated deficit irrigation (RDI) treatment which received the same irrigation as STD until fruit set (typically during November but the date varied with each season) at which time the water applied was halved by reducing irrigation duration. The RDI period commenced at fruit set and continued for several weeks until canopy indicators were visible; (3) a prolonged deficit (PD) treatment which consisted of the standard irrigation schedule with the RDI treatment plus a period of extreme deficit (no water) after the RDI stress until veraison.

Trunk Carbohydrate analysis

Trunk samples were taken using an increment borer, approximately mid-way between the graft union and the training wire during key physiological development stages throughout the season and analysed for carbohydrate content. Samples were snap frozen in liquid nitrogen and stored at –70 ^{O}C before being freeze dried and ground (Coffee 'n Spice grinder, Breville, NSW, Australia). The carbohydrates (100 mg) were extracted in 10 ml 80 % ethanol (v:v) at 90 ^{O}C (developed from the method by Ruhl and Clingeleffer 1993) and an internal standard, sorbitol (Sigma) was added. The starch component was separated from the soluble sugars by centrifugation (10,000 g for 15 minutes). The pellet (starch) was washed with 2 ml 80 % ethanol (w:w) and centrifuged as above. The supernatants were pooled and stored at 4 ^{O}C before being analysed. The starch in the remaining pellet was determined using a Megazyme (Bray, Co. Wicklow, Ireland) starch analysis kit. Starch was treated with dimethyl sulphoxide (Sigma-Aldrich, St Louis, MO, USA) to remove resistant starch and then broken down into glucose equivalents using α-amylase and amylo-gluconidase. The absorbance of glucose equivalents was measured at 510 nm. The supernatants (containing soluble sugars) were dried down in a water bath (100°C), suspended in MilliQ water (300µl) and filter using Sephadex G-25-80 (Sigma) columns. Columns were made by activating a solution of Sephadex (twice the volume of 1M NaOH 1 hour), washing the sephadex with twice the volume of water until neutral pH was obtained. Activated sephadex (500µl) was then packed into a Nanospec 0.45µm centrifugal device (Pall Life Sciences) and spun at 500g for 2 minutes. The samples were added and spun out from the column (500g for 1 min). A 100 µl aliquot of water was used to wash the column (procedure developed by Gerhard Dutoit, CSIRO Plant Industry, personal communication). HPLC analysis of sucrose, glucose and fructose was conducted. The standards sucrose, glucose, fructose, mannitol and sorbitol were obtained from Sigma. The HPLC system (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) consisted of an LC1610 auto-sampler, LC1150 pump with in-line degasser, LC1210 UV-Vis detector, RI detector and WinChrom chromatography manager software. The mobile phase was 5 mg L^{-1} Ca- disodium-EDTA (Aldrich Chemical Company) prepared in MilliQ water, vacuum filtered through a Millipore HA 0.45 mm and degassed. Flow rate was set at 0.6 ml min^{-1}. A Waters Sugar-Pak I HPLC column (6.5 x 300 mm) with a sulfonated styrene divinylbenzene resin stationary phase was used. The column was protected with a Sugar-Pak guard column and a C18 (0.45 µm) pre-column insert. Column temperature was maintained at 90°C. After use, the column was reversed and regenerated overnight with an aqueous solution of 500 mg L^{-1} calcium disodium EDTA, at 90°C and a flow rate of 0.5 ml min^{-1}. Detection was at 195 nm. Sample injection volume was 20 µl. Sample concentration was

determined using peak area. All reagents used in the extraction were HPLC grade and sourced from Sigma-Aldrich Co. (USA).

Physiology treatments and leaf starch analysis

Leaves were selected at the 5th leaf position and tagged for gas exchange measurements. Transpiration (E), photosynthesis (A), stomatal conductance (Gs) and internal CO_2 were measured using a CIRAS-1 Photosynthesis System (PP Systems, Amesbury, MA, USA) on 23 January in season 2003. Minimum and maximum air temperatures on this day were 13°C and 32°C respectively, and minimum and maximum relative humidity were 15 and 74%, respectively. A single exposed leaf per plot from the standard irrigation and prolonged deficit treatments (total n=16) were measured from 9:00 am until 6:00 pm at approximately 30 minute intervals. At the same time-points, five exposed leaves per vine from the 5th leaf position were collected from the 12 replicate blocks. Leaves were snap frozen in liquid nitrogen, wrapped in foil and placed on dry ice until they could be stored at -70°C. Leaf samples were ground in liquid nitrogen using a mortar and pestle. Starch analysis was conducted using the Megazyme (Bray, Co. Wicklow, Ireland) starch analysis kit.

Experimental design and statistics

A randomized block experimental design with 3 irrigation treatments and 12 replicate blocks was implemented. Each irrigation treatment plot consisted of 3 rows of 12 vines per row with data collected from the centre row of each plot. One-way analysis of variance (ANOVA, Genstat) assuming 95% confidence and the LSD multiple-comparison method was used to determine the significance between time points and between treatments.

Results

Leaf starch concentration, at the end of the extreme deficit stress on a warm day (maximum 31°C), in both the STD and PD increased significantly from the pre-dawn measurement to early evening (Figure 1). The PD treatment vines had significantly lower leaf starch concentration at most time points ($p > 0.05$) throughout the day compared to the STD. Leaf water potential became more negative throughout the day for both STD and PD (Figure 2). Water potential for the PD treatment by 1:00 pm was near -1.0 MPa and remained at this level until 5:00 pm, whereon it became less negative. Water potential for the PD treatment was significantly more negative than the STD treatment. There was no significant relationship between water potential and starch concentration.

Significant reductions in photosynthetic rate and stomatal conductance were found with the PD treatment compared to the STD treatment throughout the day (Figure 3). An exponential rise to max (y = a (1 - b x) SigmaPlot version 9) could be fitted to the STD photosynthesis rate and starch concentration (Regression P = 0.04, $R^2 = 0.53$). This relationship was not found for the PD treatment photosynthesis rate and starch concentration. No significant relationships between stomatal conductance, transpiration or internal CO_2 concentration were found (data not shown). Increases in leaf starch concentration occurred during the afternoon for both STD and PD (Figure 3). Leaf temperature also increased during this time (Figure 4).

Trunk carbohydrates were measured at the time of water stress, post water stress and at leaf fall over three seasons (Table 2). Starch concentrations during season 2003 were lower in the trunks of RDI and PD treated vines at the time of water stress and post water stress, but were lower only in the PD treatment at leaf fall. Trunk starch concentrations were significantly lower during the early part of season 2004 relative to 2003, and were lower again at around 100 days after budburst during 2005 (Figure 5). Significant seasonal variation ($P < 0.001$) was found with most carbohydrates measured (starch, sucrose and glucose) during the periods of applied water deficit (Figure 6).

Variation in total soluble solids (TSS) of berries was found with each season (data not shown). Deficit irrigation resulted in a reduction in TSS but not in all seasons. A severe deficit reduced the accumulation of berry total soluble solids but not in all seasons. Season to season impacts on berry TSS were greater than the effect of water stress treatments (data not shown).

Discussion

Highly significant reductions to A with the PD treatment during season 2003 were found and have also been reported in other grapevine varieties (Bota et al. 2004). The magnitude of the response is known to depend on the severity of the water stress as well as the response mechanism (Lawlor & Cornic 2002). Different mechanisms causing reductions to A potential have been described as Type 1 and Type 2 (Lawlor & Cornic 2002 see references therein). During the stress period, the PD treatment had lower A and Gs, and A potential (A at saturated CO_2, data not shown) and a reduction in the A to Gs ratio, suggesting a Type 2 response. Reductions to Ci have been suggested to lead to down regulation of electron transport, for example reductions to ATP synthesis which can then result in reductions to RUBISCO activity (Lawlor & Cornic 2002). Increases in electron transport oxygenation under severe drought stress suggest that photorespiration is important for photoprotection under drought stress (Guan et al. 2004). Energy is required for the process of photorespiration and therefore a transfer of potential energy from carbohydrate partitioning to photorespiration reduces the stress induced by drought.

Changes to the Ci concentration can result in changes to carbohydrates and nitrates (Lawlor & Cornic 2002) and may explain reductions in starch concentration found in the leaf with the PD treatment. Reductions to leaf starch and sucrose concentrations were found during a water stress but there were no differences to glucose and fructose concentration (Rodrigues et al. 1993). Reductions to starch and sucrose accounted for significant reductions to the dry weight to leaf area ratio in water-stressed vines (Rodrigues et al. 1993). It has been suggested that both young and fully mature leaves are an important source of carbohydrate during berry ripening (Iacono & Sommer 2000).

As different source sink ratios are found across seasons, different degrees of water stress are incurred. If differences in the degree of water stress result in switching between a Type 1 and Type 2 response (both within and across seasons) this may explain some of the seasonal variations found with both A and sink reserves (trunk carbohydrates). Other environmental factors, such as temperature, may also be causing reductions to A as well as compounding seasonal effects (Camejo et al. 2005).

Evidence has shown that increases to ambient CO_2 concentration can negate reductions to A during water deficits (Lawlor & Cornic 2002; Widodo et al. 2003). Interactions with osmotic potential, cell turgor and the hormone ABA have also been suggested to influence responses to A during water stress (Loveys 1984; Pan et al. 2005). It has been suggested that sucrose accumulation in the leaf may inhibit photosynthesis (Plaut et al. 1987; Krapp et al. 1991, 1993), whereas evidence presented by Quick et al. (1992) contradicts this (although it should be noted that it was total leaf sucrose that was measured and not an organelle specific concentration).

Leaf A rate was reduced in the PD stress period during season 2003 and some significant reductions in berry TSS were recorded during early berry development in all seasons (data not shown). A reduction in berry TSS with the PD treatment was found at harvest during season 2003 but not during the other seasons. It may be possible that total seasonal source size was limited during season 2003 and not in other years and/or that other environmental impacts (such as high temperatures) confounded the water stress effect. High ambient temperature in conjunction with a water stress can change the translocation of carbohydrates or reduce phloem translocation. Similar photosynthetic rates were found in Tempranillo whether fruit was present or not (Bota et al. 2004) and it is phloem loading which results in sucrose transport into the fruit. Conflicting evidence has been found regarding stress effects on phloem translocation (Bota et al. 2004; Wang et al. 2003) and as it was not measured here we cannot speculate on its contribution. Tempranillo vines under water stress allocated a lower proportion of carbohydrates to the main shoot compared to those not subjected to a water stress (Bota et al. 2004).

Growth activities of both the source and sink tissues are regulated by the modulation of gene expression and enzyme activities. It has been shown that a low source sugar status will enhance the rate of photosynthesis, reserve mobilization and export; whereas growth and carbohydrate reserves increase with abundant sugar reserves (Koch 1996).

It may be concluded from this work that deficit irrigation practices have the potential to impact on both source and sink reserves in grapevines but this response is subject to significant season to season variation. The seasonal variation may be due to differences in the response of sink reserves to the water stress which may then impact on the source reserves or alternatively there may be interactions occurring between water stress and high ambient temperatures which significantly effect the canopy's ability to produce carbohydrate and partition it according to sink demand.

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References:

BOTA J., STASYK O., FLEXAS J., MEDRANO H., 2004. Effect of water stress on partitioning of C-14-labelled photosynthates in *Vitis vinifera*. *Functional Plant Biology* 31, 697-708.

CAMEJO D., RODRIGUEZ P., MORALES A., DELL'AMICO J.M, TORRECILLAS A., ALARCON J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of Plant Physiology* 162, 281-289.

CIFRE J., BOTA J., ESCALONA J. M., MEDRANO H., FLEXAS J., 2005. Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.). An open gate to improve water-use efficiency? *Agriculture, Ecosystems and Environment* 106, 159-170.

DE SOUZA C. R., MAROCO J.P., DOS SANTOS T.P., RODRIGUES M.L., LOPES C., PEREIRA J.S., CHAVES M.M., 2005. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agriculture, Ecosystems and Environment* 106, 261-274.

ESCALONA J. M., FLEXAS J., MEDRANO, H., 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Australian Journal of Plant Physiology* 26, 421-433.

FLEXAS J., ESCALONA J.M., MEDRANO H., 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant Cell and Environment.* 22, 39-48.

GOMEZ-DEL-CAMPO M., BAEZA P., RUIZ C., LISSARRAGUE J.R., 2004. Water-stress induced physiological changes in leaves of four container-grown grapevine cultivars (*Vitis vinifera* L.). *Vitis* 43, 99-105.

GUAN, X. Q., ZHAO S. J., LI, D. Q.,SHU, H. R., **2004**. Photoprotective function of photorespiration in several grapevine cultivars under drought stress. *Photosynthetica* 42, 31-36.

IACONO F., SOMMER K.J., 2000. Response of electron transport rate of water stress-affected grapevines, Influence of leaf age. *Vitis* 39, 137-144.

KOBLET W., CANDOLFIVASCONCELOS M.C., KELLER M., 1996. Stress and stress recovering by grapevines. *Botanica Helvetica* 106, 73-84.

KOCH K. E., WU Y., XU J., **1996.** Sugar and metabolic regulation of genes for sucrose metabolism, potential influence of maize sucrose synthase and soluble invertase responses on carbon partitioning and sugar sensing. *Journal of Experimental Botany* 47, 1179-1185.

KRAPP A., QUICK W.P., STITT, M., 1991. Ribulose-1,5-Bisphosphate Carboxylase-Oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. *Planta* 186, 58-69.

KRAPP A., HOFMANN B., SCHAFER C., STITT M., **1993.** Regulation of the expression of Rbcs and other photosynthetic genes by carbohydrates - a mechanism for the sink regulation of photosynthesis. *Plant Journal* 3, 817-828.

KRIEDEMANN P.E., GOODWIN I., **2004**. Irrigation Insights No3. Regulated deficit irrigation and partial rootzone drying. Land and Water Australia, ACT, Australia.

LAWLOR D. W., CORNIC G., **2002.** Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell and Environment* 25, 275-294.

LOVEYS B.R., 1984. Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars. III. The influence of xylem-derived abscisic acid on leaf gas exchange, *New Phytologist* 98, 563-573.

MOUTINHO-PEREIRA J. M., CORREIA C.M., GONCALVES B.M., BACELAR E.A., TORRES-PEREIRA J.M., 2004. Leaf gas exchange and water relations of grapevines grown in three different conditions. *Photosynthetica* 42, 81-86.

PAN Q. H., LI M.J., PENG C.C., ZHANG N., ZOU X., ZOU K.Q., WANG X.L., YU X.C., WANG X.F., ZHANG D.P., **2005.** Abscisic acid activates acid invertases in developing grape berry. *Physiologia Plantarum* 125, 157-170.

PATAKAS A., NOITSAKIS, B., 2001. Leaf age effects on solute accumulation in water-stressed grapevines. *Journal of Plant Physiology* 158, 63-69.

PENMAN F., TAYLOR J.K., HOPPER P.D., MARSHALL T.J., **1939.** A soil survey of the Merbein Irrigation District, Victoria. Bulletin no. 123 (Council for Scientific and Industrial Research: Melbourne).

PLAUT Z., MAYORAL M.L., REINHOLD L., 1987. Effect of altered sink - source ratio on photosynthetic metabolism of source leaves. *Plant Physiology* 85, 786-791.

QUICK W. P., CHAVES M.M., WENDLER R., DAVID M., RODRIGUES M.L., PASSAHARINHO J.A., PEREIRA J.S., ADCOCK M.D., LEEGOOD R.C., STITT M., **1992.** The effect of water-stress on photosynthetic carbon metabolism in 4 species grown under field conditions. *Plant Cell and Environment* 15, 25-35.

RODRIGUES M. L., CHAVES M.M., WENDLER R., DAVID M.M., QUICK W.P., LEEGOOD R.C., STITT M., PEREIRA S.J., 1993. Osmotic adjustment in water-stressed grapevine leaves in relation to carbon assimilation. *Australian Journal of Plant Physiology* 20, 309-321.

RUHL E. H., CLINGELEFFER P.R., 1993. Effect of minimal pruning and virus inoculation on the carbohydrate and nitrogen accumulation in Cabernet Franc vines. *American Journal of Enology and Viticulture* 44, 81-85.

SINCLAIR T. R., TANNER C.B., BENNETT J.M., 1984. Water-use efficiency in crop production. *Bioscience* 34, 36-40.

WANG Z. P., DELOIRE A., CARBONNEAU A., FEDERSPIEL B., LOPEZ F., 2003. Study of sugar phloem unloading in ripening grape berries under water stress conditions. *Journal International Des Sciences De La Vigne Et Du Vin* 37, 213-222.

WIDODO W., VU J.C.V., BOOTE K.J., BAKER J.T., ALLEN L.H., **2003.** Elevated growth CO2 delays drought stress and accelerates recovery of rice leaf photosynthesis. *Environmental and Experimental Botany* 49, 259-272.

Tables and Figures

Irrigation treatment	RDI period Irrigation applied				PD period irrigation applied				Season total water applied (ML per ha)
	ET[1] (%)	ON	OFF	Water applied (mm per ha)	ET[1] (%)	ON	OFF	Water applied (mm per ha)	
Standard	100	21-Nov-02	13-Dec-02	50	100	24-Dec-02	31-Dec-02	25	4.5
RDI	50	21-Nov-02	13-Dec-02	25	100	24-Dec-02	31-Dec-02	25	4.3
PD	50	21-Nov-02	13-Dec-02	25	0	24-Dec-02	31-Dec-02	0	4.0
Standard	100	25-Nov-03	2-Jan-04	134	100	2-Jan-04	18-Jan-04	42	6.5
RDI	50	25-Nov-03	2-Jan-04	67	100	2-Jan-04	18-Jan-04	42	5.1
PD	50	25-Nov-03	2-Jan-04	67	0	2-Jan-04	18-Jan-04	0	4.9
Standard	100	22-Nov-04	09-Dec-04	34	100	20-Dec-04	03-Jan-05	50	4.5
RDI	50	22-Nov-04	09-Dec-04	18	100	20-Dec-04	03-Jan-05	50	4.4
PD	50	22-Nov-04	09-Dec-04	18	0	20-Dec-04	03-Jan-05	0	3.8

Abbreviations, **RDI** regulated deficit irrigation, **PD** prolonged deficit irrigation and **ET** evaporative transpiration. [1] **ET** was based on soil water.

Table 1. *Irrigation treatment durations and volumes applied during managed stress periods along with total season irrigation applied in Cabernet Sauvignon grape.*

	Starch concentration (% dw)		
	Water stress	**Post water stress**	**Leaf fall**
Standard	5.38 a	6.93 a	7.95 a
RDI	4.55 b	6.58 b	7.38 a
PD	4.81 b	5.79 b	5.29 b

Table 2. *The effect of standard, RDI and PD irrigation on trunk starch concentration at various stages during season 2003 (water stress 03rd Dec 2002, post water stress 20th Jan 2003 and leaf fall 8th May 2003).*

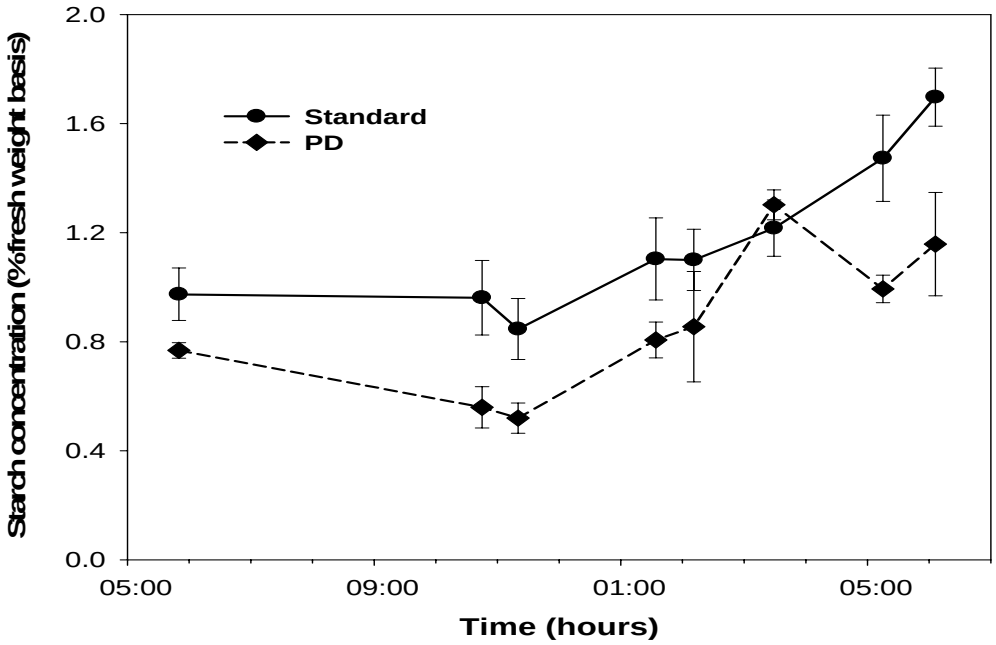


Figure 1. *The effect of standard and prolonged deficit irrigation on leaf starch concentration throughout a day at the end of the prolonged deficit stress period. Bars are ± standard error.*

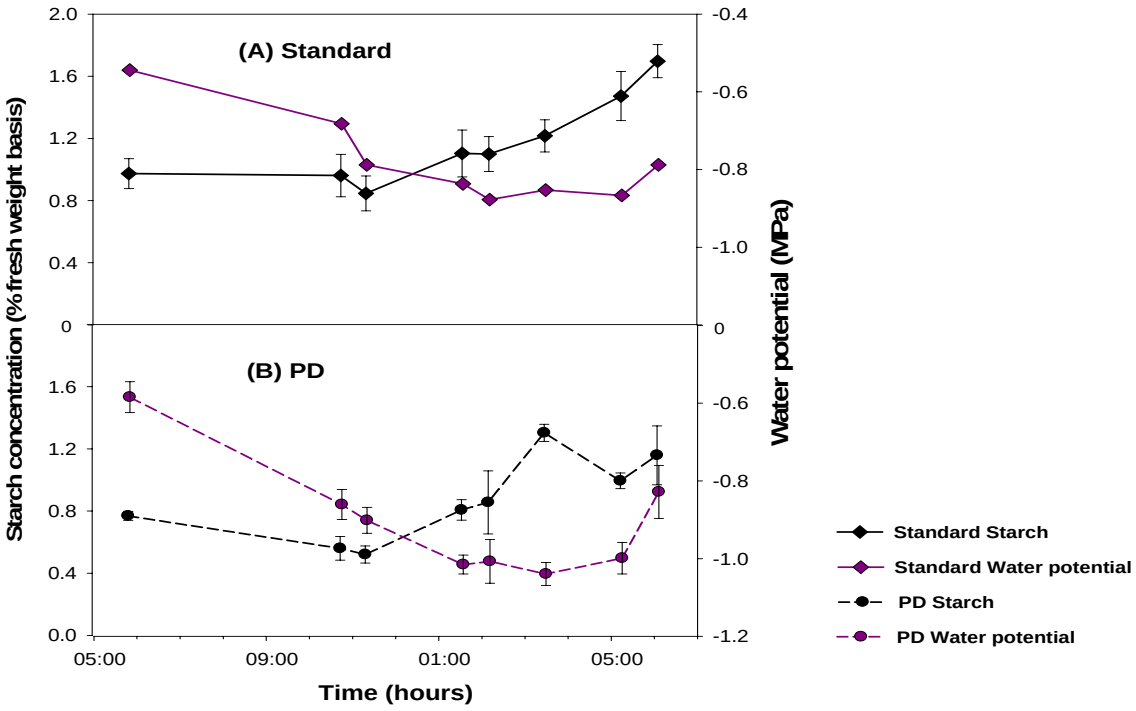


Figure 2. *The effect of standard (A) compared to prolonged deficit (B) irrigation on leaf starch concentration and leaf water potential throughout a day at the end of the prolonged deficit stress period. Bars are ± standard error.*

