Management of carbohydrate reserve accumulation as a tool for regulating vine productivity and fruit quality



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSU 05/01**

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Research Organisation: **NWGIC**

Date: **30 September 2009**

Grape and Wine Development Corporation

Final Report, September 2009

Project Code **CSU05/01**

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ABSTRACT

This project investigated the importance of carbohydrates reserves for the growth and reproductive development of grapevines, and evaluated practical methods of modifying reserve accumulation for deliberate effect on vine productivity and fruit quality. Results suggest that carbohydrate reserves are used to support root growth in the month prior to bud-break, and confirm the importance of reserves for supporting shoot growth between bud-break and flowering. Reserve status alone did not appear to have a major influence on inflorescence initiation or fruit-set, and the supply of carbohydrates from current photosynthesis is probably more important for these processes. Attempts to manipulate reserve accumulation were less successful than expected, but significant responses of berry development amongst treatments suggest carbohydrate availability immediately before and after veraison may play a role in determining relative rates of sugar accumulation and colour development in Shiraz. A key conclusion of the project is that carbohydrate reserves play an integral role in the overall carbon economy of the vine, and that by understanding factors that influence internal competition for carbohydrates we may improve our ability to produce grapes at desired quality specifications.

EXECUTIVE SUMMARY

Carbohydrates play a critical role in grapevine growth and development, and many viticultural practices such as crop load regulation, canopy management and deficit irrigation are essentially modifying the relationship between carbohydrate sources and sinks. However, the role of carbohydrate reserves in the overall carbon economy of the vine has not received much attention despite the importance of stored carbohydrates for early canopy development, and the demonstrated capacity of reserves to contribute to berry ripening. This work aimed to provide a better understanding of the role of carbohydrate reserves in grape production through four complimentary studies. The first involved the excavation of whole grapevines to quantify the size of various reserve pools, and the concentration dynamics during the season. The second study involved a survey of 34 Shiraz and Chardonnay vineyards over three years to characterise the seasonal variation in carbohydrate reserves, and the third evaluated practical methods for manipulating reserve accumulation and fruit composition in Shiraz and Chardonnay. The final experiment was conducted with pot grown Chardonnay using controlled environment conditions to clarify the relative importance of carbohydrate reserves and current photosynthesis for fruit-set in grapevines.

The seasonal pattern of carbohydrate reserve dynamics and biomass partitioning of 12-year-old Shiraz grapevines was characterized with a series of whole vine excavations at the Charles Sturt University vineyard. The amount of non-structural carbohydrates stored in the perennial tissues at leaf-fall was 1194 g, compared to the total vine dry weight of 5648 g. The roots and rootstock contained 64% of these carbohydrates, indicating the importance of root resources for the next seasons development. The concentrations of carbohydrates declined in all tissues until shortly after flowering resulting in a net loss of 632 g, and then increased from veraison to harvest when they were largely replenished. The post-harvest period was not critical for reserve replenishment in this season, as the 14.6 t/ha yield did not appear sufficient to prevent the restoration of reserves prior to harvest. The pattern of carbohydrate reserve mobilisation and replenishment was strongly related to the internal supply and demand of carbon, with mobilization occurring during periods of root growth and shoot growth, and replenishment as the canopy reached is maximum photosynthetic capacity. The work suggests that the dynamics of carbohydrate reserve concentrations may provide a useful physiological indicator of internal assimilate availability.

Carbohydrate status of perennial tissues was assessed over three years in 34 Shiraz and Chardonnay vineyards in Southern New South Wales. Bud-dissections were also undertaken to investigate possible links between carbohydrate reserve status and bud-fruitfulness. Half of the vineyards sampled were located near Griffith in the Riverina, and the other half in the Hilltops, Gundagai and Tumbarumba grape growing regions (collectively referred to here as the South-West slopes). Carbohydrate reserve concentrations differed significantly between vineyards, and to a lesser extent seasons, with roots showing the highest variability. Root reserves were lower for Ramsey, and where vineyards had experienced prolonged water shortages. Average fruitfulness was higher in the Riverina than SW Slopes, and within both regions, Chardonnay was more fruitful than Shiraz. However, both varieties initiated the same number of inflorescences, with the difference in average fruitfulness related to a high incidence of primary bud necrosis (PBN) in Shiraz. The was no relationship between winter reserve status with initiated inflorescences, although there was some evidence that high wood carbohydrate reserves were associated with a higher incidence of PBN. However, there was some indication of an effect of nutrient status on fruitfulness and PBN.

Practical techniques for manipulating carbohydrate reserves were evaluated in a Shiraz and a Chardonnay vineyard in the Riverina. These included 33% and 66% crop removal prior to veraison to increase carbohydrate availability in the vine, and extended RDI, post-harvest deficit irrigation, and post-harvest hedging to reduce reserve storage. Relative to the broader seasonal fluctuations, the effects of these treatments on carbohydrate reserve concentrations appeared small. At times during the season concentrations were higher for the crop removal treatments in the wood, and to a lesser extent the roots, while post-harvest hedging caused a slight decrease. However, there was no evidence of any real impact of the two deficit irrigation treatments. Conversely, a number of these treatments had a significant impact on fruit development. For Shiraz, both of the crop removal treatments increased the rate of sugar accumulation, and to a greater extent colour development, with the effect becoming more pronounced over seasons. Extended RDI resulted in lower berry weights and yields, but higher colour. However, post-harvest deficit irrigation had the opposite effect and delayed colour development and sugar accumulation in the next season without affecting yield. This is the first time such an effect has been reported, and in the absence of an effect on carbohydrates, suggests there may have other effects on nutrient uptake or vine metabolism that carried through to the next season. For Chardonnay, the same treatments appear to have very little effect on fruit development or the performance of the vine. Evaluation of experimental wines also showed that the treatments had a significantly greater influence on wine quality for Shiraz than Chardonnay. The study findings point to the importance of internal carbohydrate availability around the time of veraison for fruit quality in Shiraz, and the possibility that a more detailed evaluation of reserve dynamics during this period may provide a physiological indicator of vine carbon balance.

The relative importance of current assimilate and stored carbohydrate reserves for fruit-set was investigated in potted Chardonnay vines, as stored reserves may provide an alternative source of carbohydrates during weather conditions that reduce the supply of carbohydrates from photosynthesis. This was achieved by placing vines firstly under warm and cold root temperature regimes from just after bud-break until to flowering, producing high and low carbohydrate reserves in the perennial structure. During the flowering to fruit-set period the vines were exposed to ambient or low CO_2 to alter the production of photoassimilate while maintaining vines in the same light and temperature environment. The study highlighted the importance of photosynthesis and the supply of current assimilate for maximising fruit set. Carbohydrate reserves may play a role in fruit set, but possibly an indirect one whereby carbohydrate reserves that are already replenished by flowering will not act as a competing sink for current assimilate with the developing inflorescence.

The overall all findings of the study highlight the need to consider carbohydrate reserves as an integral part of the overall carbon balance of the vine, and not just as a single independent factor. Through improving our knowledge of the relative importance of

carbohydrates supplied from current photosynthesis or mobilized from stored reserves, there may be an opportunity to significantly improve our capacity to grow and supply grapes at the desired yield and quality specifications.

PROJECT BACKGROUND

Extensive work on carbohydrate reserves in the Northern Hemisphere did not specifically address the post harvest-period, although some information was obtained with studies in Australia (Scholefield et al. 1978, Sommer & Clingeleffer 1996). Recent work carried out at the NWGIC suggested the importance of vine reserves accumulated after harvest for early shoot growth and grape production (Smith & Holzapfel 2003). These latest results show the effect of treatments which lower vine reserves also appear to be different between vineyards. In the highest yielding vineyard, the shoots have only half the length of the control if leaves were removed after harvest. In addition, bunch numbers were decreased by 30 to 40%, flower numbers were reduced by about 30%, and berry set was lowered. These findings show that vine reserves can have a dramatic effect on the following seasons vegetative and reproductive development if amount of stored reserves changes sufficiently. The starch levels in the wood were increased by early crop removal and significantly reduced by leaf removal after harvest. While starch levels in roots were much higher in the wood and increased 5-fold in the post-harvest period, the other two treatments (crop and leaf removal) underwent only a small change. The dynamics of root starch reserves appear to be particularly important in the higher yielding vineyards of the Riverina.

Further understanding of reserve accumulation during the post-harvest period could have a significant impact on vineyard management during this period. Reserve levels will be managed to optimise early vine growth and canopy development. Reserve levels impact on reproductive development, vine yield and berry composition. Vines appear to be most susceptible to the influence of reserves during inflorescence initiation, differentiation, flowering and fruit set. Recent studies (Sanchez & Dokoozlian 2004) have shown that carbohydrate supply is correlated with fruitfulness. Bennett et al. (2005) found that yield variations are significantly influenced by carbohydrate reserves. On the other hand, Zapata et al. (2004) suggested that bad weather at flowering has less impact on fruit set if the vine has appropriate reserve levels. Key objectives of the current project were to determine the importance of carbohydrates reserves for growth and reproductive development, and evaluate practical methods of modifying reserve accumulation for deliberate effect on vine productivity and fruit quality.

Project code:	CSU 05/01
Project title:	Management of carbohydrate reserve accumulation as a tool for regulating vine productivity and fruit quality
Research Organisation:	National Wine & Grape Industry Centre
Project Supervision:	Bruno Holzapfel

Chief Investigators:	Jason Smith (project appointment) John Gray (left project in December 2007) Michel Meunier (left project in December 2007) Bruno Holzapfel (commitment increased early 2008) Suzy Rogiers Leo Quirk (in addition to original proposal)
Project sites:	Griffith, Canberra, Wagga Wagga
Commencement date:	July 2005
Completion date:	June 2009
Project funding:	$581,170

PROJECT AIMS AND PERFORMANCE TARGETS

- Understand the interactive effects of crop load and carbohydrate reserve level on the seasonal dynamics of reserves for two varieties in warm climate irrigated vineyards.
- Investigate the effects of carbohydrate reserve levels on the interaction between shoot growth rates, canopy micro-climate and inflorescence initiation at flowering.
- Examine the effect of carbohydrate reserves on inflorescence differentiation prior to bud-break.
- Investigate the relative importance of current assimilate and stored carbohydrate reserves for fruit set under unfavourable environmental conditions.
- Provide a better understanding of factors that influence the relative strength of reproductive (fruit) and storage sinks (trunk, roots) and during the ripening process.
- Evaluate methods for altering vine carbohydrate reserve accumulation during the post-harvest period as a tool for managing vine vigour, yield and fruit composition.
- Investigate the potential for a standard carbohydrate test to be used in conjunction with bud-fruitfulness for yield estimation, yield regulation and post-harvest vine management.

Outputs	Performance Targets
1. Information on variety and crop load effects on the seasonal dynamics of carbohydrate reserves.	Analysis of root, wood and spur samples from Shiraz and Chardonnay vineyards in the Riverina completed.
2. Information on the within vine distribution of carbohydrate reserves.	Distribution and variability of carbohydrate concentration in the root system and above-ground parts determined.
3. Method for assessing the rate of starch synthesis or storage in vine tissue.	Development of analytical method for qualitative or quantitative assessment of enzyme activity associated with starch metabolism completed.
4. Method for assessing inflorescence initiation in relation to carbohydrate supply and canopy development.	Establishment of microscopic techniques for assessing bud-fruitfulness and carbohydrate supply during the growing season. Construction and validation of data logging system for monitoring canopy microclimate..
5. Information on the effects of carbohydrate reserves on inflorescence development and fruit set.	Microscopic assessment of inflorescence development at bud-break following post-harvest treatments in the previous season. Assessment of flower numbers and fruit set.
6. Information on the effects of carbohydrate reserve levels on inflorescence initiation.	Pattern of inflorescence initiation determined for Shiraz and Chardonnay at high and low carbohydrate reserve levels.
7. Knowledge on the role of reserves in improving fruit-set under cool weather conditions.	Pot experiment conducted under controlled environment conditions to determine the effect of reserve levels on fruit under conditions conducive to poor fruit set.
8. Understanding of the importance of carbohydrate reserves for reproductive development in grapevines.	Effect of carbohydrate reserve levels on bud fruitfulness, flower numbers and fruit set determined following second consecutive season of post-harvest treatments.
9. Evaluation of practical methods for managing the accumulation of reserves after harvest.	Effects of post-harvest deficit irrigation treatments on carbohydrate accumulation, vegetative growth and yield determined over two consecutive seasons
10. Development of a wood reserve test as a tool for crop forecasting and yield regulation.	Carbohydrate analysis of all root and wood samples completed, and standard concentration ranges defined for effects on crop load in the following season.
11. Understanding interactive effects of crop load and carbohydrate reserves on fruit composition.	Fruit composition and quality attributes monitored during ripening over two final seasons. Small scale winemaking from high and low reserve treatments at all field sites in final year of study.
12. Transfer of technology and information to industry.	One industry publication per year, and carbohydrate reserve workshop at the Australian Wine Industry Technical Conference. Preparation and submission of papers for refereed scientific journals. Linkage with Viticare/RITA on-farm trials for grower evaluation.

1. This work was conducted in 34 vineyards over three years in the Riverina, but also in Hilltops, Tumbarumba and Gundagai vineyards (collectively referred to here as the South-West slopes), significantly exceeding the initially proposed target (see section 3). The seasonal dynamics of carbohydrates and N was determined in two Shiraz vineyards in the Riverina and Canberra region (see appendix 4).
2. Carbohydrate concentrations were determined in below (three root sizes, rootstock) and above ground organs (trunk, cordon, spurs) in the CSU vineyard. This work was conducted throughout the whole growing season (winter 2007 to winter 2008) in Shiraz vines through monthly destructive harvests (see section 2). This output has been undertaken together with a project on vine nutrition and therefore was considerably enhanced.
3. This target has been altered to investigate vine carbohydrate transport, accumulation and mobilisation. A C_{14} method has been assessed, and an experiment that will combine this technique with our CO_2 control system is being established at present to characterise sink activity of various vine parts during ripening. This work will enhance our investigations on grapevine partitioning and vine balance in the current season.
4. The microscopic method to assess bud-fruitfulness was developed, despite the early departure of the principal investigator, and the canopy microclimate monitoring system was validated and utilised at two experimental sites (see appendix 4).
5. The relationship between bud fruitfulness and carbohydrate reserves were assessed in a survey of 34 vineyards (see section 3) and in two management trials (see section 4). The impact of vine reserves on inflorescence development and fruit set was assessed in potted vines (see section 5).
6. The pattern of inflorescence appearance was assessed in two Shiraz vineyards in the Riverina and Canberra wine growing regions (see appendix 4). However, the assessment of inflorescence size in the bud influenced by carbohydrate levels would require the assessment of a larger number of buds to provide a meaningful result.
7. A pot experiment on Chardonnay vines was conducted and the effect of reserve levels on fruit set under conditions conducive to poor fruit set was assessed (see section 5). This was achieved by altering vine carbohydrate reserves by varying root temperature and photosynthesis by the differing CO_2 levels.
8. The effect of post-harvest treatments on carbohydrate reserve levels and reproductive development was assessed over two consecutive seasons in a Shiraz and Chardonnay trial in the Riverina (see section 4).
9. As part of these trials above, the impact of post-harvest deficit irrigation on carbohydrate accumulation was determined over two consecutive seasons. The vine vegetative growth and yield was also assessed (see section 4).
10. Carbohydrate reserve levels in root and wood tissues were determined from several trials of the project. Concentration ranges have been established for the Riverina and SW slopes with information on crop load (see section 3).
11. The berry composition was assessed over two seasons in Chardonnay and Shiraz from the carbohydrate management trials. Wine was made and assessed from four key treatments from these management trials (see section 4).
12. Eight technical publications and five scientific publications have been produced during the project. In addition, eight international and 24 national presentations were given in the area of this research since the middle of 2005 (see appendix 1). An expression of interest for a workshop presentation at the AWITC is currently considered.

DETAILED PROJECT REPORT

1 General Introduction

Vine reserves are crucial for new shoot growth and development in early spring until photosynthesis becomes the primary source of carbon (Glad et al. 1992). Vine reserves are also important for reproductive development. Yield variations are significantly influenced by carbohydrate reserves (Bennett et al. 2005, Smith & Holzapfel 2003). Variation in berry size (and other physical attributes) is dependent on events that occur prior to flowering. Gray (2002) suggests that the developmental morphology of the inflorescence primordia during initiation and differentiation is critical to final berry size. Variation in berry size makes a significant contribution to variation in the size of the annual grape crop (6-25%) (Martin & Clingeleffer 2003). Variation in bunch number and bunch size are also significant components of annual yield variation.

Carbohydrate source-sink relationships are complex and dynamic throughout the growing season and winter reserves are important for shoot development until bloom. Grapevine leaves need to reach about one third of their final size before they begin to export carbohydrates (Koblet 1977) but continue to import carbohydrates until half their final size. Retranslocation of carbon reserves begins with budburst, reaches a maximum at the 6 to 8 leaf stage and eases by the flowering stage (Yang & Hori 1979). On the newly developed shoots, the basal leaves begin to export at the 5-leaf stage, and the export from leaves increases with age (Yang & Hori 1980). Leaves between 30 and 50 days after leaf unfolding have the highest photosynthetic rate (Kriedemann 1968, Intrieri et al. 1992). Before flowering, translocation of photosynthates from the leaves is towards the shoot tips (Koblet 1969, Hale & Weaver 1962, Yang & Hori 1980). The first flush of root growth peaks at anthesis, indicating its importance as a sink (McKenry 1984, Van Zyl 1984). Carbohydrates are seen as the major determinate of fruit set in grapes (Caspari et al. 1998). After fruit set the berry starts to become a very large sink (Koblet 1969). The carbohydrates in fruit originate mainly from leaf photosynthesis. The mobilisation of reserves from the permanent structure only occurs if supply of photoassimilate to the fruit is insufficient due to stress situations (Candolfi-Vasconcelos et al. 1994, Koblet et al. 1996), or if the crop load is beyond the capacity of the vine (Balasubrahmanyam et al. 1978).

Although the fruit forms a major sink for assimilated carbon, it is clear that the vine has other major carbon-requiring processes or carbon stores to conserve assimilates for future requirements in the following seasons (Chaumont et al. 1994, Miller et al. 1997). These seem to become more important after mid-berry ripening (Candolfi-Vasconcelos et al. 1994, Koblet et al. 1996, Keller 1993). It is important that photosynthesis after harvest continues for carbohydrate storage (Hunter et al. 1994). Scholefield et al. (1978) showed that the month after harvest is of particular importance. Root growth can be reduced by defoliation during berry ripening (Candolfi-Vasconcelos et al. 1994) and carbohydrate concentrations in the wood are highest in winter (Sommer & Clingeleffer 1996), indicating that the photosynthetic activity after harvest is important for the second major flush of roots and for storage (McKenry 1984, Van Zyl 1984). A large part of the reserves can be converted to soluble forms in connection with cold winter temperatures or to support spring growth (Glad et al. 1992). The reserves also vary between parts of the vine, roots have been reported to have much higher levels (Uys & Orffler 1983). The absolute amount of non-structural carbohydrates in vines differs according to cultivar, vine size, age, crop load, environmental conditions, presence of viral disease (Mullins et al. 1992), cultural practices, such as nutrition (Holzapfel & Treeby 1998, Keller et al. 1995, Keller & Koblet 1995, Korkas et al. 1994a, b, 1996a, b), canopy management (Hunter et al. 1995), vine spacing (Hunter 1998) and irrigation (Petrie et al. 2004, Rühl & Alleweldt 1990).

Initiation of the inflorescence primordia in latent buds begins in summer. Timing is coincident with flowering for the current growing season. The efficiency of this process depends on the availability and distribution of photosynthates in the developing shoot. The allocation of resources (carbohydrates and nutrients) to the inflorescence primordia is governed by source-sink relationships at flowering and competing sinks (shoot tip, flowers) might inhibit inflorescence initiation. Sink strength is a function of both sink size and sink activity (Ho 1988). Sink size and sink activity are likely to be key factors in the initiation process. Vascular morphology is closely linked to both sink size and activity. The impact of vine reserves on inflorescence primordia development is unknown, but recent findings by Smith & Holzapfel (2003) indicate that vine reserves can affect bunch number and size.

The differentiation of flowers from the inflorescence primordia begins after the dormant latent buds are activated in spring (Mullins et al. 1992). All assimilates required for differentiation must be derived from vine reserves since the photosynthetic apparatus of the vine is not yet functional. Smith and Holzapfel (2003) found a positive relationship between reserve levels in the cordon/trunk during dormancy and vine yield in the following season. The availability of reserves is likely to depend on both environmental factors (soil temperature, air temperature, day length) and plant factors (root activity, storage capacity), although conclusive research is scarce.

The amount of winter reserves impacts on canopy development in the spring, bunch and flower numbers, fruit set and final yield (Bennett et al. 2005, Smith & Holzapfel 2003). The amount of reserves will contribute to either vigorous spring growth (VSG) or restricted spring growth (RSG). The later has been discussed in connection with a lack of carbohydrate reserves, production or partitioning (Hackett & Holzapfel 2002). This work will further reveal the important role of carbohydrate reserves and metabolism on reproductive development at the different stages, and will direct vineyard management to optimise carbohydrate reserves (particularly from harvest to bloom) to achieve balanced vegetative and reproductive development for premium grape production.

2. Whole vine distribution of carbohydrate reserves

2.1 Introduction

Published information on the whole vine distribution of carbohydrate reserves is limited to a few studies in Australia (Rühl & Clingeleffer 1993, Sommer & Clingeleffer 1996), the United States (Mullins et al. 1992, Williams 1996, Bates et al. 2002), and South Africa (Hunter 1998). The majority of other carbohydrate reserve research, including that presented in the following sections of this report, have been based on concentration measurements due to the practical difficulties of undertaking whole vine excavations. However, whole vine information is important because it provides a quantitative measure of carbohydrate storage capacity, and is essential for any research that aims to understand the relative importance of different vine storage tissues for the overall carbon economy of the vine.

Determining the concentration of non-structural carbohydrates in both wood and root tissue is, by contrast, more straightforward. Where research trials are undertaken within a vineyard, and vines are a similar size at the start of the experiment, relative differences that subsequently develop due to applied treatments should be similar on a whole vine or concentration basis providing the latter is representative of the vine part being sampled. Comparisons between vineyards are more difficult, but measurements of above ground vine biomass or volume can be made to estimate quantitative differences in carbohydrate storage capacity. However, there is no way to determine how changes in root carbohydrate concentrations influence the overall storage capacity of the vine without knowing, or being able to estimate, the below ground biomass.

Prior to the 2007/2008 season, the opportunity arose (due to a planned vineyard replanting) to excavate whole vines from the Charles Sturt University commercial vineyard. As one of the planned outputs of the project was to provide information on the within vine distribution of carbohydrate reserves, a study was planned whereby whole vines were excavated at monthly intervals from dormancy through to leaf-fall. This work was undertaken jointly with Stewart Field and other staff from the NWGIC with overlapping interest in carbohydrate and nutrition research, and will be reported in further detail as part of the NWGIC futures program. For the current project, our aim was to obtain data on the whole distribution of carbohydrate reserves, and look at the relative changes in the carbohydrate content of perennial vine parts during the growing season. This in turn would provide a context in which concentration based data from the survey and field trials described in following chapters could also be interpreted in terms of consequences for total carbohydrate storage.

2.2 Materials and methods

2.2.1 Site details

The vineyard used for the trial was located at the Charles Sturt University vineyard (35°03’38”S, 147°21’50”E), and consisted of own-rooted Shiraz grapevines (clone PT23) planted in 1997 and trained to a single bilateral cordon. Vine and row spacing were 2 and 3 m respectively. During the 2007/2008 the vineyard received approximately 3.5ML/ha of irrigation or rainfall, and carried a yield of 14.6 t/ha.

2.2.2 Vine excavations

During winter in 2007 the trunk circumference of all vines in the 1.1 ha trial area were measured, and 44 uniformly sized vines were tagged to give 4 replicates for each of the 11 excavation dates. The first excavation commenced on Aug 21 2007, with the others following on Sep 20, Oct 17, Nov 14, Dec 13, Jan 9, Feb 6, Mar 3, Apr 2, Apr 30 and May 29 throughout the 2007/2008 season. At each excavation the top half of the vine was cut off at ground level and separated into leaf, shoot (stem or dormant cane depending on date), last

seasons spur (1 yr wood), older wood above the cordon (2 yr + wood), cordon and trunk. All of the wood was cut into smaller pieces, representative sub-samples collected, and washed. The sub-samples were then freeze dried for later analysis, and the dry weight recorded. The remaining wood tissue was oven dried at 70°C, and the dry weight also recorded. On the same day as the wood samples were collected, the area allocated to each vine (6m^2) was marked out, and excavated with a backhoe to a depth of 80 to 100 cm (Figure 2.1). Excavation below this depth was prevented due to high density sub-soils and the size of machinery that could be used between the vine rows.

Immediately after each excavation the majority of large roots and the rootstock were collected and placed in a cool room. Over the following three days, the soil from each of the four vine excavations was manually sieved through 10 × 10 mm mesh to remove all remaining roots (Figure 2.1). All of the roots from each vine were then separated into three size classes which corresponded approximately to the diameter range of the main structural roots (large, > 7 mm), secondary roots (medium, 3 to 7 mm), and tertiary roots (small, < 3 mm). Based on random measurements made after the roots from each vine were separated, the average diameter in each class 10.7, 4.0 and 1.1 mm respectively. This did not include any of the new seasons fine root growth, as these were either too fine to be caught in the sieve, or too delicate to survive the excavation process. After separation into the three size categories, the roots were sub-sampled and dried as described for the wood.



Figure 2.1 Excavation of whole Shiraz grapevines (left), and soil sieving for fine root collection (right), in the CSU vineyard during 2007/2008 season to determine whole vine biomass partitioning and carbohydrate reserve distribution between above and below ground parts of the vine.

2.2.3 Carbohydrate analysis

For analysis of total non-structural carbohydrates (TNC), a 20 mg sub-sample of each tissue was weighed after grinding to 0.12 mm using a heavy duty cutting mill (Retsch ZM2000, Haan, Germany), and then an ultra-centrifugal mill (Retsch ZM100). Soluble sugars extracted using two 1 ml washes of 80% aqueous ethanol at 80°C for 10 min, and then a single 1 ml wash of 80% ethanol at room temperature. After centrifuging between each wash, the three aliquots were combined, diluted to 10 ml, and the concentration of sucrose, D-fructose and D-Glucose determined by enzymatic assay (Megazyme International, Bray, Ireland).

For starch analysis, the remaining wood sample was re-suspended in 200 μL of dimethylsulfoxide and heated at 98°C for 10 minutes. The remainder of the analysis was then performed using commercial enzymes and glucose assay kits (Megazyme International). Briefly, 300 μL of thermostable ∝-amylase in MOPS buffer was added, mixed, and incubated for 15 min in a 98°C water-bath. After cooling, 400 μL of amyloglucosidase in sodium acetate buffer was added and incubated at 50°C for 60 min. The samples were mixed at 20 min

intervals, and then centrifuged at 10,000 rpm for 2 min. Supernatant from root samples was diluted 1:11, and trunk and shoot samples 1:6 in Ultra-pure® water. Glucose concentration of the diluted samples was then determined colorimetrically and the amount of starch in the original 20 mg sample calculated.

2.3 Results and discussion

The information collected during this trial has created a significant data set on whole vine growth and biomass accumulation, and together with association nutrient, photosynthesis, canopy light interception, and berry development data, will provide a basis for ongoing work on vine nutrition and vine carbon balance under the NWGIC futures program. For this report we have presented the reserve concentration data for perennial tissues at all excavation dates, and then the whole vine carbohydrate distribution data for the final excavation date at leaf-fall. We have also included some fine root growth data from a minirhizotron study in the same vineyard, as well as shoot growth and berry development data from the excavated vine. This is shown for simple comparative purposes at present, but it is intended that this information be formally incorporated in an existing or new grapevine carbon balance model.