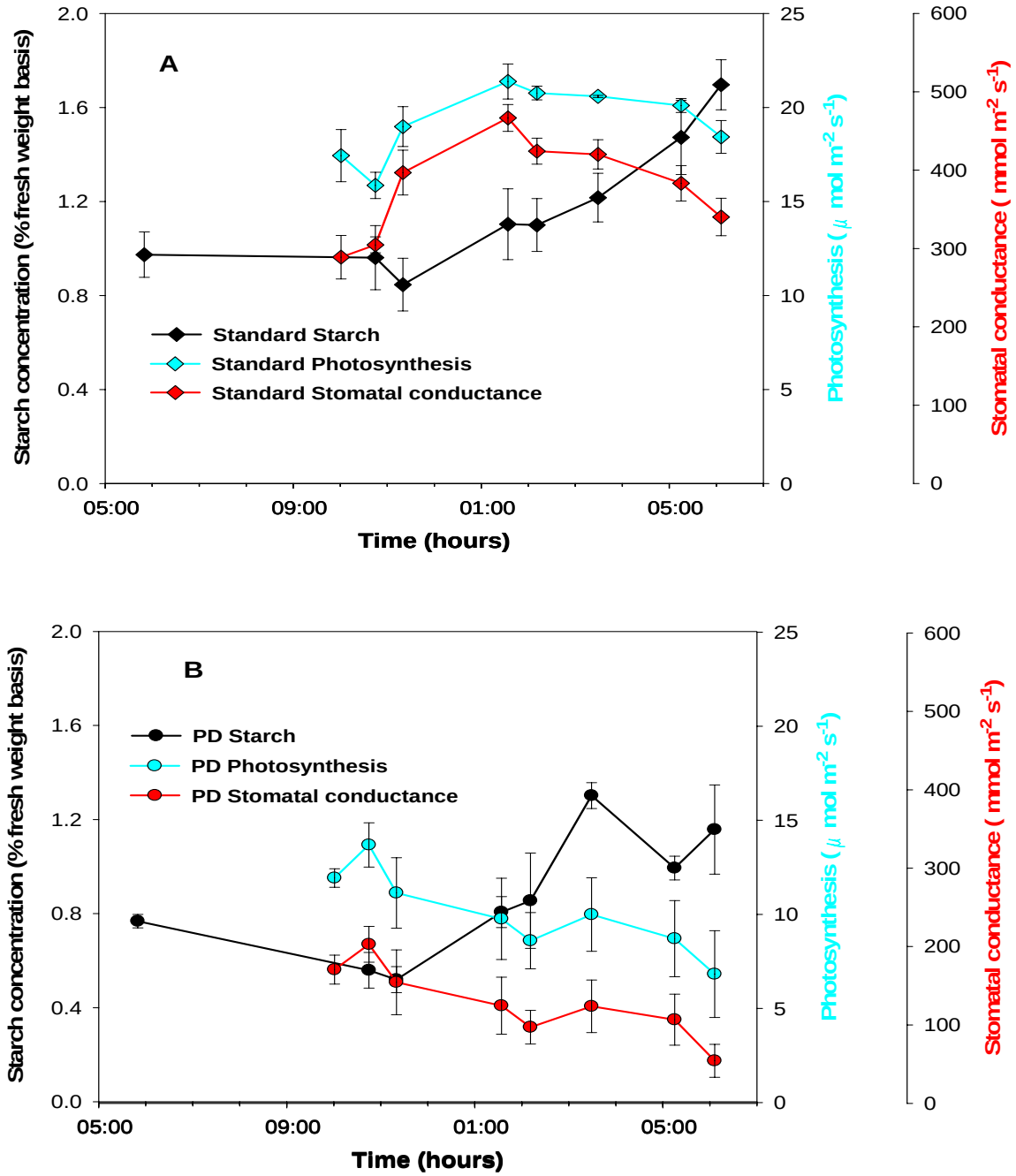


Figure 3. *Comparisons of leaf starch concentration with photosynthesis and stomatal conductance for the standard (A) and prolonged deficit (B) irrigation treatments throughout a day at the end of the prolonged deficit stress period. Bars are ± standard error.*

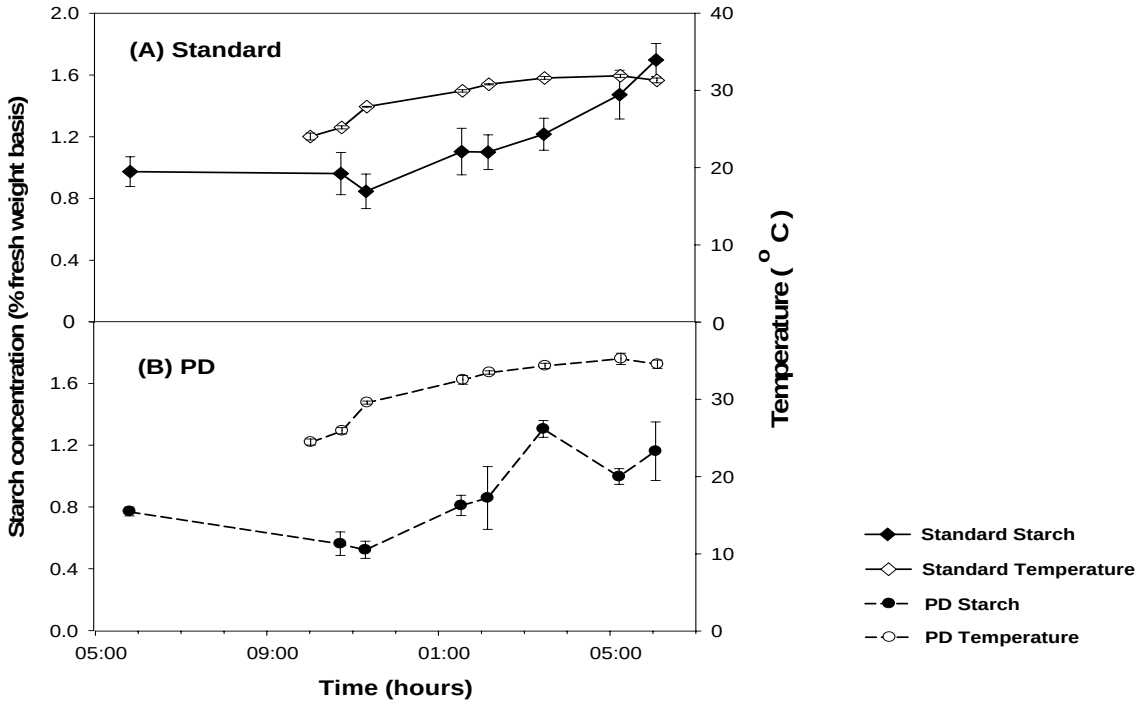


Figure 4. *Comparisons of leaf starch concentration with leaf temperature for the standard (A) and prolonged deficit (B) irrigation treatments throughout a day at the end of the prolonged deficit stress period. Bars are ± standard error.*

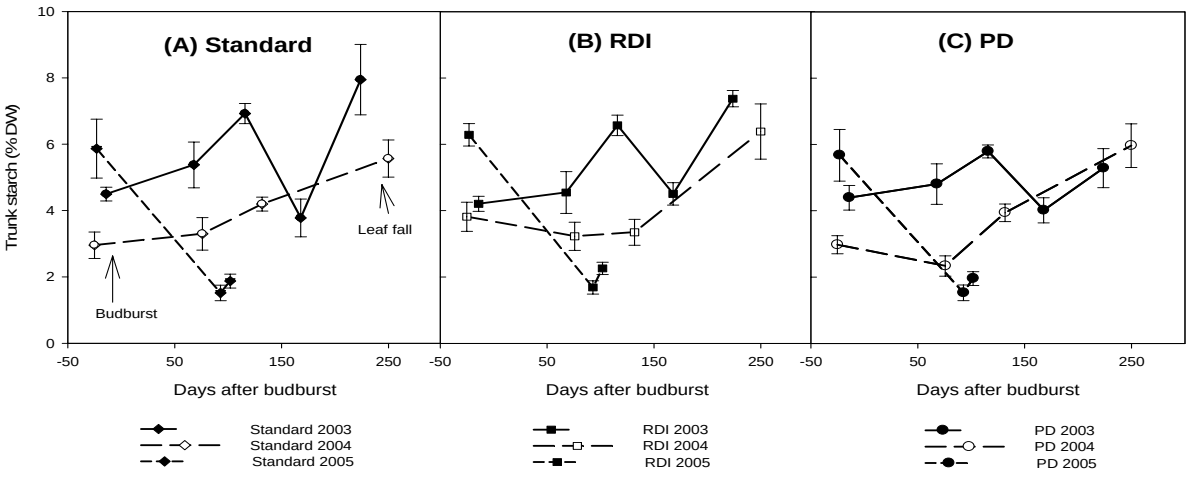


Figure 5. *The effect of standard (A), RDI (B) and PD (C) irrigation on trunk starch concentration from season 2003 to season 2005. Bars are ± standard error.*

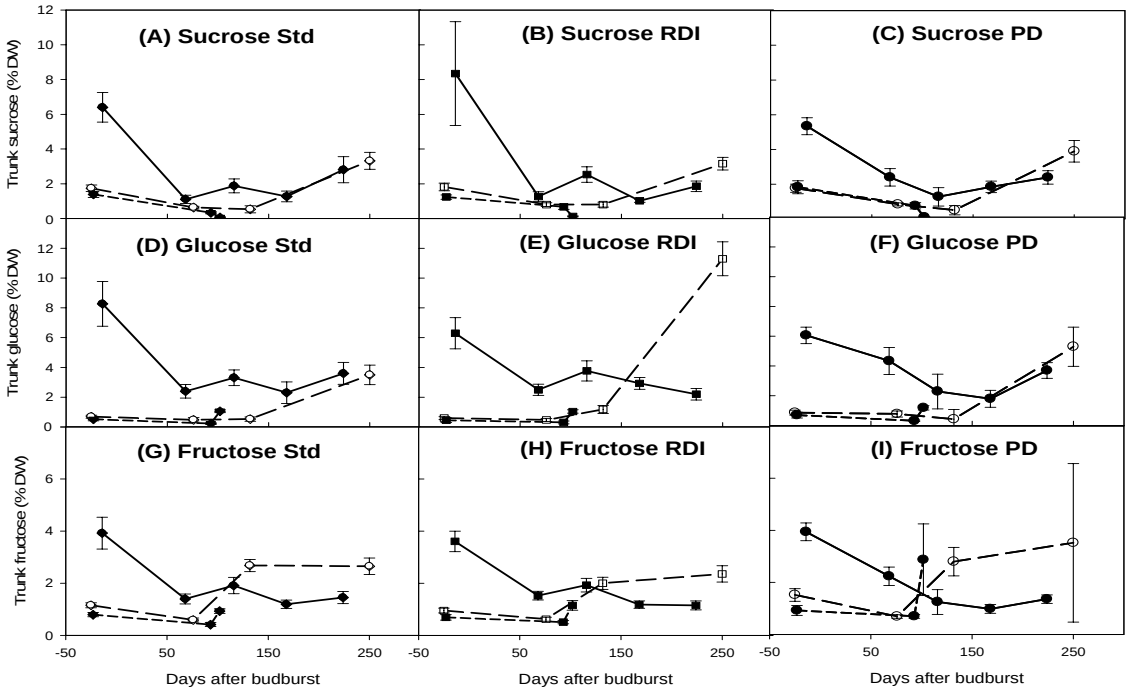


Figure 6. *The effect of standard (A, D & G), RDI (B, E & H) and PD (C, F & I) irrigation on trunk sucrose (A-C), glucose (D-F) and fructose (G-I) concentrations from season 2003 to season 2005. Key: solid line (season 2003), large dash line (season 2004) and small dash line (season 2005). Note: 2005 data is missing leaf fall measurements. Bars are ± standard error.*

Insert:

6.2.3. Carbohydrate status of Cabernet Sauvignon as influenced by irrigation treatment and time of season (adapted from Clingeleffer and Pellegrino 2006)

To determine the effects of deficit irrigation treatments on the carbohydrate status of own-rooted Cabernet Sauvignon, trunk starch concentrations were determined at key phenological stages in season 2004/05. The irrigation treatments were a control (Con) irrigation treatment, scheduled by the operators of the vineyard, a Regulated Deficit Irrigation (RDI) where a soil water deficit was applied in the post fruit set to pre-veraison period and Prolonged Deficit (PD) treatment which was similar to the RDI but had an extended period of extreme deficit (no water) after the RDI stress period until veraison.

Trunk starch concentrations were highest at leaf fall in autumn (7-8% dry matter) and reached very low levels at 10% veraison (1%) and 50% veraison (2%) (Fig. 6.2.3.1). There was a significant reduction in trunk carbohydrates with the PD treatment at fruit set, presumably due to carry over effects from the previous season and development of a smaller canopy. At the completion of veraison carbohydrate levels of all treatments had increased considerably from the low levels at the commencement of veraison. At that time there was however a very significant reduction in trunk starch levels due to deficit irrigation treatments (ie. 6%, 5% and 3% of dry matter for the Con, RDI and PD treatments respectively). It should be noted that yields were very high (21 kg vine^{-1}) and only slightly reduced with deficit irrigation treatments, particularly PD.

The low levels of trunk starch in the period between fruit-set and veraison indicate that this period is critical to carbohydrate partitioning between storage organs and the developing fruit. The unexpected rapid increase in trunk starch concentration at the end of veraison suggests that the vine has commenced redirection of carbohydrate assimilate toward storage organs rather than to the fruit and that this process was significantly affected by responses to deficit irrigation treatments. Current

research aims to further elucidate these processes and investigate the seasonal trend effects on total carbohydrate concentration and composition and to assess the long term effects of deficit irrigation on the development of fruit and sustainability of production.

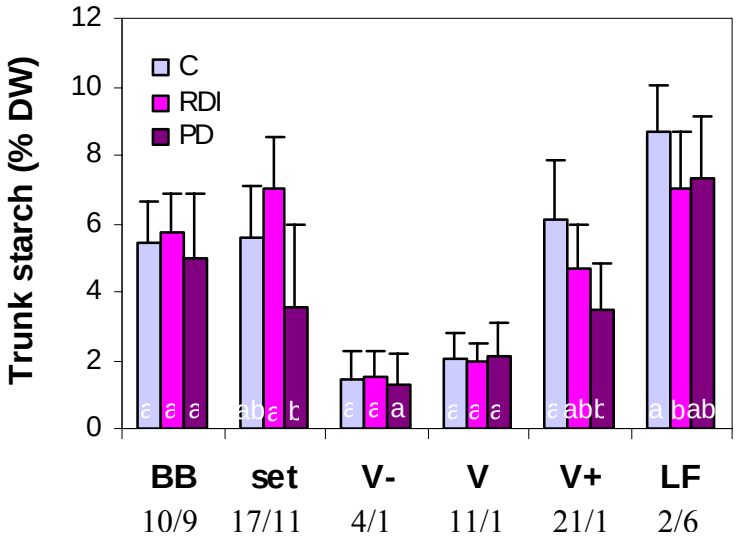


Figure 6.2.3.1. *Effect of control (C), regulated deficit irrigation (RDI) and prolonged deficit irrigation (PD) on trunk starch concentrations at budburst (BB), fruit set (set), 10% veraison (V-), 50% veraison (V) and 100% veraison (v +) and leaf fall. Vertical bars indicate the standard deviation. Sampling date is also included. At each date of sampling letters indicate significant differences between treatment means (P<0.05).*

6.3 Berry development and composition

6.3.1 Main experiment

Fruit characteristics at harvest

Harvest dates were determined when berry total soluble solids reached 24.5 (°Brix) during stage III of berry development (ie. 11th March 03, 15th March 2004 and 02nd March 2005). Seasonal and irrigation treatment effects on bunch and berry characteristics, yield and water use efficiency are shown in table 6.3.1.1 Irrigation treatments did not significantly affect bunch fresh weight at harvest whereas reductions to berry fresh and dry weights were found with the RDI (9-10%) treatment and PD (13-14%) treatment compared to the control irrigation. A significant reduction in exocarp (skin) weight was found with the PD treatment but not with the RDI treatment compared to the control treatment. No significant reductions were found in mesocarp (flesh) or seed dry weight with deficit treatments. While reductions were found in the exocarp dry weight with the PD treatment no significant effect was found to berry mid-point diameter compared to the control. No significant effect of irrigation treatment was found on berry fresh to dry weight ratio. A reduction in berry size did not affect the yield in the RDI treatment but did significantly reduce the yield with the PD treatment. Irrigation treatment did not significantly alter water use efficiency due to reductions in yield with deficit irrigation treatments.

Bunch fresh weight at harvest was not significantly different over the three seasons (6.3.1.1). Berry fresh weights at harvest were smallest in 2003 and largest in 2004 whereas berry dry weights were similar during seasons 2003 and 2005 but significantly reduced during season 2004 (26 and 34% respectively) at harvest. Berry diameter was larger in season 2005 compared with season 2004 (not measured in season 2003). The ratio of fresh to dry weight ratio was lowest in 2003 and highest in 2005. Lighter exocarp weights were found during season 2004 but there were no significant differences between seasons in mesocarp or seed dry weights. No change in seed number was found with any season measured (data not shown) although seeds were significantly larger in 2005. The ratio of exocarp: mesocarp did not change with season. Crop yield was 31% higher in season 2005 compared to seasons 2003 and 2004. WUE varied with each season studied and ranged from 10.4 t per ML (2003) to the low efficiency of 3.7 t per ML in season 2004.

Berry development

Berry development was monitored during stage III in season 2003 and stages II and III in seasons 2004 and 2005. Berry weight development for all treatments across all seasons was calculated to fit a continuous function of time and where a typical exponential relationship with growth was found (Figure 6.3.1.1). There were significant effects of season and irrigation treatment on berry development. In general seasonal differences were larger than irrigation treatment responses. Berry weights were largest in season 2005 when their development commenced at an earlier date and smallest in 2004. Plotting berry development against degree day after flowering did not reduce the seasonal differences, suggesting that post-flowering thermal time is not a key driver of berry weight. Similarly the plots of berry weight against degree day after veraison suggest that post-veraison thermal time is not a key driver controlling the final stages of berry development. Interestingly there was no significant berry weight loss in any season. Significant reductions to berry fresh weight, where time was recorded as day of year, were observed with both the RDI and PD treatments compared to the control treatment, the response being larger for PD. Where time was recorded as degree days after flowering or degree days after 50% veraison reductions to berry weight for both the RDI and PD treatments are also observed. The results also clearly show that seasonal differences in berry weight were reduced by the deficit irrigation treatments, particularly PD.

	Irrigation treatment			
	Control	RDI	PD	p-value
Bunch FW (g)	70.4	66.1	63.9	ns
Berry FW (g)	0.90 a	0.82 b	0.79 b	**
Berry DW (g)	0.22 a	0.19 b	0.18 b	**
Berry FW DW ratio	4.19	4.24	4.28	ns
Exocarp DW (g)	0.096 a	0.088 ab	0.085 b	*
Mesocarp DW (g)	0.082	0.072	0.069	ns
Seed DW (g)	0.052	0.05	0.047	ns
Exocarp **:** mesocarp	0.12	0.13	0.13	ns
Diameter (mm)[1]	10.8	10.7	10.6	ns
Yield (t per ha)	23.7 a	22.1 ab	20.2 b	**
Water use efficiency (t per ML)	7.2	7.1	6.9	ns
	Season			
	2003	2004	2005	p-value
Bunch FW (g)	66	65.5	69	ns
Berry FW (g)	0.77 a	0.81 a	0.94 b	***
Berry DW (g)	0.21 a	0.15 b	0.23 a	***
Berry FW DW ratio	3.73 b	5.27 a	4.01 c	***
Exocarp DW (g)	0.091 a	0.081 b	0.098 a	**
Mesocarp DW (g)	0.073 a	0.072 a	0.078 a	**
Seed DW (g)	0.043 a	0.048 a	0.058 b	***
Exocarp **:** mesocarp	0.13	0.12	0.13	ns
Diameter (mm)[1]	-	10.3	11.2	***
Yield (t per ha)	17.9 a	20.3 a	27.8 b	***
Water use efficiency (t per ML)	10.4 a	3.7 c	7.0 b	***

Note: Mean values were calculated (n=12) and treatment differences tested (ANOVA). Significant treatment difference are indicated * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$ and dissimilar letters show treatment differences. No significant interaction of treatment by season was found (data not shown). [1]Berry diameter was not recorded in season 2003. Abbreviations: **ns** not significant, **DW** dry weight and **FW** fresh weight.

Table 6.3.1.1 The effect of irrigation treatments and season on berry growth characteristics and water use efficiency at harvest (24.5 °Brix during phase III berry development).

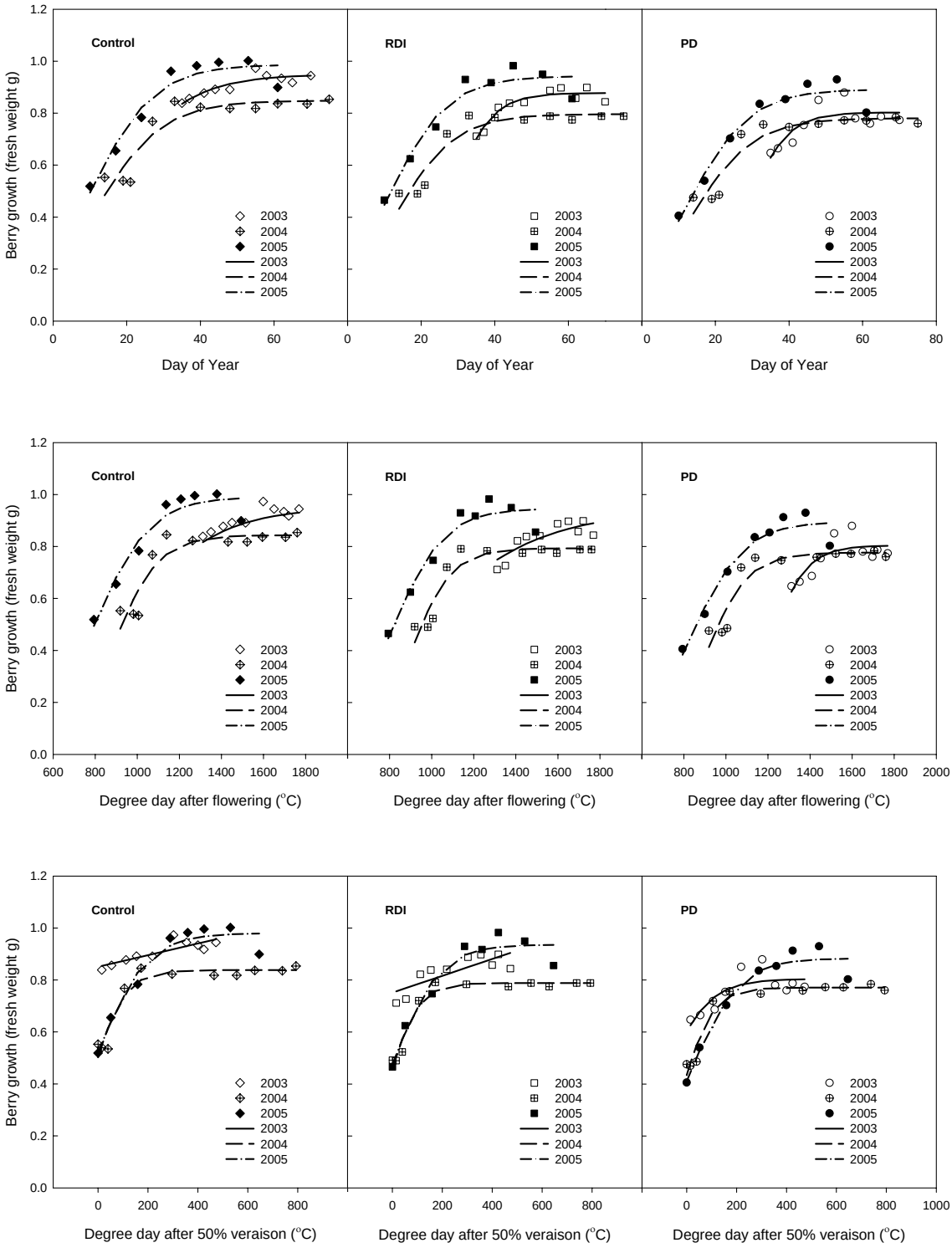


Figure 6.3.1.1 Changes in berry fresh weight of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005). Day of year, degree day after flowering and degree day after 50% veraison are included as the X axis for comparative purposes.

Sugar development

Seasonal and irrigation treatments had little impact on the rate of ripening (ie. change in total soluble solids concentration over time) (Figure 6.3.1.2). In all seasons and across all treatments total soluble solids increased rapidly for about 20 days from mid January (veraison) until early February followed by a decline in the rate of change in total solids until harvest. While not obvious in the plots against date, in the plots against thermal time it is clear that the there was little increase in total soluble solids at the later stages of berry development in season 2004 in all treatments, possibly due to very high temperatures in mid February. Based on thermal time from flowering, the increase in total soluble solids appeared to be delayed in 2003 although there are insufficient data points during the early stages of ripening to make detailed comparisons with the other seasons. The increase in total soluble solids was linear in 2003 and 2005 until harvest based on post-veraison thermal time (ie. during ripening), although in 2003 higher total solids were reached in all irrigation treatments at lower levels of thermal time and the rate of change was less than in 2005. Compared to the control, RDI and in particular the PD treatment delayed the increase in total soluble solids, particularly when plotted against post-veraison thermal time.