2.3.1 Seasonal carbohydrate reserve dynamics

The seasonal dynamics of carbohydrate reserves in the perennial parts the Shiraz vines used in the present study was consistent with general pattern observed in previous work (eg. Winkler & Williams 1945, Goffinet 2004, Bennett et al. 2005). That is, that reserve concentrations declined between dormancy and flowering as carbohydrates were mobilized to support new shoot and root growth, and then progressively replenished during the season (Figure 2.2). The reported interconversion of starch and sugars in above ground tissue as the vines moves into and out of dormancy, and the lack of an equivalent response in the roots, is also consistent with the findings of the current study.

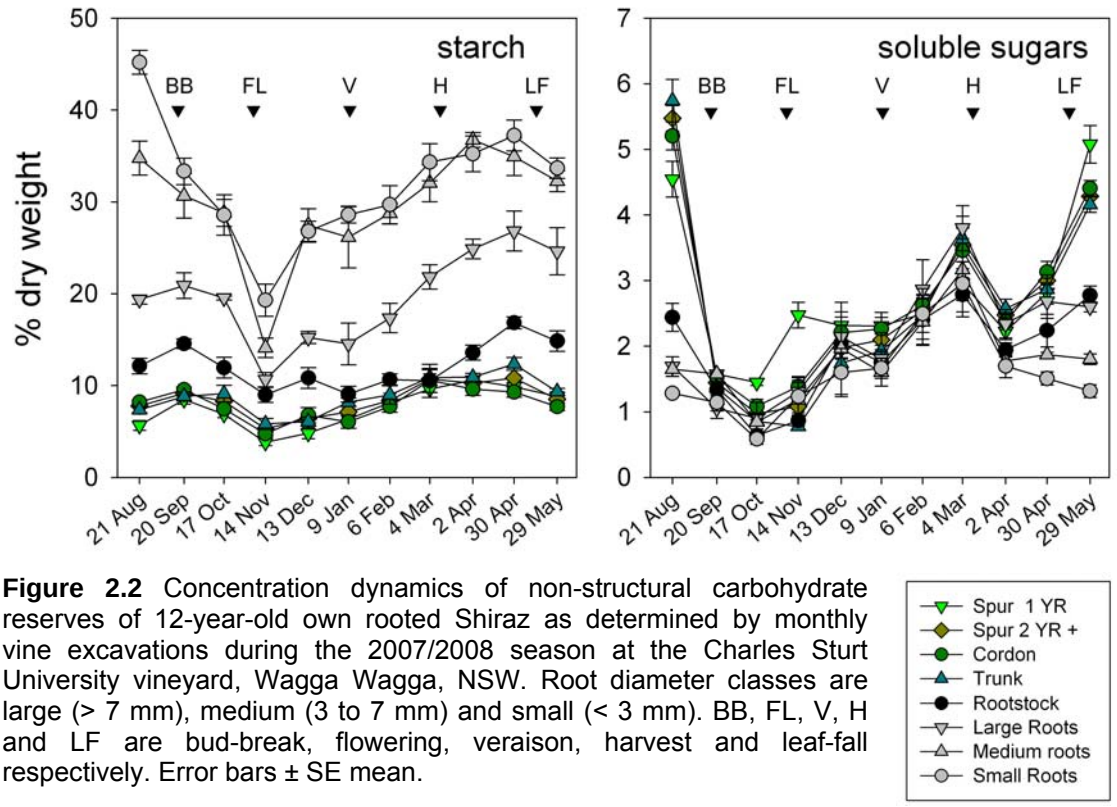


Figure 2.2 Concentration dynamics of non-structural carbohydrate reserves of 12-year-old own rooted Shiraz as determined by monthly vine excavations during the 2007/2008 season at the Charles Sturt University vineyard, Wagga Wagga, NSW. Root diameter classes are large (> 7 mm), medium (3 to 7 mm) and small (< 3 mm). BB, FL, V, H and LF are bud-break, flowering, veraison, harvest and leaf-fall respectively. Error bars ± SE mean.

Between the first and fourth excavation dates, which spanned the period from 26 days before bud-break to 8 days after flowering, the decrease in carbohydrate concentrations equated to a loss of 471 g from the root system, and 133 g from the wood. When the rootstock and spurs are included, the net mobilization was 632 g per vine during the period, or just under 60% of the total vine reserves at the start of the season. In an earlier project with high yielding Semillon vineyards in the Riverina (Smith & Holzapfel 2009), we found that vines were highly dependant on the post-harvest period for replenishing carbohydrate reserves in the root system. This was attributed to the high yields typical of this variety under full irrigation in the Riverina, which exceeded 40 t/ha in some cases and prevented the replenishment of reserves until after the fruit was harvested. However, at 14.6 t/ha the yield was significantly lower for the Shiraz vines in the present study, and carbohydrates were largely restored by harvest.

The relative differences between the carbohydrate reserve concentrations of the various perennial tissues as shown in Figure 2.2, were largely maintained throughout the season. For TNC reserve concentrations, all correlations between all combinations of perennial tissue were significant at $p \leq 0.05$ (data not shown). Therefore, based on these results, the carbohydrate reserve status of any of these tissues may be broadly reflective of the status of the rest of the vine within the growing season. However, for starch, the relationship between small roots and the above ground parts of the vine was weaker or not significant in some cases (Table 2.1). Likewise, correlations with the rootstock shank were weaker, but this may be a reflection of sampling variability between dates (ie. variation in the relative amounts of root and trunk tissue included as the vines were divided into biomass components).

Table 2.1 Significance of the relationship between the internal carbohydrate concentrations of different vine parts during the season as based on data from monthly vine excavations.

	Spur 1 yr	Spur 2 yr +	Cordon	Trunk	Rootstock	Large roots	Medium roots
Starch (%DW)							
Spur – 2 yr +	***[a]						
Cordon	***	***					
Trunk	***	***	**				
Rootstock	*	*	ns	*			
Large roots	***	***	**	***	***		
Medium roots	**	**	**	*	*	***	
Small roots	ns	*	*	ns	ns	*	***
Sugars (%DW)							
Spur – 2 yr +	***						
Cordon	***	***					
Trunk	***	***	***				
Rootstock	*	**	***	**			
Large roots	ns	ns	ns	ns	***		
Medium roots	ns	ns	ns	ns	**	***	
Small roots	ns	ns	ns	ns	*	***	***

[a] *,**,*** and ns indicate significance at $p < 0.05$, < 0.01, < 0.001, and not significant, respectively.

For sugars, none of the correlations between the roots and above ground parts of the vine were significant due the interconversion of starch and sugars at bud-break and leaf-fall. However, if these dates are excluded, sugars in the roots and above ground parts of the vine follow each other closely in relative terms and absolute concentration.

With the exception of the fruit-set experiment in Section 4, which used potted vines, all of the studies described in the remainder of this report used root and wood sub-sampling to determine carbohydrate concentrations of vines within and between seasons. The wood samples were collected with a 3/16" (4.8 mm) drill bit to a depth visually estimated as the centre of the cordon or trunk. For the dynamics study (Section 2) samples from six vines were combined as replicate, and for the reserve variability survey (Section 3) and reserve management trials (Section 4), four vines were used per replicate. Root samples ranging in diameter from approximately 3-7 mm, or medium size root class as defined in the current study, were collected from near the base of the vine and combined for each treatment panel as for the wood samples. Neither samples were separated into xylem and phloem tissue, and therefore represent the carbohydrate reserve status of the bulk tissue.

The close relationship seen between the carbohydrate reserve concentrations of the three root diameter classes used in the present study indicate that the root sub-sampling method used at other project trial sites would satisfactorily reflect the overall concentration and seasonal carbohydrate reserves dynamics of the root system as a whole. However, as the medium root class stores higher concentrations of carbohydrates than the large roots, total reserve storage would be overestimated if this value was used as a representative value for the whole root system. For the wood tissue, the trunk and cordon make up the majority of above ground biomass of a spur pruned vine, and are strongly correlated with the younger wood. Therefore, sub-samples of trunk and cordon should reflect the reserve status of the above ground biomass with reasonable accuracy.

2.3.2 Whole vine carbohydrate reserve distribution

For the 12-year-old Shiraz grapevines used in the present study, 57% of the total vine carbohydrates were stored in the root system prior to pruning (Table 2.2). When the contribution from canes is excluded, as would be the case after pruning, then 64% of the 1193 g of total reserves potentially available for next season would have been located in the roots. This compares to 29% of 1956 g for 16-year-old spur pruned bilateral cordon Cabernet Franc on own-roots (Rühl & Clingeleffer 1993), 43% of 798 g for 12-year-old spur pruned bilateral cordon Chenin Blanc on own-roots (Mullins et al. 1992), and 51% of 536 g for 14-year-old spur pruned unilateral cordon Pinot Noir grafted on 99 Richter rootstock (Hunter 1998).

Within the three root diameter classes, large roots had the lowest concentration of carbohydrates, but stored 43% of the total root reserves due to the higher total biomass of these roots. The concentrations of carbohydrates in the medium and small roots were similar, but in both cases higher than the large roots. However, due the smaller biomass, net storage by the medium root fraction was 26% of total root reserves, and 31% for the small root fraction. Of the studies mentioned previously, only Hunter (1998) divided the roots into diameter classes. Although five categories were used that partly overlap the three used in our study, the findings were remarkably similar given the vines used in the South African work were much smaller and were grafted rather than grown on own-roots. In that study, the larger roots (> 10 mm), had the lowest carbohydrate concentrations but stored 51% of the total root reserves. Medium roots (2 to 10 mm) and smaller roots (< 2 mm combined) had higher carbohydrate concentrations, but only stored 25% and 24% of the total root reserves respectively. In our study, as with the results reported by Hunter (1998), starch was the predominant storage carbohydrate in all root diameter classes.

For the above ground parts, 12% of the total vines reserves were found in the canes, which is comparable to the value of 10% obtained by (Rühl & Clingeleffer 1993). The canes also

had the highest concentration of carbohydrates after the roots, which was due to both high starch and soluble sugars. For the trunk, cordon and wood above the cordon, the carbohydrate concentrations and relative amounts of starch and sugar were very similar, and collectively represented 26% of the total vine reserves. The rootstock shank, which contains the transitional tissue between root and wood, made up the remaining 5%.

Table 2.2 Concentration and distribution of carbohydrate reserves in 12 year old own-rooted Shiraz grapevines excavated at leaf-fall in May 2008.

		starch	sugars	dry weight	Total CHO	CHO distribution
		% dry weight		(g)	(g)	(%)
Individual tissue						
Canes		15.1	6.0	785	165	12
Spur – 1 yr		8.8	5.1	82	11	1
Spur – 2 yr +		8.5	4.3	517	66	5
Cordon		7.7	4.4	979	118	9
Trunk		9.3	4.2	1162	157	12
Rootstock		14.8	2.8	421	73	5
Large roots		24.6	2.6	1216	329	24
Medium roots		32.3	1.8	593	202	15
Small roots		33.7	1.3	680	239	18
Combined*	Canes	15.1	6.0	785	165	12
	Wood	8.6	4.5	2739	351	26
	Rootstock	14.8	2.8	421	73	5
	Roots	30.2	1.9	2489	769	57
Total biomass or reserves				6433 g	1358 g	100 %

* data combined according general tissue type

2.3.3 Carbohydrate reserve dynamics and vine sink:source relations

The seasonal changes in the concentration of TNC reserves in the main perennial parts of the excavated vines appeared to correlate well with the pattern of carbohydrate supply and demand that would be anticipated with the timing of root growth and berry development in relation to shoot growth and canopy establishment (Figure 2.3).

In the one month period between the first excavation at dormancy, and second excavation four days after bud-break, there was a decrease in total carbohydrate concentrations in the small and medium roots that resulted in the loss of 128 g of reserves from these root fractions. Measurements of fine root growth on nearby vines in the same vineyard showed significant new fine root production in the month prior to bud-break, suggesting that carbohydrates from the small and medium roots may have been used to support this growth. In the above ground tissues, TNC reserve concentrations also declined in the trunk, cordon and younger wood fractions resulting in the net loss of 36 g of reserves. As the second excavation was after bud-break, some of these carbohydrates would have been used to support the newly developing shoots. For both root and wood tissue, there would also have

been losses due to maintenance respiration, metabolic costs associated with mobilizing carbohydrates, and possibly renewed nutrient uptake and assimilation.

One interesting finding is that the concentration of carbohydrates in the large roots remained relatively stable until the third excavation 20 days after bud-break. Although we have no way of knowing if this was due to re-distribution within the vine, or whether carbohydrates were still transported from the smaller roots to the developing shoots or *vice versa*, it may imply that above ground reserves are preferentially used for shoot growth, and reserves from smaller and medium sized roots for fine root growth. Carbohydrate concentrations in the large roots did subsequently decline, but this occurred around flowering when there was a strong concurrent demand from growing shoots and a renewed period of fine root production.

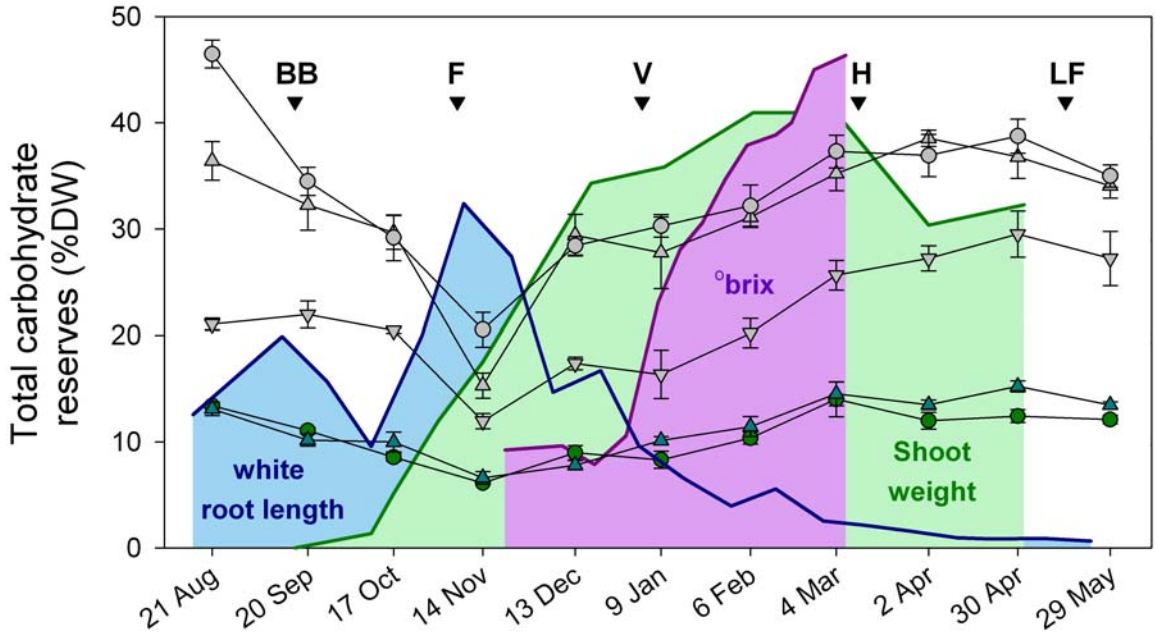


Figure 2.3 Seasonal carbohydrate reserve dynamics of the larger perennial biomass components in relation to fine root growth, vegetative shoot growth and berry ripening. The root length data (Stewart Field, unpublished), shoot weights and berry sugar concentrations have been adjusted to an arbitrary common scale for comparative purposes.

Previous studies have suggested that the maximum demand for carbohydrates is reached at the 8 to 10 leaf stage (Yang & Hori 1979, 1980), and that canopy carbon autotrophy is reached by approximately flowering (Lakso 2009). In our study, the fourth excavation was 8 days after full bloom, so we can not be sure that TNC reserves were not already recovering by this point. However, in the first half of the period between flowering and veraison there was a rapid increase in carbohydrate concentrations in the roots, and to a lesser extent, also in the wood. This fourth excavation corresponded to the end of the rapid shoot growth phase and a period of reduced fine root production, but proceeded after the onset of berry sugar accumulation. Photosynthesis rates and canopy light interception were also at a seasonal maximum (data not shown), with an overall result that nearly 40% or 247 g of the perennial reserves used during the preceding 3 months were replaced within 4 weeks.

As discussed earlier, carbohydrate concentrations in the wood and root tissue continued to increase through the ripening period, albeit at a slower rate than immediately after flowering, and were largely replenished by harvest. In our earlier work (Holzapfel et al. 2006, Smith & Holzapfel 2009) yields of 30 to 40 t/ha, or yield to pruning weight ratios of around 15, were

found to be sufficient to delay the replenishment of root reserves until after harvest. The results of the present study, where the vine yields and pruning weight ratio were approximately 14.6 t/ha and 7 respectively, the competition from ripening fruit did not appear sufficient to prevent the replenishment of reserve before harvest. However, between December 13 and January 9 there was a negligible 2.5 g increase in total perennial reserves, which contrasts strongly with the 247 g gained in the previous month. The two week period leading up to January 9 was characterized by a run of high temperatures, and also corresponded to a period of rapid sugar accumulation in the berries. Consequently, while reserves were restored later in the ripening period, this finding suggests that these vines may have experienced a deficit or carbohydrate supply during the critical stages of berry development shortly before and after veraison.

2.4 Summary of key findings.

1. The total dry weight of 12-year-old own rooted Shiraz grapevines excavated from a irrigated hot climate vineyard at leaf fall was 6433 g. The amount of non-structural carbohydrates stored by the same vines was 1358 g, of which 57% was found in the roots, 26% in the wood, 12% in the canes and 5% in the rootstock. Following pruning the contribution of the roots to overall vine carbohydrate reserve storage would have risen to 64%, suggesting a key role for root reserves in the overall carbon economy of the vine.

2. Carbohydrate reserve concentrations of the various perennial tissues, although differing in absolute concentrations, generally followed a similar pattern over the season. That is, that concentrations declined in all tissues to a minimum shortly after flowering, and then increased again through season with only a slight delay around veraison to be largely replenished by harvest. Unlike findings from previous research with heavily cropped Semillon, the post-harvest period did not appear to be critical for carbohydrate reserve accumulation in the season of the present study, and suggests that the importance of the post-harvest period is very much dependant on crop load and conditions that influence reserve replenishment prior to harvest.

3. The pattern of carbohydrate reserve mobilization and replenishment appeared to strongly reflect the seasonal changes in carbon demand and supply as fine root growth, developing shoots utilized stored carbohydrates, and then later, ripening fruit and storage sinks appeared to compete for carbohydrates supplied from recent assimilation. The seasonal dynamics of carbohydrates, even on concentration basis, may therefore provide a useful physiological indicator of internal carbon availability.

4. For the subsequent survey and reserve management studies presented in this report, the results of this whole vine study suggest that sub-sampling wood and root tissue in the vineyard can provide a reasonable reflection of the overall carbohydrate reserve status, or relative changes during the season. However, while the importance of the root system for overall carbohydrate storage is also demonstrated, the variability amongst published reports on root biomass allocation suggest that obtaining an accurate measure of reserve storage capacity would be impossible without excavating whole vines.

3 Carbohydrate reserve status survey of commercial vineyards

3.1 Introduction

Carbohydrate reserve concentrations in the perennial tissues of grapevines vary according to developmental stage, with the timing of mobilization and replenishment broadly reflecting the seasonal pattern of internal carbon availability as determined by photosynthetic output and demand from sinks such as growing shoots and ripening fruit. However, it has been demonstrated that cultural factors such as pruning practice (Sommer & Clingeleffer 1996), leaf removal (Bennett et al. 2005), crop removal (Goffinet 2004), harvest date and water deficit (Smith & Holzapfel 2009) can all impact on the capacity of the vine to replenish or store carbohydrates. Consequently, while it may be reasonable from a physiological perspective to assume that a vine be at least able to replace the carbohydrate reserves that were used during the season, this may not always occur where environmental conditions or production objectives change between seasons.

Elsewhere in this report we have presented results from two studies. In the first of these, whole Shiraz vines were excavated to determine the within vine partitioning of carbohydrate reserves, and the relationship between the reserve content of various vine tissues during the season (Section 2). In the second study, the concentrations of carbohydrate reserves in perennial tissues were assessed on a monthly basis in two Shiraz vineyards with differing yield levels (Appendix 4). The results from these studies provide an understanding of within season carbohydrate reserve dynamics, but for the broader objectives of the project, which were to investigate the relationship between reserves and reproductive development, a better understanding of factors that influenced between vineyard and season variation in carbohydrate reserve accumulation was also required.

The approach taken to address these questions was to undertake a carbohydrate reserve survey of vineyards from a range of locations across South Eastern NSW that differed in climatic conditions and/or management practices. A key objective of the survey was to characterize the variability of winter carbohydrate status between vineyards and seasons, look at the possible contribution of nutrition and irrigation to any observed variation, and establish if the reserve status of a vineyard can be characterized in a similar manner to the standard ranges developed for plant nutrient analysis. This information would also provide a basis for interpretation of other research findings, particularly in regard trials where reserve accumulation is deliberately manipulated. A second objective was to look at potential relationships between carbohydrate levels, fruitfulness and yield in the following season with a view to determining if there is evidence of any link between winter carbohydrate reserve status and seasonal yield fluctuations.

3.2 Materials and methods

3.2.1 Site locations and sampling strategy

A total of 34 Shiraz and Chardonnay blocks in commercially operated vineyards were selected across the Riverina, Gundagai, Tumbarumba and Young GI regions as summarized in Table 3.1. These blocks were considered representative of the range of vines, production objectives and management practices that are found across each of these regions. Within each of these survey blocks, five panels of four vines were selected and tagged after leaf fall in 2005. The position of each replicated panel was not chosen based on any pre-existing spatial information, but where possible, one panel of vines was located near the centre of the block, and the other four approximately within each quarter of the block, or towards the corners of the block as shown in Figure 3.1.

Prior to pruning in 2005, 2006 and 2007 wood, root and 4-node cane samples were collected from each panel of four vines. The wood samples were collected with a 4.8 mm drill bit to a depth visually estimated as the centre of the cordon or trunk, and pooled across the four vines. Root samples ranging in diameter from 3 to 7 mm were collected from near the base of each vine, and again pooled across the four vines. Two 4-node canes were collected from each vine, with four canes from the total of eight collected from each panel used for bud fruitfulness assessments and the other four used for carbohydrate analysis. The latter canes cut in half between nodes 2 and 3, such that the carbohydrate content of nodes 1 and 2 and nodes 3 and 4 could be determined separately. Only data for nodes 1 and 2 are presented here, and are subsequently referred to as spurs. All samples collected for carbohydrate and nutrient analysis were oven dried at 70^{o}C and ground to 0.12 mm. Roots were washed in phosphate free detergent and rinsed with de-ionized water prior to oven drying.



Figure 3.1 Aerial view showing the approximate arrangement of the five replicate panels of four vines monitored over three years at each survey site (a), and (b), collecting cane, wood and root samples during winter 2005 for bud-fruitfulness assessments and carbohydrate analysis.

3.2.2 Bud-fruitfulness assessments

Bud-fruitfulness was determined by dissection under stereo microscope for buds at nodes 1 to 3, with node 1 defined as the first bud with more than 5 mm separation from the base of the cane. The number of inflorescences primordia in the primary bud was counted, but if the primary bud was dead as a result of primary bud necrosis (PBN), the number of inflorescence primordia in the largest secondary bud was recorded instead. Where both primary and secondary buds were dead, this was also noted.

For this report, fruitfulness data has been presented as maximum potential fruitfulness (average number of inflorescences in living primary buds) and average fruitfulness (average of primary or secondary buds, allowing for PBN or dead buds). The distinction between these values has been made to separate the propensity to initiate inflorescences (and contributing physiological factors at the time), from the average fruitfulness of buds, which does not allow a separation of the processes of inflorescence initiation and maintenance.

Table 3.1 Details of Shiraz and Chardonnay vineyards used in the survey. Blank values still being confirmed at the time of writing.

	Location	Variety	Rootstock	Year planted	Pruning method	Irrigation method	Density (ha^{-1})
R-CHA-1	Riverina	Chardonnay	Ramsey	1996	hedge	furrow	1190
R-CHA-2	Riverina	Chardonnay	Ramsey	2002	spur	furrow	1126
R-CHA-3	Riverina	Chardonnay	Ramsey	1997	spur	drip	904
R-CHA-4	Riverina	Chardonnay	Ramsey	2002	spur	furrow	1208
R-CHA-5	Riverina	Chardonnay	Ramsey	2002	spur	furrow	1208
R-CHA-6	Riverina	Chardonnay	Own roots	2003	spur	furrow	1709
R-CHA-7	Riverina	Chardonnay	Ru-140	2001	spur	drip	1333
R-CHA-8	Riverina	Chardonnay	Ramsey	1997	spur	drip	1086
S-CHA-1	Gundagai	Chardonnay	Own roots	1995	spur	drip*	1970
S-CHA-2	Hilltops	Chardonnay	Own roots	1995	hedge	drip*	1450
S-CHA-3	Hilltops	Chardonnay	Own roots	1995	hedge	drip*	1287
S-CHA-4	Tumbarumba	Chardonnay	Own roots	1995	spur	drip	1819
S-CHA-5	Hilltops	Chardonnay	Own roots	2004	spur	drip*	1521
S-CHA-6	Gundagai	Chardonnay	Own roots	1998	spur	drip	1621
S-CHA-7	Tumbarumba	Chardonnay	Own roots	-	spur	drip	3010
S-CHA-8	Gundagai	Chardonnay	Own roots	-	spur	drip*	1982
R-SHZ-1	Riverina	Shiraz	Own roots	1997	hedge	furrow	1736
R-SHZ-2	Riverina	Shiraz	Own roots	-	spur	drip	1502
R-SHZ-3	Riverina	Shiraz	Own roots	1997	spur	drip	1226
R-SHZ-4	Riverina	Shiraz	Own roots	1988	spur	furrow	1543
R-SHZ-5	Riverina	Shiraz	Grafted	1995	hedge	furrow	1077
R-SHZ-6	Riverina	Shiraz	Own roots	-	hedge	furrow	1684
R-SHZ-7	Riverina	Shiraz	Ramsey	-	spur	drip	1333
R-SHZ-8	Riverina	Shiraz	Own roots	1995	spur	drip	999
S-SHZ-1	Gundagai	Shiraz	Own roots	1997	spur	drip*	1634
S-SHZ-2	Hilltops	Shiraz	Own roots	2002	hedge	drip	1754
S-SHZ-3	Hilltops	Shiraz	Own roots	1995	hedge	drip*	1467
S-SHZ-4	Tumbarumba	Shiraz	Own roots	1995	spur	drip	2222
S-SHZ-5	Hilltops	Shiraz	Own roots	1999	spur	drip	1628
S-SHZ-6	Hilltops	Shiraz	Own roots	1995	spur	drip*	1413
S-SHZ-7	Gundagai	Shiraz	Own roots	2000	spur	drip	1800
S-SHZ-8	Gundagai	Shiraz	Own roots	1997	spur	drip	1852
S-SHZ-9	Tumbarumba	Shiraz	Own roots	-	spur	drip	993
S-SHZ-10	Gundagai	Shiraz	Own roots	-	spur	drip*	1982

* drip irrigated, but limited water applied or available through the survey period.

3.2.3 Carbohydrate and nutrient analysis

Carbohydrate concentrations for all collected root, wood and spur samples to the replicate level were determined using the methodology described in section 2.2.3. However, due to time and cost limitations, nutrient analysis was only determined at the vineyard level using a bulked sample with equal proportions of root, wood or spur tissue from each replicate. Total N content was determined with a total combustion gas chromatograph using a 50 mg sub-sample of the bulked tissue (Vario Macro, Elementar, Hanau, Germany). The other macro nutrient analysis was undertaken with an inductively coupled plasma optical emission spectrometer (ICP-OES) (ARL 3580B, Applied Research Laboratories, Ecublens, Switzerland) on a 300-400 mg sub-sample.

3.2.4 Statistical analysis

Data was fitted using a linear mixed model in ASReml 2 (VSN International Ltd, Hemel Hempstead, UK). Region (Riverina, SW Slopes), sub-region (Riverina, Gundagai, Tumbarumba, Hilltops), year (2005, 2006, 2007), variety (Shiraz, Chardonnay), and two way interactions within year were fitted as fixed effects. All other two and multi-way interactions were fitted as random effects (irrigation, rootstock, pruning method, and site). After testing each term, predicted values for each variate were determined from a reduced model of significant random and fixed effects.