Seasonal and irrigation treatment effects on total sugar accumulation, expressed as total soluble solids per berry are presented in Figure 6.3.1.3. Across all seasons and irrigation treatments sugar accumulation followed an asymptotic pattern generally reaching a maximum level in mid February, quite early in the ripening process. This indicates that the increase in total soluble solids concentration in the later stages of ripening is associated with loss in berry weight rather than net sugar accumulation. It is clear that accumulation of sugar ceased at a much lower level in early to mid February in 2004 when very high temperatures were recorded. Total sugar accumulation was reduced by deficit irrigation treatments, particularly PD. These differences become more apparent when plotted against thermal time. The differences in the relationships when plotted against post-flowering thermal time and post-veraison thermal time indicate that sugar accumulation is affected by both pre- and post-veraison climatic conditions.

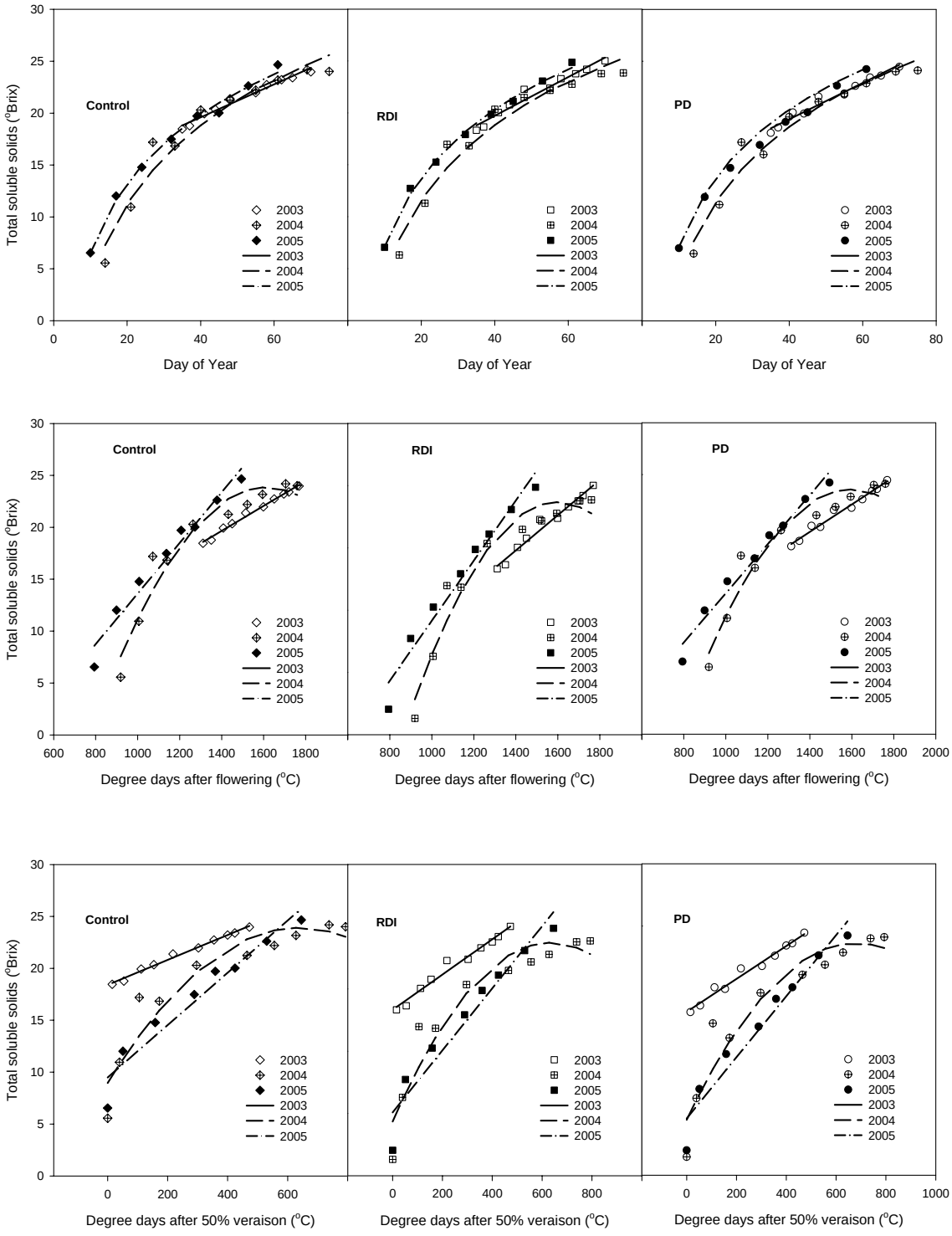


Figure 6.3.1.2. Changes in total soluble solids (^{0}Brix) of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005). Day of year, degree day after flowering and degree day after 50% veraison are included as the X axis for comparative purposes.

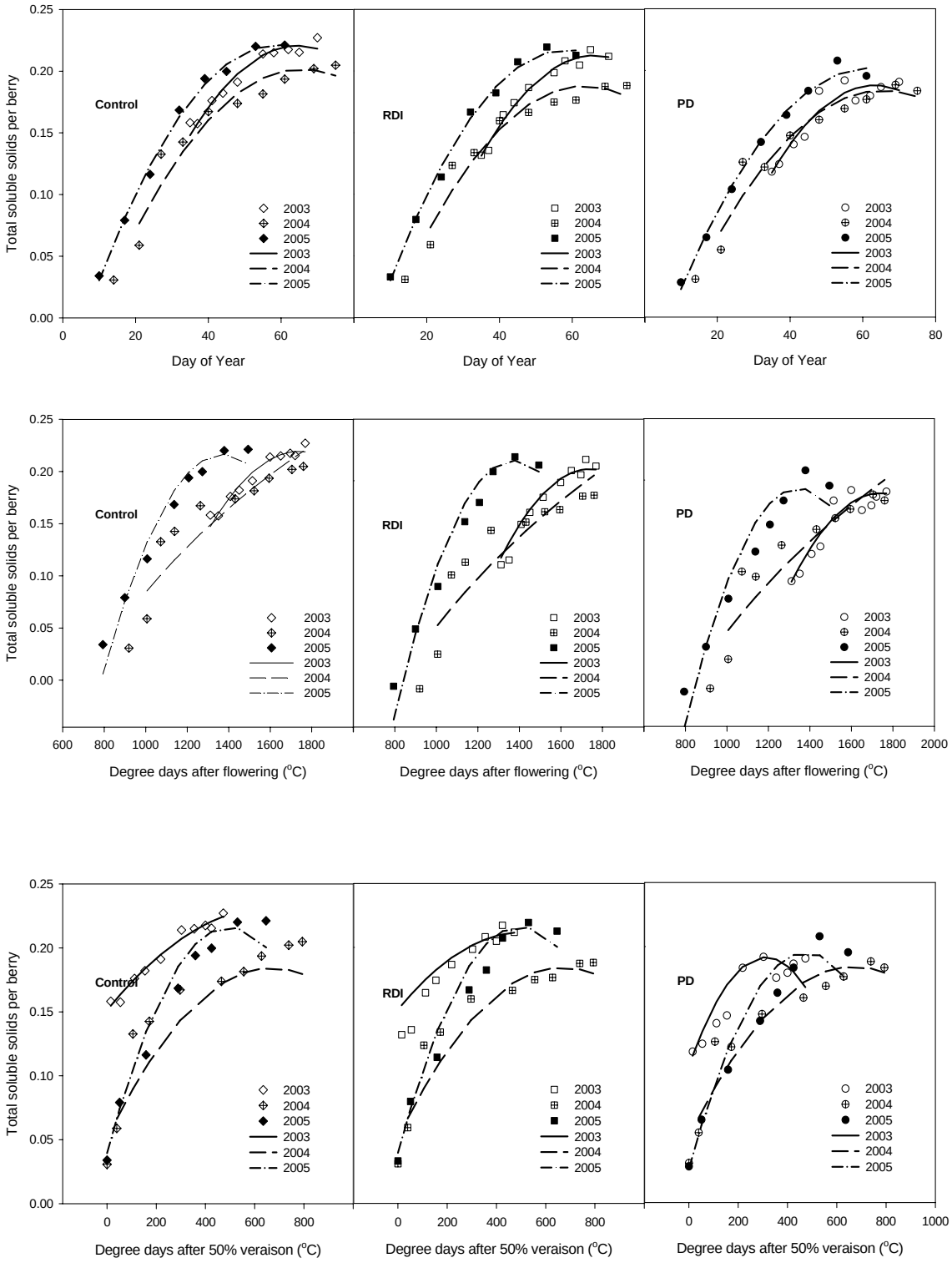


Figure 6.3.1.3. Changes in total soluble solids per berry (g) of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005). Day of year, degree day after flowering and degree day after 50% veraison are included as the X axis for comparative purposes.

Berry anthocyanin concentrations

Berry anthocyanins increased rapidly from mid January (veraison) generally reaching a maximum after about 20 days in early February, well before harvest with the control and RDI treatment (Figure 6.3.1.4). Anthocyanin concentration tended to increase for an extended period up to harvest with the PD treatment. There were large seasonal differences in anthocyanin development with both the control and RDI treatments which showed similar patterns of anthocyanin accumulation, with lowest levels occurring in 2003. The PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons.

There were large seasonal differences, but no apparent irrigation treatment effects on the development of degraded anthocyanins (Figure 6.3.1.4). Degraded anthocyanins, believed to be largely responsible for the brown coloured pigments in berries and potentially wine, were highest in 2003 and lowest in 2005. They tended to increase slowly from veraison to harvest. By contrast, non-degraded anthocyanins tended to increase until harvest, with season 2003 having the lowest concentrations. Control and RDI treatments followed similar trends in each year, whereas with PD, seasonal differences were less apparent.

Berry phenolic concentrations

Trends for changes in berry phenolic concentrations, although similar for all irrigation treatments, were inconsistent between seasons (Figure 6.3.1.5). Phenolic concentrations were lowest in 2005 and remained relatively stable over the ripening period. In 2004, phenolic concentrations dropped during the middle of the ripening period and then increased until harvest. In 2003, phenolic concentrations tended to drop at the later stages of ripening. In that season deficit irrigation treatments, particularly PD, had higher phenolic concentrations during ripening.

Berry titratable acidity and pH

The decline in titratable acidity over the ripening period was largely unaffected by season or irrigation treatment (Figure 6.3.1.6). There were however differences between seasons in the change in pH over time. Season 2005 had the most rapid increase and reached the highest pH level while season 2003 had the slowest increase and lowest pH at harvest in all irrigation treatments. Irrigation treatment had no effect on the rate of change in pH or the final level at harvest.

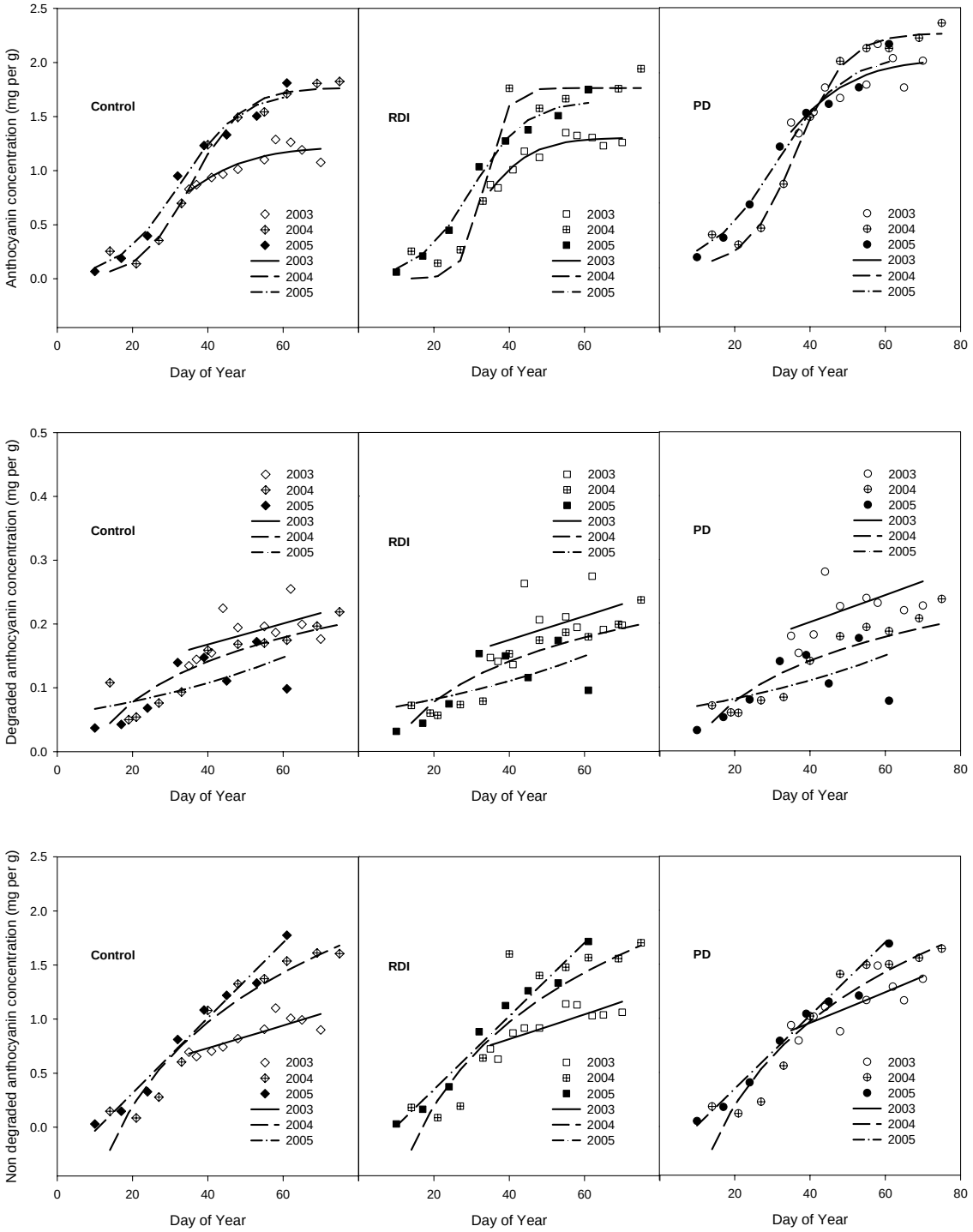


Figure 6.3.1.4. Changes in total berry anthocyanins (top), degraded anthocyanins (middle) and non-degraded anthocyanins (bottom) of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005).

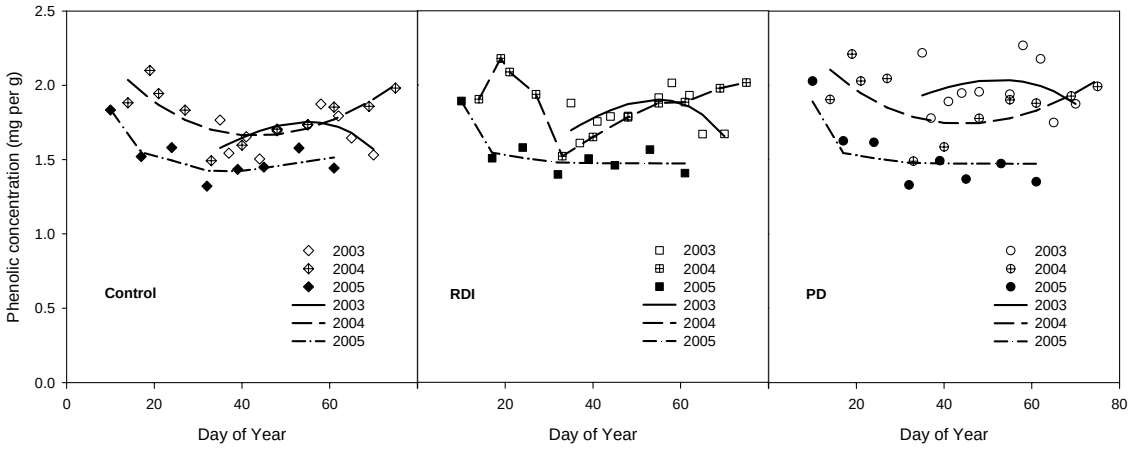


Figure 6.3.1.5. Changes in total berry phenolics of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005).

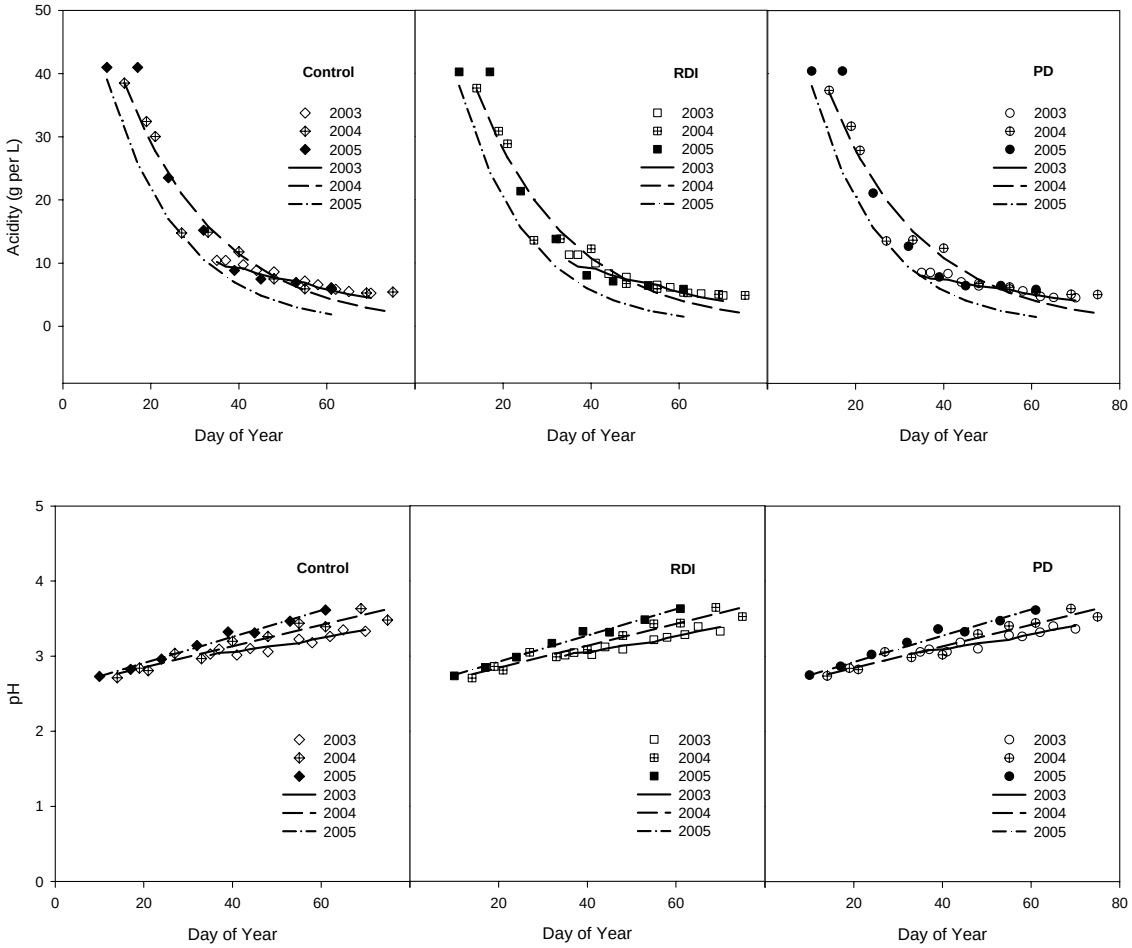


Figure 6.3.1.6. Changes in berry juice titratable acidity (top) and pH (bottom) of the three irrigation treatments (control, RDI and PD) during ripening in three seasons (2003, 2004, 2005).

Changes in organic acids during berry development

HPLC techniques were used to monitor changes in berry organic acids (ie. tartaric, malic, succinic and citric acid) during berry development and over the ripening period for the 3 seasons and irrigation treatments. There were no seasonal and irrigation treatment effects on the pattern of tartaric acid concentration decline during berry development (Figure 6.3.1.7). Similarly there was no seasonal effect on malic acid decline during berry development (Figure 6.3.1.8). However deficit irrigation treatments, in particular PD had reduced malic acid levels compared to the control. As a consequence the tartrate : malate ratio was higher in all seasons during the ripening phase with both deficit treatments (Figure 6.3.1.9). During ripening in season 2003 the tartrate : malate to ratio was relatively static for all irrigation treatments whereas in both 2004 and 2005 it continued to increase up to harvest. Citric acid levels were detected at low concentrations and generally showed a decline during berry development but were unaffected by season or irrigation treatments (Figure 6.3.1.10). Changes in total acid concentration (ie. tartaric + malic + succinic + citric) was unaffected by season or irrigation treatment (Figure 6.3.1.11).

Changes in glucose and fructose during berry development

HPLC techniques were used to monitor changes in berry glucose and fructose during berry development in seasons 2004 and 2005. Glucose concentrations tended to be higher at most stages of berry development in season 2005 compared to 2004 (Figure 6.3.1.12). There was no effect of irrigation treatment on glucose concentration. Maximum glucose levels were reached about 95-100 days after flowering. In season 2004 fructose concentration of the control treatments reached a maximum about 90 days after flowering then declined whereas with the irrigation stress treatments it continued to increase until harvest (Figure 6.3.1.13). In season 2005 fructose concentration reached a maximum at 100 days and then declined in all treatments.

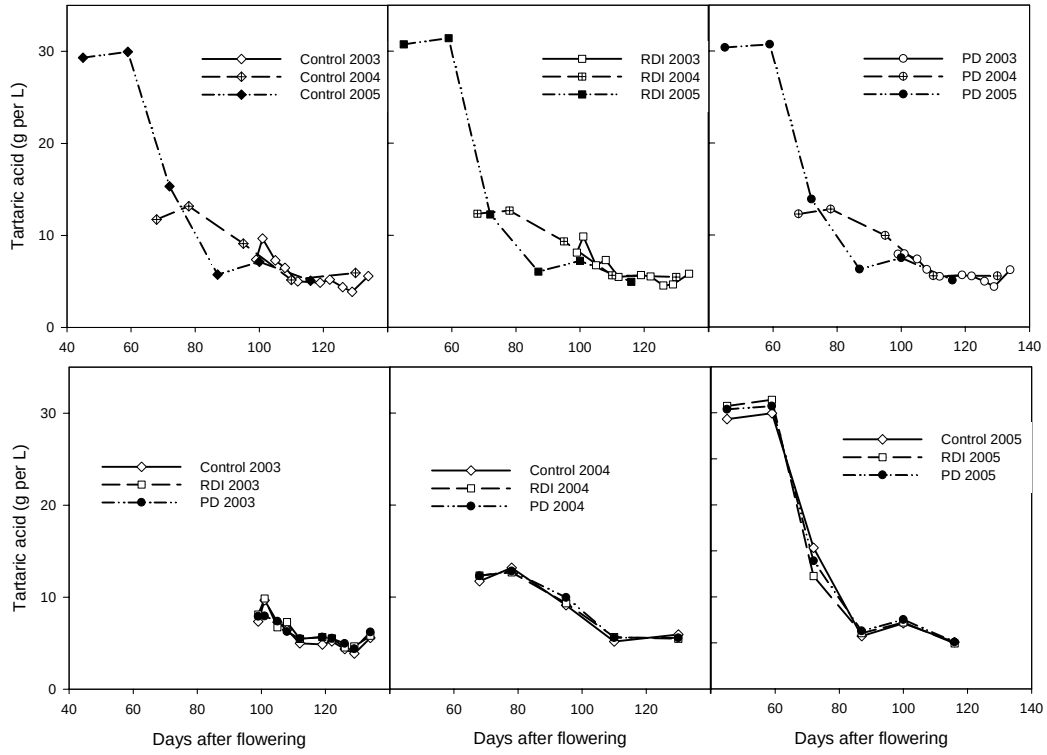


Figure 6.3.1.7. Changes in berry juice tartaric acid concentration during berry development and ripening for the three seasons (2003, 2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

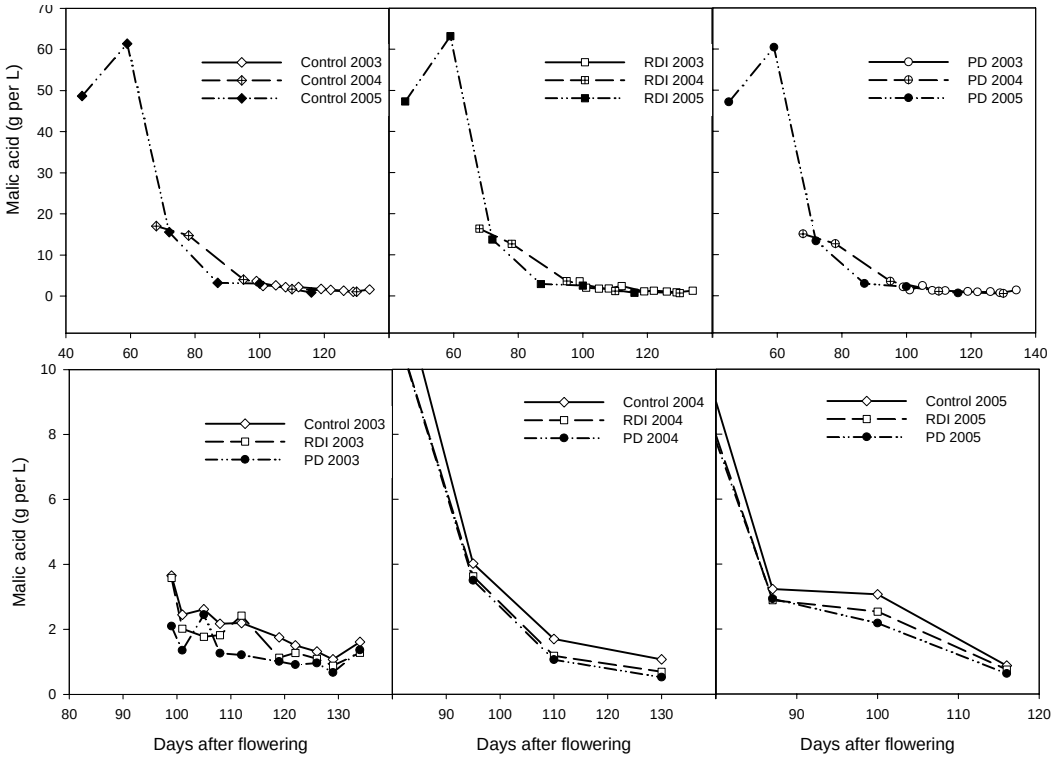


Figure 6.3.1.8. Changes in berry juice malic acid concentration during berry development and ripening for the three seasons (2003, 2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

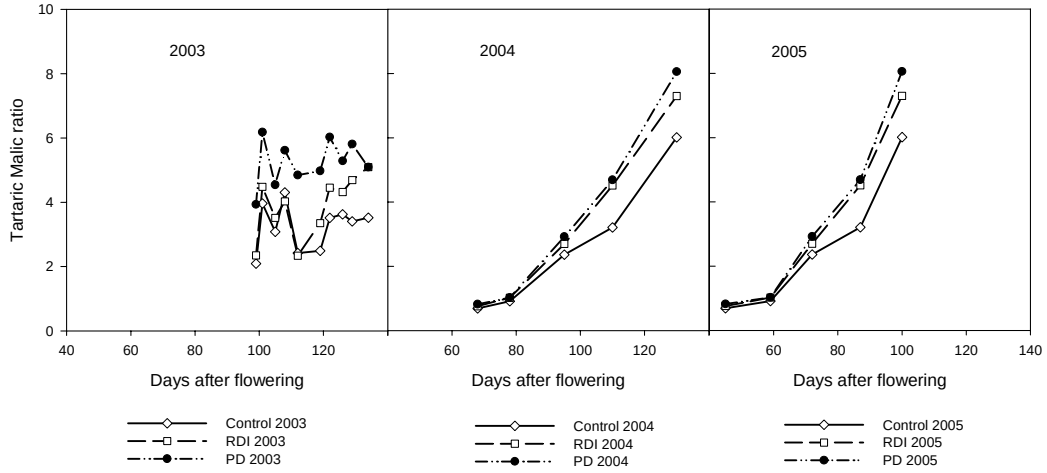


Figure 6.3.1.9. Changes in berry juice tartaric : malic acid ratio during berry development and ripening over the three seasons (2003, 2004, 2005) and three irrigation treatments (control, RDI and PD, bottom).

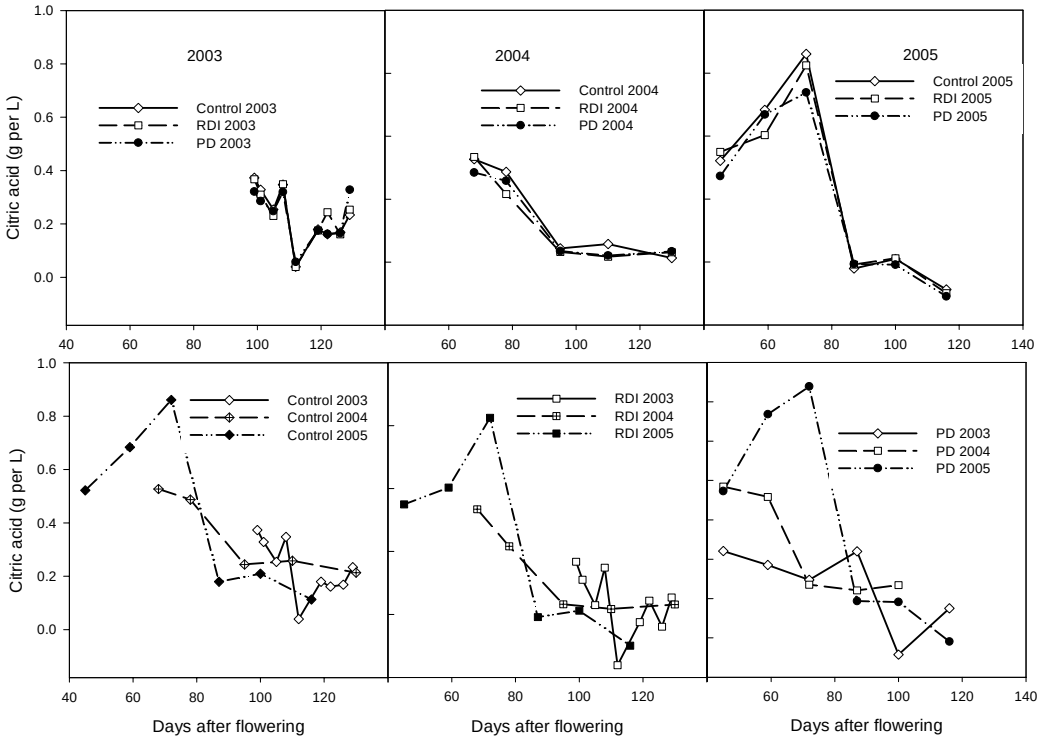


Figure 6.3.1.10. Changes in berry juice citric acid concentration during berry development and ripening for the three seasons (2003, 2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

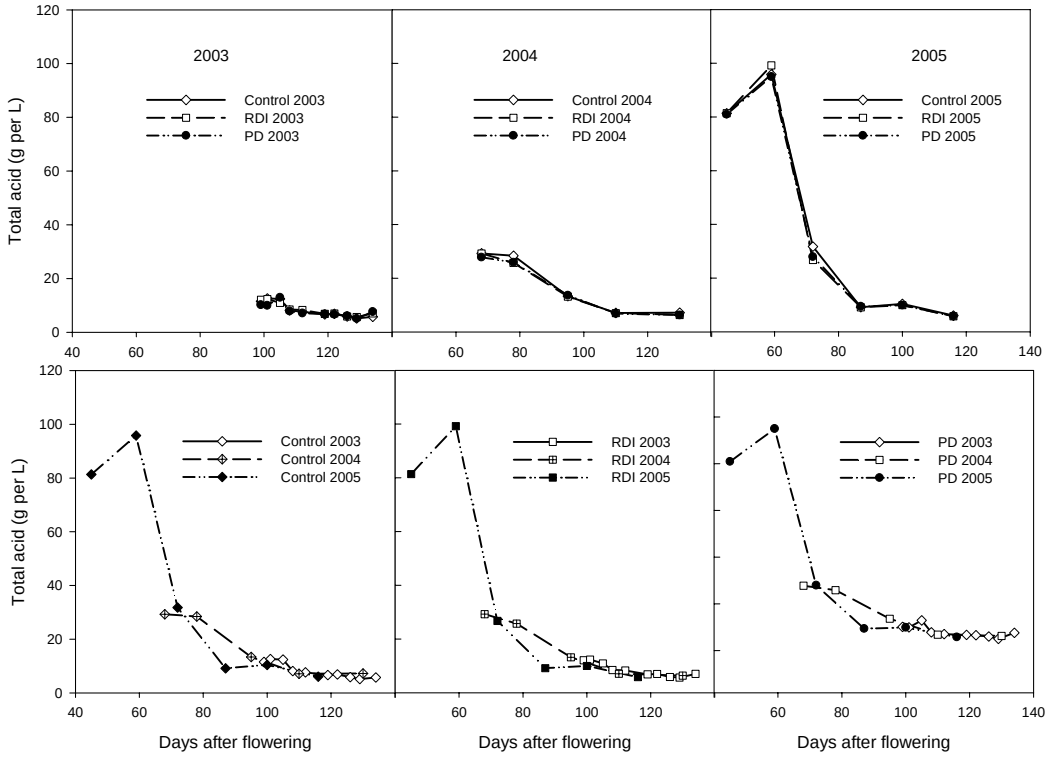


Figure 6.3.1.11. Changes in berry total organic acid concentrations during berry development and ripening for the three seasons (2003, 2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

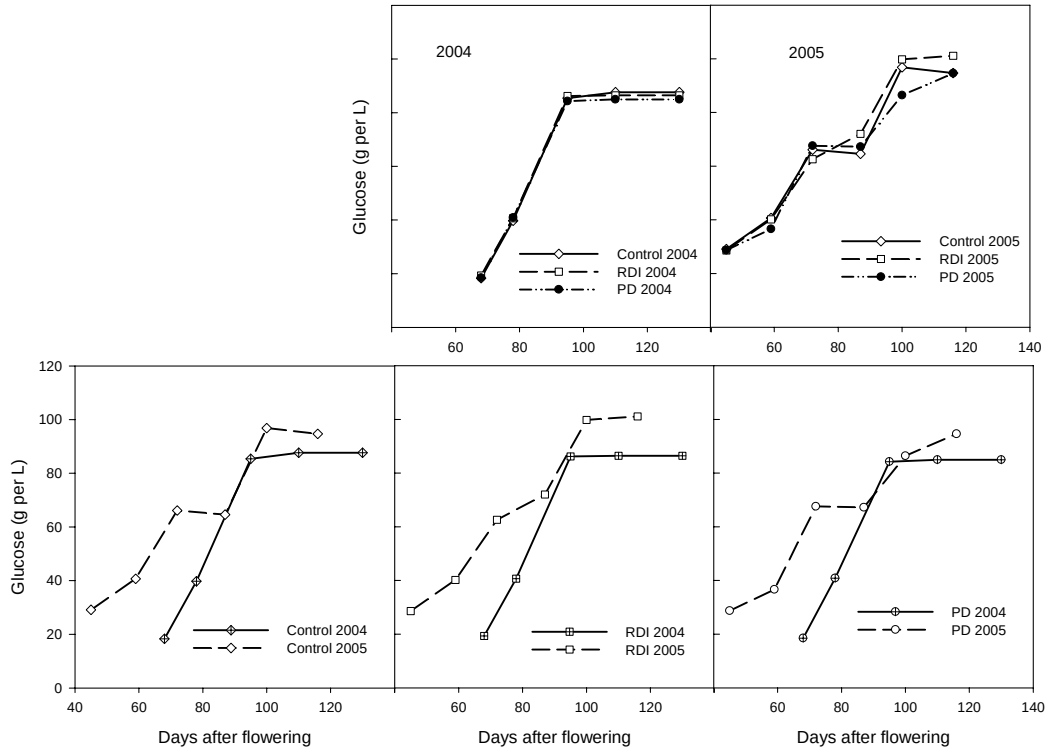


Figure 6.3.1.12. Changes in berry juice tartaric acid concentration during berry development and ripening for the three seasons (2003, 2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

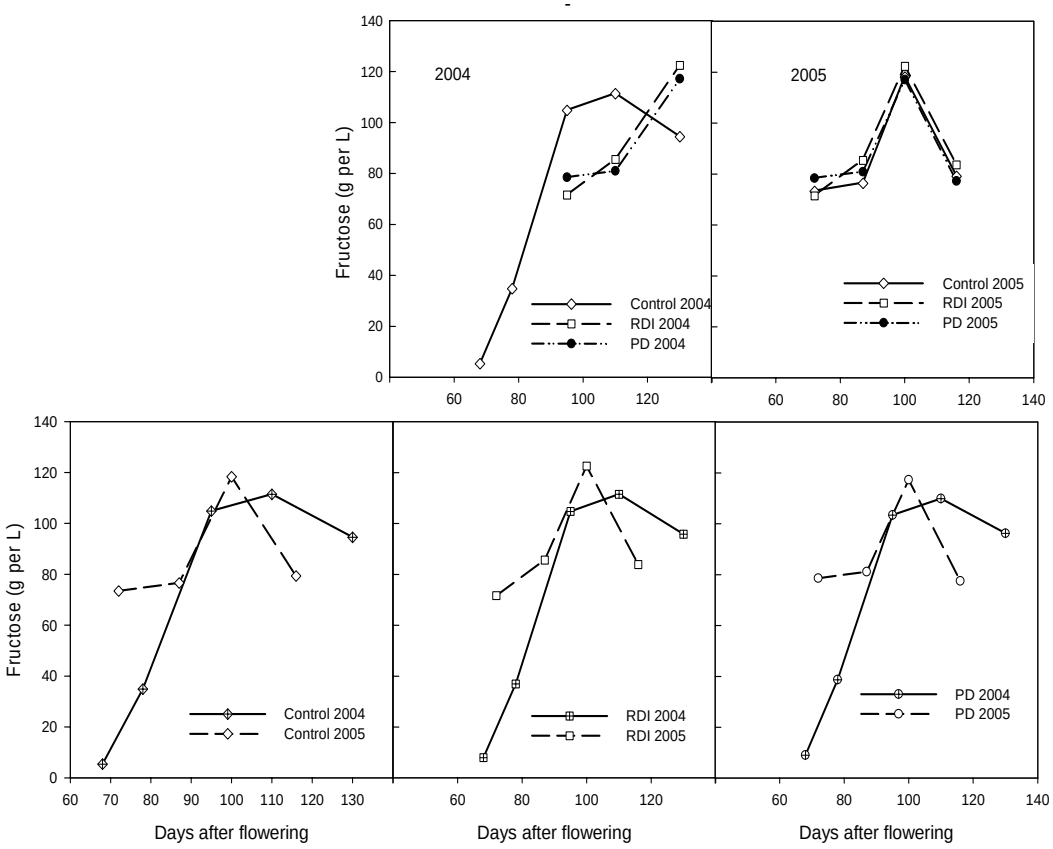


Figure 6.3.1.13. Changes in juice fructose concentration during berry development and ripening for the two seasons (2004, 2005, top) and three irrigation treatments (control, RDI and PD, bottom).

Berry composition at harvest

Harvest dates were determined when anticipated total soluble solid reached 24.5 °Brix (ie. 11th March 2003, 15th March 2004 and 02nd March 2005). The effect of season, irrigation treatment and the season x irrigation interaction on berry composition are shown in Table 6.3.1.2. The results for total soluble sugars indicate that desired maturities were largely achieved although the fruit was slightly under-ripe in 2004 and over-ripe in 2005. In general, differences between seasons were much larger than between irrigation treatments for all variables. The only significant effects of irrigation treatments were reduced total soluble solids per berry with PD, due largely to differences in berry weight (Table 6.3.1.1) and higher levels of degraded anthocyanins with both irrigation treatments. By contrast all variables differed significantly between seasons. Of particular note are the very significant seasonal differences in pH (lowest in 2003), phenolics (highest in 2004 and lowest in 2005), total anthocyanins (lowest in 2003), non-degraded anthocyanins (lowest in 2003) and degraded anthocyanins (lowest in 2005). It is interesting to note that phenolics and anthocyanins had different seasonal trends. The significant interactions between season and irrigation were due to the high level of acidity with PD in 2003 and the low levels with PD in 2004 and 2005, the high levels of phenolics with PD in 2003 and low levels in 2005, the low level of non-degraded anthocyanins with the control and RDI in 2003 and the low levels of degraded anthocyanins with PD in 2005.

Wine composition

The effects of season, irrigation treatment and the season x irrigation interaction on wine quality are shown in Table 6.3.1.3. Tartaric acid was added during winemaking with the aim to adjust wine pH values to 3.5. This was largely achieved although significant effects show that control irrigation had the highest wine pH and PD the lowest pH. Similarly, season 2003 had the highest pH and 2004 had the lowest pH. Titratable acidity of the wine was unaffected by irrigation treatment although there were significant seasonal effects with 2003 having the lowest acid level and 2005 the highest acid level. Over the seasons there were significant irrigation treatment effects on wine spectral properties with the wines of the control treatment having the lowest colour density, total anthocyanins, ionised anthocyanins, total phenolics and the highest hue indicating lower brightness. By contrast, wines from the PD treatment had the highest colour density, total anthocyanins, ionised anthocyanins, total phenolics and the lowest hue. Wines from the RDI treatment tended to be intermediate for all spectral parameters except that total phenolic levels were similar to PD. Colour density and ionised anthocyanins were lowest in 2003 and highest in 2004, total anthocyanins were lowest in 2004 and highest in 2003 while phenolics were lowest in 2005 and highest in 2003. Wines from 2003 had the highest hue. The significant interactions between season and irrigation treatment for colour density, total anthocyanins and ionised anthocyanins can largely be attributed to differences in the magnitude of the response to irrigation treatments, particularly in season 2004 when all treatments produced wines with high values. This result suggests that benefits in wine spectral properties associated with stress will only be achieved in seasons when wine colour is likely to be low and that there is a maximum level of colour extractability during the winemaking process.

Over the seasons and irrigation treatments there were significant positive correlations between colour density and ionised anthocyanins ($r = 0.93$) and between total anthocyanins and phenolics ($r = .77$). Significant negative correlations were found between hue and both colour density and ionised anthocyanins ($r = -0.82$ and -0.95 respectively) indicate that wines with more colour were also brighter, ie. had lower hue values. There were strong correlations over the seasons and irrigation treatments between total berry total anthocyanins and colour density and between total anthocyanins and ionised anthocyanins ($r = 0.85$ and 0.86 respectively). A significant negative correlation between berry total anthocyanins and colour hue indicates that berries with high levels of anthocyanins produce brighter wines. The correlation between berry anthocyanins and berry phenolics was not significant, but there was a correlation between berry phenolics and colour density of the wine.

Irrigation treatment	TSS (°Brix)	TSS per berry (g)	Acidity (g per L)	pH	Phenolics (mg per g)	Total anthocyanins (mg per g)	Non degraded anthoycanins (mg per g)	Degraded anthoycanin (mg per g)
Control	24.2	0.22 a	5.3	3.5	1.65	1.57	1.43	0.16 a
RDI	24.7	0.21 a	5.1	3.5	1.68	1.65	1.49	0.18 b
PD	24.3	0.19 b	5.1	3.5	1.69	1.72	1.55	0.18 b
p-value	ns	***	ns	ns	ns	ns	ns	*
Season								
2003	24.5 a	0.21 a	5.2 b	3.3 a	1.69 b	1.31 a	1.11 a	0.20 a
2004	24.1 b	0.19 b	5.0 b	3.5 b	1.93 a	1.86 b	1.64 b	0.22 b
2005	25.6 a	0.21 a	5.3 a	3.6 c	1.40 c	1.76 b	1.73 b	0.10 a
p-value	**	**	*	***	***	***	***	***
Irrigation by season interaction	ns	Ns		ns		Ns		
Control 2003	24	0.23	5.0 abc	3.3	1.53 ab	1.08	0.90 a	0.18 c
RDI 2003	25	0.21	5.2 bcd	3.3	1.67 b	1.26	1.06 a	0.20 cd
PD 2003	24.5	0.19	5.4 cd	3.4	1.88 bc	1.59	1.36 b	0.23 de
Control 2004	24	0.2	5.3 cd	3.5	1.97 c	1.81	1.61 c	0.21 de
RDI 2004	24.2	0.19	4.7 a	3.5	1.97 c	1.93	1.70 c	0.23 de
PD 2004	24.1	0.18	4.8 ab	3.5	1.85 bc	1.83	1.61 c	0.23 de
Control 2005	24.7	0.22	5.5 d	3.6	1.44 a	1.81	1.78 c	0.09 ab
RDI 2005	24.9	0.21	5.2 cd	3.6	1.41 a	1.75	1.72 c	0.11 b
PD 2005	24.2	0.2	5.1 abc	3.6	1.35 a	1.72	1.69 c	0.08 a
p-value			*		*		**	***

Table 6.3.1.2 The effect of irrigation treatments and season on berry composition at harvest (24.5 °Brix during phase III berry development). Mean values were calculated (n=12) and treatment differences tested (ANOVA). Significant treatment difference are indicated * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and dissimilar letters show treatment differences.

Irrigation treatment	Acid (g per L)	pH	Colour density (A.U.)	Colour hue (A_{420} / A_{520})	Total anthocyanins (mg per L)	Ionised anthocyanin (mg per L)	Total phenolics (mg per L)
Control	7.81	3.53 a	11.0 a	0.54 a	686 a	91 a	57 a
RDI	7.86	3.5.0 b	12.1 b	0.53 ab	784 b	102 b	64 b
PD	7.86	3.47 c	14.0 c	0.51 b	895 c	126 c	65 b
p-value	ns	***	***	**	***	***	*
Season							
2003	7.34 c	3.55 a	9.4 a	0.54 a	944 a	85 a	73 a
2004	7.55 b	3.45 b	16.1 c	0.51 b	678 c	125 c	62 b
2005	8.4 a	3.50 c	11.7 b	0.52 ab	755 b	109 b	55 c
p-value	***	***	***	*	***	***	***
Irrigation by season interaction							
Control 2003	7.5	3.58	6.8 e	0.58	804 c	56 g	64
RDI 2003	7.31	3.55	8.8 d	0.54	942 b	77 f	73
PD 2003	7.22	3.53	12.7 b	0.5	1084 a	121 ab	82
Control 2004	7.54	3.48	15.6 a	0.52	641 de	119 ab	59
RDI 2004	7.52	3.46	16.0 a	0.51	682 de	126 a	62
PD 2004	7.59	3.41	16.6 a	0.51	712 de	131 a	64
Control 2005	8.24	3.54	10.7 c	0.53	632 e	97 e	51
RDI 2005	8.53	3.48	11.5 bc	0.53	742 cd	103 be	59
PD 2005	8.53	3.47	13.0 b	0.51	891 b	127 a	54
p-value	ns	ns	**	ns	**	**	ns

Table 6.3.1.3 The effect of irrigation treatments and season on wine composition and spectral properties. Mean values were calculated (n=4) and treatment differences tested (ANOVA). Significant treatment difference are indicated * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and dissimilar letters show treatment differences.

6.3.2. Summary paper.

The balance of berry sugar accumulation, colour and phenolic concentration under deficit irrigation strategies

The following paper which was prepared for the 12th Wine Industry Technical Conference by Cooley et al. (2004) provides an overview of the work undertaken with regard to berry development and composition.