3.3 Results and discussion

For this report, the presentation of data has been divided into two sections. The first section provides an overview of the raw data, and a discussion of general trends without statistical analysis. This has been included for the benefit of growers involved in the study who may be interested in individual block data, or those wishing to compare the results to other published studies. The second section then deals with the separate statistical analysis of the carbohydrate reserve and bud-fruitfulness data, and some of the factors contributing to the vineyard to vineyard variability observed in these parameters. A preliminary analysis of the relationship between carbohydrate reserves, bunch initiation and bud necrosis has also been included, as a key broader objective of the project was understanding the importance of carbohydrate reserves for reproductive development. However, the extension of the survey into a second and third season from the single season survey initially proposed has provided an opportunity for a much more comprehensive analysis of possible factors underlying seasonal yield variability, and these will be presented when the collection of management and yield data from all sites is completed.

3.3.1 Carbohydrate reserves

Across all sites and seasons, root TNC reserve concentrations were on average higher than for wood or spur tissue (Table 3.2), making up an average of 21.5% of the root dry weight compared to 14.3% for wood and 14.0% for the spurs. The corresponding concentration ranges were 9.9 to 41.8%DW for the roots, 3.4 to 20.7%DW for the wood, and 10 to 17.4%DW for the spurs. Root TNC reserves concentrations also showed the greatest variability between vineyards with average coefficient of variation within seasons of 31.7% compared to 20.9% for the wood, and 9.2% for spur tissue. For within vineyard, or between replicate variability, the corresponding values were 19.8%, 11.0% and 7.6% respectively. The variability in carbohydrate reserve status between vineyards was therefore greater than the within vineyard variation.

A significant finding of the study, and one consistent with other research undertaken at the NWGIC (Field et al. 2008), is that roots of vines grafted to Ramsey were found to have generally lower starch concentrations than roots of comparable diameter from own-rooted *Vitis vinifera*. It has previously shown that potted Shiraz on Ramsey produced vines with lower carbohydrate reserve concentrations and a smaller root system than own rooted vines

(Smith 2004), and the current findings support the suggestion that part of the vigour promoting characteristic of this rootstock may be related to a grafting induced shift in carbon partitioning to the shoot. Another finding consistent with earlier work (Smith & Holzapfel 2009), is that carbohydrate reserves were often lower in vineyards affected by limited water supply over several seasons. However, this effect was more apparent in the Hilltops region than Gundagai region, where water availability was also limited in some seasons, suggesting an additional influence of environmental conditions or cultural practice on carbohydrate reserve accumulation in this region.

One of the original reasons for undertaking the survey was to determine if the carbohydrate reserves status of vineyards varies substantially from season to season, and if there is any evidence of a link between reserve accumulation and seasonal yield fluctuations. Although this question is addressed in more detail later with the accompanying statistical analysis, the trends in the data do provide some insight. Firstly, that there is evidence of an overall regional effect on the change in carbohydrate reserve concentrations between seasons, as the relative changes in spur and wood reserves over the three years are similar within each of the Riverina and broader 'SW slopes' regions (Figure 3.2). This is consistent with the region-wide response typical of seasonal yield fluctuations, so raises the possibility of a link between the two. Secondly, that some vineyards exhibited fluctuations in root reserves of sufficient magnitude that might be expected to influence reproductive development based on results of earlier studies (Bennett et al. 2005, Smith & Holzapfel 2009).

3.3.2 Bud-fruitfulness

Across all of the surveyed blocks, the maximum number of inflorescences initiated per primary bud was slightly higher for Shiraz than Chardonnay at 1.80 and 1.77 respectively, and higher in the Riverina than the Tumbarumba, Gundagai and Hilltops regions at 1.90, 1.76, 1.76 and 1.59 respectively. However, high levels of PBN were seen in Shiraz (Table 3.3), and many were above the 20% level considered sufficient to impact on yield (Rawnsley & Collins 2005). This was particularly evident across the SW slopes regions where average level of PBN were 38% compared to 26% in the Riverina. On average, 6% of the 6120 buds assessed were found to be completely dead, but at an individual vineyard level, the incidence ranged between 0 and 35% (data not shown). At four Shiraz blocks in the SW slopes the combined incidence of PBN and dead buds reached more than 60% in some seasons. The loss of inflorescence primordia due to these effects reduced the average fruitfulness of Shiraz to a greater extent than Chardonnay, so that the overall average for these two varieties across all regions and seasons was 1.42 and 1.25 respectively. Within regions, the values were reduced to 1.41. 1.18, 1.17 and 1.12 for the Tumbarumba, Gundagai and Hilltops regions respectively. The final fruitfulness of these vineyards was therefore not only determined by the rate of inflorescence initiation, but also the extent to which these were able to be maintained through the rest of the season.

The possible relationships between carbohydrate reserves and fruitfulness will be discussed in more detail in the following section. However, reserves were not associated with inflorescence initiation, but did appear to influence the subsequent loss of inflorescences due to PBN or complete bud death. Previous authors have suggested a link between phosphorous (P) nutrition and reproductive development (Skinner & Matthews 1989), and in the present study high P concentrations in the wood and root were associated with higher fruitfulness, and high P concentrations in the root, root and spur tissue were associated with a lower incidence of PBN in that season. Vines in the SW Slopes regions were generally lower in P, which may be a consequence of low nutrient mobility and reduced uptake under low irrigation and rainfall. However, there did not appear to be any link between water availability and inflorescence initiation, nor evidence that water stress increased the incidence of PBN. The worst affected vineyards appeared to be the Shiraz vineyards in the SW Slopes that were not affected by water stress, at least during the earlier part of the season. The was also a clear interactive effect of variety and region across seasons, as the average rate of PBN for Shiraz in the SW Slopes regions was substantially higher than the

Riverina in 2005, but less so in 2006 and 2007. In contrast, there was no difference in Chardonnay PBN between regions in 2005, but a higher incidence amongst the SW Slopes vineyards in 2006 and 2007.

Table 3.2 Average tissue concentration of starch, soluble sugars and TNC reserves from 34 Shiraz and Chardonnay blocks surveyed across Southern NSW over winter 2005, 2006 and 2007.

	Root CHO %DW			Wood CHO %DW			Spur CHO %DW		
Site ID	starch	sugars	total	starch	sugars	total	starch	sugars	total
R-CHA-1	13.2	2.7	15.9	6.6	8.5	15.1	5.9	8.0	13.9
R-CHA-2	12.0	2.6	14.6	8.9	7.9	16.9	6.4	7.6	14.0
R-CHA-3	14.2	2.6	16.8	9.2	7.0	16.3	7.2	7.5	14.7
R-CHA-4	12.2	2.6	14.7	8.3	7.3	15.6	6.7	6.4	13.2
R-CHA-5	14.1	2.6	16.7	7.3	7.7	15.0	6.3	7.6	13.9
R-CHA-6	21.4	2.4	23.8	6.7	7.9	14.6	6.3	7.0	13.3
R-CHA-7	22.4	3.3	25.7	8.3	6.9	15.3	7.3	7.3	14.6
R-CHA-8	14.6	2.4	17.0	7.9	8.0	15.9	6.2	8.1	14.2
S-CHA-1	18.2	2.7	20.9	6.3	7.5	13.7	7.0	7.1	14.1
S-CHA-2	11.0	2.7	13.7	8.2	4.9	13.2	7.1	5.5	12.5
S-CHA-3	9.6	2.8	12.4	6.4	5.3	11.7	7.3	5.7	13.0
S-CHA-4	23.3	2.5	25.7	9.7	6.6	16.4	7.9	5.8	13.7
S-CHA-5	8.8	2.8	11.6	8.8	5.0	13.8	7.9	5.0	12.9
S-CHA-6	17.7	2.8	20.5	8.1	6.5	14.5	6.7	6.5	13.2
S-CHA-7	17.1	2.0	19.0	4.3	9.5	13.8	4.9	8.8	13.7
S-CHA-8	18.8	2.6	21.3	3.5	5.1	8.6	6.2	7.8	14.0
R-SHZ-1	25.3	2.7	27.9	4.2	6.5	10.7	5.5	8.5	14.0
R-SHZ-2	34.5	2.6	37.1	8.8	7.2	16.0	6.2	9.2	15.4
R-SHZ-3	24.0	2.7	26.7	8.5	7.6	16.1	5.9	8.8	14.7
R-SHZ-4	22.9	2.2	25.0	7.5	6.1	13.6	6.0	8.1	14.1
R-SHZ-5	13.4	2.3	15.7	11.4	6.6	18.0	7.8	7.4	15.2
R-SHZ-6	25.4	2.6	28.0	4.2	6.3	10.5	6.1	7.5	13.6
R-SHZ-7	14.3	3.0	17.3	11.9	7.5	19.5	7.5	8.1	15.6
R-SHZ-8	33.3	3.1	36.4	10.2	7.9	18.1	6.5	9.6	16.1
S-SHZ-1	21.9	2.6	24.5	7.4	7.6	15.0	6.5	7.9	14.5
S-SHZ-2	21.5	2.6	24.1	9.5	5.3	14.8	7.9	5.2	13.0
S-SHZ-3	9.6	2.5	12.1	5.3	5.2	10.5	5.9	5.7	11.6
S-SHZ-4	23.3	1.9	25.2	6.2	8.9	15.2	6.3	9.1	15.4
S-SHZ-5	22.8	2.3	25.1	7.1	7.5	14.5	7.2	7.5	14.7
S-SHZ-6	17.1	2.5	19.5	8.7	4.8	13.5	7.8	5.4	13.2
S-SHZ-7	19.7	2.9	22.6	8.3	6.7	15.1	6.6	6.8	13.4
S-SHZ-8	25.4	2.5	28.0	6.6	7.1	13.7	7.3	6.8	14.1
S-SHZ-9	22.7	1.8	24.5	7.7	8.1	15.8	6.0	8.9	14.9
S-SHZ-10	17.7	2.1	19.8	1.9	3.1	5.0	6.3	7.5	13.7

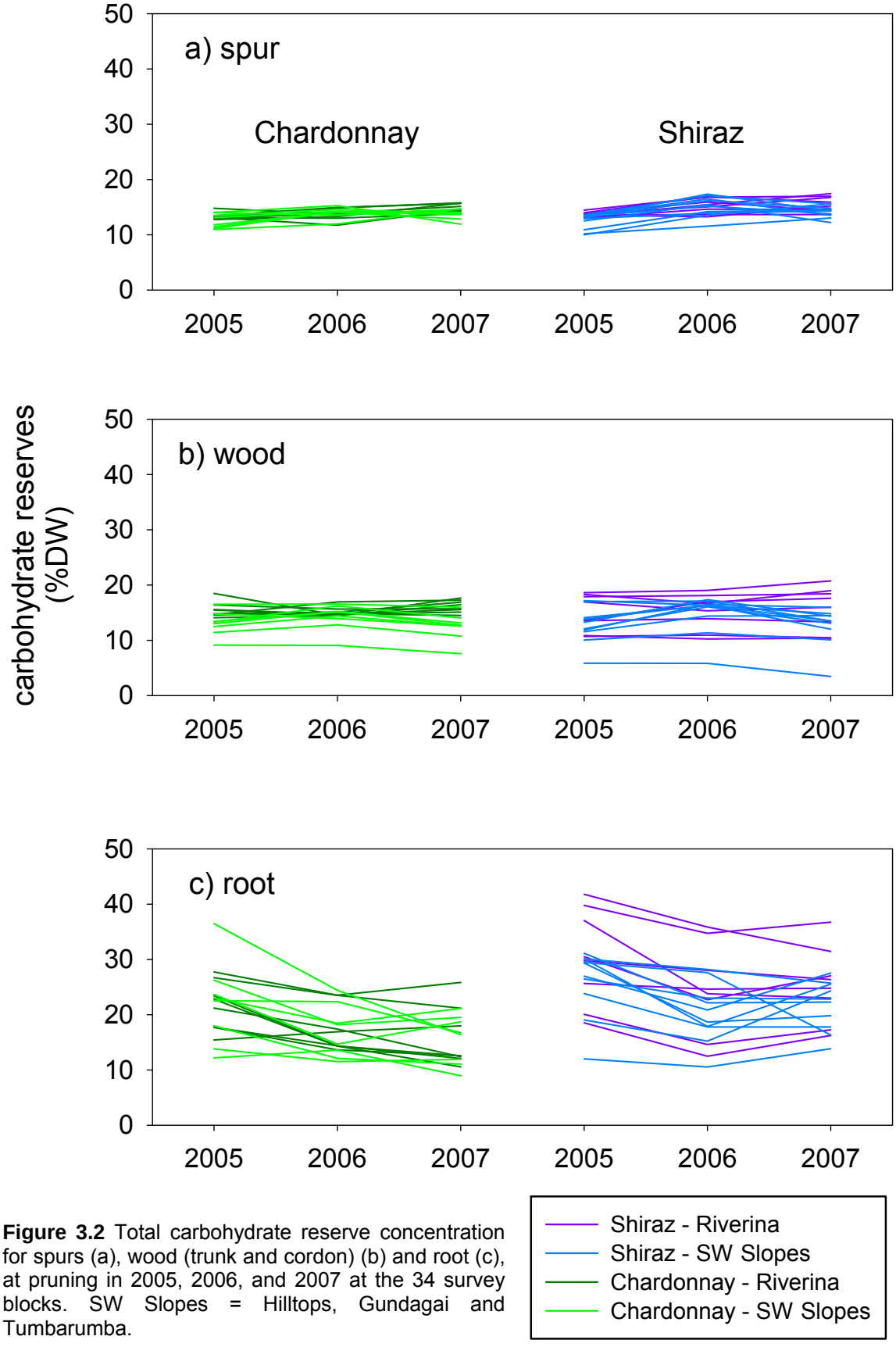


Figure 3.2 Total carbohydrate reserve concentration for spurs (a), wood (trunk and cordon) (b) and root (c), at pruning in 2005, 2006, and 2007 at the 34 survey blocks. SW Slopes = Hilltops, Gundagai and Tumbarumba.

Table 3.3 Average tissue concentration of starch, soluble sugars and TNC reserves from 34 Shiraz and Chardonnay blocks surveyed across Southern NSW over winter 2005, 2006 and 2007.

	Initiated bunch #			Average bunch #			PBN %		
Site ID	2005	2006	2007	2005	2006	2007	2005	2006	2007
R-CHA-1	1.96	1.81	1.96	1.57	1.37	1.68	22	15	15
R-CHA-2	2.02	1.99	1.97	1.63	1.78	1.83	17	12	7
R-CHA-3	1.77	1.77	1.98	1.47	1.58	1.50	23	5	23
R-CHA-4	2.06	1.88	2.00	1.73	1.58	1.50	18	12	23
R-CHA-5	1.98	1.97	2.01	1.67	1.67	1.62	13	15	18
R-CHA-6	1.98	1.91	1.96	1.57	1.38	1.52	17	25	18
R-CHA-7	1.90	1.84	1.89	1.48	1.60	1.55	22	7	13
R-CHA-8	1.97	1.71	1.74	1.58	1.53	1.28	20	7	28
S-CHA-1	1.77	1.62	1.83	1.45	1.32	1.53	13	12	15
S-CHA-2	1.41	1.26	1.46	0.85	0.93	1.13	23	18	25
S-CHA-3	1.43	1.13	1.18	1.08	0.87	0.88	25	20	23
S-CHA-4	1.73	1.82	1.81	1.55	1.52	1.27	15	22	30
S-CHA-5	1.51	1.61	1.80	1.08	1.38	1.28	30	10	30
S-CHA-6	1.67	1.64	1.59	1.17	1.35	0.87	28	10	47
S-CHA-7	1.76	1.83	1.71	1.57	1.63	1.53	12	8	12
S-CHA-8	1.78	1.70	1.91	1.62	1.10	1.43	10	23	17
R-SHZ-1	1.84	1.94	1.96	1.53	1.58	1.60	13	13	17
R-SHZ-2	1.78	1.75	2.00	1.43	1.40	1.52	28	12	37
R-SHZ-3	1.93	1.64	1.95	1.53	1.10	1.68	20	38	17
R-SHZ-4	1.96	1.84	1.88	1.68	1.45	1.38	20	18	37
R-SHZ-5	1.94	1.79	1.98	1.58	1.12	1.23	23	40	38
R-SHZ-6	1.91	1.62	1.95	1.37	1.10	1.52	25	22	20
R-SHZ-7	1.86	1.71	2.00	1.33	0.98	1.55	38	35	28
R-SHZ-8	1.94	1.93	1.88	1.43	1.57	1.23	28	13	38
S-SHZ-1	1.80	1.63	1.98	0.95	1.33	0.98	50	27	50
S-SHZ-2	1.47	2.00	1.76	1.23	1.40	1.32	32	30	27
S-SHZ-3	1.51	1.56	1.48	0.80	1.03	1.25	50	32	28
S-SHZ-4	1.45	1.84	1.51	0.72	1.17	1.10	53	33	25
S-SHZ-5	1.73	1.82	1.98	1.02	1.20	1.57	55	33	32
S-SHZ-6	1.76	1.84	1.53	0.98	1.08	1.18	50	33	27
S-SHZ-7	1.86	1.44	1.88	0.85	0.80	1.23	58	40	40
S-SHZ-8	1.73	1.74	1.86	1.05	0.97	1.03	58	45	47
S-SHZ-9	1.87	1.83	1.96	1.32	0.95	1.80	28	50	12
S-SHZ-10	1.95	1.77	1.86	1.37	0.78	1.28	48	25	23

3.3.3 Nitrogen and phosphorous concentrations

The concentrations of N and P in perennial tissues were determined to provide a surrogate measure of nutritional status, as petiole analysis were not undertaken during the survey, and records of fertilizer applications were not available for all sites. These nutrients are also carried through the winter in perennial tissue, and approximately 50% of the following seasons requirements for these nutrients thought to come from stored reserves. They therefore play a similar role to carbohydrate reserves, and provide an alternative source of nutrients to support rapidly growing shoots if the uptake from roots is not able to meet this

demand. The potential influence of P nutrition and bud fruitfulness has already been mentioned, but previous studies also suggest a link between N reserves and the growth and fruiting response of grapevines (Cheng et al. 2004, Weyand & Schultz 2006). However, carbohydrates are used for N uptake and assimilation, and also for the growth of new roots that absorb soil N in the first place. Therefore, it may be difficult to separate the effects of the two on vine growth and development, and may depend on which is most limiting.

For this report we have presented the individual average site data for comparison of N and P concentrations with carbohydrate reserves (Table 3.4), but have not continued beyond this point with a detailed statistical analysis. It is intended that analysis of samples at the replicate rather than vineyard level can be completed in the future, so that a comprehensive multivariate analysis can be undertaken to fully investigate the interactions between carbohydrate reserves and other stored nutrients.

The average concentration of N in the root tissue was 1.3% on a dry weight basis, followed by spur and wood at 0.63% and 0.29% respectively. The concentrations of P were eight times lower across all tissues, but the ranking was the same with root, spur and wood concentrations 0.18%, 0.04% and 0.07% respectively. This compares to 21.5%, 14.0% and 14.3% for total carbohydrates in the same tissue. Therefore, relative to carbohydrate reserves, a higher proportion of total N and P reserves are likely to be stored in the roots. The concentrations of N and P were positively correlated in all tissues, but there did not appear to be any relationship between N and carbohydrate reserves. Consequently there was considerable variation between sites in the ratio of total N to total carbohydrates. This is a very crude measure of the relative supply of stored carbon and nitrogen in each tissue, as relative availability of the two may differ according to storage pool and form. However, the findings suggest that vineyards can vary significantly in their relative storage of carbohydrates and nitrogen.

3.3.4 Factors contributing to variation in winter carbohydrate reserve status

Of all the carbohydrate data collected during the survey, the factor accounting for the largest percentage of variation was tissue type. There was also significant variation between seasons and GI regions within tissue type, and between rootstock and irrigation within regions. Overall, root reserve concentrations were significantly higher than for wood and spur tissue ($p < 0.001$), while wood and spur did not differ from each other. When the varieties are separated, total carbohydrate reserves concentrations also did not differ in the above ground tissues of Shiraz and Chardonnay (Table 3.5). However, Shiraz roots were significantly higher than those of Chardonnay. Between carbohydrate reserve fractions, the differences in total reserves were largely determined by the starch concentrations. Small differences in soluble sugar concentrations in the spurs were seen between varieties, but this was not sufficient to reflect in the overall reserve status of the tissue.

At the level of GI region, there was no difference between total reserve concentrations in the above ground tissues, but root reserves were significantly lower for the Hilltops vineyards than other three regions ($p < 0.001$). This difference in storage of root reserves may be related to water stress, as a number of vineyards in the Hilltops region had been affected by water shortages over consecutive seasons. With the Hilltops regions reserve concentrations were also significantly lower on drought affected vineyards than those with more reliable water supplies (not shown). However, at other sites in the Gundagai region, where water availability was also limited by drought or deliberate irrigation strategy, reserve concentrations did not differ significantly from vineyards with higher water inputs.

It is difficult to explain these contrasting effects before the collection of yield, irrigation and rainfall data is finalized, and the extent of water deficit defined more clearly for each vineyard over the three seasons. We have previously shown that carbohydrates reserves, although lower in vineyards affected by prolonged water shortages, are not depleted to the same extent within a season due to reduced canopy growth and lower yields (Smith & Holzapfel 2009). The timing of any water deficit may also be important, as a vine that establishes a

large canopy and crop early in the season and then runs out of water may be more prone to a depletion of reserves than one that can adapt to lower water availability from the start of the season through reduced canopy growth and reduced flower numbers or fruit-set. In general, we did not find any evidence to suggest that reserves will be completely depleted under water stress, as there are adaptive processes to ensure this does not happen. On a concentration and bulk tissue basis at least, it also appears that water stress over a shorter term is not likely to cause large decline in total carbohydrate reserve storage.

Table 3.4 Average concentrations of nitrogen and phosphorous in the root, wood and spur tissue during winter in 2005, 2006 and 2007, and corresponding ratio of total N to total CHO.

	Root (%DW)			Wood (%DW)			Spur (%DW)		
Site ID	N	C/N	P	N	C/N	P	N	C/N	P
R-CHA-1	1.2	13.3	0.18	0.25	60.0	0.039	0.61	23.0	0.064
R-CHA-2	1.5	9.7	0.32	0.37	48.2	0.051	0.63	22.4	0.093
R-CHA-3	1.7	9.9	0.26	0.36	45.2	0.050	0.70	21.3	0.089
R-CHA-4	1.7	9.0	0.28	0.38	42.5	0.062	0.69	19.5	0.100
R-CHA-5	1.6	10.7	0.22	0.34	45.0	0.046	0.68	21.3	0.068
R-CHA-6	1.4	16.9	0.29	0.38	40.4	0.047	0.68	21.0	0.067
R-CHA-7	1.2	21.0	0.21	0.32	48.4	0.046	0.73	21.1	0.085
R-CHA-8	1.5	11.7	0.27	0.29	55.8	0.043	0.62	24.3	0.068
S-CHA-1	1.0	20.8	0.12	0.26	53.1	0.036	0.66	22.7	0.059
S-CHA-2	0.9	15.8	0.08	0.24	58.1	0.031	0.61	20.6	0.061
S-CHA-3	1.0	13.0	0.10	0.21	56.4	0.030	0.62	21.3	0.059
S-CHA-4	1.4	19.3	0.20	0.35	46.5	0.043	0.64	21.4	0.085
S-CHA-5	1.0	11.9	0.16	0.26	53.2	0.035	0.61	21.1	0.074
S-CHA-6	1.0	20.3	0.14	0.24	62.6	0.034	0.49	27.0	0.057
S-CHA-7	1.1	17.3	0.19	0.24	59.4	0.039	0.64	24.4	0.094
S-CHA-8	1.6	13.7	0.27	0.25	34.6	0.046	0.63	22.6	0.097
R-SHZ-1	1.4	20.8	0.12	0.30	36.5	0.034	0.59	24.0	0.060
R-SHZ-2	1.4	26.9	0.18	0.31	51.1	0.040	0.63	24.5	0.068
R-SHZ-3	1.1	24.1	0.20	0.30	53.8	0.038	0.55	27.1	0.064
R-SHZ-4	1.5	17.0	0.23	0.33	41.1	0.041	0.69	20.7	0.073
R-SHZ-5	1.4	11.5	0.22	0.34	52.8	0.043	0.65	23.7	0.070
R-SHZ-6	1.5	19.4	0.14	0.27	39.4	0.036	0.68	20.1	0.065
R-SHZ-7	1.0	17.0	0.17	0.27	74.6	0.040	0.65	25.1	0.075
R-SHZ-8	1.2	29.6	0.18	0.30	61.5	0.041	0.62	27.3	0.072
S-SHZ-1	1.2	20.8	0.13	0.28	54.8	0.034	0.54	27.5	0.053
S-SHZ-2	1.2	20.6	0.18	0.32	47.5	0.040	0.63	20.8	0.066
S-SHZ-3	1.1	11.0	0.07	0.25	42.7	0.032	0.70	17.1	0.057
S-SHZ-4	0.9	31.4	0.07	0.31	48.2	0.030	0.59	28.0	0.048
S-SHZ-5	1.1	22.5	0.13	0.30	48.0	0.036	0.59	25.3	0.059
S-SHZ-6	1.0	19.0	0.13	0.28	49.0	0.036	0.68	19.8	0.068
S-SHZ-7	1.3	17.7	0.14	0.29	54.5	0.034	0.58	23.7	0.054
S-SHZ-8	1.2	22.9	0.10	0.29	49.0	0.032	0.59	24.6	0.048
S-SHZ-9	1.5	17.6	0.19	0.26	64.3	0.039	0.62	26.6	0.078
S-SHZ-10	1.6	12.7	0.16	0.26	20.7	0.048	0.75	18.9	0.076

Table 3.5 Predicted carbohydrate concentrations in perennial tissues of 34 Shiraz and Chardonnay vineyards in Southern NSW surveyed between 2005 and 2007.

		Carbohydrate concentration (%DW)					
Tissue	Variety	Starch		Sugars		Total	
Spur	Shiraz	6.8	a	7.4	b	14.3	a
	Chardonnay	6.8	a	7.0	a	13.7	a
LSD		1.76		0.35		1.79	
Wood	Shiraz	7.5	a	6.7	a	14.3	a
	Chardonnay	7.3	a	7.0	a	14.1	a
LSD		1.74		0.36		1.77	
Root	Shiraz	19.6	b	2.3	a	22.4	b
	Chardonnay	16.6	a	2.6	a	19.3	a
LSD		1.75		0.35		1.78	

At the level of season, there were significant changes in carbohydrate reserve concentrations between years for all tissues ($p < 0.01$). For root tissue, the concentration was significantly higher in 2005 than 2006 or 2007, while the spurs showed the opposite response with lower concentrations in 2005 than 2006 and 2007. For wood tissue, concentrations were higher in 2006 than the other two seasons, so effectively these findings indicate that root, wood and spur tissue will not necessarily respond in the same manner to seasonal factors that can influence carbohydrate reserve accumulation. There were some significant interactions between GI region and season (Table 3.6), such as the relatively high concentrations of root reserves at Tumbarumba in 2005, or lack of difference in wood reserve concentrations between regions in 2006. However, they do not substantially change the interpretation provided by the mean regional or seasonal effects.

Table 3.6 Predicted carbohydrate concentrations in perennial tissues for GI region and season for 34 Southern NSW vineyards surveyed between 2005 and 2007.