Abstract

The relationship between sugar concentration, colour and phenolic concentration was assessed in berries of *Vitis vinifera* (Cabernet Sauvignon) subjected to deficit irrigation treatment in a warm climate region. A control treatment (standard drip) was compared with a regulated deficit irrigation (RDI) treatment and a prolonged deficit irrigation (PD) treatment during seasons 2002/2003 and 2003/2004. Photosynthesis, stomatal conductance and leaf water potential were significantly reduced during season 2002/2003, whereas only occasional reductions in mid-day photosynthesis, stomatal conductance and leaf water potential were found during season 2003/2004. Berry size was significantly reduced in the deficit irrigation treatments (RDI and PD) during both seasons. Season 2002/2003 resulted in an increase in anthocyanin and phenolic concentration with both RDI and PD compared to the control treatment whereas no significant change was recorded during season 2003/2004. Harvest date was set when the total soluble sugars reached 24° Brix. Optimum anthocyanin (colour) and phenolic concentrations were reached before required sugar accumulation was obtained in season 2002/2003. Significant colour and phenolic concentration loss was observed (17 and 18% respectively), during days of the year 58 to 70 while a relatively small increase in sugar accumulation occurred (1.24 °Brix). No undesirable green herbaceous flavours were detected in small-scale wine-making from the various irrigation treatments. To conclude, in warm climate regions where irrigation stress management is implemented, sugar accumulation, colour, phenolic concentration and seed ripening should be monitored and a careful balance of these parameters used to determine harvest date, thus ensuring achievement of optimum potential wine quality.

Introduction

In recent years, several winegrape producing areas of Australia have experienced problems with grapes maturing within reasonable time frames. Season 2000/2001 was a worse case scenario with grape maturation being significantly delayed in the latter part of the season. Fruit was often low in acid with high pH. Combinations of heat stress and water stress are believed to compound the slow down in maturation. This project was set up to research the interaction of heat stress with water stress using regulated deficit irrigation (RDI) and prolonged deficit irrigation (PD) and to investigate its interaction on berry maturation and quality. The relationship among sugar concentration, colour and phenolic concentration was assessed over two seasons in berries of *Vitis vinifera* subjected to deficit irrigation treatment in a warm climate region.

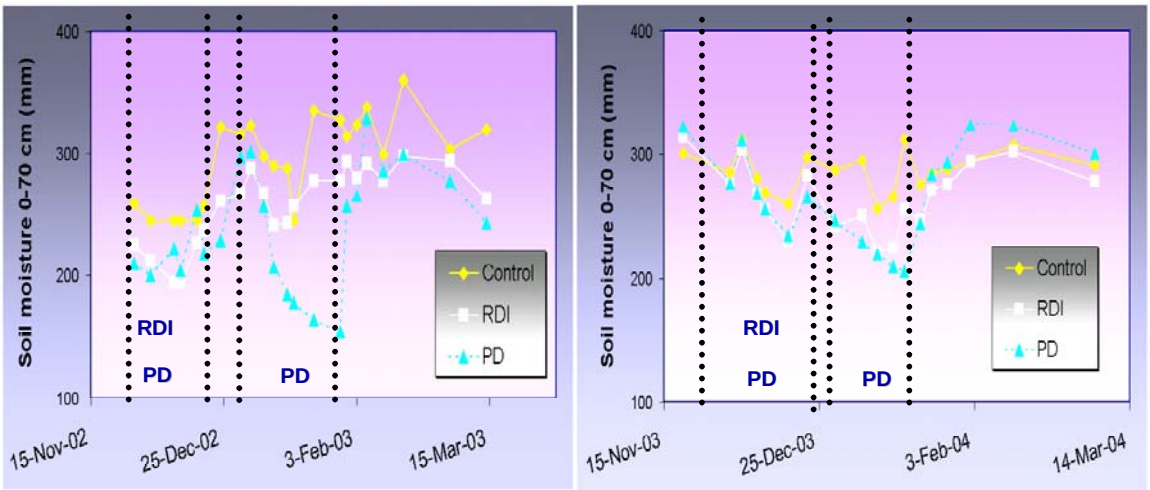
Materials and methods

Cabernet Sauvignon vines on own roots were planted in 1995 at a density of approximately 1366 vines per ha (2.44 m within row and 3 m between rows) as part of a 5 ha commercial vineyard on Nookamka sandy loam (Hubble and Crocker 1941) located in the Sunraysia region of Victoria, Australia. Vines were trained in east-west orientated rows, on a two wire vertical trellis and mechanically hedged. The experiment was conducted over two seasons (2002/2003 and 2003/2004). A control treatment (standard drip), which was irrigated to full point, was compared with a regulated

deficit irrigation (RDI) treatment and a prolonged deficit irrigation (PD) treatment during the season. Treatments were arranged in a random design down the rows. The duration of the RDI was determined based on visual assessment of tendrils, canopy leaf area, vine canopy to crop ratio and soil water monitoring data (Table 1). Additional information on RDI use and implementation has been reviewed by Kriedemann and Goodwin (2004). The PD treatment was imposed after the RDI period and presents an extreme deficit irrigation treatment (Figure 1).

2002/2003	Irrigation description	Irrigation date Date ON	Date OFF	Irrigation duration (days)	Irrigation applied (ML/ha)
Control	Full point	21-Nov-02	13-Dec-02	22	6
RDI	Commerical RDI	21-Nov-02	13-Dec-02	22	4.6
PD	Commerical RDI	21-Nov-02	13-Dec-02	22	3.2
	No water	24-Dec-02	31-Dec-02	7	
2003/2004					
Control	Full point	25-Nov-03	2-Jan-04	38	6.5
RDI	Commerical RDI	25-Nov-03	2-Jan-04	38	5.1
PD	Commerical RDI	25-Nov-03	2-Jan-04	38	4.9
	No water	2-Jan-04	18-Jan-04	16	

Table 1. Description of control, regulated deficit irrigation (RDI), and prolonged deficit irrigation (PD) treatments.



2002-2003 2003-2004

Figure 1. Soil moisture content (0 – 70 cm profile) measured by neutron moisture meter for the control, regulated deficit irrigation (RDI), and prolonged deficit irrigation (PD) treatments

The maturation period was monitored in 2003 from the onset of veraison (defined here as the beginning of a change in colour of the berries from green to red) until the harvest date which was determined by an optimal total soluble solids reading of 24º Brix. In 2004, the observation period was extended to include pre-veraison berry development. Bunches (n=5) taken from different positions on both sides of the canopy (Krstic et al. 2002) were collected at bi-weekly intervals throughout the observation periods; from 27 February until 11 March 2003, and from 14 January until 15 March 2004. Bunches were transported on ice from the field where berries were separated from the rachis, mixed and fresh weight recorded. A 100 berry sub-sample was collected. The berry sub-sample was

homogenised in a mortar and pestle, filtered through a sieve and the total soluble solids (TSS) concentration (ºBrix) determined on the berry juice using a temperature compensating digital refractometer (Atago, Tokyo, Japan). The juice was centrifuged at 3000 rpm (1500 g) for 10 min. to separate the solid matrix from the liquid. An aliquot (5 mL) was taken for pH and titratable acidity analysis employing an auto-titrator (SAC 80 Sample Changer, ABU91 Autoburette with a VIT90 Video titrator, Radiometer-Copenhagen, Linsellesstr, Germany) with tartaric acid equivalents determined to an end point of pH 8.2.

Anthocyanin and phenolic concentrations were determined using the spectrophotometric method described by Iland et al. (1996) on a frozen berry sub-sample (n=100) defrosted in the dark overnight at 2°C. Berries were homogenised using an Ultra-Tarax T25 basic (IKA Labortechnik, Staufen, Germany) 19000 rpm 1/min. A sub-sample was centrifuged (at 1500 g for 15 min.). Acidified ethanol was added to the supernatant and incubated at room temperature for 1 h. 1M HCL was added and incubated for 3 h. Anthocyanins were determined by measuring the absorbance at 520 nm while the phenolic concentration was measured at 280 nm.

Small-scale wines were made as described by Kerridge (1983) with tartaric acid addition to adjust all wines to a constant pH of 3.5. Berries sub-sampled from each vine produced three field wine replicates for each treatment. Quality measurements, both chemical and sensory, were conducted on the wine.

Results and discussion

Water stress indicators were measured during deficit irrigation periods. In season 2002/2003, significant reductions (up to approximately 30%) were observed in photosynthesis, stomatal conductance, transpiration, leaf water potential and leaf turgor (data not shown). These findings have been observed by other groups investigating water stress and physiological indicators (Poni et al. 1993, 1994; Escalona et al. 1999; Flexas et al. 1999; Bota et al. 2001). Where physiological stress described above was observed, an increase in deficit treatment leaf temperature was found when compared to the control leaf temperature. Vines in the RDI treatment recovered from the water stress after a couple of weeks, whereas the PD treated vines took approximately three weeks to recover.

Season 2003/2004 was climatically a contrasting year to season 2002/2003. October, November and, particularly, January were cooler in 2003/2004 (Figure 2) with more overcast days. These different environmental conditions were reflected in the water stress indicator measurements, with only occasional reductions in photosynthesis, stomatal conductance, transpiration, leaf water potential and leaf turgor.

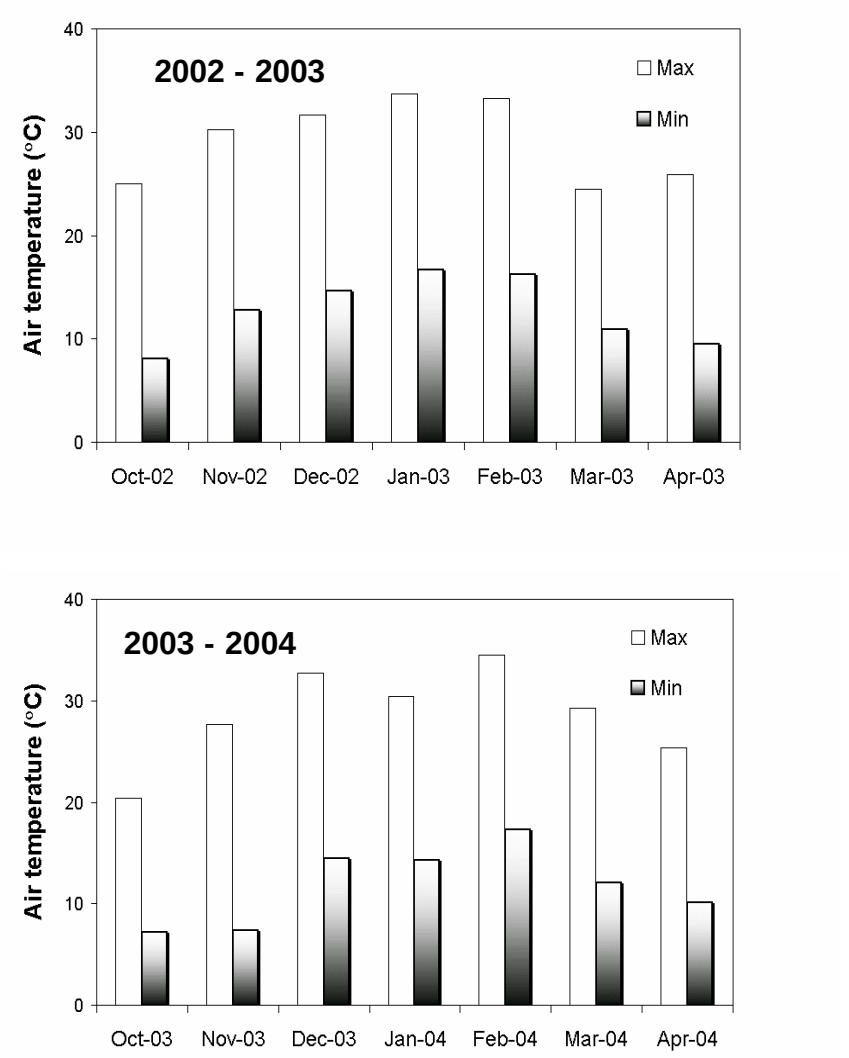


Figure 2. Maximum and minimum air temperatures for seasons 2002-2003 and 2003-2004 monitored by Nangiloc and Mildura weather stations

Berry development was assessed during both seasons. Berry size was significantly reduced in the deficit irrigation treatments (RDI and PD) and berry juice total soluble solids (°Brix, Baumé, etc.), along with whole berry anthocyanin and phenolic concentrations, increased with deficit irrigation treatments during season 2002/2003 (data not shown). These observations have been previously recorded (Van Zyl 1984). Berry development was significantly different during season 2003/2004. Anthocyanin concentrations in the control treatments during season 2003/2004 were much higher (approximately 30% higher) than in season 2002/2003 (Table 2). Season 2003/2004 showed no significant differences with these development parameters (not including berry weight where a reduction was found) and reflected the seasonal differences observed with water stress indicators.

Accumulation period Day of the Year 30 to 70	Anthocyanin (g mg^{-1} day^{-1})	TSS (°Brix day^{-1})
2003	0.007	0.16
2004	0.027	0.17
% difference	41	9

Table 2. Comparison of seasonal differences in the rate of anthocyanin and total soluble solids (TSS) accumulation calculated from the control data for days of year 30 to 70 in 2003 and 2004 season.

Sugar concentration measurements are often used in viticulture as an important determinant for harvest date. Harvest date was set in this experiment when the total soluble solids reached 24°Brix. It was found during season 2002/2003 that optimum colour and phenolic concentration was reached before required sugar accumulation was obtained. Significant colour and phenolic concentration loss was observed (17 and 18% respectively) during days of the year 58 to 70, while a relatively small increase of 1.24°Brix in sugar accumulation occurred (Table 3). Therefore, it is important to monitor both colour and TSS simultaneously to ensure that optimum wine quality is achieved, especially during seasons of heat stress.

	Value of parameter at	
	Day of the year 58	**Day of the year 70**
Anthocyanins (mg g^{-1})	1.29 ± 0.1	1.07 ± 0.05
Phenolics (mg g^{-1})	1.87 ± 0.1	1.53 ± 0.05
TSS (°Brix)	22.7 ± 0.28	24.0 ± 0.28

Table 3. Whole berry anthocyanin and phenolic concentrations, and total soluble solids (TSS) for days of the year 58 and 70. Results were calculated from the control treatment in season 2003.

During season 2002/2003, highly significant increases in colour density, ionised anthocyanins, total anthocyanins and total phenolics were found with deficit irrigation treatments compared to the control treatment (Table 4). It should be noted that the increase in colour and phenolic concentration with the deficit irrigation treatments were lower in the berry juice than the wine and could suggest co-pigmentation. Wine was also made from season 2003/2004 vintage but the quality measurements were not available at the time of publication.

Previous researchers have noted that delayed seed ripening might result in lower wine quality, which is thought to be caused by undesirable sensory characteristics such as the commonly described 'green/herbaceous' flavours (tannins are thought to be responsible). Wine tasting was conducted by the CSIRO (Plant Industry) internal sensory panel (wine made from season 2002/2003 harvest) and no herbaceous flavours were detected with the irrigation treatments.

		% increase with deficit irrigation	
	Control	RDI	PD
Wine pH	3.6	n/a	n/a
Wine titratable acidity	7.5 (g/L)	n/a	n/a
Colour density	6.8 (A.U.)	22%	46%
Ionised anthocyanins	56 (mg/L)	27%	54%
Total anthocyanins	804 (mg/L)	15%	26%
Total phenolics	94 (mg/L)	12%	22%

Table 4. Wine pH, total titratable acids, colour density, ionised and total anthocyanins and total phenolics for the control treatment and the % increase for RDI and PD treatments. Measurements are the means from three replicates. Note that % increase in pH and wine titratable acidity were not applicable (n/a) due to acid adjustments.

Summary

The contrasting environmental conditions found with both seasons (the months October, November and January were cooler during season 2003/2004 than season 2002/2003), and might explain the vine physiology and wine quality treatment differences. During the warmer season, where a heat stress might have occurred (2002/2003), reductions in photosynthesis, stomatal conductance and leaf water potential (vine physiology) were found in the water deficit treatments in conjunction with an increase in wine-grape quality (increased TSS, phenolic and anthocyanin concentration etc.) compared to the control treatment. During the cooler season (2003/2004), which was probably below average for the region, the vine physiology was not as affected with deficit irrigation, which resulted in no significant change in winegrape quality compared to the control treatment.

To achieve the best wine quality, in years where heat stress occurs and a water deficit is implemented, it is important to monitor both sugar accumulation and anthocyanin concentration to determine harvest date, as they can reach optimum levels at different rates.

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References

Bota, J.; Flexas, J.; Medrano, H. (2001) Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. Ann. Appl. Biol. 138(3): 353-361.

Escalona, J.M.; Flexas, J.; Medrano, H. (1999) Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. Aust. J. Plant Physiol. 26(5): 421-433.

Hubble, G.D.; Crocker, R.L. (1941) A soil survey of the Red Cliffs Irrigation District, Victoria. Bulletin (Council for Scientific and Industrial Research (Australia)) ; no. 137. by Hubble, G. D.

Melbourne, Council for Scientific and Industrial Research, 1941.

Flexas, J.; Badger, M.; Chow, W.S.; Medrano, H.; Osmond, C.B. (1999) Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. Plant Physiol. 121(2): 675-684.
Iland, P.G.; Cynkar, W.; Francis, I.L.; Williams, P.J.; Coombe, B.G. (1996) Optimisation of methods for the determination of total and red-free glycosyl-glucose in black grape berries of *Vitis vinifera*. AJGWR 2, 171-178.

Kerridge, G.H. (1983) Small-scale winemaking in varietal assessment. Lee, T.H. (ed.) Fermentation technology : Proceedings of a seminar organised by the Aust. Soc. Vitic. Oenol. Inc. 14 Oct. 1982, Mclaren Vale, S.A. Adelaide, S.A. Aust. Soc. Vitic. Oenol. Inc. 71-77.

Kriedemann, P.E.; Goodwin, I. (2004). Irrigation Insites No. 3. Regulated deficit irrigation and partial rootzone drying. Land and Water Australia, ACT.

Krstic, M.P.; Leamon, K.; DeGaris, K.; Whiting, J.; McCarthy, M.; Clingeleffer, P. (2002) Sampling for wine grape quality parameters in the vineyard: variability issues. Blair, R.J.; Williams, P.J.; Høj, P.B. (eds). Proc. 11th Aust. Wine Ind. Tech. Conf., 7-11 October 2001, Adelaide, S.A. Adelaide, S.A. Aust. Wine Ind. Tech. Conf. Inc. 255.

Poni, S.; Lakso, A.N.; Turner, J.R.; Melious, R.E. (1993) The effects of pre- and post-veraison water stress on growth and physiology of potted Pinot Noir grapevines at varying crop levels. Vitis 32(4): 207-214.

Poni, S.; Lakso, A.N.; Turner, J.R.; Melious, R. E. (1994) Interactions of crop level and late season water stress on growth and physiology of field-grown Concord grapevines. Am. J. Enol. Vitic. 45(2): 252-258.

Van Zyl, J.L. (1984). Response of Colombard grapevines to irrigation as regards quality aspects of growth. S. Afr. J. Enol. Vitic. 5(1): 19-28.

6.3.3. Studies with particle film technology

Fruit from the comparative studies where particle film technology treatments were superimposed on the irrigation treatments was sampled for measurement of berry composition. Larger quantities of fruit were also harvested and small scale wine lots produced in seasons 2004 and 2005. These studies were designed to assess whether the reduced stress associated with PFT had an impact on yield or fruit and wine composition.

Yield components and berry composition

The results for berry composition and yield components in season 2004 are presented in Table 6.3.2.1. In 2004 berry weight, titratable acidity and sugar per berry were significantly reduced by water deficits. There were no significant effects of irrigation treatment on total soluble solids, pH, anthocyanins, phenolics, yield, bunch number and bunch weight, although yield and bunch weight tended to reflect the significant reduction in berry weight. The particle film treatment (PFT) produced a significant reduction in total soluble solids and an increase in acidity and phenolics across all irrigation treatments but had no effect on berry weight, pH, sugar per berry, anthocyanins, yield, bunch number or bunch weight.

In season 2005, irrigation deficits significantly reduced total soluble sugars, sugar per berry, and yield but had no effect on berry weight, pH, acidity, anthocyanins, phenolics, bunch number or bunch weight (Table 6.3.2..2). Across all irrigation treatments the PFT treatment produced a significant increase in berry weight, sugar per berry and bunch weight but had no effect on total soluble solids, pH, titratable acidity, anthocyanins, phenolics or yield. The significant increase in bunch number which could be associated with reduced bunch abortion, a possible result of less stress, requires verification, as it is not consistent with the non-significant effect on yield or the higher bunch weights.

Must and wine composition

The results for must and wine composition are shown for season 2004 in Table 6.3.2.1. In all cases during the winemaking tartaric acid was added to adjust wine pH. Hence there were no treatment effects on wine pH (mean 3.47) or titratable acidity (7.6 g/L) (data not shown). Irrigation deficits produced a significant reduction in total soluble sugars (PD only) but wines were brighter as shown by the lower hue and had a higher colour density. There were no significant irrigation effects on must pH or acidity, total anthocyanins, ionised anthocyanins or phenolics. Across the irrigation treatments the PFT treatment had significantly lower acid in the must while the wines had a higher hue, an increased degree of ionization of the anthocyanins, a higher colour density despite lower level of total anthocyanins. PFT treatment had no effect on must total soluble solids or wine pH, ionized anthocyanins or phenolics. For ionised anthocyanins there was a significant interaction between irrigation treatment and PFT treatment which showed that PFT produced wines with higher levels of ionized anthocyanins with the deficit irrigation treatments but not with the control treatment.

In season 2005, the adjusted wine pH (mean 3.50) and titratable acidity (mean 8.4 g/L) were unaffected by treatment (detailed data not shown). Deficit irrigation treatments significantly reduced must acidity and produced wines with significantly higher colour density (PD only), total anthocyanins and ionised anthocyanins (PD only)(Table 6.3.2.4). Irrigation treatments had no effect on total soluble sugars or pH of the must or wine hue and phenolics in the wine. Across the irrigation treatments PFT treatment increased total soluble solids in the must and total anthocyanins in the wine. PFT treatment had no effect on pH and acidity of the must or wine hue, colour density, ionised anthocyanins or total phenolics. For total anthocyanins there was a significant interaction between irrigation and PFT treatment which showed that with PD, the PFT reduced total anthocyanins, albeit from a high level (ie. 891 and 836 mg/L respectively) compared to increases in total anthocyanins when PFT was applied to the control and RDI treatments.

Treatment	Berry weight (g)	TSS (^{0}Brix)	pH	Acidity (g/L)	Sugar per berry (g)	Antho. (mg/g)	Phenolics au	Yield (t/ha)	Bunch Number	Bunch weight (g)
Irrigation										
Control	**0.854 b**	24.0	3.48	**5.42 c**	**0.205 b**	1.76	1.50	20.0	275	65.0
RDI	**0.789 a**	23.9	3.52	**4.86 a**	**0.188 a**	1.87	1.51	18.0	295	63.0
PD	**0.761 a**	24.1	3.52	**5.03 b**	**0.184 a**	1.81	1.51	18.5	299	59.9
Particle film										
-PFT	0.799	**24.2**	3.52	**4.99**	0.193	1.81	**1.00**	19.2	284	64.7
+PFT	0.803	**23.8**	3.50	**5.22**	0.191	1.81	**2.01**	18.5	296	60.6

Table 6.3.2.1. Effect of particle film treatments (-PFT and +PFT) and irrigation treatments (Control, RDI, PD) on yield components and berry composition in 2004. Significant treatment effects are shown in bold. Means followed by different letters are significantly different.

Treatment	Berry weight (g)	TSS (^{0}Brix)	pH	Acidity (g/L)	Sugar per berry (g)	Antho. (mg/g)	Phenolics au	Yield (t/ha)	Bunch Number	Bunch weight (g)
Irrigation										
Control	0.879	**24.6ab**	3.61	5.31	**0.216b**	1.63	1.35	**30.4b**	288	79.6
RDI	0.882	**24.9b**	3.63	5.18	**0.211a**	1.68	1.35	**27.8ab**	277	77.9
PD	0.851	**24.4a**	3.63	5.11	**0.208a**	1.58	1.31	**26.5a**	290	80.9
Particle film										
-PFT	**0.853**	24.6	3.62	5.27	**0.204**	1.68	1.40	29.8	**232**	**69.0**
+PFT	**0.889**	24.7	3.62	5.13	**0.219**	1.50	1.27	26.7	**338**	**90.1**

Table 6.3.2.2. Effect of particle film treatments (-PFT and +PFT) and irrigation treatments (Control, RDI, PD) on yield components and berry composition in 2005. Significant treatment effects are shown in bold. Means followed by different letters are significantly different.