		Carbohydrate concentration (%DW)					
Year	GI region	Root		Wood		Spur	
2005	Riverina	25.6	de	15.6	abcd	13.7	ab
2005	Gundagai	26.2	e	11.8	ab	13.0	ab
2005	Hilltops	16.6	ab	12.2	abc	11.3	a
2005	Tumbarumba	30.5	f	16.4	d	13.5	ab
2006	Riverina	20.6	bc	15.6	bcd	14.5	ab
2006	Gundagai	21.5	cd	13.8	abcd	14.6	ab
2006	Hilltops	13.7	a	14.5	abcd	13.8	ab
2006	Tumbarumba	20.1	bc	16.0	cd	16.0	b
2007	Riverina	20.0	bc	16.4	d	15.3	ab
2007	Gundagai	19.8	bc	11.5	a	13.8	ab
2007	Hilltops	15.1	a	13.0	abcd	14.3	ab
2007	Tumbarumba	21.0	c	13.6	abcd	14.0	ab
LSD		4.2		4.2		4.1	

Within the Riverina, as mentioned in Section 2.3.1, a significant amount of variability was also associated with rootstock. For both Shiraz and Chardonnay vines grafted to Ramsey, root starch concentrations were significantly lower than own rooted vines, and resulted in lower total reserve concentrations (Table 3.7). This did not appear to be an effect of grafting *per se*, as the concentration of carbohydrates in the roots of Ruggeri 140 when grafted to Chardonnay were similar to that of Chardonnay vines on own roots. In other studies we have also found that the concentration of carbohydrates in the roots of Ramsey is lower than for own-roots or Ruggeri 140, so it appears that there may be a real difference in the capacity or propensity for reserve storage between *Vitis* species or their hybrids.

In the whole vine study described in the previous chapter, it was found that 64% of total reserves were stored in the root system of own-rooted Shiraz. In the survey we have shown that root reserve concentrations are also the most variable of perennial tissues, whether due to environmental responses or differences between species, so root the root system appears to the most important tissue in terms of total reserve capacity, but also the one most likely to be affected by changes in cultural practice or environmental conditions. The collection of wood and spur tissue is easier than for roots, but the variability in reserve concentrations between vineyards and seasons is less. Within a season, the carbohydrate concentrations of various perennial tissues were shown to be strongly correlated (Table 2.1 in section 2), but the results of the survey indicate that the accumulation of reserves in the root, wood and spur tissue can be differentially affected between seasons. While a question remains as to the relative or absolute importance of the various reserve pools for vine growth and productivity, these findings suggest that carbohydrate concentrations of both above and below ground tissue need to be determined to properly characterize the reserve status of a vine.

Table 3.7 Predicted total winter carbohydrate concentrations in the perennial tissues of 16 grafted and own-rooted vines in the Riverina over three seasons (2005-2007)

		Carbohydrate concentration (%DW)		
		Root	Wood	Spur
Shiraz	Own-roots	28.5 d	13.8 a	14.3 a
Shiraz	Ramsey	17.7 ab	18.4 a	15.4 a
Chardonnay	Own-roots	23.0 bc	15.8 a	14.0 a
Chardonnay	Ramsey	15.9 a	15.6 a	14.1 a
Chardonnay	Ruggeri 140	23.7 cd	15.6 a	14.5 a
LSD		5.4	5.3	5.3

Despite the variation associated with GI region, season, rootstock and irrigation, a final but important finding of the study with respect to carbohydrates is that the same relative difference between root and wood reserve concentrations of the 34 surveyed vineyards were maintained across seasons. For the roots, the correlation between 2005 and 2006 concentrations was 0.74, and for 2006 to 2007 the correlation coefficient was 0.79. For the wood, the respective values were 0.76 and 0.84. A strong correlation was also maintained between 2005 and 2007. From the results of the whole dynamics study presented earlier, and findings of previously published work (eg. Winkler & Williams 1945, Bennett et al. 2005), it is clear that reserve concentrations can fluctuate considerably during the season according the relative supply and demands for carbon within the vine. However, the results of the survey suggest that vines may reach some sort of equilibrium with respect to the amount of carbohydrates stored each season, but this may not necessarily be the same from vineyard to vineyard. Therefore, absolute concentrations or amounts of reserves may say less about a

vines cropping capacity than the extent of changes within a season. Overall, there may be region wide or season wide effects on reserve accumulation, but relative differences between individual sites will be maintained. In some vineyards we did observe large changes between seasons that were not reflected across the broader region, and possible reasons for this, such as frost or large changes in crop load, are being investigated.

3.3.5 Factors contributing to variation in bud-fruitfulness

For maximum potential fruitfulness, as defined here by the fruitfulness of primary buds not affected by PBN or complete bud death, there was no significant difference between Shiraz and Chardonnay. Neither were there significant effects of irrigation method or rootstock. Instead, the variation in maximum potential fruitfulness was largely related to region and year, and interactions between the two. For the main effects, fruitfulness in the Riverina was higher than the collective SW Slopes regions ($p < 0.001$), and in 2006 fruitfulness was lower on average than 2005 and 2007. However, there were significant region interactions, with Gundagai vineyards having lower fruitfulness in 2006, while fruitfulness was higher at Tumbarumba in that season (Table 3.8). At the Riverina and Hilltops sites there were no differences between seasons. Previous work has indicated a clear link between carbohydrate supply from photosynthesis and inflorescence initiation (eg. Sanchez & Dokoozlian 2005), but our earlier study with Semillon (Smith & Holzapfel 2009) also suggested a possible influence of carbohydrate reserves on fruitfulness when the canopy environment was otherwise similar. However, we found no evidence of any relationship between carbohydrate reserves and the number of initiated inflorescences in the same season, or reserves and the number of inflorescences initiated in primary bud in the following season in the current study. Although the carbohydrate concentrations of bulk tissue collected during winter do not necessarily relate to the carbohydrate reserve status at the time of inflorescence initiation around flowering, these findings tend to support suggestions that the photosynthetic capacity of the shoot is the most important factor for inflorescence initiation.

Table 3.8 Predicted regional values for number of inflorescences initiated per primary buds not subsequently affected by primary bud necrosis, or 'maximum potential fruitfulness'

Year	Region	GI region	Inflorescences initiated in the primary bud	
2005	Riverina	Riverina	1.92	f
2006	Riverina	Riverina	1.82	def
2007	Riverina	Riverina	1.95	f
2005	SW Slopes	Tumbarumba	1.70	bcd
2006	SW Slopes	Tumbarumba	1.83	ef
2007	SW Slopes	Tumbarumba	1.75	cde
2005	SW Slopes	Gundagai	1.79	de
2006	SW Slopes	Gundagai	1.65	abc
2007	SW Slopes	Gundagai	1.85	ef
2005	SW Slopes	Hilltops	1.55	a
2006	SW Slopes	Hilltops	1.61	ab
2007	SW Slopes	Hilltops	1.60	ab
LSD			0.13	

Average bud-fruitfulness values for each of the surveyed vineyards is shown in Table 3.2, and as previously described, average bud fruitfulness was significantly higher in the Riverina than SW Slopes regions ($p < 0.001$), and Chardonnay was significantly higher than Shiraz ($p < 0.001$). The appearance of a significant varietal difference in average fruitfulness, when the number of inflorescences initiated by each variety was initially the same, was due the higher

rate of PBN amongst Shiraz vineyards in both the Riverina and SW Slopes. The incidence of PBN was negatively correlated with P concentrations in the root and wood, and was one of the factors most strongly associated with disorder across all sites. However, there was also evidence of a relationship between carbohydrate reserves and PBN. In all seasons there was a positive correlation between total carbohydrate concentrations in the wood at dormancy and the incidence of PBN, and this was significant in 2005 ($p = 0.022$) and 2007 ($p = 0.002$). High wood carbohydrates were also associated with higher incidence of PBN in the next season, and this was significant from 2006 to 2007 ($p = 0.004$), although not for 2005 to 2006 ($p = 0.071$). There was no relationship between spur carbohydrates and PBN, even though this tissue was in closest proximity to the buds, and might have been expected to more closely reflect the carbohydrate supply experienced by the buds during the previous season.

We have previously found that higher carbohydrate reserves can be associated with increased shoot growth rates (Smith & Holzapfel 2009), so it is possible that the higher wood reserves promoted higher vigour, and turn led to a higher incidence of PBN (Lavee et al. 1981, Dry & Coombe 1994). Primary bud necrosis can in some cases progress to secondary buds (Collins et al. 2006), but in the present study the high levels of PBN did not correspond to high levels of completely dead buds. In 2005 there was no difference between varieties, despite the high rates of PBN seen in Shiraz in that season. In 2006 Shiraz was higher at 9.8% compared to 6.3% for Chardonnay, but in 2007 Chardonnay had significantly more dead buds than Shiraz.

Table 3.9 Predicted values for incidence of primary bud necrosis (PBN) in relation to season, region and variety.

Year	Region	Variety	%PBN	
2005	Riverina	Chardonnay	18.3	b
2006	Riverina	Chardonnay	11.9	a
2007	Riverina	Chardonnay	18.5	b
2005	Riverina	Shiraz	25.1	cd
2006	Riverina	Shiraz	24.0	c
2007	Riverina	Shiraz	28.8	de
2005	SW Slopes	Chardonnay	21.8	bc
2006	SW Slopes	Chardonnay	17.2	b
2007	SW Slopes	Chardonnay	26.3	cde
2005	SW Slopes	Shiraz	46.8	g
2006	SW Slopes	Shiraz	33.7	f
2007	SW Slopes	Shiraz	30.1	ef
LSD			4.6	

The relationship between carbohydrate reserves and yield is obviously of key importance for understanding if the carbohydrate reserve status of a vine in winter can influence the yield in the next season. At the time of writing we are still collecting and verifying yield data for each block, as this has proved more difficult than expected where vineyards have changed ownership during the course of the study, have not been harvested due to severe water stress, or the fruit has been combined across blocks of the same variety within a vineyard. However, based on a very preliminary analysis of the data collected so far, there does appear to be a positive relationship between total carbohydrate concentrations in the wood and yield in the following season. Average bud-fruitfulness is also correlated with yield, although not with wood reserves, suggesting that carbohydrate reserve concentrations of the wood could be used to refine current yield predictions or yield targets based on bud-dissections and retained bud numbers.

3.4 Summary and conclusion

A survey of 34 Shiraz and Chardonnay blocks in Southern NSW was undertaken between 2005 and 2007 to characterize the variability in carbohydrate reserve status between vineyards and look at the relationship between carbohydrate status and reproductive development. Some of the key outcomes were:

1. Carbohydrate reserves were found to vary significantly between vineyards, and to a lesser extent between seasons. Roots stored the highest concentrations of carbohydrate reserves, but also had the highest variability between vineyards. Some of the factors contributing to this variability were rootstock and water stress, where vines grafted to Ramsey, or those experiencing prolonged periods of drought, had significantly lower root reserves. Wood and spur concentrations did not differ from each other, and were also less likely to vary significantly between vineyards.

2. Despite the overlying variability associated with rootstock and irrigation it was found that the relative differences in perennial tissue reserve concentrations between sites was maintained from one season to the next. This finding suggests that once established, vine carbohydrate reserves may reach an equilibrium if cropping level and management practice remain relatively stable from season to season.

3. No relationship was found between inflorescence initiation and carbohydrate reserve status during winter in the same season, or carbohydrate reserves in winter and the number of inflorescences initiated in the next season. However, there did appear to be a positive correlation between wood reserves and the incidence of PBN. Nutritional factors may have played a more significant role in the differences in fruitfulness and PBN seen at some sites, but further analysis of samples at the replicate level is needed before the nature of any relationships can be confirmed statistically.

4. As the final collection a verification of yield data was still in progress at the time of writing, we have not yet been able to undertake a thorough statistical analysis of the relationship between carbohydrate reserve status and yield. However, preliminary results suggest that knowledge of the vines winter carbohydrate reserve status may be a useful refinement for yield estimates based on bud-fruitfulness and retained bud numbers.

4. Evaluation of practical techniques for manipulating carbohydrate reserves

4.1 Introduction

In an earlier project undertaken in the Riverina and Hilltops regions of NSW, it was found that significant replenishment of carbohydrate reserves can occur in Semillon during the post-harvest period. However, this is dependent on maintaining canopy function and photosynthesis for a sufficient length of time after harvest. In the Riverina, a combination of irrigation, warm temperatures, and a long post-harvest period provides such conditions, and allow high amounts of carbohydrate reserves to be stored before leaf-fall. Although the post-harvest period in the Hilltops region should be of sufficient length to allow a similar recovery of reserve levels, this appeared to be prevented by drought conditions and a lack of water for irrigation after harvest. When treatments were applied to change the amount of reserves stored after harvest, more than two-fold differences in vegetative growth (shoot growth rates and pruning weights) were generated between vines with high and low carbohydrate reserves. The effect was particularly strong in the Riverina, where high crop loads prevent the full replenishment of reserves before harvest, and vines are dependent on favourable post-harvest conditions to maximise reserve storage before leaf-fall.

The relative importance of stored versus current assimilate for reproductive development is not well understood, but carbohydrates are known to be important at key stages. Recent work at the University of California (Sanchez & Dokoozlian 2004) has shown that bud carbohydrate content is directly correlated with fruitfulness, so mobilized reserves could potentially influence inflorescence initiation. Carbohydrate reserves have also been linked to inflorescence development and flower numbers (Bennett 2005), and may reduce the impact of adverse weather conditions on fruit-set (Zapata et al. 2004). In our previous study with Semillon we found that the increased yields on high reserve vines were due to improved bud-fruitfulness, higher flower numbers, and a higher percentage fruit-set. High reserve levels were also associated with faster shoot growth rates and dense canopy development, but this did not appear detrimental to inflorescence initiation or fruit-set.

The relative ease with which reserve levels could be altered in the Riverina, and the strong effect this had on yield, suggested that carbohydrate reserve accumulation after harvest could be managed for deliberate effect on vine productivity. In this section of the project we evaluated practical methods for controlling the amount of reserves stored in the vine after harvest, namely post-harvest deficit irrigation and post-harvest defoliation. However, we also used crop thinning and deficit irrigation to target reserve storage earlier in the season. The effect of these treatments on carbohydrate reserve dynamics, and response of vegetative growth, reproductive development and berry development, was then assessed. The broader aim of the study was to provide a better understanding of factors that determine yield in the following season, and if reserve level could be managed for specific effects on yield, fruit composition and wine quality.

4.2 Material and methods

4.2.1 Site location and experimental details

The field trials were located near Hanwood in the Riverina grape growing region of NSW. The soil type was a heavy clay loam and the elevation 125m. In the Riverina (34ºS, 146ºE) the mean January temperature (MJT) is 23.8ºC and the average annual rainfall is 406mm. However, the MJT was significantly higher (26.9/27.6°C) and the annual rainfall lower (486.5/238.0mm) in the last two years (Table 4.1). The vines of the Shiraz trial (SA1654) were on own roots planted in 1997, while the vines of the Chardonnay trial (I10V1) were on Ramsey and planted in 1994 (Figure 4.1a). The row width in both trials was 3.6m, the vines were planted 2.7m for Shiraz and 1.8m for Chardonnay apart. The phenological dates of the

two varieties indicates that the season is slightly longer for Chardonnay vines in the second season due to the earlier bud-break and the post-harvest period is longer because of the earlier harvest. These differences between areas were also apparent in heat accumulation (growing degree days, GDD, base 10°C) before and after harvest (Table 4.1). The GDD were calculated from budburst to the end of the growing season (leaf-fall). The rain and irrigation amounts were lower in the Shiraz vineyard, due to the RDI normally practiced in the surrounding vineyard. However, additional drip lines were installed in the trial area with switchable bypasses for the deficit irrigation treatments to allow irrigation to maintained for the other treatments. The vines were trained to a single cordon and mechanically pruned and harvested. Both varieties were drip-irrigated, and soil moisture was monitored with GBug loggers (MEA, Magill, South Australia) connected to pairs of gypsum blocks and watermark sensors installed at 30 and 60 cm in 15 cm square around emitter (one per corner) in centre of each treatment panel.

Six treatments aimed at either increasing or decreasing reserve accumulation were applied to four replicated panels of eight vines in a fully randomised block design. These treatments included 33% and 66% bunch removal at veraison (or shortly before), deficit irrigation between fruit-set and veraison, or from harvest to leaf-fall, and post-harvest hedging. Untreated vines were used as controls. For the crop removal treatments bunches were removed on January 5 (2006/07), December 20 (2007/08) and December 22 (2008/09) at °Brix levels for Shiraz/Chardonnay of 10.9/12.5, 4.9/4.9 and 4.8/5.6 respectively at these times. The two thinning treatments are aimed to increase storage of carbohydrate reserves. The fruit-set to veraison treatment was essentially an extended version of RDI normally used with Shiraz, but was applied to both varieties with the aim of reducing photosynthesis, and therefore reserves replenishment in the period when carbon availability within the vine is assumed to be high. For the second deficit irrigation treatment the water supply was stopped for approximately six weeks immediately following harvest, with a similar intent of reducing photosynthesis during a period when the vine is normally replenishing reserves. The final hedging treatment was applied on the day of harvest, with all shoots cut back to approximately five nodes (Figure 4.1b). The aim of this treatment was to reduce post-harvest carbohydrate reserve storage by reducing leaf area and total photosynthesis, but have enough leaves remaining for continued transpiration and nutrient uptake.



Figure 4.1a The carbohydrate reserve management sites in the Riverina showing the Chardonnay in Spring 2007 with minirhizotron tubes (left), and Shiraz prior to harvest in February 2008 (right).

4.2.2 Vine performance determination, sampling and analysis

Of the eight treated vines, only vines two, three, six and seven were used for monitoring carbohydrate reserve concentrations. Vines one and eight acted as buffers, and vines four and five in the centre of the panel were left undisturbed by wood or root sampling for soil

moisture monitoring and minirhizotron tubes (see below). Flower numbers were determined by collecting ten random inflorescences per treatment panel a week prior to the start of cap-fall and counting the number of flowers. Yield and bunch numbers were determined the day before commercial harvest by hand harvesting four vines in each plot. Prior to harvest, 100 berry samples were collected randomly from each side of the treatment panel. Pruning weights were determined prior to commercial pruning, and the shoots of the vines were also counted to allow calculating the average shoot weight. In addition, fruitfulness was assessed in the first three buds from eight shoots per plot, these shoots were sent shortly after collection to a commercial service. For this determination the bud was dissected under a microscope and the number of inflorescences counted. Trunk circumference was recorded in winter 2009 at the end of the study by measuring each vine 25cm above the ground and 25cm below the cordon wire.

Table 4.1 Phenological stages and sampling dates at for two seasons (2007/08 and 2008/09) for Shiraz and Chardonnay trials in the Riverina.

	Shiraz	Chardonnay
2007-2008 Season		
Phenology		
Dormancy (Pruning)	11 July	11 July
Bud-break	17 September	11 September
Flowering	8 November	3 November
Veraison	30 December	24 December
Harvest date	19 February	30 January
Leaf-fall	14 May	14 May
Seasonal		
GDD (ºC)	2861	2861
Rain/irrigation (mm)[x]	311/324	311/446
Post-harvest		
GDD (ºC)	798	1061
Rain/irrigation (mm)[x]	23/107	35/178
2008-2009 Season		
Phenology		
Dormancy (Pruning)	7 July	7 July
Bud-break	18 September	10 September
Flowering	3 November	31 October
Veraison	31 December	23 December
Harvest date	10 February	27 January
Leaf-fall	13 May	13 May
Seasonal		
GDD (ºC)	2706	2835
Rainfall + irrigation (mm)[x]	187/490	187/535
Post-harvest		
GDD (ºC)	894	1224
Rainfall + irrigation (mm)[x]	37/194	37/230

[x] Irrigation amounts relate to vineyard management application, but to harvest date of trials

To monitor the seasonal pattern of root activity in the treatments expected to induce the largest variation in carbohydrate reserve accumulation, namely the control, 66% crop removal and post-harvest hedging treatment, minirhizotron tubes were installed in early April 2007. The minirhizotron tubes were made from 1 m lengths of 50 mm diameter polycarbonate tube, and installed at a 30^{o} angle to pass under the vine and through an area of high root density. Images at approximately 1.3 cm depth increments down the tube were taken fortnightly from August to July using a BTC100 minirhizotron camera (Bartz Technology, Santa Barbara, CA, USA). During the 2007/2008 season images were collected from the control treatment only, but tubes from all three treatments were monitored in the 2008/2009 season. The total length of white roots was calculated for each tube using WinRHIZO Tron (Régent instruments Inc., Nepan, ON, Canada).

Root and wood sampling and the subsequent analysis of carbohydrates was undertaken as described in sections 3.2.1 and 2.2.3 respectively. Sampling commenced at veraison in 2006, and continued through to winter in 2009 with other sampling times harvest, leaf-fall, pruning, bud-break and flowering. These were the same as listed in Table 4.1, except for the flowering sampling dates which were November 1 in 2007 and October 27 2008, and veraison dates which were December 20 2007 and December 21 2008.

4.2.3 Assessment of berry ripening and composition

Berry samples were taken throughout the berry ripening period for both varieties at approximately weekly intervals from veraison to harvest in the last two seasons. Each 100 berry sample taken was divided into two lots of 50 in the laboratory. Juice from one 50 berry sample was extracted using a domestic juice extractor, and total soluble solids (TSS) determined with a bench refractometer (ATAGO PR-101, Tokyo, Japan), and juice pH and TA (g/L) measured with an automatic titrator (TitraLab 80, Radiometer Copenhagen, Lyon, France). For the Shiraz grapes, the other 50 berries were homogenized to uniform consistency using an ultra-turrax at 16,000 rpm. Berry total anthocyanin content was determined from this sample by the method described by Williams et al. (1995) using a 96 well plate reader (µQuant, Bio-Tek Instruments Inc., Vermont, USA).

4.2.4 Winemaking, analysis and assessment

Small lot winemaking was undertaken in 2008 and 2009 at the NWGIC experimental winery using approximately 50 kg of fruit collected from each replicate of the extended RDI, post-harvest deficit irrigations, 66% crop removal, and control treatments. After overnight storage at 4°C following harvest, the fruit was destemmed and crushed (Shiraz), or pressed (Chardonnay), prior to transfer to 100L stainless steel variable capacity fermentation tanks.

The pH of the Chardonnay juice was adjusted to 3.2 to 3.4 with tartaric acid and then cold settled at 4°C for up to two days. Following racking off the gross lees, sugar levels were adjusted to ± 0.5 °Baume, and yeast and enzymes (DV10, GoFerm Danstar, Ferment Ag, Zug Schwitzerland) added at 0.3g/L. The juice was fermented at 16^{o}C, with yeast assimilable N adjusted to 250mg/L after two days with DAP. The fermentation was monitored until residual sugar levels were below 2g/L. Bentonite was used for protein stabilization, and then the wine was cold settled for three days at 4°C. After racking off, the wine was further cold stabilised for 14 days, the pH (3.0 to 3.4) and TA (6.0 to 8.0) was adjusted. Prior to bottling the wine was filtered twice and pH and TA were determined. Alcohol content was also measured at this point using an alcohol analyser (DMA 4500, Anton Paar, Graz, Austria). Post bottling spectral measurements were conducted to determine the phenolics in the wine (UV 1700 PharmaSpec, Shimadzu Corporation, Kyoto, Japan).

The red grapes wine making process differed in the adjustment and overall procedures. The pH was adjusted to 3.5 to 3.6, and yeast (EC1118) added at rate of 0.25g/L. The fermentation was conducted on the skins at 20°C, and allowed to complete after pressing off at day six. The wines were then held at 20 to 25°C until malolactic fermentation was completed (<0.05g/L). After racking off the lees the wine was cold stabilised for 21 days.

After three further rackings and following the bottling of the wine, spectral measurements were conducted to assess wine colour, pigmentation and phenolics (UV 1700 PharmaSpec, Shimadzu Corporation, Kyoto, Japan). The fermentation was monitored for both wine making procedures by assessing temperature and Baume levels twice a day (8am/4pm) with a portable density meter (DM35, Anton Paar, Graz, Austria). For both trials from two years initial wine tasting was conducted and involved seven trained persons for Chardonnay and six for Shiraz wines. The Roseworthy 20-point scorecard was used which provides for marks to be allocated for appearance (colour and clarity; maximum 3), aroma (intensity and faults; maximum 7), palate (intensity, acid balance and faults; maximum 8), and overall quality and balance (maximum 2).

4.2.5 Data analysis

Data analysis was conducted by using the statistical program Genstat release 6.1 (Rothhamsted Experimental Station, Harpenden, Herts, United Kingdom). Analysis of variance and Fisher's LSD test was used to locate the significant differences between means ($P < 0.05$). For graphical representation of data, results are shown with error bars for ± standard error of the mean ($n = 4$).

4.3 Results and discussion

4.3.1 Reproductive and vegetative development

The treatments imposed in the Shiraz and Chardonnay trials were designed to alter vine carbohydrates and productivity. The study was based on previous work on Semillon, which indicated a potential link between carbohydrate reserves, reproductive development and fruit quality. The current study aimed to expand on this work and find practical management options to manipulate reproductive and vegetative production in following seasons, but included also the implications on grape and wine composition. Therefore the work was conducted on the white variety Chardonnay and on Shiraz to ensure an expanded relevance of the overall grape production in Australia.

In general, yield, pruning weights, vine balance and sugar per vine was altered by the treatments similarly in both varieties, but were more pronounced in the last season (Table 4.2). The yield was reduced by the thinning treatments as expected, although for the Chardonnay vines in particular, not to the same extent as intended. For instance, in 2009 for Shiraz the 66% crop removal treatment resulted in 59% and in Chardonnay in only 46% yield reduction. This was due to the difficulties of accurately counting all the bunches in a crowded fruiting zone, rather than any compensation effect. In addition, the yield was reduced by the extended RDI treatment in both seasons and by the post-harvest hedging in the last season in Shiraz. However, a reduction of yield was present in all treatments in Chardonnay in that season other than in the post-harvest DI treatment.

The impacts of the other treatments compared to the control were only significant in the 2008/09 season in Shiraz. Vines had higher pruning weight under the high crop removal treatment and it was lower if water deficit was implemented after harvest. The changes in pruning weight and yield altered the vine balance, not only in the treatments which directly effected a lowering by the crop or the pruning weights (crop removal, post-harvest hedging), but also by irrigation management in Shiraz. Total berry sugar per vine was lowered by crop removal and the extended RDI treatment in both years in Shiraz. In Chardonnay, a significant reduction of total berry sugar per vine in the first season was only seen in the high crop removal treatment, while in the second season a decrease was present in all treatments except post-harvest DI. The results show that several of the implemented practical approaches to alter vine performance and balance were effective, particularly after two or three years of implementation.

The observed differences in pruning weight were mostly due to the shoot weight, while differences in yield appeared to be mainly due to shoot fruitfulness and fruit set, since no

significant difference was present for flower numbers. However, the flower numbers were variable between seasons in both varieties, Shiraz had 250 flowers per inflorescence in spring 2007 and 288 in spring 2008, for Chardonnay they were 299 and 256 respectively. The shoot numbers were only reduced in the last season in Chardonnay by post-harvest hedging (Table 4.3). The shoot weight was increased by crop removal in both varieties, while late season water management affected the shoot weight only in Shiraz. Post-harvest hedging was the only treatment other than crop removal that lowered bunches per shoot in the last season in both varieties. The shoot length assessed in Spring 2008 tended to be affected in Shiraz vines by crop load and irrigation management, while no changes appear to be present in the trunk size measured as circumference in either variety. The total bunch number per vine was altered by post-harvest hedging and extended RDI in Shiraz, in both varieties the crop removal treatments were present in the bunch numbers per vine (Table 4.4). Bunch weight was increased by all treatments in Chardonnay in the last season, except post-harvest DI. In Shiraz only the high crop removal treatment decreased the bunch weight. However, the berries per bunch were only affected by the treatments in Shiraz, particularly the number of berries was reduced if crop was removed around veraison, the post-harvest hedging led to the same in the last season. The berry weight was generally reduced by the irrigation treatments in both years in Shiraz and in Chardonnay in 2008. The assessment of the fruitfulness conducted in winter over three years did not show an impact of the treatments on the number of inflorescences initiated or average bud fruitfulness (Tables 4.5 and 4.6).

Table 4.2 The impact of crop thinning, irrigation management and post-harvest hedging on vine performance and balance in Shiraz and Chardonnay.