Treatment	Must TSS (^{0}Brix)	Must pH	Must Acidity (gL^{-1})	Wine Hue	Wine Colour Density (au)	Wine % ionized antho.	Wine Total antho. (mgL^{-1})	Wine Ionised antho. (mgL^{-1})	Wine Total phenolics au
Irrigation									
Control	**23.7b**	3.59	5.20	**0.53b**	**16.3a**	19.6	603	117	**58.5a**
RDI	**23.6b**	3.63	4.76	**0.52a**	**17.8b**	21.5	624	133	**61.5b**
PD	**23.3a**	3.61	4.92	**0.52a**	**17.6b**	21.1	651	136	**62.1b**
Particle film									
-PFT	23.4	3.60	**5.22b**	**0.51a**	**16.1a**	**18.5a**	**678b**	125	61.5
+PFT	23.6	3.61	**4.83a**	**0.53b**	**17.8b**	**21.8b**	**600a**	130	60.3

Table 6.3.2.3. Effect of particle film treatments (-PFT and +PFT) and irrigation treatments (Control, RDI, PD) on must and wine composition in 2004. Significant treatment effects are shown in bold. Means followed by different letters are significantly different.

Treatment	Must TSS (^{0}Brix)	Must pH	Must Acidity (gL^{-1})	Wine Hue	Wine Colour Density (au)	Wine Total antho. (mgL^{-1})	Wine Ionised antho. (mgL^{-1})	Wine Total phenolics au
Irrigation								
Control	24.0	3.44	**6.40c**	0.53	**11.4a**	**705a**	**104a**	54.0
RDI	24.4	3.44	**6.21b**	0.53	**11.8a**	**780b**	**107a**	56.6
PD	24.3	3.43	**6.08a**	0.51	**13.0b**	**868c**	**125b**	53.6
Particle film								
-PFT	**23.7a**	3.43	6.19	0.52	11.7	**752a**	109	54.7
+PFT	**24.9b**	3.45	6.27	0.53	12.5	**827b**	116	54.8

Table 6.3.2.4. Effect of particle film treatments (-PFT and +PFT) and irrigation treatments (Control, RDI, PD) on must and wine composition in 2005. Significant treatment effects are shown in bold. Means followed by different letters are significantly different.

6.3.4. Conclusions from treatment effects on berry development and composition

In general it can be concluded that effects of season on berry development, fruit composition and final wine quality attributes are greater than the effects of the imposed deficit irrigation treatments within a season.

Effect of season

- a variation in harvest date of 2 weeks, 2 -15th March with maturities ranging from 24.1- 25.6 ^{0}Brix. Interestingly, the season with the ripest fruit (2005) was also the earliest.
- 18%, 35% and 29% differences in berry fresh wt., dry wt. and the ratio of fresh to dry weight respectively

- 17%, 8% and 26% differences in exocarp, mesocarp and seed dry weight respectively. (Note, there were no significant difference in the exocarp: mesocarp ratio indicating that the skin to flesh ratio did not change with smaller berries which must have thinner skins)
- a 36% difference in yield
- large differences in pH (3.3-3.6) despite there being only small differences in titratable acidity (5.0-5.3 g/L)
- significant differences in sugar per berry (0.19 - 0.21g)
- large differences in total phenolics (1.4 -1.9 mg/g)), total anthocyanins (1.3-1.9mg/g), non-degraded anthocyanins (1.1-1.7 mg/g) and degraded anthocyanins (0.10 -0.22 mg/g) in berries
- large differences in wine spectral properties, ie. colour density (9.4-16.1 a.u.), hue (0.51-0.54), total anthocyanins (678-944 mg/L), ionised anthocyanins (85-125 mg/L) and total phenolics (55-73 mg/L)

Effect of irrigation deficit treatments

- compared to the control, 6% and 9% reductions in bunch weight
- compared to the control, 9% and12 % reductions in berry fresh weight for RDI and PD, 14% and 18% reductions in dry weight for RDI and PD but no effect on the fresh to dry weight ratio
- compared to the control, 8% and 11% reduction in exocarp dry weight with RDI and PD respectively, but no significant effect on mesocarp weight, seed weight, or the exocarp: mesocarp ratio
- compared to the control, 7% and 15% reductions in yield with RDI and PD respectively
- significant differences in sugar per berry with PD (0.19 - 0.22g)
- no effect on titratable acidity, pH, phenolics, total anthocyanins and non-degraded anthocyanins, but increased degraded anthocyanins with both stress treatments (ie. 0.18 mg/g cf 0.16mg/g) in berries
- a significant reduction in wine pH with both deficit irrigation treatments (ie.3.53, 3.50, and 3.47 for Con, RDI and PD respectively)
- compared to the control, significant enhancement of wine spectral properties particularly with PD, ie. colour density (16.3 for Con to 17.6 au for PD in 2004 and 11.4 for Con to 13.0 for PD in 2005), hue (0.53 for Con to 0.52 for PD in 2004), total anthocyanins (705 for Con to 868 mg/L for PD in 2005), ionised anthocyanins (104 for Con to 125 mg/L for PD in 2005) and total phenolics (58.5 for Con to 62.1 mg/L for PD in 2004)

Effect of particle film (PFT)

- effects of PFT on berry composition were minimal while effects on must composition and wine spectral properties varied between seasons. In season 2004 PFT produced higher wine colour density and % ionised anthocyanins but lower total anthocyanins. In season 2005 berry weight, total soluble solids in must and total wine anthocyanins were increased by PFT

Overview of ripening responses

- Berry weight reached a maximum in early February (ie. Julian day 40), about two weeks after 50% veraison and at least one month before harvest, regardless of season. From that time berry weight remained relatively constant up to harvest. Differences between seasons and irrigation treatments were obvious at maximum berry weight
- Total soluble solids concentration increased rapidly for two weeks after 50% veraison reaching 20 ^{0}Brix in early February (ie. Julian day 40) followed by a much slower rate of increase in concentration until harvest. Effects of season or irrigation treatment on the pattern of change in total soluble solids was small.

- The pattern of total sugar accumulation by the berry (total soluble solids per berry) largely reflected berry development and varied between season and with irrigation treatment. In season 2004 there was a tendency to plateau soon after maximum berry weight was reached and after the heat wave in early February, whereas in 2003 and 2005 sugar accumulation continued to harvest. The patterns show differences between seasons and irrigation treatments, pre- and post the time when maximum berry weight was reached. Total sugar accumulation was reduced by deficit irrigation treatments, particularly PD.
- Berry anthocyanins increased rapidly from mid January (veraison) generally reaching a maximum after about 20 days in early February (Julian day 40), well before harvest with the control and RDI treatment. Anthocyanin concentration tended to increase for an extended period up to harvest with the PD treatment. There were large seasonal differences in anthocyanin development with both the control and RDI treatments which showed similar patterns of anthocyanin accumulation, with lowest levels occurring in 2003. The PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons.
- There were however differences between seasons in the change in pH over time. Season 2005 had the most rapid increase and reached the highest pH level while season 2003 had the slowest increase and lowest pH at harvest in all irrigation treatments. Irrigation treatment had no effect on the rate of change in pH or the final level at harvest.
- Deficit irrigation treatment reduced malic acid levels and enhanced the tartrate to malate ratio during the ripening period.

6.4 Maturation data set analyses

A number of existing experimental and a commercial data set have been analysed to study seasonal, varietal and regional effects on fruit maturation (ie. sugar, pH, titratable acidity and colour). Examples from these studies are provided below.

6.4.1 Experimental data

Data collected by Petrie and Clingeleffer over 3 seasons (Clingeleffer, final report to GWRDC, project CSP002, 2005) for mechanically hedged Shiraz grown in Sunraysia was assessed to study seasonal impacts on fruit maturation. Measured variables are plotted against accumulated daily thermal time (post flowering) to allow valid comparisons to be made across the seasons (Fig. 6.4.1). A summary of yield components and temperature data during ripening is provided in Table 6.4.1.

	2002	**2003**	**2004**
Bunch number per metre	127	56	187
Fruit set (%)_	16.1	42.8	16.9
Bunch weight (g)	59.7	91.3	57.2
Berry weight (g)	1.22	1.39	1.01
Yield (kg/m)	7.3	5.1	10.5
Total soluble solids (^{0}Brix)	24.6	24.5	23.2
Temp. (^{0}C, mean Jan. max)	31.9	33.7	30.5
Temp. (^{0}C, mean Feb. max)	30.6	33.2	34.5
Temp. (^{0}C, mean Jan. min)	14.7	16.6	14.3
Temp.(^{0}C, mean Feb. min)	14.2	16.2	17.3

Table 6.4.1. Yield components and mean January and February temperature data, seasons 2002-2004. Adapted from Petrie and Clingeleffer (Clingeleffer, GWRDC final report 2005)

Results presented in Table 6.4.1 highlight the complexities in undertaking studies to assess seasonal impacts on maturation due to confounding factors such as crop load (ie. more than two-fold). In season 2003, very low fruitfulness produced low bunch numbers, with significant compensation occurring at fruit set and berry development to produce large, tight clusters with large berries compared to seasons 2002 and 2003. Difference in % fruit set and bunch weight were minimal between seasons 2002 and 2004 despite the high fruitfulness in 2004, although berry weights were smaller. Similar levels of sugar were achieved at harvest in 2002 and 2003 but in 2004 sugar levels were lower despite a delay in harvest. The January maximum and minimum temperatures followed similar trends, being were highest in 2003 and lowest in 2004. The February maximum and minimum temperatures were highest in 2004 and lowest in 2002. In particular, there were 10 consecutive days during ripening in early-mid February 2004 when the daily maximum temperature exceed 35^0C, culminating in 2 days of extreme temperature on 15th and 16th February (ie. $>46^0$C).

Berry development (Figure 6.4.1) appears to be a function of function of crop load (Table 6.4.1) with the largest and smallest berries produced in 2003 and 2004 respectively. Maximum berry weights were achieved around 1000 DTT, followed by significant berry weight loss until harvest. The rate of loss in berry weight also appears to be a function of crop load, being smallest in 2003 and highest in 2004.

Commencement of ripening also appears to be a function of crop load being earliest in 2003 and delayed in 2004 although a temperature response cannot be discounted, as mean January temperatures were highest in 2003 and lowest in 2004. However, the linear increase in sugar concentration, at least in the early stages of ripening, ie. up to 1200 DTT, appears to be unaffected season as the slopes are all similar. There was a considerable retardation in sugar accumulation in 2004 after 1200 DTT presumably due to the effects of the severe heat wave. When expressed on a per berry basis, there were significant seasonal effects on sugar accumulation by berries which appears to be a function of crop load, being highest in 2002 and lowest in 2004. Despite loss in berry weight at the later stages of ripening in all seasons, there was a net increase in sugar per berry until harvest in 2002 and 2003 whereas in 2004, following the severe heat wave in early-mid February there was no net gain from 1300 DTT. In fact the loss of sugar per berry at the later stages of ripening may indicate sugar loss due to respiration or back flow under high stress conditions at around 1300 DTT.

There appeared to be little effect of season on the rate of anthocyanins accumulation (Figure 6.4.1) although anthocyanin development was slightly advanced in the low crop year, 2003. Interestingly anthocyanin concentration increased at a constant rate until harvest in 2002, the season when maximum temperatures in February were lowest. By contrast, there was no net increase in anthocyanins in 2004 following the heat wave, ie. after 1300 DTT. Despite the low crop, anthocyanin levels in 2003 showed little increase after 1200 DTT. There is a possibility that this may be linked to the higher temperatures in January having an impact on anthocyanin precursors (Table 6.4.1). In season 2004 anthocyanin development appeared to be on track to achieve very high levels until the heat wave despite the high crop load, possible reflecting effects of low January temperature on anthocyanin precursors.

It can be concluded that seasonal differences in berry development and the commencement of sugar accumulation can be related to effects of crop load. Severe stress during ripening, associated with high temperatures in February can inhibit accumulation of sugar by berries with ripening (ie. increase in sugar concentration) a function of dehydration. Anthocyanin accumulation is largely unaffected by crop load but is inhibited by stress during the ripening period. Potential anthocyanin levels appear to be negatively related to mean January temperature. Further studies are required to remove crop load effects from seasonal climatic effects to fully

understand the season to season effects of climate on berry development and fruit maturation. As part of the CRCV Vitisys project, Anne Pellegrino is currently undertaking further analyses of the Shiraz data set to develop algorithims for berry development, sugar and anthocyanin accumulation to account for seasonal and crop load effects. Significant more study is required to fully understand the season to season effects on these processes particularly as they relate to temperature effects on sugar accumulation and anthocyanin development.

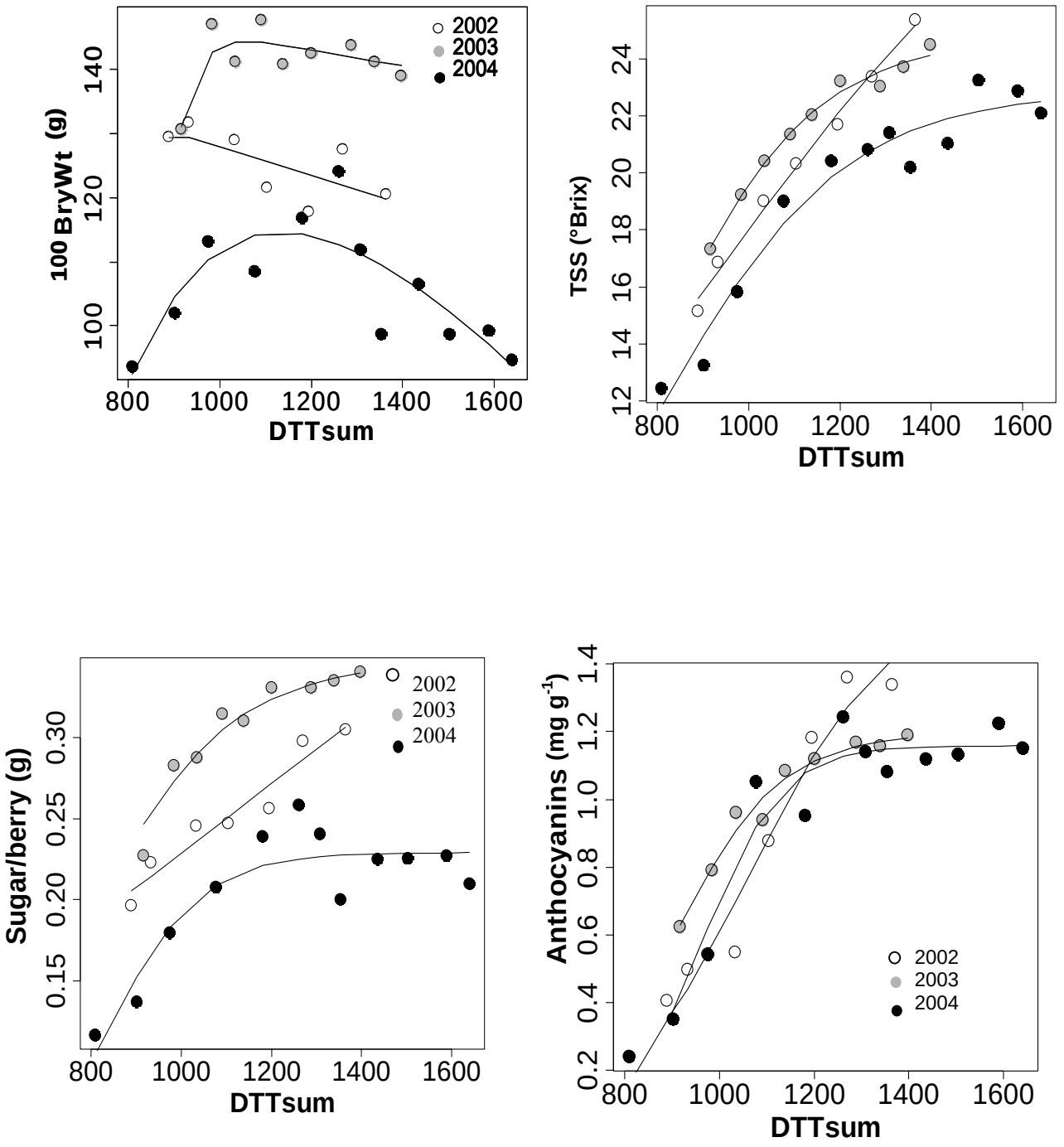


Figure 6.4.1. Changes in Shiraz berry weight, total soluble solids, sugar per berry and berry anthocyanins with accumulated daily thermal time from flowering over the ripening period (seasons 2002, 2003 and 2004).

6.4.2 Commercial data

A commercial data base from season 2002 was interrogated to assess regional, within region, varietal and specific vineyard characteristics (pruning system, rootstock, trellis characteristics, yield) effects on fruit ripening processes (ie. total soluble solids, titratable acidity, pH). For simplicity only data on sugar accumulation will be included in this report. The data base included 24 regions and 12 key varieties. The analyses for season 2002 showed that in the majority of cases (ie. each grape planting) sugar accumulation over time was linear with high R^2 values (ie. >0.9). Hence for each block of grapes it was possible to use the linear relationship to calculate the rate of sugar accumulation and predict harvest date. Harvest date was calculated on the basis of attainment of 21.6 ^{0}Brix to cater for both red and white grape varieties.

It was possible to classify each region from early to late on the calculated harvest date for each variety (Table 6.4.2.1). Notable exceptions were the late harvest of Semillon in the Lower Murray, the early ripening of Riesling in McLaren Vale, the early and late ripening of Cabernet Sauvignon in lower Murray and Cowra respectively, the late ripening of Grenache in Langhorne Creek and the early ripening of Merlot in the Clare Valley. It is likely that the date of harvest was influenced by both the suitability of the variety (genotype) to the regional conditions (environment) and the imposed management/crop load. For example without exception for the varieties available, harvest date in the Big Rivers (Riverina) region was earlier than in the Lower Murray (Riverland). This probably reflects higher crop loads in the latter case associated with larger trellises and lighter pruning. It is interesting to note that harvest dates in McLaren Vale and Mudgee tended to be slightly earlier than in the Lower Murray region. In general, across all regions where valid comparisons could be made, the earliest ripening variety was Pinot Noir, closely followed by Chardonnay, then Shiraz and Merlot, Riesling, Semillon and Cabernet Sauvignon, Grenache, Muscat Gordo Blanco and Colombard. The results highlight the significant issues of harvest scheduling to achieve optimum ripeness and use of winery capacity. For example the median date of harvest for the major Chardonnay regions in Australia, ie. Big Rivers and the Lower Murray would fall across a seven day time slot, ie. fom 52-59 Julian days. Similarly, for Shiraz and Merlot the median date of harvest across key regions (ie. Big Rivers, McLaren Vale, Mudgee, Lower Murray, the Barossa and Langhorne Creek) fall in an eleven day period (ie. 61-72 Julian days).

There was a considerable variation in the rate of sugar accumulation across both region and variety (Table 6.4.2.2). These variations did not reflect harvest date although there was a general trend to lower rates of sugar accumulation in the later ripening, cooler regions. The very large variations between varieties in a region are indicative of strong Genotype x Environment (x Management) interactions. For example in the Hunter Valley, Chardonnay and Semillon had high rates of sugar accumulation compare to the very low values of Shiraz. By contrast, sugar accumulation of Shiraz in McLaren Vale was higher than Chardonnay or Semillon. Notable features from a regional perspective were the high rates of sugar accumulation across all varieties except Riesling in McLaren Vale while in the Barossa Valley white varieties and Shiraz had high rates whereas Cabernet Sauvignon, Grenache and Merlot had relatively low rates. Interestingly, rates of sugar accumulation in the Big Rivers and Lower Murray regions were similar with most varieties, despite the differences in harvest date suggesting that there must have been significant effects on the timing of veraison. In general, ripening rates of the white varieties were similar for Chardonnay, Semillon and Riesling and lower for Colombard and Muscat Gordo Blanco. Similarly for the red varieties, Pinot Noir had the highest ripening rate followed by Shiraz with Cabernet Sauvignon, Grenache and Merlot having much lower values.

	Chardonnay	Semillon	Riesling	Colombard	Sultana	Gordo	Pinot	Shiraz	Cabernet Sauvignon	Grenache	Merlot	Ruby Cabernet
HUNTER	36	54						46				
RUTHERGLEN								48	62			
COWRA	47	64						55	81		55	
RIVERINA	52	66	61	71		60	50	61	63	72	62	72
MCLAREN VALE	57	66	77				55	67	71		66	
MUDGEE	57	76						67	74		67	
LOWER MURRAY	59	84		97	81	88		67	67	78	68	80
BAROSSA VALLEY	69	78	73					71	76	79	72	
LANGHORNE CRK	67		75				64	72	77	110	78	
BORDERTOWN								72			77	
PADTHAWAY	74							74	79		76	
CLARE VALLEY	74	91	76					76	83	83	71	
COONAWARRA								89	92		91	
WRATTONBULLY								95	99			
EDEN VALLEY	83		91					96				
ADELAIDE HILLS	94						89		107		103	
MANSFIELD	118						92					
TUMBARUMBA									113			

Table 6.4.2.1. Median harvest date (Julian day on which total soluble solids achieved 21.6 ^{0}Brix) for each variety in each region (min. n = 3, max. n = 149).

	Chardonnay	Semillon	Riesling	Colombard	Sultana	Gordo	Pinot	Shiraz	Cabernet Sauvignon	Grenache	Merlot	Ruby Cabernet
HUNTER	.253	.262						.148				
RUTHERGLEN								.173	.167			
COWRA	.213	.227						.191	.135		.140	
RIVERINA	.217	.207	.211	.172		.147	.253	.210	.177	.199	.152	.178
MCLAREN VALE	.223	.232	.185				.275	.272	.246		.266	
MUDGEE	.215	.201						.167	.129		.173	
LOWER MURRAY	.216	.164		.133	.177	.131		.209	.192	.149	.133	.137
BAROSSA VALLEY	.253	.264	.243					.241	.170	.173	.154	
LANGHORNE CRK	.242		.266				.288	.215	.172	.122	.175	
BORDERTOWN								.275			.173	
PADTHAWAY	.265							.191	.158		.168	
CLARE VALLEY	.205	.145	.231					.223	.172	.159	.157	
COONAWARRA								.166	.125		.106	
WRATTONBULLY								.179	.143			
EDEN VALLEY	.223		.207					.157				
ADELAIDE HILLS	.219						.175		.145		.177	
MANSFIELD	.109						.192					
TUMBARUMBA									.114			

Table 6.4.2.2. Median rate of ripening (^{0}Brix day $^{-1}$) for each variety in each region (min. n = 3, max. n = 149).

A more detailed analysis of the ripening and harvest information across varieties, regions, growers within a region and grower sites is provided in Figures 6.4.2.1-8. Most varieties (ie. Chardonnay, Colombard, Semillon, Pinot Noir, Shiraz, Cabernet Sauvignon, Ruby Cabernet and Grenache,) grown in the Riverina had similar rates of sugar accumulation, the exceptions being the slower ripening of Merlot and Muscat Gordo Blanco (Fig 6.4.2.1). Varietal differences in harvest date, taking into account end use and harvest at different maturities, can largely be attributed to differences in maturity at the commencement of sampling (January 30th), presumably due to differences in the occurrence of veraison.

In the Riverland, varieties fell into two distinct groups based on the rate of ripening (Figure 6.4.2.2). Chardonnay, Cabernet Sauvignon and Shiraz had fast ripening while Colombard, Muscat Gordo Blanco, Merlot, Grenache and Ruby Cabernet had slow ripening. Sultana and Semillon had intermediate ripening rates. Consequently, varietal differences in harvest date did not necessarily reflect differences at the commencement of sampling (January 30th). In McLaren Vale, the limited number of varieties had similar ripening rates (Figure 6.4.2.3). Hence, differences at the first sampling date reflected final harvest dates.

Regional differences in Shiraz ripening are shown in Figure 6.4.2.4. Despite the large differences in regional climates, ripening of Shiraz in the Hunter Valley, Coonawarra and the Riverland occurred at similar rates. Hence large differences in sugar level at the first sampling were maintained through to harvest. By contrast, ripening of Shiraz in the Barossa Valley occurred at a higher rate, presumably a result of lower crop loads. Within the

Riverland region, differences in ripening rate or harvest date between the various sub-regions were small (Figure 6.4.2.5).

In some regions there were considerable differences between growers in rates of ripening and harvest date. This is clearly shown for Shiraz grown in the Riverland and the Barossa (Figures 6.4.2.6 and 6.4.2.7 respectively). Interestingly, in the Barossa example, rates of ripening were similar with harvest date generally reflecting differences at early sampling dates. Across most varieties and regions grower differences could not be linked to differences in crop load, rootstock or pruning practices.

In some cases it was also possible to assess grower site effects. For Chardonnay grown by one grower in the Barossa Valley, differences in ripening rate and harvest date were very small (Figure 6.4.2.8).

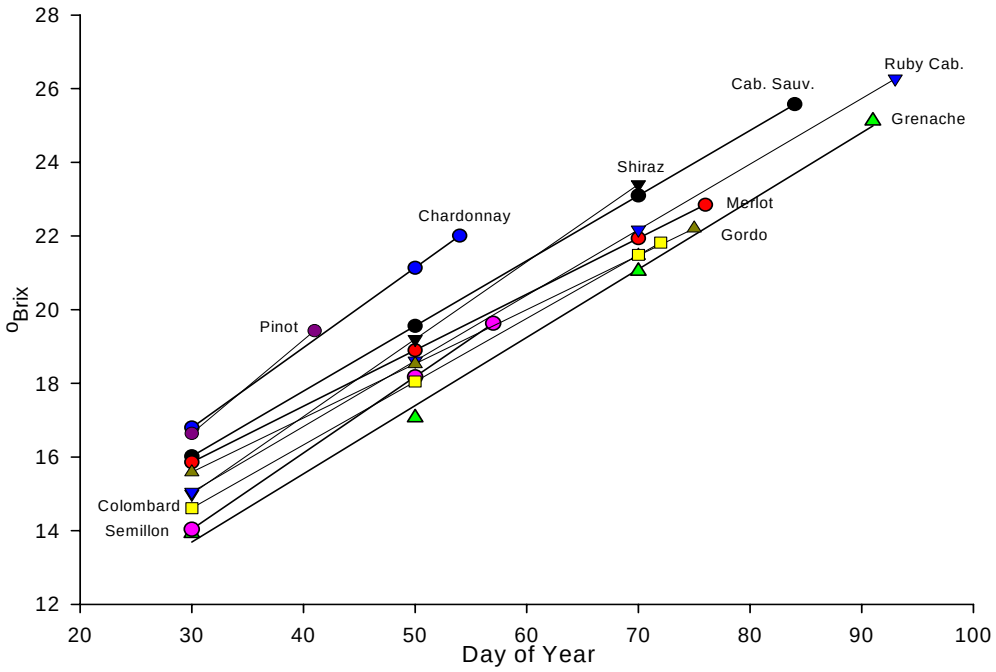


Figure 6.4.2.1. Ripening (ie. increase in total soluble solids, ^{0}Brix) of varieties grown in the Riverina region

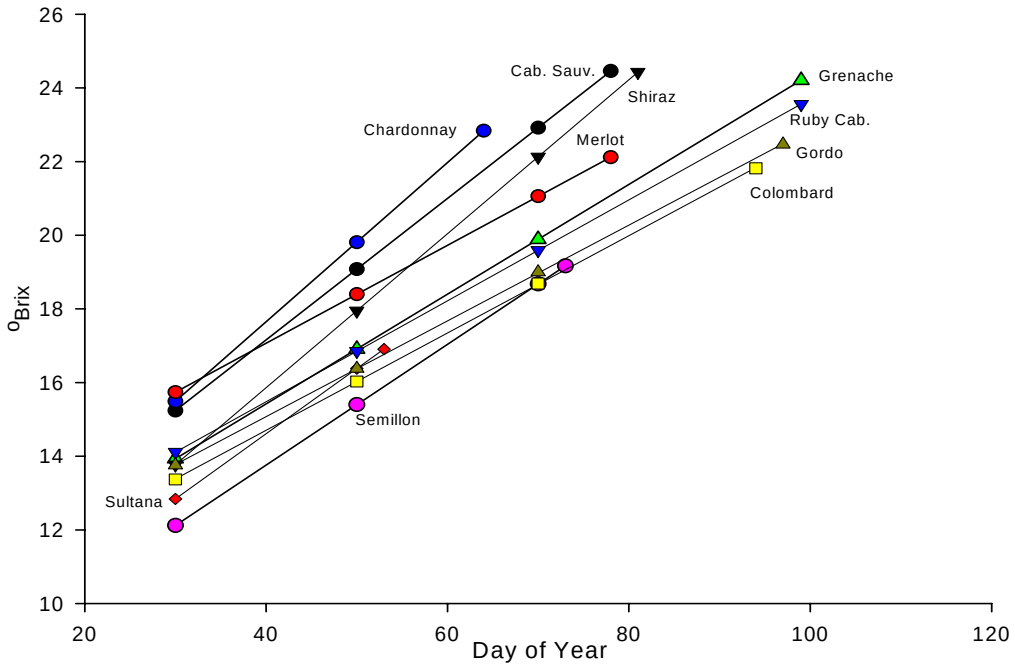


Figure 6.4.2.2. Ripening (ie. increase in total soluble solids, ^{0}Brix) of varieties grown in the Riverland

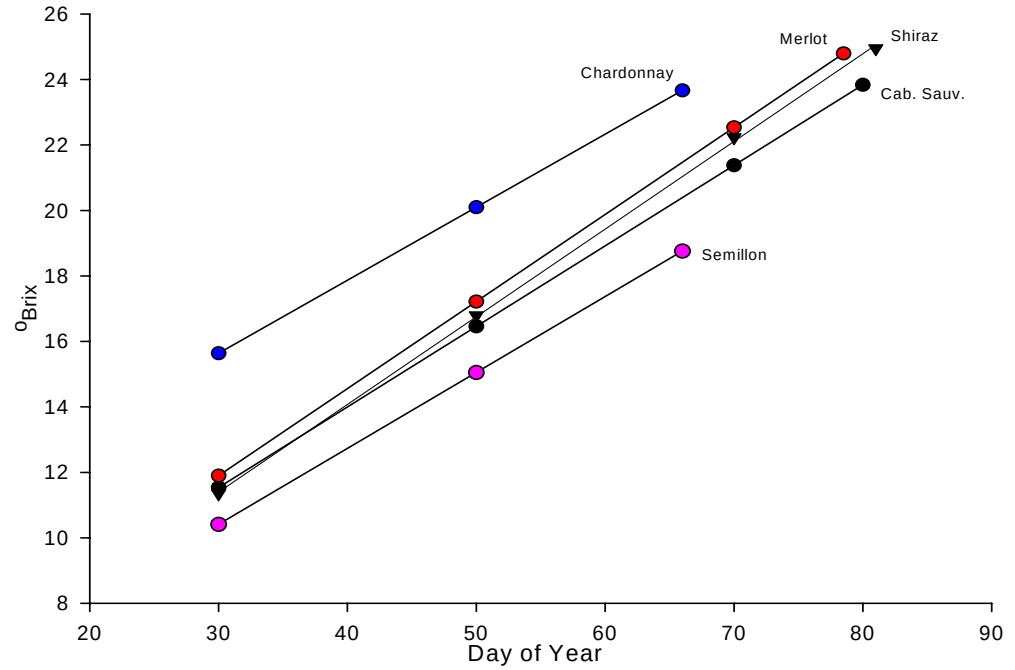


Figure 6.4.2.3. Ripening (ie. increase in total soluble solids, ^{0}Brix) of varieties grown in McLaren Vale.

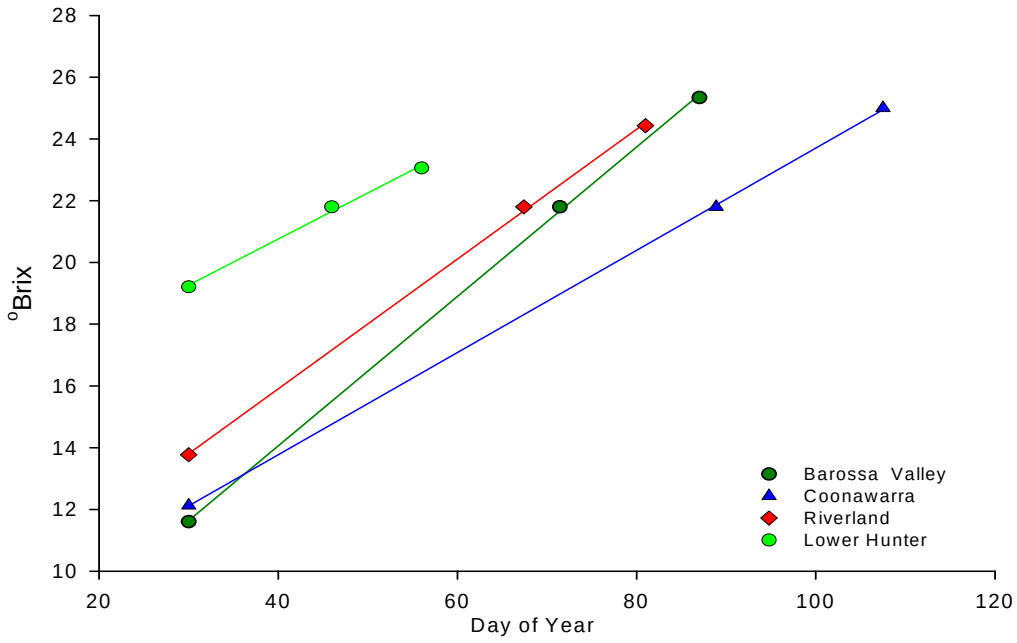


Figure 6.4.2.4. Ripening (ie. increase in total soluble solids, ^{0}Brix) of Shiraz in different regions.

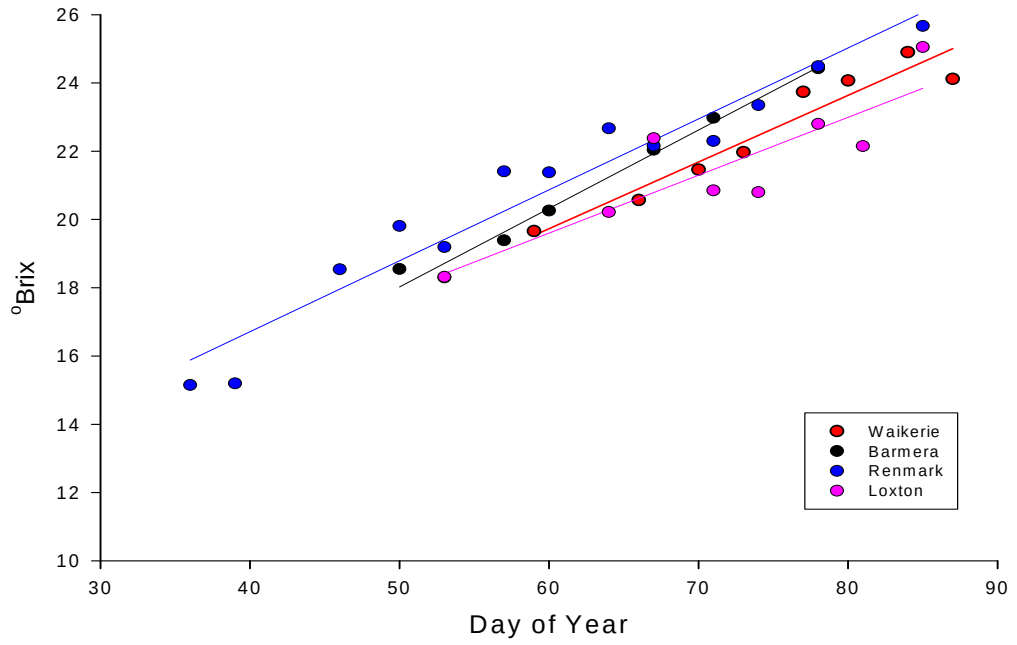


Figure 6.4.2.5. Ripening (ie. increase in total soluble solids, ^{0}Brix) of Shiraz in different sub-regions within the Riverland.

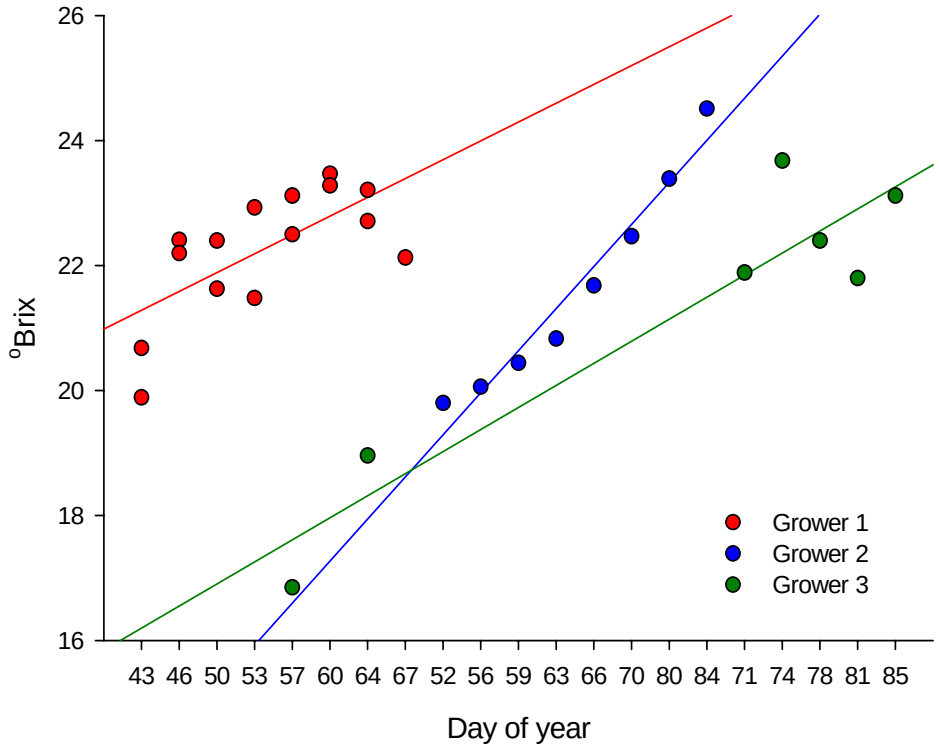


Figure 6.4.2.6. Ripening (ie. increase in total soluble solids, ^{0}Brix) of Shiraz between different growers in the Riverland.

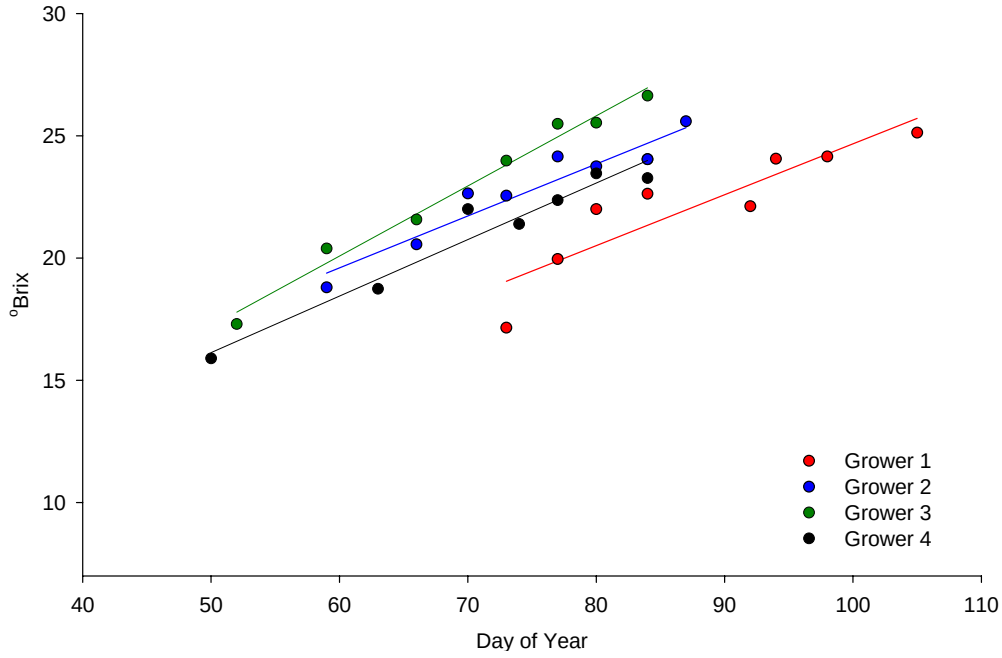


Figure 6.4.2.7. Ripening (ie. increase in total soluble solids, ^{0}Brix) of Shiraz for different growers within the Barossa.

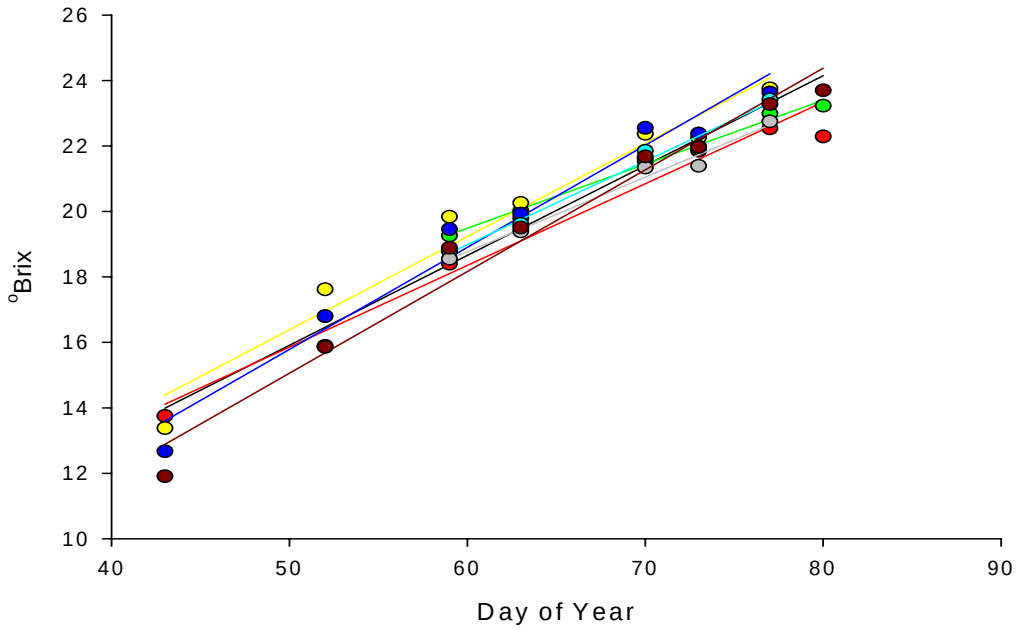


Figure 6.4.2.8. Ripening (ie. increase in total soluble solids, ^{0}Brix) of Chardonnay across different sites of the one grower in the Barossa Valley.

6.4.3. Conclusions from analyses of existing data sets.

It can be concluded that:-

- assessment of seasonal impacts on maturation are highlighted by confounding factors such as fruitfulness and crop load.
- seasonal differences in berry development and the commencement of sugar accumulation can be related to effects of crop load. Berry size and development was a function of crop load reaching a maximum around 1000 DTT followed by significant loss at the later stages of maturation.
- severe stress during ripening, associated with high temperatures in February can inhibit accumulation of sugar by berries with ripening (ie. increase in sugar concentration) a function of dehydration.
- anthocyanin accumulation is largely unaffected by crop load but is inhibited by heat stress during the ripening period. Potential anthocyanin levels appear to be negatively related to mean January temperature.
- seasonal comparisons based on daily thermal time over the period of berry development (ie. from flowering to harvest) provides a useful tool to compare season to season effects on berry development and fruit maturation. Despite the adjustment to a common denominator based on daily thermal time there were large seasonal differences in berry development, sugar accumulation on a concentration and per berry basis and colour development.
- under severe heat stress in the latter stages of ripening net loss of sugar per berry occurred. This warrants further investigation as it may be attributed to respiration or back flow from the berry.
- analysis of commercial data sets showed that average ripening rates were relatively constant across most varieties and regions, except in the very cool regions. There were differences between growers in a region which could not be accounted for by crop load, rootstock or pruning system.

- differences in harvest date between regions and varieties can largely be linked to differences when samples are first processed soon after veraison. Field assessment or prediction of the timing of veraison based on phenological models such as those incorporated in VineLOGIC should enable the industry to accurately predict varietal harvest dates based on known rates of ripening, with adjustment for grower differences based on previous history.
- further studies are required to remove crop load effects from seasonal climatic effects to fully understand the season to season effects of climate on berry development and fruit maturation.

6. 5 Discussion

The project had two main components. The first investigated the effects of water deficits imposed through irrigation management on vine performance, in particular berry development and fruit and wine composition of own rooted Cabernet Sauvignon. The irrigation treatments imposed over three seasons were a control (Con) irrigation treatment, scheduled by the operators of the vineyard based on soil moisture and vine physiology data, a Regulated Deficit Irrigation (RDI) where a soil water deficit was applied in the post-fruit set to pre-veraison period and Prolonged Deficit (PD) treatment which was similar to the RDI but had an extended period of extreme deficit (no water) after the RDI stress period until veraison. The effect of superimposing particle film treatment (PFT) on the above irrigation treatments was also assessed in some seasons with the aim to reduce canopy stress. Linkages between treatment effects on vine physiology, carbohydrate status, yield components, fruit maturation and fruit and wine composition were explored. For all parameters measured there were also very large differences between seasons. In the second component of the project, existing data sets from previous research and commercial sources were analysed focusing on seasonal, regional and varietal effects on grape berry maturation. The project has identified a number of strategies for the management of vineyards to both enhance and improve the consistency of fruit quality from season to season in irrigated inland areas.

Due to climatic effects there were large differences between seasons in the ability to achieve moisture deficits, in the amount of water applied (ie. 4.5-6.5 ML/ha applied to the control) and in the subsequent physiological responses, berry development and composition and final wine quality attributes. Major differences between seasons were determined for yield (36%); maturity (2 weeks); fresh and dry berry weight and the ratio of fresh to dry weight (ie. 18%, 35% and 29% respectively); exocarp, mesocarp and seed dry weight (ie. 17%, 8% and 26% respectively); juice pH (3.3-3.6) despite there being only small differences in titratable acidity (5.0-5.3 g/L), total soluble solids (24.1-25.6 ^{0}Brix) and sugar per berry (0.19 - 0.21g); berry phenolics (1.4 -1.9 mg/g)), total anthocyanins (1.3-1.9 mg/g), non-degraded anthocyanins (1.1-1.7 mg/g) and degraded anthocyanins (0.10 -0 .22 mg/g) and wine spectral properties, ie. colour density (9.4-16.1 a.u.), hue (0.51-0.54), total anthocyanins (678-944 mg/L), ionised anthocyanins (85-125 mg/L) and total phenolics (55-73 mg/L). It is likely that the large seasonal effects on berry characteristics, including structural properties may have contributed to the seasonal differences in fruit composition and wine quality attributes. More research is required to investigate the relationships between climatic conditions, berry structural characteristics and fruit composition and wine quality.

Frequently the effects of season were greater than the effects of the irrigation treatments on berry development, fruit composition and final wine quality attributes. However the imposed irrigation treatments (RDI and PD) produced soil moisture deficits which compared to the control had significant effects on vine physiology, berry development, composition and final wine quality attributes. A number of the findings have significant ramifications for industry. Water deficits affected vine physiology, leading to reduced photosynthesis, leaf water potential and turgor, transpiration and stomatal conductance and increased leaf temperature up to 3 weeks after the end of the stress period. Water deficits had a long term impact on shaded leaf water potential for up to 2 months after return to normal irrigation during fruit ripening. Furthermore, post-stress measurements of soil moisture indicate that vines subjected to deficit treatments adapted to the conditions over time

and had lower requirements for water during ripening and post-harvest periods in latter seasons. This may be a result of the development of vines with smaller canopies carrying smaller crops and possibly disrupted root function. As expected, the lower photosynthesis impacted on berry development, maturation, carbohydrate partitioning and storage and hence, potentially, the long term sustainability of production. Application of particle film PFT lowered leaf temperature and the impact of soil moisture deficits on leaf physiology and may increase the sustainability of deficit irrigation treatments.

Deficit irrigation practices impacted on both source and sink reserves in grapevines but this response was subject to significant season to season variation. The seasonal variation may be due to differences in the response of sink reserves to the water stress which may then impact on the source reserves or alternatively there may be interactions occurring between water stress and high ambient temperatures which significantly affect the canopy's ability to produce carbohydrate and partition it according to sink demand. The low levels of trunk starch in the period between fruit-set and veraison indicate that this period is critical to carbohydrate partitioning between storage organs and the developing fruit. The unexpected rapid increase in trunk starch concentration at the end of veraison suggests that the vine has commenced redirection of carbohydrate assimilate toward storage organs rather than to the fruit and that this process was significantly affected by responses to deficit irrigation treatments. Further research is required to investigate the long term impacts of water deficits on vine carbohydrate status with reference to the long term sustainability of wine grape production.