Treatment	Yield (kg/vine)		Pruning weight (kg/vine)		Pruning weight ratio		Berry sugar (kg/vine)	
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	20.4^{a}	19.5^{a}	2.34^{ab}	1.86^{b}	8.9^{b}	10.6^{b}	4.90^{a}	4.55^{a}
Post-harvest hedging	21.9^{a}	15.5^{b}	0.51^{c}	0.81^{c}	43.2^{a}	19.2^{a}	5.19^{a}	3.47^{bc}
Extended RDI	16.6^{b}	15.3^{b}	2.11^{b}	1.76^{b}	7.9^{b}	8.7^{bc}	3.99^{b}	3.57^{b}
Post-harvest DI	19.9^{a}	18.8^{a}	2.00^{b}	1.05^{c}	9.8^{b}	18.3^{a}	4.73^{a}	4.12^{ab}
33% Crop removal	14.9^{b}	12.3^{c}	2.27^{b}	1.82^{b}	6.9^{bc}	6.7^{c}	3.57^{b}	2.96^{c}
66% Crop removal	10.4^{c}	8.0^{d}	2.74^{a}	2.13^{a}	3.8^{c}	3.8^{d}	2.48^{c}	1.96^{d}
	***	***	***	***	***	***	***	***
Chardonnay								
Control	27.3^{a}	21.8^{a}	1.43^{ab}	1.28^{ab}	20.1^{b}	17.5^{bc}	5.11^{a}	4.58^{a}
Post-harvest hedging	24.7^{ab}	17.1^{bc}	0.53^{c}	0.49^{c}	50.1^{a}	34.6^{a}	4.54^{a}	3.60^{b}
Extended RDI	23.9^{ab}	17.5^{bc}	1.17^{b}	1.09^{b}	21.4^{b}	16.9^{bc}	4.50^{a}	3.71^{b}
Post-harvest DI	25.6^{ab}	23.2^{a}	1.50^{ab}	1.29^{ab}	17.3^{b}	19.2^{b}	4.71^{a}	5.03^{a}
33% Crop removal	24.0^{ab}	15.8^{c}	1.48^{ab}	1.28^{ab}	16.3^{b}	12.3^{cd}	4.68^{a}	3.35^{b}
66% Crop removal	17.1^{b}	11.7^{d}	1.68^{a}	1.53^{a}	10.3^{b}	7.8^{d}	3.51^{b}	2.44^{c}
	***	***	***	***	***	***	**	***

The results show that several of the treatments to alter vine performance and balance were effective, particularly after two or three years of implementation. Yield was not only reduced by crop removal, but also by irrigation management in spring. Similarly the pruning weights and consequentially vine balance were altered. However, the post-harvest pruning altered canopy size in Shiraz, but not in Chardonnay, suggesting differences in varietal response to this management option. In addition the total berry sugar per vine was reduced by all

treatments except post harvest irrigation management. The differences in vegetative development was due to the size of the shoots assessed in average weight, while all yield components were influenced by the implemented treatments. However, a difference in shoot fruitfulness of dormant canes was only present on one occasion in Chardonnay, similarly no differences were detected in the flower numbers per inflorescence. This indicates the major impact of the implemented treatments happened during the current season, when vine productivity was assessed. Most of the selected treatments are used in vineyard management or are necessary due lack of irrigation water remaining after harvest. This work clarifies the various options for the production of grapes.

Table 4.3 The impact of crop removal, irrigation management and post-harvest hedging on shoot number and weight, bunches per shoot, shoot length and trunk size.

Treatment	Shoot number		Shoot weight		Bunches per shoot		Shoot length (cm)	Trunk diameter (cm)
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	76.3	78.6	30.8^{ab}	27.5^{a}	2.49^{ab}	2.72^{a}	8.0	8.0
Post-harvest hedging	72.6	74.4	7.0^{c}	11.1^{d}	2.67^{a}	2.09^{c}	114.0	8.0
Extended RDI	77.4	73.4	27.7^{b}	24.0^{b}	2.10^{bc}	2.39^{b}	117.6	7.9
Post-harvest DI	76.5	70.9	26.8^{b}	15.0^{c}	2.52^{ab}	2.68^{a}	107.3	8.2
33% Crop removal	77.2	74.5	29.9^{b}	24.5^{ab}	1.95^{c}	1.86^{c}	127.6	7.9
66% Crop removal	77.8	78.9	35.3^{a}	27.5^{a}	1.45^{d}	1.31^{d}	139.4	8.4
	ns	ns	***	***	***	***	ns	ns
Chardonnay								
Control	84.3	70.8^{b}	16.8^{ab}	18.0^{b}	3.45^{a}	2.89^{a}	7.2	7.2
Post-harvest hedging	93.4	83.8^{a}	5.7^{c}	5.8^{c}	2.78^{ab}	2.25^{b}	91.1	7.3
Extended RDI	80.4	68.5^{b}	14.6^{b}	15.6^{b}	3.20^{ab}	2.90^{a}	92.8	7.0
Post-harvest DI	81.3	75.4^{ab}	18.4^{a}	16.9^{b}	3.51^{a}	3.04^{a}	92.2	7.4
33% Crop removal	87.4	74.9^{b}	17.0^{ab}	17.1^{b}	2.96^{b}	2.27^{b}	98.5	7.3
66% Crop removal	86.8	70.9^{b}	19.3^{a}	21.4^{a}	2.24^{c}	1.78^{c}	98.9	7.3
	ns	*	***	***	***	***	ns	ns



Figure 4.1b The carbohydrate reserve management sites in the Riverina showing the Shiraz post-harvest deficit irrigation treatment in April 2007 (left) and harvesting the post-harvest hedging treatment in the Chardonnay immediately after pruning the shoots back to 5 nodes (right).

Table 4.4 The impact of crop thinning, irrigation management and post-harvest hedging on bunches per vine, bunch and berry weigh, and berries per bunch.

Treatment	Bunches per vine		Bunch weight (g)		Berries per bunch		Berry weight (g)	
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	190^{ab}	213^{a}	109	93^{ab}	77.4^{ab}	179.5^{a}	1.41^{a}	1.20^{ab}
Post-harvest hedging	194^{a}	156^{cd}	114	100^{a}	80.3^{ab}	125.5^{b}	1.42^{a}	1.24^{a}
Extended RDI	162^{bc}	176^{bc}	102	87^{b}	85.6^{a}	166.8^{a}	1.19^{b}	1.05^{c}
Post-harvest DI	192^{ab}	191^{ab}	104	99^{a}	80.4^{ab}	169.2^{a}	1.31^{ab}	1.13^{bc}
33% Crop removal	150^{c}	138^{d}	100	89^{ab}	69.0^{bc}	119.8^{b}	1.44^{a}	1.16^{ab}
66% Crop removal	113^{d}	105^{e}	91	76^{c}	63.2^{c}	90.8^{c}	1.43^{a}	1.16^{ab}
	***	***	ns	**	*	***	**	**
Chardonnay								
Control	288^{a}	204^{ab}	95	108^{a}	76.9	75.8	1.23^{a}	1.43^{a}
Post-harvest hedging	260^{a}	190^{ab}	96	89^{bc}	76.0	62.4	1.27^{a}	1.44^{a}
Extended RDI	257^{a}	198^{ab}	93	88^{c}	85.1	63.9	1.09^{b}	1.39^{a}
Post-harvest DI	285^{a}	227^{a}	91	102^{ab}	83.3	75.7	1.10^{b}	1.35^{a}
33% Crop removal	258^{a}	170^{b}	93	93^{bc}	72.4	66.5	1.29^{a}	1.41^{a}
66% Crop removal	194^{b}	126^{c}	87	93^{bc}	66.8	73.9	1.33^{a}	1.25^{b}
	***	***	ns	*	ns	ns	***	**

Table 4.5 The impact of crop thinning, irrigation management and post-harvest hedging on the number of inflorescences initiated per primary bud in nodes 1 to 3.

Treatment	Bunch number per primary bud								
	Node 1			Node 2			Node 3		
	2007	2008	2009	2007	2008	2009	2007	2008	2009
Shiraz									
Control	1.78	1.81	1.92	1.98	1.88	1.95	1.97	1.96	1.97
Post-harvest hedging	1.87	1.60	1.93	2.00	1.98	2.00	1.94	1.91	2.00
Extended RDI	1.73	1.67	1.87	1.96	1.86	1.95	1.96	1.91	1.94
Post-harvest DI	1.85	1.76	1.96	1.91	1.86	2.00	2.00	1.89	2.00
33% Crop removal	1.71	1.56	1.97	1.95	1.81	1.98	1.97	1.96	1.97
66% Crop removal	1.83	1.69	1.94	1.98	1.82	1.92	1.95	2.00	1.98
	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chardonnay									
Control	1.79	1.92	1.96	2.00	2.00	2.00	2.00	2.00	2.03
Post-harvest hedging	1.83	1.93	1.96	1.98	2.00	2.04	2.00	2.03	2.03
Extended RDI	1.92	1.96	1.76	2.00	2.00	1.98	1.98	2.00	1.97
Post-harvest DI	1.96	1.93	1.93	1.98	2.01	1.96	1.97	2.00	1.98
33% Crop removal	1.90	1.98	1.90	1.97	1.95	2.02	1.97	1.98	2.03
66% Crop removal	1.91	1.87	2.00	1.95	1.98	2.03	2.00	2.04	2.03
	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 4.6 The impact of crop thinning, irrigation management and post-harvest hedging on average bud fruitfulness for nodes 1 to 3.

Treatment	Bunch number per bud								
	Node 1			Node 2			Node 3		
	2007	2008	2009	2007	2008	2009	2007	2008	2009
Shiraz									
Control	1.31	1.58	1.60	1.60	1.60	1.77	1.58	1.88	1.79
Post-harvest hedging	1.54	1.38	1.48	1.69	1.60	1.65	1.33	1.67	1.54
Extended RDI	1.48	1.48	1.52	1.58	1.58	1.60	1.44	1.75	1.56
Post-harvest DI	1.33	1.69	1.60	1.40	1.63	1.63	1.69	1.77	1.56
33% Crop removal	1.42	1.44	1.65	1.42	1.67	1.50	1.69	1.92	1.44
66% Crop removal	1.40	1.38	1.48	1.69	1.54	1.52	1.69	1.94	1.63
	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chardonnay									
Control	1.25	1.58[a]	1.52	1.73	1.54	1.42	1.63	1.56	1.46
Post-harvest hedging	1.42	1.29[ab]	1.27	1.71	1.29	1.17	1.77	1.52	1.46
Extended RDI	1.56	1.48[a]	1.10	1.71	1.58	1.54	1.69	1.71	1.60
Post-harvest DI	1.29	1.29[ab]	1.31	1.52	1.65	1.54	1.77	1.63	1.40
33% Crop removal	1.42	1.58[a]	1.27	1.75	1.58	1.65	1.67	1.65	1.44
66% Crop removal	1.46	1.17[b]	1.44	1.73	1.77	1.63	1.75	1.40	1.60
	ns	*	ns	ns	ns	ns	ns	ns	ns

4.3.2 Root growth dynamics and nutrient status

The impact of treatment on N reserves in root and wood tissue was determined at leaf-fall for the last two seasons. The root activity assessments in the Chardonnay were undertaken in the control for two seasons, and the control, 66% crop removal and post-harvest hedging treatment in the second season. Both assessments were also conducted to obtain information on vine nutrient accumulation and root dynamics, which are likely to be altered by carbohydrate allocation to the roots. Since the root activity determination was an additional assessment, we were limited to conduct these only in one variety and not all treatments.

Root N concentrations were more than double the concentrations found in the wood tissues, while the difference between years was relatively small. The roots of the Chardonnay vines, which are in fact grafted on Ramsey, had about 20% higher N concentration, but wood levels were similar (Figure 4.2). Although there were no significant differences in the roots between the treatments in Shiraz, at leaf-fall 2008 the extended RDI treatment had the highest N concentrations in the roots, while in 2009 this was the control. However, wood N concentrations were significantly higher in the crop removal treatments and extended RDI compared to post-harvest hedging and DI (Figure 4.2a, b). This indicated the negative impact of these treatments on nutrient uptake in the post-harvest period and/or reduced mobilisation from shoots and leaves due to reduced irrigation and shoot length. The Ramsey root system of the Chardonnay vines showed significantly higher N concentrations in autumn 2008, if crop was removed and a water deficit was implemented after harvest. However, in 2009 there was no treatment effect on leaf-fall root or wood N concentration in both years (Figure 4.2c, d). The increase in root N concentration could be due to more allocation to the roots under the crop removal treatments and to less allocation of N to the areal part of the vine implemented by the reduced irrigation after harvest.

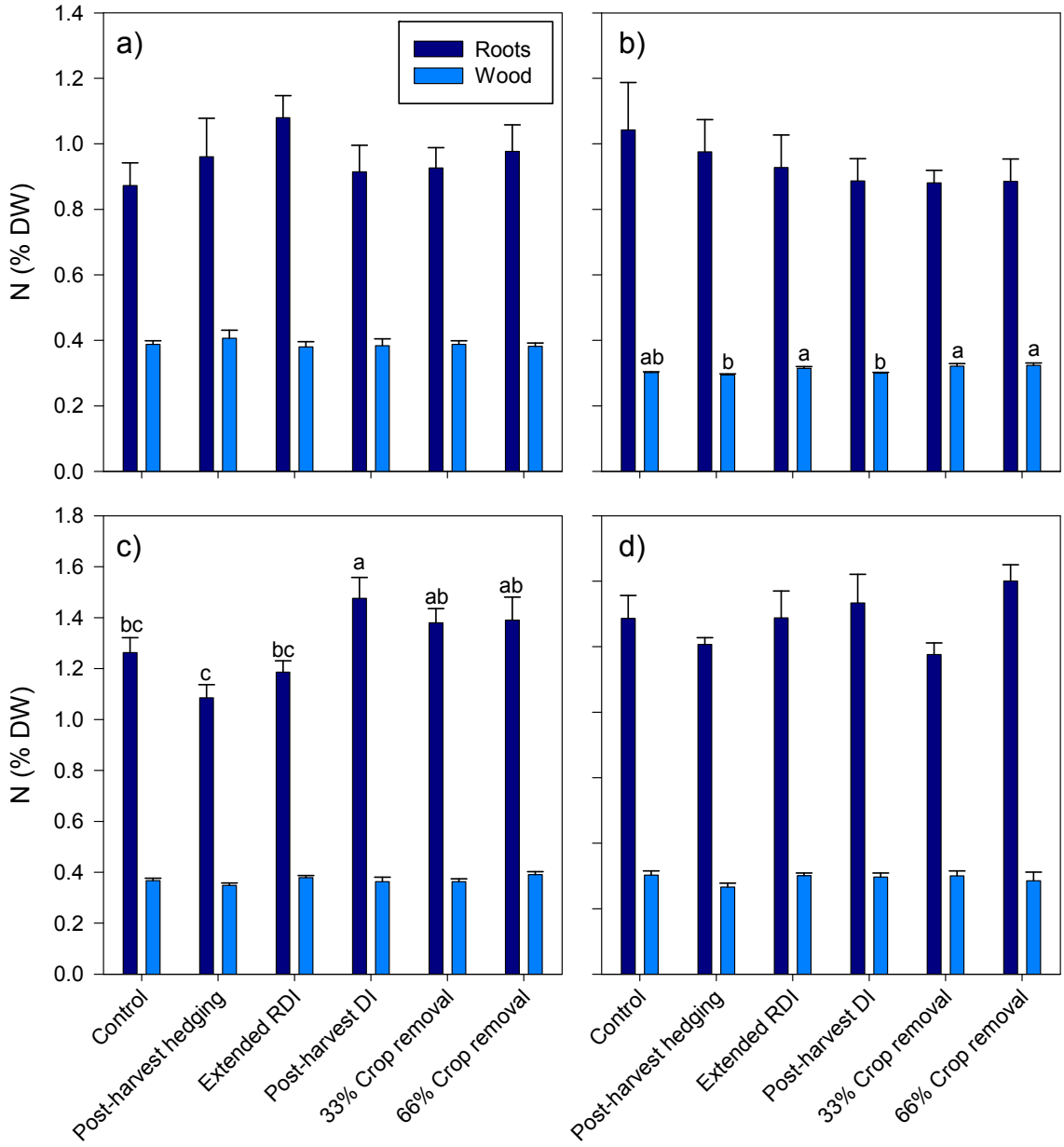


Figure 4.2 The impact of crop thinning, irrigation management and post-harvest hedging on wood and root N concentrations in Shiraz (a = 2008, b = 2009) and Chardonnay (c = 2008, d = 2009).

The first assessment of fine root growth was conducted during the 2007/08 season for the control treatment in the Chardonnay block, with images collected every two weeks (Figure 4.3). This was expanded in 2008/2009 to include the 66% crop removal and post-harvest hedging treatments, with a total of 34,800 images collected over the two seasons. The results from the post-harvest hedging treatment are still being processed, so have not been included here. It also needs to be noted that more information can be extracted from the images with further analysis than the simple standing fine root length presented here. However, this still offers a significant advance on our understanding of root growth dynamics in hot climate vineyards.

In the 2007/2008 season, fine root activity was already evident in the control treatment when the first images were collected at bud-break (Figure 4.4). Root growth subsequently peaked around flowering, and then declined through the remainder of the season. In the 2008/09

season, when measurements were started earlier, there was already a significant amount of new fine roots in the control treatment three weeks prior to bud-break. However, visual observations suggested that these roots were very 'lightly' constructed, and they rapidly disappeared leading into bud-break. The length of roots then remained constant through to harvest when there was a renewed flush of root activity, and thus demonstrated two different growth patterns for the two seasons.



Figure 4.3 The collection of images using a minirhizotron camera in the Chardonnay trial following leaf-fall in 2008 (left) and example of an image taken at 50 cm on September 17 2007.

Contrary to expectations, root activity was reduced by the 66% crop removal treatment in the two proceeding seasons, and did not show any response as the treatment was repeated again at veraison in 2008 (Figure 4.4). Neither did the reduced root activity appear to have been reflected in root or wood N concentrations at leaf-fall 2009. The lower crop load could have reduced the overall vine demand for N, although additional canopy growth would have counteracted this response. Since these are only concentrations, it is difficult to estimate the potential shift in partitioning in the vine, and would require further assessment of the N accumulated in the crop and in the canopy as well as the determination of concentrations at different key stages during the growing season.

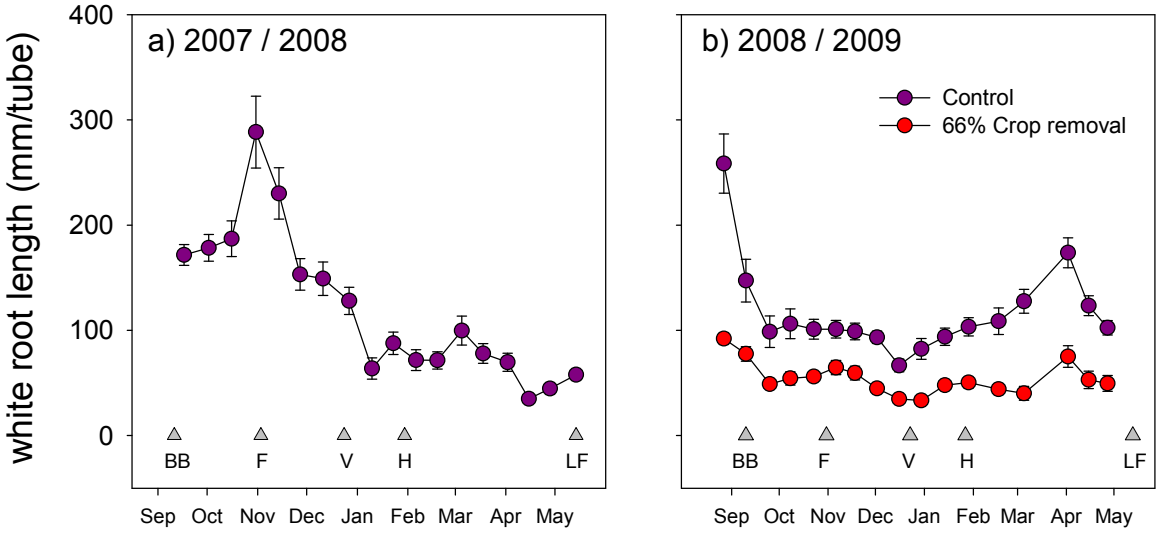


Figure 4.4 Fine root growth dynamics of Chardonnay on Ramsey during the 2007/2008 and 2008/2009 seasons, and the response to crop thinning treatments applied to the same vines at veraison in 2006, 2007 and 2009. Dates on x-axis indicate the start of each month.

4.3.3 Carbohydrate reserve dynamics

Irrigation and crop management and, to a lesser extent, post-harvest hedging treatments were found to alter productivity. The assessment of the effects of the various treatments on the seasonal dynamics in carbohydrate reserves in the perennial structure was an important part of the study, since vine reserves can influence canopy development early in the season and impact on the reproductive performance. Both have been shown in an earlier study on Semillon, although the treatments imposed in this research project would have been expected to have less severe influence on vine reserves and performance.

The pattern of carbohydrate reserve dynamics, and the relative concentrations of root and wood tissue, were generally consistent with the findings of the earlier two studies. Firstly, for the Shiraz vines root starch concentrations were significantly higher than for wood (Figure 4.5a-b), and during 2007/2008 showed a clear pattern of mobilization following bud-break and then a replenishment that commenced prior to veraison and continued to leaf-fall. In 2008/2009 the mobilization of reserves seemed to be less pronounced after bud-break, but reserves were mobilized during ripening, and then increased in concentration during the post-harvest period. For Chardonnay, which was grafted on Ramsey, the low root concentrations of starch and total carbohydrates (Figure 4.5c-d, 4.7c-d) were consistent with the findings of the survey. Reserve were also mobilized from the wood between bud-break and flowering, but the stability of root reserves between sampling dates strongly contrasted the large fluctuations seen with Shiraz, and may even raise a question as to the real importance of root carbohydrate reserves when the Chardonnay vines were able to ripen a similar crop load to Shiraz in a shorter time. Possibly this was a reflection of the reserves needed to establish the canopy, as pruning weights were on average 56% higher in the Shiraz. Alternatively, higher irrigation may have allowed for higher rates of photosynthesis. In pot experiments with grafted vines we found Ramsey causes a shift in biomass partitioning to the shoot, so the results of the current study may also suggest a significant difference in the way Shiraz, and Chardonnay grafted on Ramsey, utilize carbohydrate resources.

To simplify the assessment of the treatment impacts a focus on the harvest and leaf-fall values is appropriate for this study, since it should reflect the influence of the crop load and the management of the post-harvest-period by irrigation management and hedging. Root samples in Shiraz at harvest 2008 were significantly higher after the 33% crop removal treatment was implemented. At leaf-fall both crop removal treatments had higher carbohydrate levels than the control treatment in the wood tissue, similar differences were observed at harvest 2009. In Chardonnay wood starch concentrations were also higher at harvest and at leaf-fall 2008, when crop was removed around veraison. This indicates the impact of cropping level on vine carbohydrate reserves, since resource allocations were directed towards the perennial structure of the vines. However, in the Chardonnay vines at leaf-fall in 2007 and 2009 all treatments resulted in lower starch concentrations in the wood, except in the post-harvest DI in the last year. This indicates the complexity of the source-sink relationship in vines, due to altered movements in vine resources between the perennial structure, the shoots and the crop. For instance, the pruning weights in the last season were highest in the 66% crop removal treatment in Chardonnay, while the second lowest weights were achieved with the post-harvest deficit irrigation.

Overall, altered carbohydrate levels are related to the balance between vegetative and reproductive development as reported in the earlier work in Semillon and in the first part of this section. The assessment of carbohydrates in the perennial structure at harvest or in winter will assist in optimising vine resources for the next growing season. After harvest the reserve level can be altered by management strategies as implemented in this work. This study has also demonstrated the impact of the crop load on the concentrations of carbohydrates in the wood and root tissues. In winter the decision of the number of buds remaining after pruning could take the carbohydrate levels into account. Both of these strategies will assist in optimising vine balance for the coming growing season, with implications on grape and wine composition.

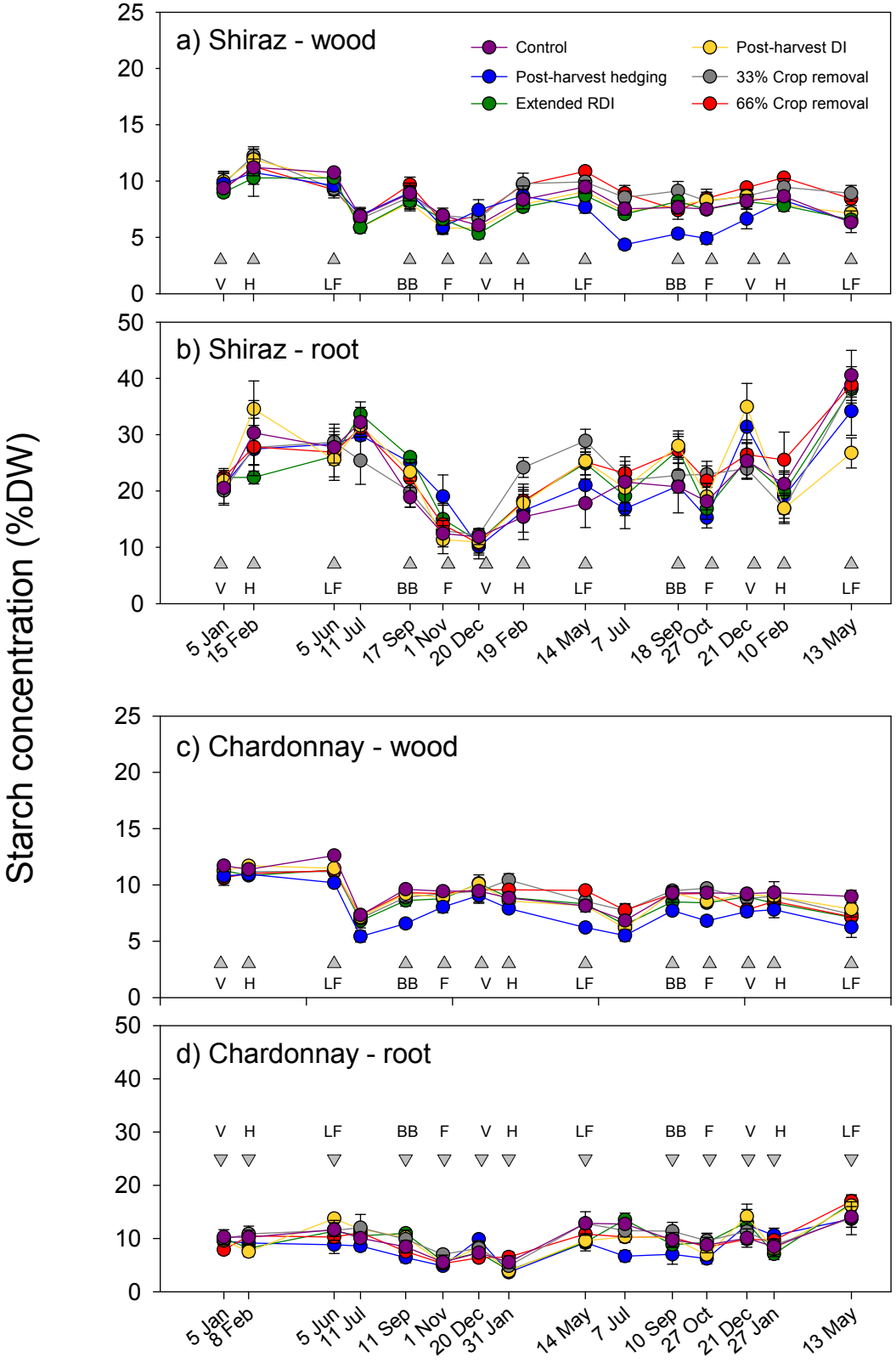


Figure 4.5 The impact of crop thinning, deficit irrigation and post-harvest hedging on wood and root starch concentrations for Shiraz (a,b) and Chardonnay (c,d). Sampling commenced on Jan 5 2007 and concluded on May 13 2009.