The negative effects of deficit irrigation treatments on vine physiology impacted on crop development, fruit composition and wine quality attributes. Compared to the control, irrigation deficit treatments (RDI and PD) had lower yields (7% and 15%), smaller bunches (6% and 9%), reduced berry size, (9% and 12% fresh weight, 14% and 18% dry weight) and reduced weight of skin (8% and 11%). The deficit treatments had no significant effect on flesh weight, seed weight, or the skin to flesh ratio or the fresh to dry weight ratio of berries. Furthermore the deficit treatments had minimal effect on fruit composition (titratable acidity, pH, phenolics, total anthocyanins and non-degraded anthocyanins) except for an increase in the level of degraded anthocyanins in berries. They did however have a significant positive effect on wine quality attributes such as lower wine pH (ie.3.53, 3.50, and 3.47 for Con, RDI and PD respectively) and resulted in a significant enhancement of wine spectral properties particularly with PD. Application of PFT had minimal effect on berry composition while effects on must composition and wine spectral properties varied between seasons. In season 2004, PFT produced higher wine colour density and % ionised anthocyanins despite having lower total anthocyanins. In season 2005, berry weight, total soluble solids in must and total wine anthocyanins were increased by PFT. These results have significant ramifications for industry with reference to batching and streaming from different vineyards and quality payments based on berry colour as the linkage between berry colour and phenolics and final wine quality attributes, in this case wine spectral properties, was not evident. More research is required to investigate this phenomena which could be related to effects of water deficits on organic acid composition, tannin levels or anthocyanin profiles in berries and subsequent effects on co-pigmentation of anthocyanins.

Both the experimental studies and the analysis of data sets have shown that the increase in sugar concentration during ripening is a highly conserved process. Indeed, analysis of the commercial data sets showed that average ripening rates were relatively constant across most varieties and regions, except in the very cool regions where photosynthesis may have been a limiting factor. In most cases differences in harvest date between regions, varieties and growers within a region could largely be attributed to differences in the date of veraison rather than the rate of ripening. There were, however, differences between growers in a region which could not be accounted for by crop load, rootstock or pruning system. More research is required to further investigate the early stages of berry development, particularly to understand the underlying causes and processes controlling the date of veraison and differences between growers in a region. Further studies are required to remove crop load effects from seasonal climatic effects to fully understand the season to season effects of climate

on berry development and fruit maturation. Field assessment or prediction of the timing of veraison based on phenological models such as those incorporated in VineLOGIC should enable the industry to accurately predict varietal harvest dates based on known rates of ripening, with adjustment for grower differences based on previous history. Analyses of commercial data sets has been useful in enhancing our understanding of ripening processes. Further analyses of such data over a number of seasons, and where possible including colour and other compositional parameters, particularly where yield components are known could be used to further understand seasonal and crop load effects on ripening processes.

In the Cabernet Sauvignon water deficit experiment maximum berry weight occurred about two weeks after 50% veraison and then remained relatively constant regardless of season until harvest one month later. Differences between seasons and irrigation treatments were obvious at maximum berry weight. Total soluble solids concentration increased rapidly for two weeks after 50% veraison reaching 20 ^{0}Brix followed by a much slower rate of increase in concentration until harvest where limited movements of sugar into the berry were offset by water loss as berry weight remained constant. Effects of season or water deficits on the pattern of change in total soluble solids was small. Consequently, differences attributed to seasons and water deficits in the pattern of total sugar accumulation largely reflected differences in berry development. It is likely that reduction in total sugar accumulation attributed to water deficits, particularly PD is most likely due to the limited supply of carbohydrates around veraison (see above). However it is interesting to note that the increase in sugar concentrations was again highly conserved compared to the very significant impact of water deficits on berry weight and hence total sugar accumulation. These results suggest that the increase in sugar concentration during the later stages of ripening, at least in Cabernet Sauvignon, is most likely a passive process associated with mass flow and likely to be influenced by seasonal conditions affecting vapour pressure deficit (ie. associated with temperature and relative humidity). Consequently it is likely that fruit maturation may be delayed during periods of rain or mild temperatures. Indeed this was the case in the trial site in season 2006 where berry weight also continued to increase up to harvest (data not shown in this report). Similar but more extreme processes occurred with Shiraz over a number of seasons where the increase in sugar concentration at the later stages of ripening was associated with significant moisture losses and berry dehydration. Indeed under severe heat stress in season 2004 during the latter stages of ripening net loss of sugar per berry occurred possibly due to respiration or back flow of sugar from the berry.

The pattern of berry anthocyanins accumulation differed with different levels of water deficit. With the control and RDI treatment anthocyanin concentration reached a maximum about 20 days after veraison, whereas with PD, anthocyanin concentration tended to increase for an extended period up to harvest. There were large seasonal differences in anthocyanin development with both the control and RDI treatments which showed similar patterns of anthocyanin accumulation, with lowest levels occurring in 2003. The PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons. Deficit irrigation treatment reduced malic acid levels and enhanced the tartrate to malate ratio during the ripening period.

In the Shiraz study, anthocyanin accumulation was largely unaffected by crop load but was inhibited by stress during the ripening period. Potential anthocyanin levels were negatively related to mean January temperature.

Seasonal comparisons based on daily thermal time over the period of berry development (ie. from flowering to harvest) provided a useful tool to compare season to season effects on berry development and fruit maturation. However, despite the adjustment to a common denominator based on daily thermal time there were large seasonal differences in berry development, sugar accumulation on a concentration and per berry basis and colour development.

It is clear from both the Cabernet Sauvignon water deficit study and the Shiraz crop load study that much more research is required to fully understand berry ripening processes, particularly to identify causes for the large seasonal effects on berry weight, accumulation of sugar and anthocyanins in relation to stress and climatic influences and differences between varieties.

The project has identified two, rather contradictory vineyard management practices which, if proven under larger commercial conditions, should provide growers with further tools to improve the consistency of grape quality and assist in meeting set specifications. Firstly, the results with PD show that extended pre-veraison water deficits enhance berry colour, particularly in seasons with low potential colour caused by high temperatures in late December and early January. It should be noted that the standard RDI treatment had minimal effect on berry colour development compared to the control. Secondly, the application of PFT can potentially reduce leaf temperature and minimise stresses associated with hot weather and/or water deficits.

7. Outcomes/conclusions.

The project had two components. The first involved a major field experiment with own-rooted Cabernet Sauvignon designed to study the effects of water deficits imposed through irrigation management on vine performance, in particular berry development and fruit and wine composition. In the second component of the project, existing data sets from previous research and commercial sources were analysed focusing on seasonal, regional and varietal effects on grape berry maturation.

Vine physiology

The imposed irrigation treatments (RDI and PD) produced significant soil moisture deficits which affected vine physiology, leading to reduced photosynthesis during key stages of vine development. Due to climatic effects there were large differences between seasons in the ability to achieve soil moisture deficits and in the subsequent physiological responses. A reduction in photosynthesis resulting from severe water deficits is likely to impact on berry development, maturation, carbohydrate partitioning and storage and hence, the long term sustainability of production. Application of particle film PFT reduced the impact of soil moisture deficits on leaf physiology. Significant findings from the plant physiology studies are listed below.

- There were large seasonal differences in the ability to both impose moisture deficits during targeted deficit periods and in recovery of soil moisture on return to normal irrigation. There were also large seasonal differences in total water applied (ie. 4.5-6.5 ML/ha).
- Post-stress measurement of soil moisture indicates that vines subjected to deficit treatments adapted to the conditions over time and had lower requirements for water during ripening and post-harvest periods in latter seasons. This may be a result of the development of vines with smaller canopies carrying smaller crops and possibly disrupted root function.
- Water deficits reduced leaf water potential and turgor, increased leaf temperature and lowered transpiration, assimilation and stomatal conductance.
- Effects of deficit treatments on leaf physiology (assimilation, stomatal conductance and transpiration) were measured up to 3 weeks after the end of the stress period.
- Water deficits had a long term impact on shaded leaf water potential (eg. for up to 2 months after the end of the RDI period). Both the RDI and the PD treatments were under stress during fruit ripening in season 2003-04, i.e. for a significant time after water application had been returned to normal.
- Addition of PFT to the deficit treatments reduced leaf temperature and stress responses and may increase the sustainability of deficit irrigation treatments.

- Deficit irrigation practices have the potential to impact on both source and sink reserves in grapevines but this response was subject to significant season to season variation. The seasonal variation may be due to differences in the response of sink reserves to the water stress which may then impact on the source reserves or alternatively there may be interactions occurring between water stress and high ambient temperatures which significantly effect the canopy's ability to produce carbohydrate and partition it according to sink demand.
- The low levels of trunk starch in the period between fruit-set and veraison indicate that this period is critical to carbohydrate partitioning between storage organs and the developing fruit. The unexpected rapid increase in trunk starch concentration at the end of veraison suggests that the vine has commenced redirection of carbohydrate assimilate toward storage organs rather than to the fruit and that this process was significantly affected by responses to deficit irrigation treatments.

Berry ripening

- Berry weight reached a maximum in early February (ie. Julian day 40), about two weeks after 50% veraison and at least one month before harvest, regardless of season. From that time berry weight remained relatively constant up to harvest. Differences between seasons and irrigation treatments were obvious at maximum berry weight.
- Total soluble solids concentration increased rapidly for two weeks after 50% veraison reaching 20 ^{0}Brix in early February (ie. Julian day 40) followed by a much slower rate of increase in concentration until harvest. Effects of season or irrigation treatment on the pattern of change in total soluble solids was small.
- The pattern of total sugar accumulation by the berry (total soluble solids per berry) largely reflected berry development and varied between season and with irrigation treatment. Total sugar accumulation was reduced by deficit irrigation treatments, particularly PD.
- Berry anthocyanins increased rapidly from mid January (veraison) generally reaching a maximum after about 20 days (ie. in early February, Julian day 40) i.e. well before harvest with the control and RDI treatment. Anthocyanin concentration tended to increase for an extended period up to harvest with the PD treatment. There were large seasonal differences in anthocyanin development with both the control and RDI treatments which showed similar patterns of anthocyanin accumulation, with lowest levels occurring in 2003. The PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons.
- There were differences between seasons in the change in pH over time. Season 2005 had the most rapid increase and reached the highest pH level while season 2003 had the slowest increase and lowest pH at harvest in all irrigation treatments. Irrigation treatment had no effect on the rate of change in pH or the final level at harvest.
- Deficit irrigation treatment reduced malic acid levels and enhanced the tartrate to malate ratio during the ripening period.

Fruit composition and wine quality attributes

The imposed irrigation treatments (RDI and PD) had significant effects on berry development, composition and final wine quality attributes compared to the control. However, it can be concluded that effects of season were greater than the effects of the irrigation treatments on berry development, fruit composition and final wine quality attributes. Notable effects of season were:-

- a 36% difference in yield
- a variation in harvest date of 2 weeks, 2 -15th March with maturities ranging from 24.1- 25.6 ^{0}Brix although the season with the ripest fruit (2005) was also the earliest.
- 18%, 35% and 29% differences in berry fresh wt., dry wt. and the ratio of fresh to dry weight respectively

- 17%, 8% and 26% differences in exocarp, mesocarp and seed dry weight respectively. There was however no significant effect of season on the exocarp: mesocarp ratio indicating that the skin to flesh ratio did not change despite significant effects of season on berry weight.
- large differences in pH (3.3-3.6) despite there being only small differences in titratable acidity (5.0-5.3 g/L)
- significant differences in sugar per berry (0.19 - 0.21g)
- large differences in total phenolics (1.4-1.9 mg/g)), total anthocyanins (1.3-1.9 mg/g), non-degraded anthocyanins (1.1-1.7 mg/g) and degraded anthocyanins (0.10-0 22 mg/g) in berries
- large differences in wine spectral properties, ie. colour density (9.4-16.1 a.u.), hue (0.51-0.54), total anthocyanins (678-944 mg/L), ionised anthocyanins (85-125 mg/L) and total phenolics (55-73 mg/L)

Compared to the control, irrigation deficit treatments (RDI and PRD) produced:-

- 6% and 9% reductions in bunch weight
- 9% and12 % reductions in berry fresh weight, 14% and 18% reductions in dry weight but had no effect on the fresh to dry weight ratio
- 8% and 11% reduction in exocarp but had no significant effect on mesocarp, seed weight, or the exocarp: mesocarp ratio
- 7% and 15% reductions in yield
- no significant effects on titratable acidity, pH, phenolics, total anthocyanins and non-degraded anthocyanins but increased the level of degraded anthocyanins in berries
- a significant reduction in wine pH with both deficit treatments (ie.3.53, 3.50, and 3.47 for Con, RDI and PD respectively)
- a significant enhancement of wine spectral properties particularly with PD, ie. colour density (16.3 for Con to 17.6 aufor PD in 2004 and 11.4 for Con to to 13.0 au for PD in 2005), hue (0.53 for Con to 0.52 for PD in 2004), total anthocyanins (705 for Con to 868 mg/L for PD in 2005), ionised anthocyanins (104 for Con to 125 mg/L for PD in 2005) and total phenolics (58.5 for Con to 62.1 mg/L for PD in 2004).

Application of PFT had minimal effect on berry composition while effects on must composition and wine spectral properties varied between seasons. In season 2004, PFT produced higher wine colour density and % ionised anthocyanins but lower total anthocyanins. In season 2005, berry weight, total soluble solids in must and total wine anthocyanins were increased by PFT

Analyses of existing data sets.

A number of existing experimental data sets and a commercial data set were analysed to study seasonal, varietal and regional effects on fruit maturation (ie. sugar, pH, titratable acidity and colour). From the experimental data collected for mechanically pruned Shiraz over three seasons it can be concluded that:-

- assessment of seasonal impacts on maturation are influenced by confounding factors such as fruitfulness and crop load.
- seasonal differences in berry development and the commencement of sugar accumulation can be related to effects of crop load. Berry size reached a maximum around 1000 DTT after flowering followed by significant loss at the later stages of maturation.
- severe stress during ripening, associated with high temperatures in February can inhibit accumulation of sugar by berries with ripening (ie. increase in sugar concentration) a function of dehydration.
- anthocyanin accumulation is largely unaffected by crop load but is inhibited by stress during the ripening period. Potential anthocyanin levels appear to be negatively related to mean January temperature.

- seasonal comparisons based on daily thermal time over the period of berry development (ie. from flowering to harvest) provides a useful tool to compare season to season effects on berry development and fruit maturation. Despite the adjustment to a common denominator based on daily thermal time there were large seasonal differences in berry development, sugar accumulation on a concentration and per berry basis and colour development.
- under severe heat stress in the latter stages of ripening net loss of sugar per berry occurred. This warrants further investigation as it may be attributed to respiration or back flow from the berry.

Analyses conducted on commercial data from season 2002 also provided further insights into regional and varietal effects on maturation. The results showed:-

- that average ripening rates were relatively constant across most varieties and regions, except in the very cool regions. However, there were differences between growers in a region which could not be accounted for by crop load, rootstock or pruning system.
- differences in harvest date between regions and varieties can largely be linked to differences when samples are first processed soon after veraison. Field assessment or prediction of the timing of veraison based on phenological models such as those incorporated in VineLOGIC should enable the industry to accurately predict varietal harvest dates based on known rates of ripening, with adjustment for grower differences based on previous history.
- further studies are required to remove crop load effects from seasonal climatic effects to fully understand the season to season effects of climate on berry development and fruit maturation.

8. Recommendations

The project has identified a number of issues which have significant ramifications for growers and future viticultural research needs. These included:

- Issues with regard to sustainability of production with adoption of water deficit irrigation treatments due to the extended effects on water status, photosynthetic capacity, carbohydrate dynamics and vine size. Water deficits reduced photosynthesis, leaf water potential and turgor, transpiration and stomatal conductance and increased leaf temperature up to 3 weeks after the end of the stress period and had a long term impact on shaded leaf water potential for up to 2 months after return to normal irrigation during fruit ripening.
- Large seasonal differences in vine physiology, vine carbohydrate status, berry development, fruit composition and final wine attributes. Note that the PD treatment minimised seasonal differences in anthocyanin concentrations and produced higher levels of anthocyanins than either the control or RDI treatments in all seasons. In general the results suggest that fruit quality benefits from the use of RDI may be limited in most seasons with Cabernet Sauvignon.
- Analyses of commercial data sets showed that that average ripening rates were relatively constant across most varieties and regions, except in the very cool regions and that differences in harvest date were largely associated with differences in the timing of veraison. Measurement or prediction of veraison may be a useful tool for estimating harvest date.
- Benefits from water deficit treatments included enhanced wine spectral properties which were not evident in berry colour measurements. These results have significant ramifications for industry with reference to batching and streaming from different vineyards and quality payments based on berry colour as the linkage between berry colour and final wine quality attributes, in this case wine spectral properties was not evident.

The project has identified two vineyard management practices which, if proven under larger commercial conditions, should provide growers with further tools to improve the consistency of grape quality and assist in meeting set specifications.

- Firstly, the results with PD show that extended pre-veraison water deficits enhance berry colour, particularly in seasons with low potential colour caused by high temperatures in late December and early January. However, this benefit is potentially offset by yield reductions and the impact of this treatment on vine performance for more than three seasons is yet to be determined.

- Secondly, the application of PFT can potentially reduce leaf temperature and minimise stresses associated with hot weather and/or water deficits.

More research is required:

- To investigate the relationships between climatic conditions, berry structural characteristics and fruit composition and wine quality and to identify and understand the causes contributing to the large differences between seasons.
- To develop a clearer understanding of vine carbohydrate dynamics, taking into account the very low levels and effects of water deficits in the pre- and post-veraison periods and at leaf fall. Such studies should investigate the seasonal trend effects on total carbohydrate concentration and fruit composition and assess the long term effects of deficit irrigation on fruit development and sustainability of production.

- To develop a clearer understanding of root development and function, particularly under water deficit and subsequent effects on vine water use, vine size and productivity.
- To investigate the long term impacts of water deficits on the long term sustainability of wine grape production.
- To further investigate the use of PFT to reduce the impacts of stress in other varieties (red and white) and regions and determine effects on productivity, fruit composition and wine quality.
- To investigate links between vineyard practices (eg. deficit irrigation), berry composition and final wine quality taking into account organic acid composition, tannin levels or anthocyanin profiles in berries and subsequent effects on co-pigmentation of anthocyanins and flavour development.
- To understand the causes for grower differences in a region in the time of harvest and ripening which could not be accounted for by crop load, rootstock or pruning system.
- To investigate the early stages of berry development, particularly to understand the underlying causes and processes controlling the date of veraison and differences between growers in a region in ripening and harvest date. Field assessment or prediction of the timing of veraison based on phenological models such as those incorporated in VineLOGIC should enable the industry to accurately predict varietal harvest dates based on known rates of ripening, with adjustment for grower differences based on previous history.
- To fully understand climatic effects, varietal impacts and processes affecting changes in berry size, sugar concentration, total sugar accumulation, anthocyanins and phenolics close to harvest; as the results indicated that most 'programming' processes involved in berry development, sugar and anthocyanin accumulation virtually ceased within 2 weeks of veraison.
- To understand the different effects of water deficits on colour development in berries, particularly the response to PD treatment which resulted in sustained increase in colour up to harvest.
- To undertake further data anlaysis of commercial data sets to enhance understanding of seasonal, regional, varietal, vine management and crop load effects on ripening processes. These analyses should be conducted over a number of seasons, and where possible include colour and other compositional parameters, particularly where yield components are known.

9. Appendix 1. Communication

Over the course of the project presentations have been made at various scientific, CRCV, technical and industry forums. These included annual CRCV Meetings, CRCV review (2005), Mildura Field Day Seminar (2003), Riverlink Workshop on warm climate Cabernet (2003), the 2nd Australian Grapevine Physiology Workshop (2003), ComBio (2003), CSIRO Food Futures Flagship team (2004), the Seventh International Symposium of Grapevine Physiology and Biotechnology, University of California, Davis, California (2004), the 12th Australian Wine Industry Technical Conference (2004), the 12th Australian Wine Industry Technical Conference Irrigation Management Workshop (2004), 'Some like it Hot' seminar (2004), Combio (2005), GWRDC Workshop on carbohydrate dynamics (2006) and the International Symposium of Irrigated Horticultural crops (2006). A list of published material is provided below.

9.1 Published Papers

Cooley NM, **Clingeleffer PR**, **Walker RR**. Integrated strategies to manage seasonal variation in wine grape maturation [abstract]. In. Riverlink Workshop on Cabernet Sauvignon. Merbein, Vic. [Mildura]: Riverlink, 2003: 11-2.

Cooley NM, **Clingeleffer PR**, **Walker RR**. Integrated strategies to manage seasonal variation in wine grape maturation [abstract]. In. 2nd Australian Grapevine Physiology Conference. Sunbury, Vic. Melbourne: University of Melbourne, 2003: 11.

Cooley NM, **Clingeleffer PR**, **Walker RR**. Integrated strategies to manage seasonal variation in wine grape maturation [abstract]. Proceedings of the Australian Society for Biochemistry and Molecular Biology, 2003:35

Clingeleffer PR, **Walker RR**, **Cooley NM**, **Ashley RM**. Irrigation for water use-efficiency and grape and wine quality [abstract]. In: Poiani A, editor. New directions in agroecology : productivity and sustainability . La Trobe University. Mildura, Vic.: La Trobe University, 2004: 15.

Petrie PR, **Cooley NM**, **Clingeleffer PR**. The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following season. Australian Journal of Grape and Wine Research 2004;10:203-15.

Cooley NM, **Clingeleffer PR**, **Walker RR**. The balance of berry sugar accumulation, colour and phenolic concentration under deficit irrigation strategies. In: Blair RJ, Williams PJ, Pretorius IS, editors. Proceedings of the Twelfth Australian Wine Industry Technical Conference. Melbourne, Vic. Urrbrae, S. Aust.: Australian Wine Industry Technical Conference Inc., 2005: 94-6.

Cooley NM, **Clingeleffer PR**, **Walker RR**. The balance of berry sugar accumulation, colour and phenolic concentration under deficit irrigation strategies [poster summary]. In: Blair RJ, Williams PJ, Pretorius IS, editors. Proceedings of the Twelfth Australian Wine Industry Technical Conference. Melbourne, Vic. Urrbrae, S. Aust.: Australian Wine Industry Technical Conference Inc., 2005: 331-2.

Cooley NM, Glenn, DM, Walker, RR, Clingeleffer, PR. Plant physiological responses to water stress applied in warm climates. Proceedings of the Australian Society for Biochemistry and Molecular Biology, 2005:124.

Cooley NM, Walker, RR, Clingeleffer, PR. Effects of water stress on grapevine carbohydrate reserves. In: Proceedings GWRDC Workshop on Grapevine Carbohydrate Dynamics. Editor, R.R. Walker, Merbein January 2006:32-44.

Cooley NM, Glenn, DM, Clingeleffer, PR, Walker, RR. Integrated management of deficit irrigation and particle film technology on maturation grape Cabernet Sauvignon. International Symposium Irrigated Horticultural crops, Mildura August 2006 (in press)

Glenn, DM, Cooley NM, Shellie, K. Walker, RR, Clingeleffer, PR. Impact of Kaolin Particle Film and Deficit irrigation on wine grape Water use efficiency and Plant Water Relations (In prep for American Journal Horticultural Science)

9.2 Grower tools

The project has identified two, rather contradictory vineyard management practices which, if proven under larger commercial conditions, should provide growers with further tools to improve the consistency of grape quality and assist in meeting set specifications.

- Firstly, the results with PD show that extended pre-veraison water deficits enhance berry colour, particularly in seasons with low potential colour caused by high temperatures in late December and early January. However, this benefit is potentially offset by yield reductions and the impact of this treatment on vine performance for more than three seasons is yet to be determined.

- Secondly, the application of PFT can potentially reduce leaf temperature and minimise stresses associated with hot weather and/or water deficits.

10. Appendix 2: Intellectual property

The main intellectual property associated with the project is the considerable body of material which will be published in the public domain.

11. Appendix 3: References

References cited in this report are included as components of the background and inserted papers and posters in the results section.

12. Appendix 4: Staff

Nicola Cooley	- Post Doctoral Fellow
Peter Clingeleffer	- Principal Investigator
Robert Walker	- Program Leader
Michael Glenn	- visiting scientist from USDA
Deidre Blackmore	- Scientific Support
Damian Watson	- Technical Support
Melissa Craig	- Technical Support
Troy Fry	- Technical Support
Joanne McLaughlin	- Casual Technical Support
Geoff Raymond	- Casual Technical Support
Louise Ziguras	- Casual Technical Support

12. Finalisation of budget