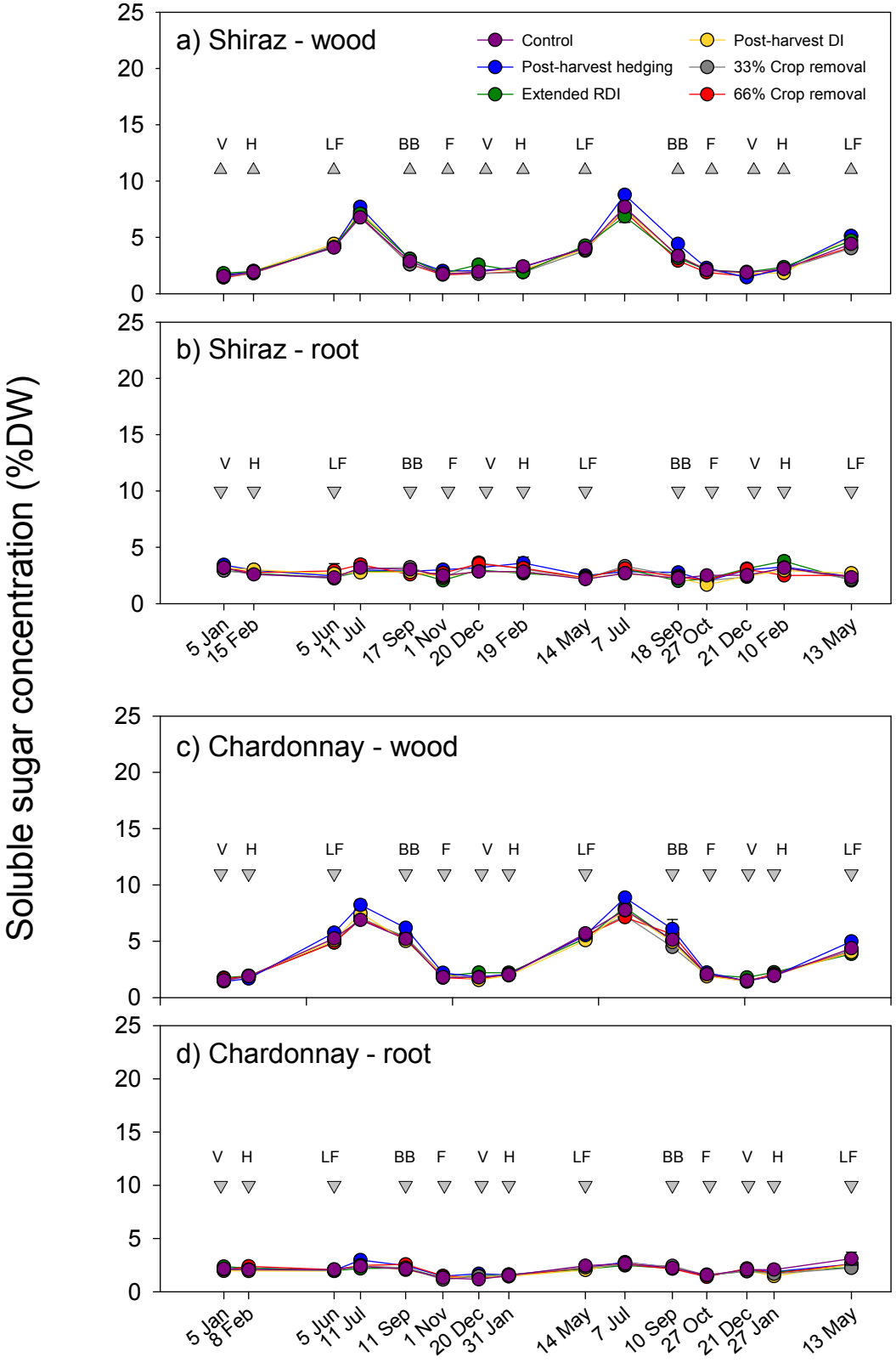


Figure 4.6 The impact of crop thinning, deficit irrigation and post-harvest hedging on wood and root soluble sugar concentrations for Shiraz (a,b) and Chardonnay (c,d). Sampling commenced on Jan 5 2007 and concluded on May 13 2009.

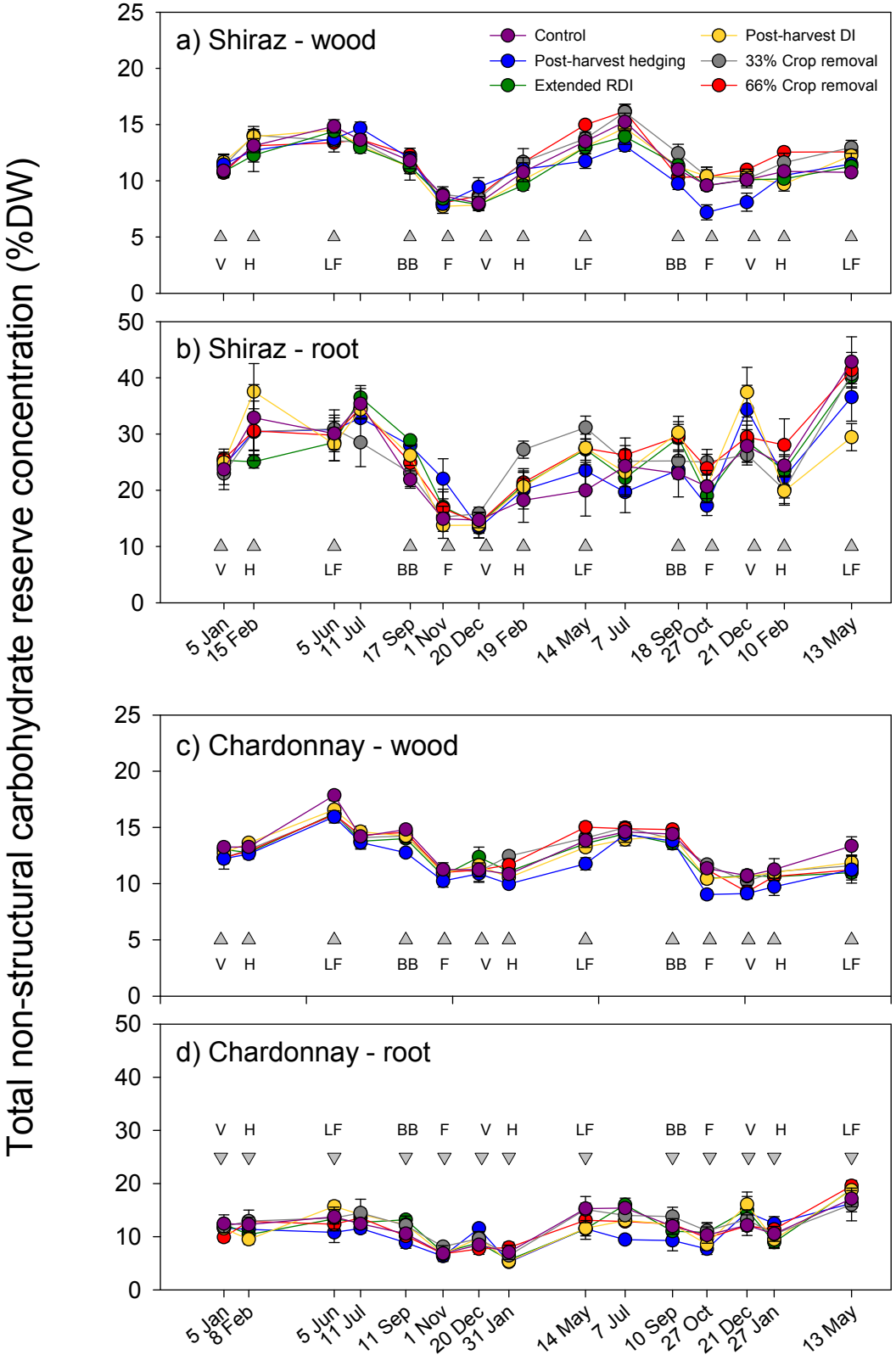


Figure 4.7 The impact of crop thinning, deficit irrigation and post-harvest hedging on wood and root total non-structural carbohydrate reserve concentrations for Shiraz (a,b) and Chardonnay (c,d). Sampling commenced on Jan 5 2007 and concluded on May 13 2009.

4.3.4 Berry ripening and fruit composition

The changes during berry maturation of sugar and anthocyanin (Shiraz only) accumulation and the juice parameters were assessed to investigate the treatments influence on fruit composition. The most pronounced effect was expected through the thinning treatments, while the impact of the early irrigation treatments was likely to have an influence on fruit composition, as shown in a number of studies. However, previous work on Semillon showed that alterations made during the post-harvest period in relation to length and effectiveness resulted in differences in berry ripeness at harvest.

The effect of the treatments on berry sugar accumulation varied between the two seasons and the varieties. In Shiraz in the 2007/08, seasonal differences were only present in the first half of the grape maturation period, while in the second year these differences were still present at harvest (Figure 4.9a, b). Both crop removal treatments ripened faster and in 2009 final Brix levels were higher at harvest with the highest sugar per berry amounts. The post-harvest DI resulted in the lowest Brix levels and the extended RDI treatment had the lowest amounts of sugar per berry, as berry size was also reduced as reported earlier (Table 4.7).

The changes in berry anthocyanin content differed between the two season, but the highest content at harvest was similar in both years for the 66% crop removal treatment (Figure 4.9c, d). The variation between treatments, however, of the final anthocyanin levels ranged from 0.96 to 1.11mg/g FW in 2008 and from 0.76 to 1.10mg/g FW in 2009. Both crop removal treatments showed significantly higher anthocyanin concentrations at harvest 2009 than the control, while the post-harvest DI treatment was significantly lower. This treatment led to the highest TA at harvest, while the final juice pH was highest in the 66% crop removal treatment (Table 4.7). In contrast to Shiraz, the sugar accumulation in Chardonnay was variable during grape maturation in 2008 (Figure 4.10). The crop removal treatments ripened faster, resulting in significantly higher Brix levels and sugar per berry in the 66% crop removal treatment at harvest (Table 4.7). The juice pH and TA was not altered by the treatment at harvest 2008, while post-harvest DI led to lower juice pH and higher TA levels in 2009. However, berry sugar accumulation and final Brix levels were not influenced by any treatment in the Chardonnay in that year.

Both varieties showed that crop load alteration, irrigation management and hedging after harvest can alter grape maturation and basic juice parameters. Most changes occurred in Chardonnay in 2008 and in Shiraz in 2009. However, the responses were more pronounced in Shiraz, where the most significant crop removal had the ripest fruit and colour. The latter would have been even higher if the fruit would have been harvested a week or two earlier. This highlights the influence of crop load on berry and final wine composition, which will be described in the next section. On the other hand, the post-harvest DI resulted in lowest berry colour and Brix levels at harvest in 2009; the later was also present for the post-harvest hedging treatment. This indicates the importance of the post-harvest period, not only for vine productivity, but also for grape composition. However, while the data is not presented in this report, berry samples (and wine making on remaining un-harvested vines) were collected from the two deficit irrigation treatments and control ten days after the first harvest. By that time colour and sugar content were similar to the control treatment from the previous harvest, and indicates that through the combined effects of crop load and post-harvest management a two to three week difference in fruit development rates could be achieved within the same vineyard. The reduced irrigation also led to the lowest starch reserves in the wood at harvest in 2009, suggesting a connection to this lower berry and sugar colour level. It is likely that the nutrient reserves were also influenced by this treatment, as several macro-nutrients are taken up in significant amounts after harvest, and the opportunity will be taken to undertake more detailed nutrient analysis around veraison in the coming season. However, the principals behind these findings require further investigation, particularly as it may become an increasingly important issue when insufficient irrigation water is available to the vineyard.

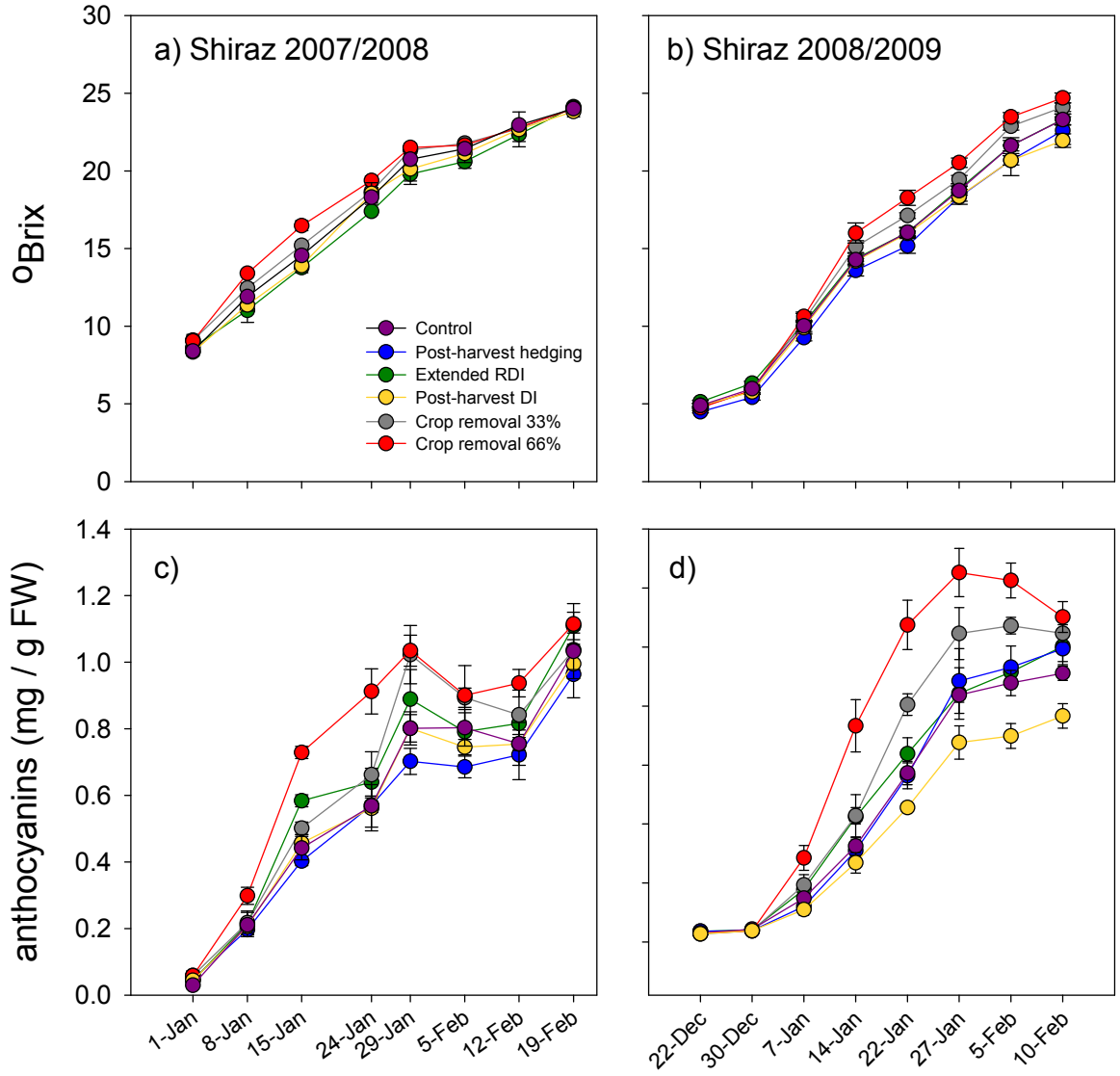


Figure 4.9 The impact of crop thinning, irrigation management and post-harvest hedging on berry sugar (a = 2008; b = 2009) and colour (c = 2008; d = 2009) accumulation in Shiraz.

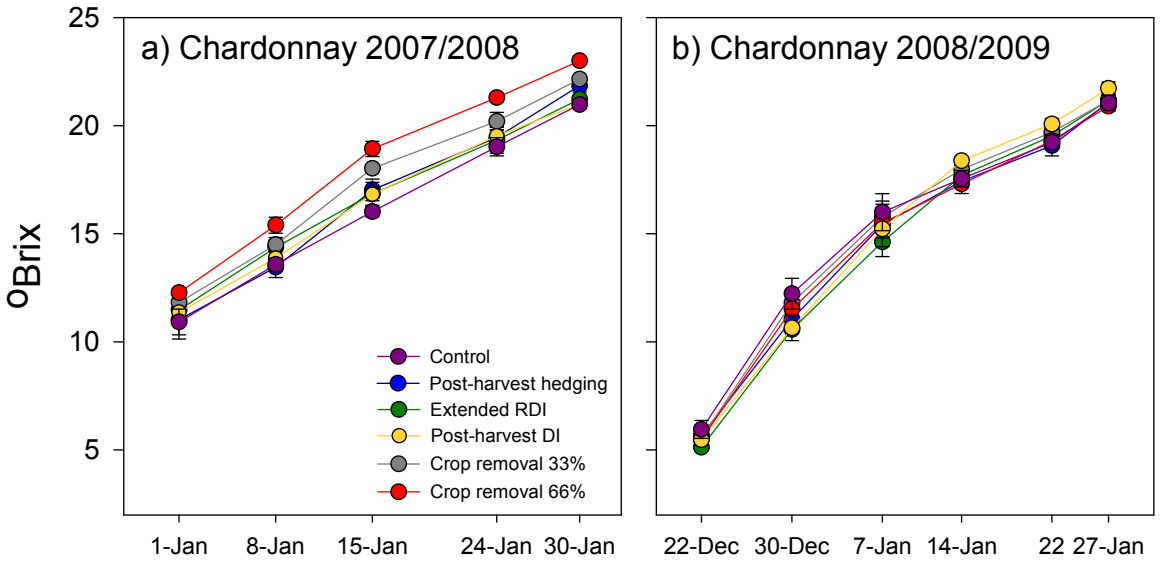


Figure 4.10 The impact of crop thinning, irrigation management and post-harvest hedging on sugar accumulation in Chardonnay in the 2008 (a) and 2009 (b) berry ripening period.

Table 4.7 The impact of crop thinning, irrigation management and post-harvest hedging on total soluble solids (TSS), sugar per berry, juice ph and total acidity (TA).

Treatment	TSS (Brix)		Sugar/ Berry (mg)		(pH)		TA (g/L)	
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	24.0	23.3^{bc}	338^{ab}	279^{a}	4.24	3.97^{c}	3.66	3.66^{ab}
Post-harvest hedging	23.7	22.6^{c}	337^{ab}	280^{a}	4.19	3.99^{c}	3.74	3.54^{bc}
Extended RDI	24.1	23.3^{bc}	288^{c}	246^{b}	4.27	4.03^{b}	3.57	3.48^{c}
Post-harvest DI	23.8	22.0^{c}	313^{bc}	248^{b}	4.23	3.98^{c}	3.72	3.81^{a}
33% Crop removal	24.1	24.1^{ab}	347^{a}	278^{a}	4.25	4.06^{ab}	3.69	3.49^{c}
66% Crop removal	24.0	24.7^{a}	343^{a}	287^{a}	4.27	4.09^{a}	3.65	3.52^{bc}
	ns	**	**	***	ns	***	ns	**
Chardonnay								
Control	18.7^{bc}	21.1	231^{c}	300^{a}	3.84	3.61^{ab}	5.28	5.45^{b}
Post-harvest hedging	18.3^{c}	21.1	233^{bc}	302^{a}	3.78	3.62^{a}	5.28	5.19^{b}
Extended RDI	18.8^{bc}	21.2	205^{d}	294^{a}	3.84	3.64^{a}	5.10	5.07^{b}
Post-harvest DI	18.4^{c}	21.7	202^{d}	294^{a}	3.84	3.56^{b}	5.36	5.88^{a}
33% Crop removal	19.5^{b}	21.2	251^{b}	298^{a}	3.87	3.62^{a}	5.35	5.38^{b}
66% Crop removal	20.5^{a}	20.9	273^{a}	262^{b}	3.91	3.64^{a}	5.40	5.29^{b}
	***	ns	***	*	ns	*	ns	**

4.3.5 Wine composition and sensory attributes

The effect of four treatments key treatments was further investigated by making wine from both Shiraz and Chardonnay grapes from each field plot. These grapes were transported from the vineyard in Griffith to the experimental winery of the NWGIC for processing as described earlier (Figure 4.11) The winemaking process included an analysis of the must and berries prior to fermentation, monitoring the process, and the determination of basic wine parameters before bottling. A tasting of all wines concluded the assessments of the impact of vine reserve management.

The assessment of the must in relation to Baume, pH and TA showed significant differences between treatments to a larger extent than the measurements conducted on 50 berry samples described in the previous section (Table 4.8). In addition, differences were present in berry phenolics and YAN levels in Shiraz 2009 (data not presented). The YAN levels were higher in Chardonnay must with 335mg/L in 2008 and 233mg/L in 2009 vintage, for Shiraz must the average values were165mg/L and 124mg/L respectively. However, juice sugar levels were adjusted to the treatments which had the highest value, the pH was also adjusted with tartaric acid as was the FAN with DAP to the appropriate levels (see section 4.2.4) to reduce the potential influence on the wine making process and the final wine perception. The fermentation proceeded faster in Shiraz than in Chardonnay must in both years, with the sugar depletion slightly slower in the 66% crop removal treatment particularly in 2009 (Figure 4.12a, c), and the sugar adjustment is visible early during the fermentation process. The fermentation curve of the Chardonnay must in 2008 shows the sugar adjustment after a couple of days for the crop removal treatment, otherwise differences between treatments are small (Figure 4.12b, d).

Table 4.8 Baume, phenolics, pH and total acidity (TA) in must of Shiraz and Chardonnay grapes that had altered crop levels and irrigation management.

Treatment	°Baume		Phenolics (mg/berry)[X]		(pH)		TA (g/L)	
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	13.6	12.8^{c}	1.01^{c}	0.43^{a}	3.92^{b}	3.64^{b}	4.38	3.52^{a}
Extended RDI	14.1	13.3^{b}	1.27^{a}	0.43^{a}	3.95^{b}	3.68^{b}	4.40	3.55^{a}
Post-harvest DI	13.6	12.2^{d}	1.12^{b}	0.31^{b}	3.94^{b}	3.66^{b}	4.15	3.63^{a}
66% Crop removal	13.9	14.7^{a}	1.02^{c}	0.51^{a}	4.03^{a}	3.92^{a}	4.38	3.15^{b}
	ns	**	***	**	***	***	ns	***
Chardonnay								
Control	13.3^{b}	11.8^{bc}	7.97	2.67^{b}	3.48^{b}	3.39^{b}	6.88^{a}	6.83^{ab}
Extended RDI	11.7^{b}	12.1^{ab}	7.57	3.18^{a}	3.47^{b}	3.44^{a}	6.03^{b}	6.13^{c}
Post-harvest DI	11.3^{b}	11.6^{c}	8.22	2.45^{b}	3.46^{b}	3.34^{b}	6.85^{a}	7.13^{a}
66% Crop removal	12.7^{a}	12.4^{a}	9.92	2.53^{b}	3.56^{a}	3.42^{a}	5.73^{b}	6.50^{bc}
	***	*	ns	*	***	**	**	**

[X]Phenolics are from whole berry analysis and in Chardonnay are in absorption units (a.u.)



Figure 4.11 Grapes harvested from Shiraz trial (left) and the processing area in the experimental winery of the NWGIC (right).

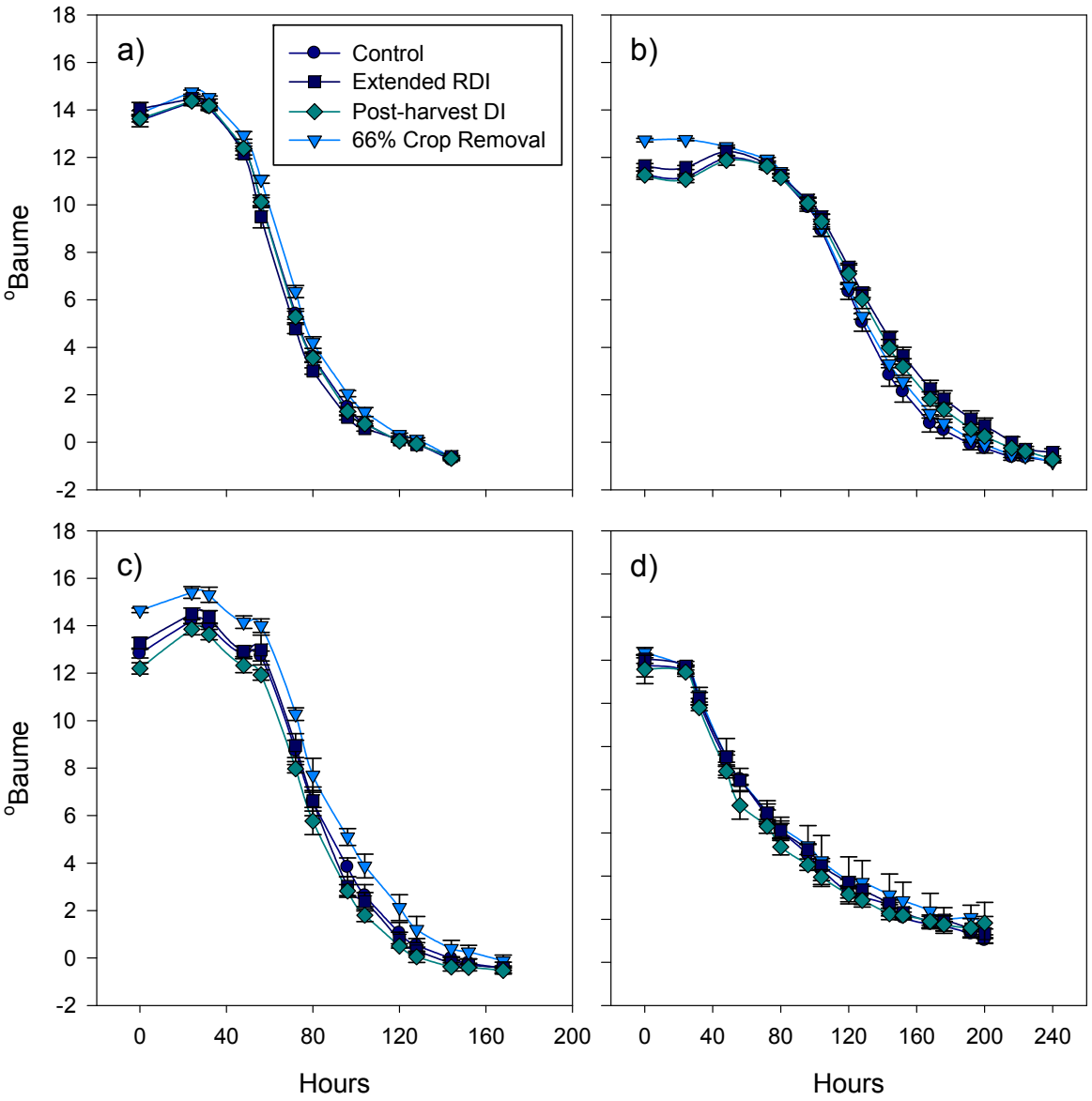


Figure 4.12 The fermentation rates of must coming from Shiraz (a = 2008; c = 2009) and Chardonnay (b = 2008; d = 2009) vines that had altered crop levels irrigation management.

The assessment of basic wine parameters showed a number of significant differences in Shiraz and a few in the Chardonnay wines. The alcohol content varied despite the sugar adjustments made at the early stage of the fermentation process, where the content is highest in the wine made from grapes coming from the 66% crop removal treatment (Table 4.9). Wines made from this treatment had also highest phenolics and TA content in Shiraz. In addition, wine colour density, red pigment colouration and total red pigments were highest in these wines, while wine colour hue was the lowest. For the post-harvest DI treatment the values were the reverse, wines made from this treatment showed low wine colour, red pigment colouration and total red pigments. However, the perception of these wines scored by a small tasting panel showed similar scores than the control, while wines made from extended RDI and particularly 66% crop removal were received much better (Figure 4.13).

Table 4.9 Alcohol concentration, phenolics, pH and total acidity (TA) in wines made from Shiraz and Chardonnay grapes that had altered crop levels and irrigation management.

Treatment	Alcohol (%)		Phenolics (a.u.)		(pH)		TA (g/L)	
	2008	2009	2008	2009	2008	2009	2008	2009
Shiraz								
Control	13.9	13.9^{b}	26.3^{b}	32.8^{b}	3.23	3.46	6.12^{bc}	6.15^{bc}
Extended RDI	13.7	14.2^{b}	30.3^{a}	34.9^{b}	3.28	3.45	6.24^{ab}	6.25^{b}
Post-harvest DI	13.7	13.2^{c}	26.4^{b}	25.2^{c}	3.30	3.47	6.02^{c}	5.88^{c}
66% Crop removal	14.1	15.2^{a}	31.6^{a}	46.5^{a}	3.30	3.49	6.33^{a}	6.78^{a}
	ns	***	*	***	ns	ns	**	***
Chardonnay								
Control	12.4^{b}	12.7^{b}	0.41	0.93	3.67	3.22	7.75	6.48
Extended RDI	12.7^{b}	12.7^{b}	0.26	1.25	3.65	3.18	7.30	6.43
Post-harvest DI	12.7^{b}	12.6^{b}	0.43	1.23	3.66	3.20	7.93	6.55
66% Crop removal	13.5^{a}	13.2^{a}	0.50	1.19	3.67	3.22	7.65	6.50
	*	**	ns	ns	ns	ns	ns	ns

Table 4.10 Wine colour density, wine colour hue, red pigment colouration and total red pigments in wines made from Shiraz grapes that had altered crop levels and irrigation management

Treatment	Wine colour density (a.u.)		Wine colour hue		Red pigment colouration (%)		Total red pigments (a.u.)	
	2008	2009	2008	2009	2008	2009	2008	2009
Control	3.63^{b}	6.94^{b}	0.85^{a}	0.64^{b}	12.3	24.7^{a}	15.9^{b}	17.2^{b}
Extended RDI	4.58^{a}	7.00^{b}	0.82^{b}	0.66^{b}	13.7	23.2^{ab}	18.4^{a}	18.2^{b}
Post-harvest DI	3.71^{b}	4.01^{c}	0.84^{ab}	0.73^{a}	12.5	18.5^{b}	16.1^{b}	12.4^{c}
66% Crop removal	5.11^{a}	11.06^{a}	0.77^{c}	0.64^{b}	14.4	26.4^{a}	20.0^{a}	25.5^{a}
	*	***	*	*	ns	*	**	***

The overall findings of this work suggest that crop load at least in Shiraz, has an impact on wine characteristics and perception. In addition, this work confirms that irrigation management between fruit set and veraison improves the final wine produced. However, a more interesting finding is that the irrigation management after harvest can be important for the wine produced in the following season. Harvesting all of the treatments at the same date suggested a more detrimental effect of post-harvest deficit irrigation on wine quality than may have been the case if the fruit was allowed to develop for longer, and this was suggested by a slight improvement in wine score for a second ferment undertaken on spare fruit from the treatment ten days later (not shown). As mentioned earlier, the processes which have resulted in the negative impact of post harvest water stress on grape and wine composition are not well understood and would require further investigation.

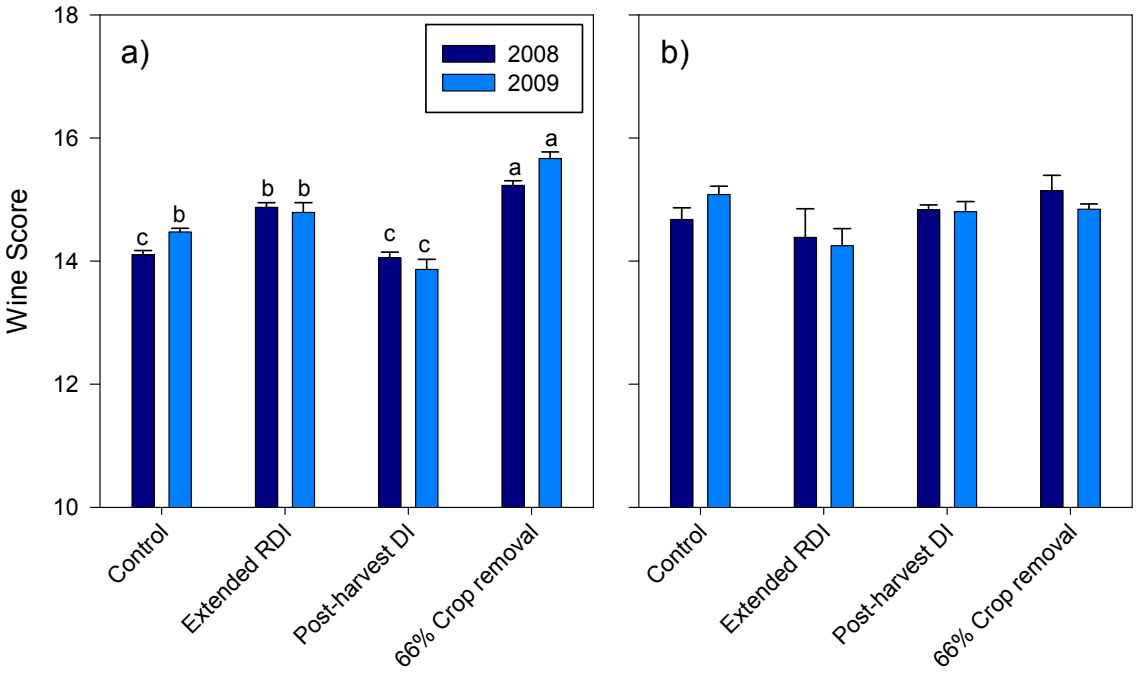


Figure 4.13 Wine scores from wines coming from Shiraz (a) and Chardonnay (b) grapes that received altered crop levels irrigation management.

4. 4 Summary and conclusion

This study investigated the response of Chardonnay and Shiraz vines in hot climate irrigated vineyards to deliberate attempts to manipulate vegetative growth, reproductive development and fruit composition by altering carbohydrate reserve accumulation. Two levels of bunch thinning (33% and 66%) were imposed shortly before veraison over three consecutive seasons to increase carbohydrate availability within the vine, while post-harvest water deficit or post-harvest hedging were used to limit the post-harvest restoration of carbohydrate reserves by reducing the photosynthetic capacity of the canopy. An extended RDI treatment was also applied between fruit-set and veraison to reduce photosynthesis in a period where photosynthetic output of the canopy is normally at a maximum. Some of the key findings of the study are as follows:

1. Attempts to manipulate carbohydrate reserve accumulation with treatments that may be considered practical in commercially operated vineyards appeared to have less impact on carbohydrate reserve concentrations than originally expected. Total carbohydrate reserves were generally higher in the 33% and 66% crop removal treatments, and lower in the post-

harvest hedging treatment, but the differences between these treatment was smaller than the magnitude of fluctuations normally season between growth stages within a season. The two deficit irrigation treatments appeared to have little effect on reserve concentrations.

2. Despite the relative small changes in bulk tissue carbohydrate reserve concentration resulting from the imposed treatments, pronounced differences in ripening rate and fruit composition were generated. Crop removal led to faster berry ripening and fast colour development in Shiraz, while post-harvest water deficit resulted in the slower ripening and less berry colour. These differences were reflected in the final wine scores. For Chardonnay, treatment effects on berry development were less obvious, and wines made from the fruit received the same average scores.

3. Root activity measured in Chardonnay and showed that significant new root growth occurs prior to bud-break, but the overall pattern of root growth differs between seasons. In 2007/2008 the highest amount of root activity was observed at flowering, while in 2008/2009 root growth peaked prior to bud-break, and again after harvest. Contrary to expectations, root growth of vines that had yield reduced at veraison by approximately 50% over three consecutive seasons was significantly lower than that of heavily cropped vines.

5 The relative contribution of carbohydrate reserves and photosynthesis to fruit-set

5.1 Introduction

Fruit set can be variable in *Vitis vinifera*, contributing to large variations in seasonal yield. Cool regions are particularly prone to poor fruit set, however warmer climates are also susceptible. Factors that influence fruit set include poor pollen germination (Staudt 1982), inadequate nutrition (Williams et al. 2004) and a limited carbon supply to the inflorescence (Lebon et al. 2008). Actively growing shoots are a strong sink for carbon and the proportion allocated to the inflorescence may be limited during vigorous vegetative growth (Hale & Weaver 1962).

Sources of carbon for ovule development include photoassimilate from recent photosynthesis and carbon from reserve areas within the vine. In spring, carbon is mobilised from the reserves to support bud-break and early shoot growth (Winkler & Williams 1945; Yang & Hori 1980). This is followed by reserve replenishment, with onset depending on variety. It has been suggested that varietal resistance to poor fruit-set during cold and overcast weather may arise from an earlier onset of reserve replenishment in relation to the time of flowering (Zapata et al. 2004). However verification of this concept is required since it is uncertain what the competing sinks for inflorescence development are and which carbon sources these different sinks are drawing from.

The objective of this study was to investigate the relative importance of stored carbohydrate reserves and newly acquired photoassimilate for fruit set in Chardonnay vines. Based on the results of a prior experiment using the same experiment system (Field et al. 2009), a soil temperature treatment was used to create vines with different reserve levels leading into flowering. Vines with high (cool soil) or low (warm soil) reserve levels were transferred at the onset of flowering to ambient or low CO_2 atmospheres to manipulate photosynthesis while maintaining the same temperature and light environment. Flowering and fruit set was monitored over several weeks.

5.2 Materials and methods

5.2.1 Root temperature treatments

At dormancy, forty 3-year-old potted Chardonnay vines (clone V5) were planted in 40 L pots with a 1 m continuous length of polyethylene irrigation tubing installed through the root-zone (Figure 5.1). The tubing was connected to a recirculating water system that allowed soil temperature to be regulated by heat exchange. The two temperature treatments included 15^{o}C (cool) or 26^{o}C (warm). Air temperatures in the glasshouse were not tightly controlled and ranged between an average minimum of 10°C and maximum of 29°C.

5.2.2 CO_2 treatments

Vines were disconnected from the root temperature system at just prior to flowering and distributed across two controlled environment chambers (Figure 5.2 and 5.3). In order to reduce photosynthesis in one chamber, a reduced CO_2 environment was created by recirculating air through scrubbers filled with a CO_2 absorbent. In the second chamber, CO_2 concentrations were not controlled. CO_2 concentrations averaged 94 ppm in the first chamber, and 336 ppm in the second chamber. Environmental conditions were otherwise kept the same, with 22°C/8^{o}C day/night temperatures, 1100 µmol m^{-2} s^{-1} PAR and 12 hour photoperiod.

5.2.3 Fruit set and photosynthesis

Fruit set was assessed by loosely tying 2 mm mesh nylon bags around inflorescences at cap-fall, and then counting the number of flower caps and abscised flowers. Photosynthesis was measured using an LI-6400 portable photosynthesis system.

5.2.4 Carbohydrate analyses

Destructive harvests were undertaken at the onset of flowering (32 days from bud-break) and with berries at peppercorn size, which for the ambient CO_2 treatment was 60 days from bud-break, and 67 days from bud-break for the low CO_2 treatment. Following oven-drying of the root, trunk, shoot, rachis and flower/berry components, samples were ground and analysed for non-structural carbohydrates as described in section 2.2.3.



Figure 5.1 Chardonnay vines were exposed to two different rootzone temperatures in a glasshouse at the NWGIC, heated or cooled water was circulated through irrigation tubing in each pot.

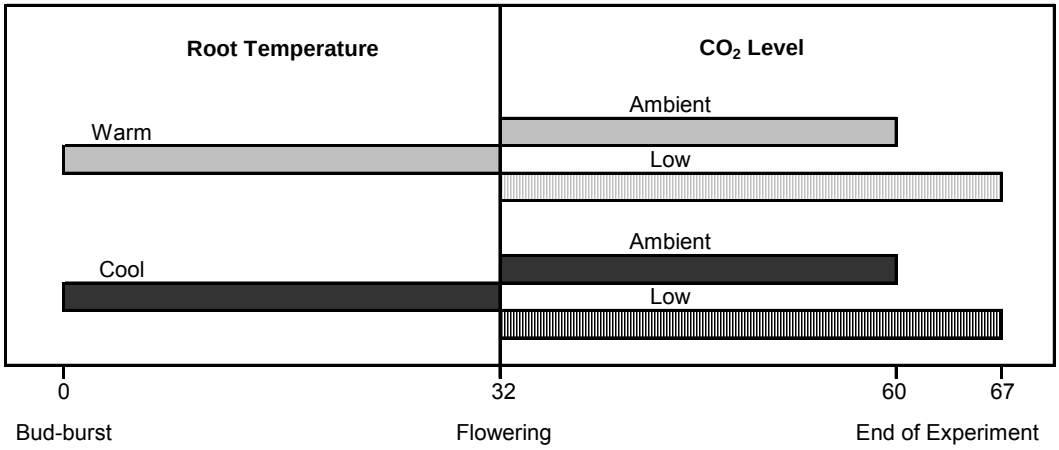


Figure 5.2 Timeline of treatment applications for warm (26°C) and cool (15°C) root temperature regimes between bud-break and flowering to alter carbohydrate reserve status, and then two concentrations of CO_2 (94 and 336 ppm) in controlled environment chambers until approximately EL-stage 29 (pepper corn size).



Figure 5.3 Potted Chardonnay vines after transfer to controlled environment chambers showing CO_2 scrubbers (left) and an inflorescence of a vine growing under low CO_2 approximately 28 days after flowering.

5.3 Results and Discussion

5.3.1 Photosynthesis

Photosynthesis of the first four emerging leaves increased as leaves expanded to full size but was not affected by the root temperature treatments between budburst and flowering. On transfer to the growth cabinets, however, single leaf photosynthesis was altered by the CO_2 treatments. A decline in CO_2 from 336 to 95 ppm reduced photosynthesis by 75% (to less than 2 µmol CO_2 m^{-2} s^{-1}). There was no effect of the previous root temperatures on photosynthesis in either the low or the ambient CO_2 atmospheres (Figure 5.4). As expected, internal CO_2 concentrations were lower in leaves of vines grown in the low CO_2 atmosphere. There was no effect of CO_2 treatment on transpiration (E) despite higher stomatal conductance (g_s) in low CO_2.

5.3.2 Carbohydrate status

Starch and sugar concentrations were unevenly distributed amongst the vine components. When compared to the trunk, the shoots and the inflorescences, the roots contained the predominant proportion of starch within the vine (Tables 5.1 and 5.2). On a % dry weight basis, the roots contained 2- fold more starch than the trunk and up to 10- fold more than the shoots. Glucose, fructose and sucrose were present in all parts of the vine, but again the roots contained the greatest amount of sugars (3-5 g or 2.5 % on a dry weight basis). This was followed by the shoots (1-3.5 g, 2-9 %) and then the trunk (less than 1 g or 1.5 %).

The warm root temperature treatment was effective at depleting root and trunk starch reserves (Tables 5.1 and 5.2). Total non-structural carbohydrate content was 26 g in the warm treatment as compared to the 38 g in the cool treatment. The total starch levels and concentrations within the trunk were not affected by the root temperatures, but the shoots had 2-fold more starch in the warm treatment as compared to the cold treatment.

5.3.3 Vegetative growth

Bud-burst was stimulated by the warm root temperature treatment with a greater number of shoots developing. Despite removing these and maintaining vines to four shoots, canopy growth was hastened (Figure 5.5). After 32 days of treatment, and with the onset of flowering, shoot length was 20% greater on vines with the warmed roots than the cold roots. This was likely the result of solubilisation of the starch in the roots and subsequent transport to the shoots as evidenced by the higher shoot sugar concentrations.

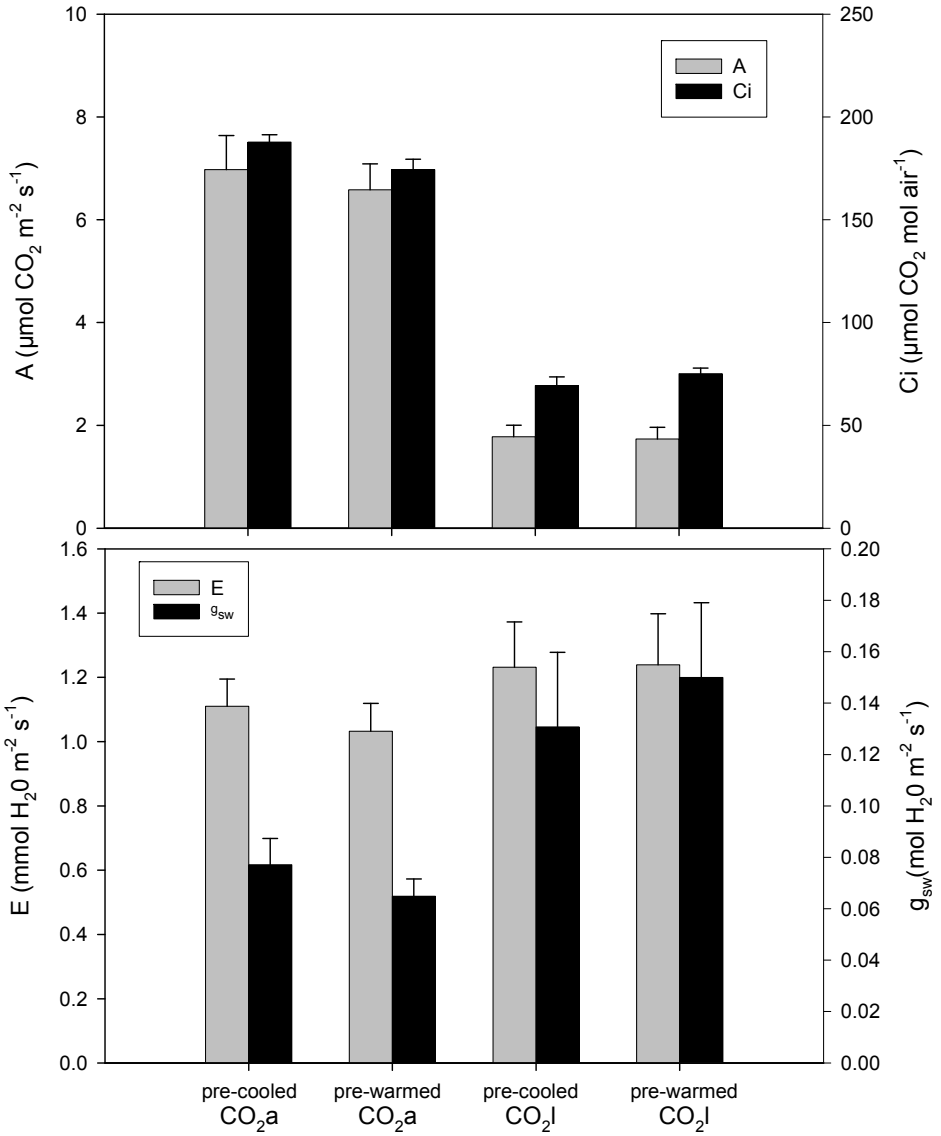


Figure 5.4 Photosynthesis (A),leaf internal CO_2 concentration (C_i), transpiration (E) and stomatal conductance (g_s) of leaves from nodes 1-4 of vines grown in a cold or warm soil pre-treatment, followed by ambient or low CO_2. The first two treatments were applied during the flower development stage, and the last 4 treatments were applied during the fruit set stage.

3.3.4 Reproductive growth

Prior to the onset of cap-fall, the inflorescences were longer and heavier after developing on vines grown in the warm root temperature treatment (Figure 5.6). This could be mainly attributed to the rachis since flower number and size were similar. At this stage, destructive harvest of a subset of inflorescences indicated that flower number (116 ± 11) and individual flower size (0.64 g ± 0.6 fresh weight and 1.08 ± 0.05 mg DW) was not different between the two root temperature treatments. The rachis, however, was heavier in the warm treatment on both a fresh (390 ± 32 and 277 ± 29 mg) and dry weight basis (62.0 ± 5 and 43.4 ± 5 mg dry weight). Upon transfer to the low CO_2 treatment the inflorescence growth rates continued to decline through until the end of the experiment.

Table 5.1 Starch and sugar concentrations (%DW) in vine roots, trunk and shoots at flowering and fruit-set. Soil temperature pre-treatments were applied until flowering and the CO_2 treatments were applied from flowering to fruit-set.

	root		trunk		shoot	
pre-treatment / CO_2	**flowering**	**Fruit-set**	**flowering**	**Fruit-set**	**flowering**	Fruit-set
			starch concentration (%DW)			
cool	19.5	22.8	8.1	9.0	1.9	1.2
warm	13.1	17.4	7.6	7.8	3.4	1.0
ambient	-	23.9	-	10.1	-	1.6
low		16.3		6.7		0.6
soil temp.	*[a]	***	ns	*	ns	ns
CO_2 conc.	-	***	-	***	-	***
			sugar concentration (%DW)			
cool	2.19	2.44	1.08	1.18	8.21	4.22
warm	3.00	2.48	1.07	1.40	9.56	3.50
ambient	-	2.48	-	1.35	-	5.39
low		2.45		1.23		2.32
soil temp.	*	ns	ns	***	*	***
CO_2 conc.	-	ns	-	*	-	***

[a] *,**,*** and ns indicate significance at $p < 0.05$, < 0.01, < 0.001, and not significant, respectively.

Flower abortion was more prevalent in the low CO_2 than in the ambient CO_2 treatment (Table 5.3). In the low CO_2 treatment, on average 78% of flowers abscised while in the ambient treatment only 46% were lost.

Even though cap-fall had occurred for most flowers within 50 days of starting the experiment, the initiation of berry development did not necessarily follow and was very dependent on the CO_2 atmosphere (Figure 5.7a). While over 90% of inflorescences held berries which had initiated expansion within 53 days in the ambient CO_2, less than 40% of inflorescences had berry expansion in the low CO_2 treatments. Vines in the low CO_2 treatments were kept for one extra week prior to the destructive harvest, so that fruit set could be attained. But even then, only 80% (cold pre-treatment) and 20% (warm pre-treatment) of the inflorescences had initiated berry development.

Once berry development initiated, berry growth was influenced by the root temperature pre-treatments and the CO_2 atmospheres. The average diameter of those berries that were initiated was greater for both high CO_2 treatments as compared to the low CO_2 treatments (Figure 5.7b). Within 24 days of the ambient CO_2 treatment mean berry diameter was 3.8 mm and 3.2 mm for the cold and warm root pre-treatments respectively. After the same number of days in the low CO_2 treatment berry diameter was less than 1.5 mm. Again, the low CO_2 combined with the warm root pre-treatment had the smallest berries. These differences in berry diameter were maintained over the next 10-20 days.

Table 5.2 Non-structural carbohydrate reserves (g), dry weight (g) and residual dry weight (g) in vine perennial components and shoots at flowering and fruit-set. Soil temperature pre-treatments were applied until the onset of flowering and the CO_2 treatments were applied from flowering to fruit-set.

pre-treatment/ CO_2	perennial		shoot		total	
	flowering	Fruit-set	flowering	Fruit-set	flowering	Fruit-set
			Non-structural carbohydrate reserves (g)			
cool	38	50	2.6	3.2	40	50
warm	26	39	5.2	2.8	31	39
ambient	-	58	-	4.4	-	62
low		31		1.5		33
soil temp.	*[a]	**	*	ns	ns	***
CO_2 conc.	-	***	-	***	-	***
			dry weight (g)			
cool	198	220	26	56	224	277
warm	187	220	39	59	226	277
ambient	-	247	-	63	-	311
low		191		52		243
soil temp.	ns	ns	ns	ns	ns	ns
CO_2 conc.	-	***	-	***	-	***
			residual dry weight (g)			
cool	160	171	23	53	184	223
warm	161	178	34	57	195	235
ambient	-	189	-	59	-	248
low		159		51		210
soil temp.	ns	ns	ns	ns	ns	*
CO_2 conc.	-	***	-	**	-	***

[a] *,**,*** and ns indicate significance at $p < 0.05$, < 0.01, < 0.001, and not significant, respectively.

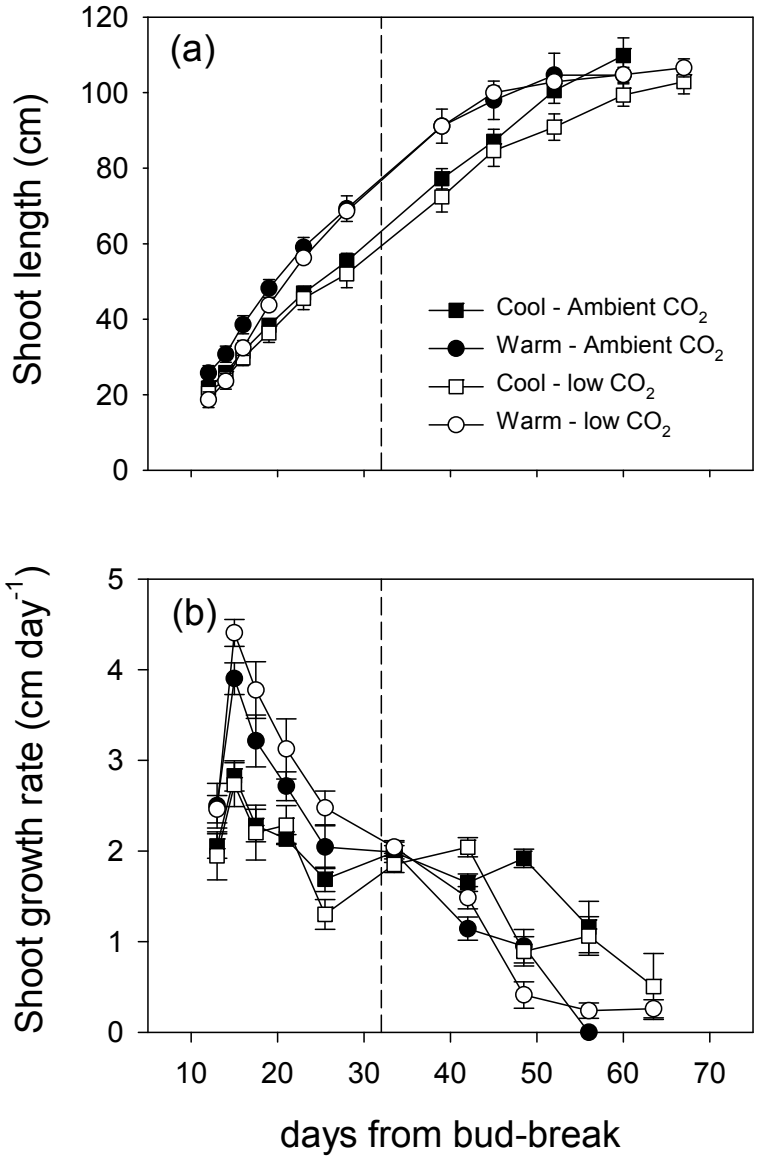


Figure 5.5 Shoot length (a) and shoot growth rate (b) from budburst, through flowering and fruit-set. The vines were exposed to warm or cool soil for 32 days. Subsequently the root temperature treatments were discontinued and plants were divided into ambient or low CO_2 atmospheres through cap-fall and fruit set.

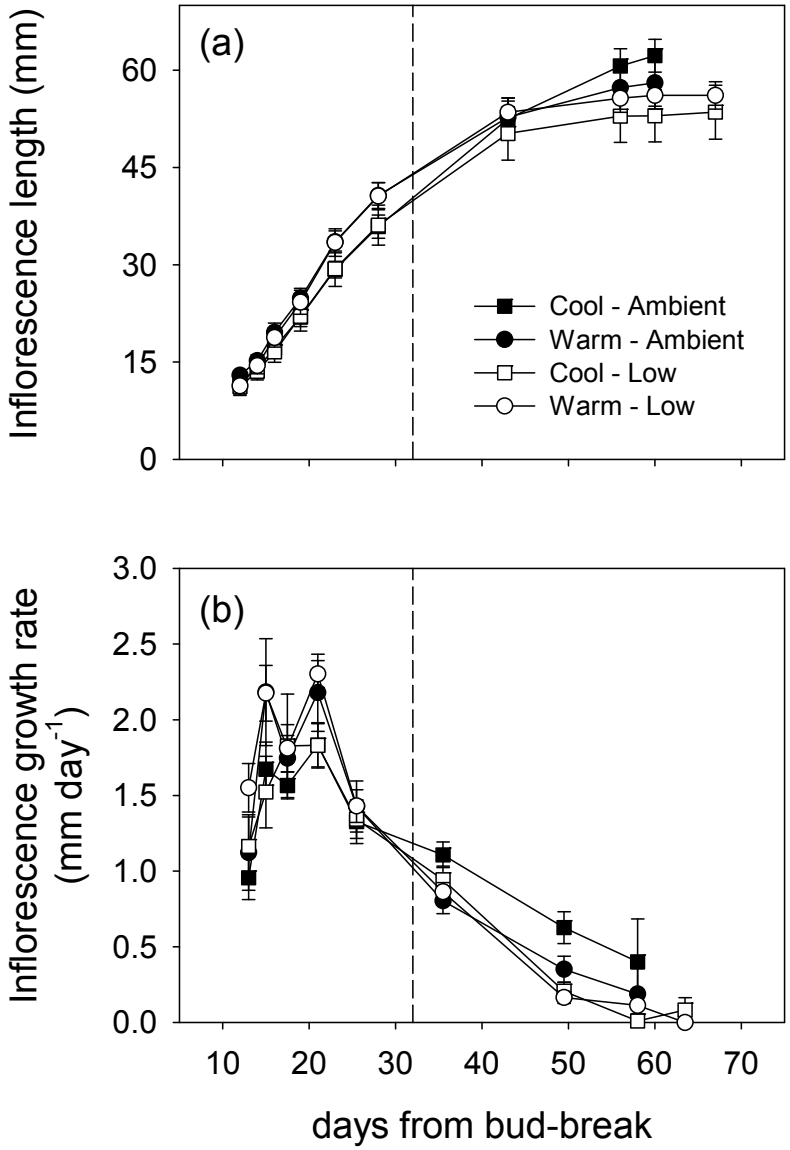


Figure 5.6 Inflorescence length (a) and growth rate (b) from budburst, through flowering and fruit-set. The vines were exposed to warm or cool soil for 32 days. Subsequently the root temperature treatments were discontinued and plants were divided into ambient or low CO_2 atmospheres through cap-fall and fruit set.

Table 5.3 Flower number, number of abscised flowers and % flower abscission of vines grown in a cold or warm soil pre-treatment, followed by ambient or low CO_2.

		flower number		abscised flowers	abscission %
Cool	**Ambient**	117	(113)[a]	46	39
	Low		(121)	98	78
Warm	**Ambient**	139	(133)	73	55
	Low		(144)	111	78
	Pre-TRT	**[b]		***	***
	CO_2TRT	-		***	***
	interaction	-		ns	***

[a] pre-CO_2 treatment population means

[b] **,*** and ns indicate significance at p < 0.01, < 0.001, and not significant, respectively.

5.4 Summary and conclusions

These results indicate that placing vines into an environment that limited photosynthesis had an inhibitory effect on inflorescence development and fruit set. In this environment, inflorescences on vines with either high or low reserve levels lagged in development compared to vines in the ambient CO_2 treatment, thus indicating that carbon from photosynthesis is the major contributor to flowering and fruit set.

Within a photosynthetically active environment, reserve levels do play a role in fruit-set as well. Leaves of vines in the high CO_2 treatment had similar rates of photosynthesis, irrespective of whether starch levels were high or low. Yet those vines with low starch levels had 20% less set than those with high starch levels. Furthermore, despite the smaller number of berries to support, berry growth in the low reserve treatment did not catch up with the high reserve treatment over the first 10 days of development. The larger amount of photoassimilate which would have presumably accumulated by the slightly larger canopies prior to fruit-set, could not compensate for the lower starch levels of these vines.

Within a photosynthetically limiting environment, reserve levels played an even larger role in fruit-set. Vines with low reserves and little carbon gain through photosynthesis had the slowest shoot growth rates and inflorescence elongation had ceased after 10 days within this environment. Most inflorescences (75%) did not reach the fruit-set stage and flower abortion along with dehydration of the rachis was common.

Starch levels increased most notably in the roots during the process of fruit-set. In the ambient CO_2 treatment, total starch increased by 2-fold in the roots for both temperature pre-treatments. In the low CO_2 atmosphere and cool pre-treatment, however, there was no increase in starch. Starch levels in roots of vines grown in this atmosphere but with the warm root temperature pre-treatment did recover to some extent, but were still lower than any of the other treatments. Vine sugar levels continued to increase when allowed to develop in ambient CO_2. This was mainly due to increases within the roots (1.5-fold) for those vines with the warm pre-treatment, and increases within the roots (1.5-fold) and shoots (1.7-fold) for the vines with the cool pre-treatment. Vines grown in the low CO_2 treatment had lower sugar levels in the shoots as compared to the onset of the treatment, while the root sugar levels remained very similar. Sugar levels in the trunk remained low, regardless of treatment.

In summary, the results indicate that fruit set in grapes is source limited and is markedly dependent on carbon flow from photosynthesis. Carbon derived from starch reserves has a less dominant role, however it is also required for optimal set. The interplay of carbon from photosynthesis and reserves will make it difficult to predict fruit set rates. Healthy vines with a good carbon pool to draw from will not have good fruit set in cool and cloudy conditions when photosynthesis is low. Similarly stressed vines with low reserve levels may have depressed fruit set, despite optimal conditions for photosynthesis.

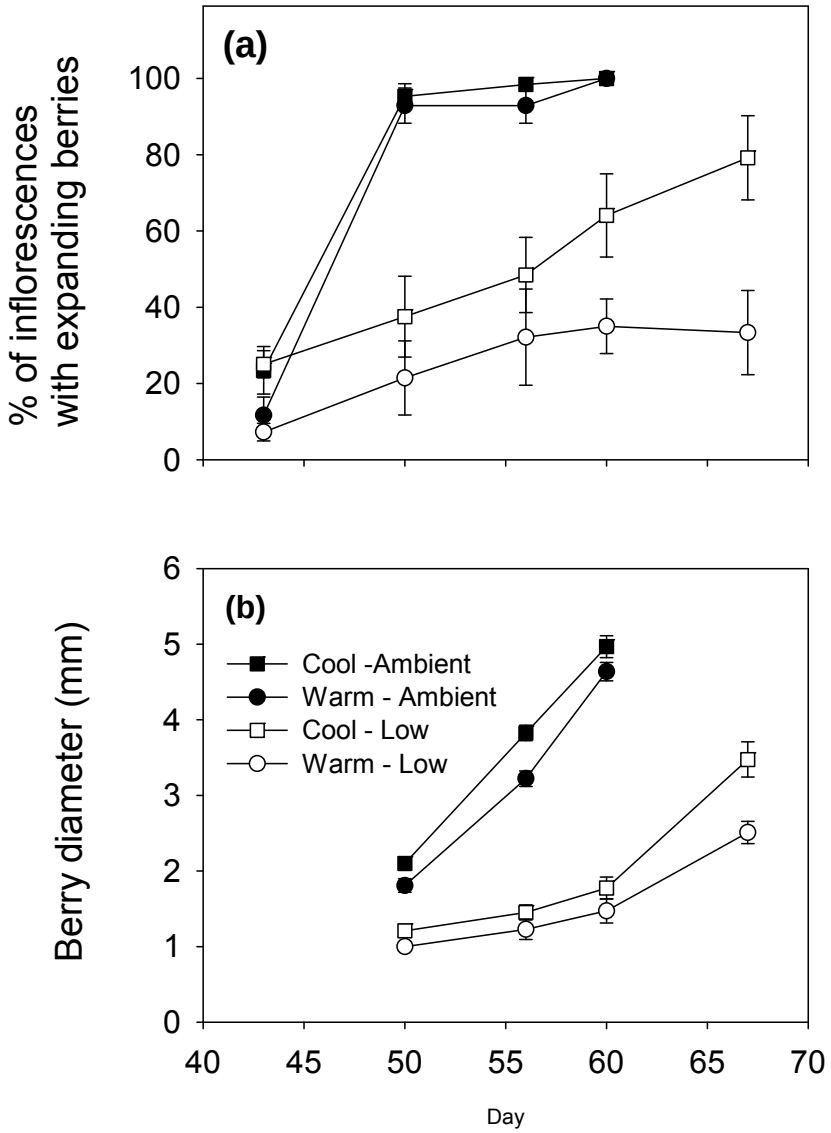


Figure 5.7 Percent of inflorescences with expanding berries (a) and berry diameter (b) of vines grown in a cold or warm soil pre-treatment, followed by ambient or low CO_2.

6. Outcomes and conclusions

6.1 The physiology of grapevine carbohydrate reserves

Due to the perennial nature of grapevines when grown in temperate climates, carbohydrate reserves are essential for the reestablishment of growth in spring. Shoot growth has been clearly demonstrated to rely on stored reserves in studies using ^{14}C to label stored carbohydrates, and similar methodology has shown reserves can also be mobilized and transported to the fruit during early ripening. In our current study we have found a consistent association between the appearance of new roots and a decline in root starch reserves in the period leading up to bud-break, indicating that at least in hot climate vineyards, stored carbohydrates reserves are also used to support early root growth and nutrient uptake. Such findings demonstrate a clear role for carbohydrates reserves as a carbon source at various points during the season.

As shoots and leaf area continue to develop in spring, the canopy reaches a point of self-sufficiency around flowering, and recently assimilated carbon is transported back towards the perennial parts of the vine. This transition to carbon autotrophy provides an opportunity for the vine to replenish the reserves used earlier in the season, and this may continue uninterrupted through berry development if crop loads are low. However, for heavily cropped vines, reserve replenishment may be delayed until later in the ripening period and through into the post-harvest period. In periods of high carbon availability reserves are therefore equally able to act as a sink for carbohydrates, although an important question remains as to whether reserve storage presents an active or passive sink, or if the relative priorities change during the season. As discussed later, this may be critical for understanding how variations in seasonal reserve dynamics influence reproductive development and fruit composition.

One key conclusion that can be drawn from the findings of previously published work, as well as those from the current project, it is that carbohydrate reserves represent an integral part of the whole vine carbon economy. This is highlighted by the results of the whole vine excavation study which showed that the pattern of reserve mobilization and storage strongly reflected the changes in carbon demand and supply as the season progressed. It also suggests that carbohydrate reserves may provide a useful physiological indicator of internal assimilate availability, and a provides a means to better understand the importance of the perennial tissues, and the seasonal patterns of shoot and root growth, for the overall growth and productivity of grapevines.

6.2 Carbohydrate reserve variability in commercial vineyards

The project provided information on between vineyard variability in carbohydrate reserve status with a winter survey of 34 commercial vineyards undertaken across Southern NSW between 2005 and 2007. Carbohydrate reserve concentrations of perennial tissues were found to vary significantly between vineyards, and to a lesser extent between seasons. Roots stored the highest concentrations of carbohydrate reserves, but were also the most likely to vary between vineyards. A significant part of this variation was associated with the consistently lower root starch concentrations of Ramsey, or reductions in reserve storage over prolonged seasons of drought. Total carbohydrate concentrations in wood and spur tissue did not differ from each other, and were also less likely to vary significantly between vineyards.

Despite the overlying variability associated with rootstock and irrigation it was found that the relative differences in perennial tissue reserve concentrations between sites was maintained from one season to the next. In some vineyards there were large fluctuations possibly associated with frost events or changes in crop load, but overall the results suggest that vine carbohydrate reserves may reach an equilibrium if cropping level and management practice

remain relatively stable from season to season. The wide range of reserve concentrations seen across vineyards, but general tendency for the relative differences between vineyards to be maintained across seasons, raises a question as the relative importance of absolute reserve concentrations compared the manner in which concentrations change between various development stages within a season. Absolute concentrations at dormancy may provide an indication of vegetative growth or reproductive development potential in the coming season. However, further work would be needed before any ideal concentration range could be suggested, as there are clear differences between rootstocks with respect to carbohydrate storage capacity, and some method to account for variations in vine size may also be required. This could be as simple as a trunk diameter measurement for own-rooted vines, but rootstock effects on biomass partitioning may need to be accounted for with grafted vines. Relative changes within a season may provide an indication of a vines capacity, given prevailing management or environmental conditions, to ripen a particular crop load to desired specifications.

6.3 Influence of carbohydrate reserves on reproductive development

Previous studies have suggested an influence of carbohydrate reserves on inflorescence initiation following treatments that dramatically reduce the amount of stored reserves. However, in the current study, no relationship was found between inflorescence initiation and carbohydrate reserve status during winter in the same season, or carbohydrate reserves in winter and the number of inflorescences initiated in the next season. Significant differences in fruitfulness at the regional and seasonal level were observed that were sufficient to influence yield, but this points to a broader climatic influence and importance of carbohydrates from current photosynthesis rather than from stored reserves.

Fruit set can contribute to seasonal yield variability, particularly in cool climate regions, where adverse weather conditions are more common during the flowering period than in warmer regions. Given the results of the survey and management trials, it is probable that carbohydrate reserves will differ at flowering between vineyards and seasons. However, the results of our controlled environment study suggests that high carbohydrate reserves during this period will not substantially offset the effects of reduced photosynthesis on fruit set. There may be a minor benefit due simply to reduced competition for carbohydrates if carbohydrate reserves are already high, but the inflorescence (and even growing shoots) did not appear to have sufficient strength to cause reserves to be mobilized during fruit set. As with inflorescence initiation, this finding points to a greater relative importance of current photosynthesis in determining fruit set in grapevines.

In earlier work we found a strong positive association between reserve status and inflorescence flower number in Semillon following crop removal and defoliation treatments, and a similar relationship has been reported by other authors. However, in the reserve accumulation management trials we found no effect of more moderate versions of the same treatments on flower numbers in Shiraz and Chardonnay, although the effects on reserve concentrations were also less marked. Flower numbers were not recorded during the carbohydrate reserve survey of commercial vineyards, but the range of carbohydrate reserve concentrations was much wider, and a preliminary analysis of yield data from the blocks suggests there may be a positive relationship between wood reserves and yield in the next season. In the absence of significant effect on inflorescence initiation, it is possible this is related to an effect on bunch weights, but further data analysis is required to confirm this.

One of the broader goals of the project was to assess the potential use of a carbohydrate reserve test as a yield forecasting tool, possibly involving the parallel analysis of carbohydrates and bud-fruitfulness from canes collected prior to pruning. Based on the results of the survey, it appears sample collection would have to include root and wood tissue the reserve status of the vine was to more accurately determined, as the canes are the least likely to vary of all tissues. However, wood tissue only may be sufficient if a relationship

can be demonstrated with bunch weight. A one off measure of winter reserves is likely to be relatively meaningless on it own as yield predicting tool, but if used over consecutive seasons in conjunction with bud-fruitfulness assessments it could indicate whether bunch weight is likely to be higher or lower than average in the coming season.

6.4 Effectiveness of techniques for altering reserve accumulation

The carbohydrate reserve status of a vineyard can be altered very quickly if vines are completely defoliated after harvest, or a significant proportion of the leaf area is removed during ripening. In these situations a pronounced impact on shoot vigour and yield can be expected in the next season, and some residual effect may still be seen in the season after that. However, with practices such as crop thinning or deficit irrigation that are more routinely practiced in commercial vineyards, the effects on reserve accumulation appear to be less pronounced in the shorter term, and suggests that within limits, vines may have some buffer capacity through adjustments to growth and photosynthesis. Any benefits for fruit development that result from such treatments may arise from a more subtle improvement in carbohydrate availability that are not easily seen in the concentrations of reserves in bulk root or wood tissue samples.

For the treatment evaluated in the current project, deficit irrigation between fruit set and veraison, which is essentially an extended version of RDI targeted at the period of reserve replenishment after flowering, reduced yield and improved berry colour in Shiraz. Conversely, deficit irrigation after harvest significantly delayed berry ripening and colour development, but had no effect on yield. Neither treatment had a major influence on carbohydrate reserves, although it is evident from the results of the survey that water stress will reduce reserve accumulation in the longer term. Both crop removal treatments increased reserves concentrations, and the rapid acceleration of colour development in Shiraz after veraison relative to control vines, suggests a positive benefit of carbohydrate supply for berry development during this period. Post-harvest hedging had the greatest impact on reserve accumulation, and within two seasons significant yield reductions were observed in both Shiraz and Chardonnay. However, the ripening and fruit quality parameters did not differ consistently from the control treatments in either variety.

6.5 Carbohydrate reserves and vineyard management: practical implications.

The possible value of a carbohydrate reserve test to compliment yield forecasting models has already been described, but there may also be an opportunity to use a carbohydrate test at key stages during the season to improve our understanding of how crop load and vineyard management practices can be used to optimise fruit quality. We have shown that carbohydrate reserve dynamics are strongly influenced by the relative changes in sink:source relations through the season, at least in response to crop load, that both reserve accumulation and fruit ripening responded to an apparent improvement in carbohydrate availability. It is therefore possible that a more detailed assessment of the changes in reserves during ripening could provide a physiological measure of vine carbon balance during this period that could be related to the development of key berry quality attributes.

The finding that post-harvest deficit irrigation can have an impact on vine growth and berry development in the following season is also important. Firstly because water shortages after harvest are becoming an increasingly important issue in some reasons, and secondly because it implies that berry development can be influenced by the way vines are managed in the previous season. This did not immediately appear to be related to carbohydrate reserve storage, but does suggest an impact on nutrient uptake or some other factor that would carry over to the next season through the perennial parts of the vine.

7 Recommendations

The project had achieved further understanding of seasonal carbohydrate dynamics in the perennial structure and the allocation to different organs of grapevines. The intra-seasonal changes and between vineyards has been assessed, this work has indentified environmental and management factors contributing to the levels of reserves in root and wood tissues. In addition, trials have shown that the reserves can be altered by crop and irrigation management and by post-harvest hedging. The role of carbohydrate reserves during the fruit-set period has also been further clarified.

The findings have identified future research directions and a proportion of the data can be utilised for further work. These existing data on carbohydrate reserves and vine carbon assimilation can be integrated to produce a vine balance tool for optimising sustainable production and grape quality for a range of production levels and climates. However, additional targeted field trials and physiological studies would be required to refine and validate this work. The role of stored carbohydrates for early growth is an important issue for vine balance, however the work suggests that vine reserves are also important for grape maturation and grape composition.

The project has further determined the importance of the roots as a storage organ, with its accumulated carbohydrates showing significant seasonal changes, between season and vineyards. These dynamics are driven by the partitioning of the grapevine and the root environment, with factors such as water supply and soil temperature. The role of the reserves in the roots for the demand of the organs above ground early in the season or during berry ripening would be an important issue to address. In addition, the requirements for the root system in relation to root growth and nutrient uptake from stored or current assimilates is an important area for further investigation.

The understanding of the response of grapevines to the range of vine atmospheres and root environments relevant to current and future climatic trends is crucial. This would allow to determination of assimilate production and requirements as well as the partitioning under different cropping levels and water availability, ensuring that optimal fruit compositions and production level can be achieved and sustained. These would be undertaken by using a combined experimental and model approach to make the work relevant to the majority of the Australian grape growing regions.

APPENDIX 1 COMMUNICATIONS

Refereed Scientific Publications

HOLZAPFEL, B., SMITH, J., MANDEL, R. and KELLER, M. (2006): Manipulating the length and effectiveness of the post-harvest period and the impact on vine productivity of Semillon grapevines. Am. J. Enol. Vitic. 57, 148-157

LEBON, G. WOJNAOWIEZ, G., HOLZAPFEL, B., FONTAINE, F., VAILANT-GAVEAU, N., and CLEMENT, C. (2008): Sugars and flowering in grapevines (*Vitis Vinifera* L.). J. Exp. Botany 59, 2565-2578

HOLZAPFEL, B.P., SMITH, J.P., FIELD, S.K. and HARDIE, W.J. (2009): Dynamics of Carbohydrate Reserves in Cultivated Grapevines. Hort. Reviews 36 (in press)

SMITH, J.P. and HOLZAPFEL, B.P. (2009): Cumulative response of grapevine (Vitis vinifera L.) to late season perturbation of carbohydrate reserve status. Am. J. Enol. Vitic. 60:(in press)

ROGIERS, S.Y., SMITH. J.P, and HOLZAPFEL, B.P (2009): The importance of photosynthesis and perennial carbohydrate reserves for flowering and fruit-set in grapevine (*Vitis vinifera L.*). J. Exp. Botany (submitted)

International Conference & Symposium Presentation

HOLZAPFEL, B. and SMITH, J. (2005): Effect of post-harvest on vine nutrient levels and the relationship to vine reserves. Poster Presentation. Proc. XV International Plant Nutrition Colloquium, Beijing, China, September 15-19

QUIRK, L., SMITH, J., GRAY, J., MEUNIER, M., ROGIERS, S. and HOLZAPFEL, B. (2006): A test for carbohydrate reserves as an additional tool of bud-fruitfulness for regulating vine yield. 6th ISCCV&O, Christchurch, New Zealand, February 6-10

HOLZAPFEL, B. and SMITH, J. (2007): Vine carbohydrate reserves at harvest and leaf fall and its importance on productivity in the next season. In: Castellucci, M.F. (Ed.), Le Bulletin de L'OIV 80, 17-29 (paper prepared for XXIX World Vine and Wine Congress, Logroño, Spain, June 25-30, 2006)

SMITH, J., QUIRK L., and HOLZAPFEL, B. (2007): Predicting yield potential for the next season with starch reserves in the perennial structure and bud fruitfulness before winter pruning. In: Sladonja, B. (Ed.), Proc. XVth International Symposium GESCO, Poreč, Croatia, 510-518

SMITH, J., QUIRK, L., FIELD, S. LAMONT, R. and HOLZAPFEL, B. (2007): Understanding the impact of environment and management practise on carbohydrate reserve accumulation and possible consequences for vine productivity. Poster Presentation. 13th AWITC, Adelaide, July 29 – August 1

HOLZAPFEL, B. and SMITH, J. (2008): The influence of management practice on vine carbohydrate reserve accumulation and vine productivity. In: Zehnder, H. (Ed.), Proc. 15th Enology Symposium, Trier, Germany, April 13-16, 101-108

ROGIERS, S.Y., SMITH, J.P. and HOLZAPFEL, B.P. (2008): The contribution of photoassimilates and carbohydrate reserves to fruit set in Chardonnay vines. 8th International Symposium on grapevine Physiology and Biotechnology, Adelaide, Australia, November 23-28

HOLZAPFEL B.P., SMITH J.P., FIELD S.K. and HARDIE W.J. (2008): The relationship between carbohydrate reserve dynamics and vine productivity. 8th International Symposium on grapevine Physiology and Biotechnology, Adelaide, Australia, November 23-28

Seminar & workshops presentations

SMITH, J. and HOLZAPFEL, B. (2005): The role of carbohydrates in the grapevine growth cycle. In: Degaris, K. Dundon, C. Johnstone, R. and Partridge S. (Eds.), ASVO Seminar 'Transforming Flowers to Fruit'. Winetitles, Adelaide, 38-42

SMITH, J. (2005): The role of carbohydrates in the grapevine growth cycle. Wine Grape Marketing Field Day, Griffith, September 8

SMITH, J. and HOLZAPFEL, B. (2005): Carbohydrate reserves - importance for yield and fruit quality. McWilliams Vintage Review, Griffith, June 20

SMITH, J., FIELD, S. and HOLZAPFEL, B. (2006): Carbohydrate reserves and vine productivity. In R.R. Walker (Ed.). GWRDC workshop 'Vine Carbohydrate Dynamics and Source-Sink Relationships'. Merbein, Victoria, Australia, January 31, 12-31

SMITH, J. (2006): Over-wintering carbohydrate reserves and vine productivity. Tumbarumba growers association meeting, Tumbarumba, July 6

SMITH, J., S., QUIRK, L.F. and HOLZAPFEL, B. (2006): Over-wintering carbohydrate reserves and vine yield potential. Griffith, September 4.

SMITH, J. (2007): Bunch initiation and bud-fruitfulness of Southern NSW vineyards. Murrumbateman Grape Growers Association, Murrumbateman, August 15

SMITH, J., QUIRK, L., FIELD, S. LAMONT, R. and HOLZAPFEL, B. (2007): Carbohydrate reserves and root growth - the hidden aspects of grapevine balance? Seminar Limestone Coast, Wynns Coonawarra Estate, Coonawarra, August 3

SMITH, J. (2008): Shoot thinning and bunch removal: effects on the fruit and whole vine. . Constellation Wines technical conference, National Wine Industry Centre, Adelaide, June 28

SMITH, J. (2008): Carbohydrate reserves and flowering: Southern NSW. Tumbarumba growers association meeting, Tumbarumba, June 2.

HOLZAPFEL, B. (2008): Relationship between carbohydrate dynamics and reproductive development. GWRDC workshop - Tumbarumba, November 18

SMITH, J. (2008): Understanding carbohydrates and carbon balance in relation to vine growth and yield. Guest lecture CSU, (VIT 303 Residential School), Wagga Wagga September 29

ROGIERS, S., SMITH, J. and HOLZAPFEL, B. (2008): Carbohydrates and fruit set in grapes. NWGIC Seminar Series, Wagga Wagga, October 23

HOLZAPFEL, B.P. and SMITH, J.P. (2008): The influence of management practice on vine carbohydrate reserve accumulation and vine productivity. Guest lecture CSU – Wagga Wagga, September 16

SMITH, J. (2009): Understanding carbohydrates reserves in relation to vine growth and balance. Southern Tablelands Winemakers Federation meeting, February 2

SMITH, J, FIELD, S., QUIRK, L.F. and HOLZAPFEL, B. (2008/09): Post-harvest care of grapevines: irrigation, nutrition and salinity. Water & Vine managing the challenge. South Australia, Victoria, NSW and ACT (9 presentations)

SMITH, J. and FARQUHAR D, (2009): Can carbohydrate reserves provide a physiological indicator of vine balance in hot climate vineyards? Riverina Field Day, Griffith, May 8.

SMITH, J. (2009): Can carbohydrate reserves provide a physiological indicator of vine balance in hot climate vineyards? NWGIC Seminar series, Wagga Wagga, August 5

SMITH, J. (2009): Can carbohydrate reserves provide a physiological indicator of vine balance in hot climate vineyards? Guest lecture CSU, (VIT 303 Residential School), Wagga Wagga, September 25

Technical publications

HOLZAPFEL, B. and SMITH, J. (2005): Vine carbohydrate production, consumption and storage. Grapevine Management Guide 2005-06, pp 103-105

HOLZAPFEL, B. and SMITH, J. (2006): Test for carbohydrate reserves and to assess bud-fruitfulness in vines. WWAI Update 2006, 54-55

HOLZAPFEL, B. and SMITH, J. (2006): Vine carbohydrate production, consumption and storage. Grapevine Management Guide 2006-07, pp 86-88

SMITH, J., QUIRK, L. and HOLZAPFEL, B. (2007): Bud-fruitfulness assessment in grapevines. Grapevine Management Guide 2007-08, pp 68-72

SMITH, J., and HOLZAPFEL, B. (2008): Seasonal pattern of grapevine root growth: a research update. Grapevine Management Guide 2008-09, pp 32-34

HOLZAPFEL, B. (2008): Impact of environment and management practice on carbohydrate reserves. WWAI Update 2008, 48-49

HOLZAPFEL, B.P. and SMITH, J.P. (2009): The relationship between vine carbohydrates and reproductive development. Australian Viticulture 1, 56-57

SMITH, J., and HOLZAPFEL, B. (2009): Understanding the importance of the post-harvest period in hot-climate vineyards. Grapevine Management Guide 2009-10, pp 13-17

APPENDIX 2 REFERENCES

Balasubrahmanyam, V., J. Eifert, and L. Diofasi. 1978. Studies on cropping level in grapes. I. Effect of differential cropping level on the organic nutrient reserves in the canes. Vitis 17:23-29.

Bates, T., R. Dunst, and P. Joy. 2002. Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. HortScience 37:313-316.

Bennett, J., P. Javis, G.L. Creasy, and M.C.T. Throught. 2005. Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. Am. J. Enol. Vitic. 56:386-393.

Candolfi-Vasconcelos, M.C., M.P. Candolfi, and W. Koblet. 1994. Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in *Vitis vinifera L*.. Planta 192:567-573.

Caspari, H.W., A. Lang, and P. Alsbach. 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Am. J. Enol. Vitic. 49:359-366.

Chaumont, Murier, Jean-François Morot-Gaudry, and Christine H. Foyer. 1994. Seasonal and diurnal changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with and without fruit. Journal of Experimental Botany 45:1235-1243.

Cheng, L., G. Xia, and D.J. Bates. 2004. Growth and fruiting of young 'Concord' grapevines in relation to reserve nitrogen and carbohydrates. J. Amer. Soc. Hort. Sci. 129:660-666.

Collins, C., R. Coles, J.G. Conran, and B. Rawnsley. 2006. The progression of primary bud necrosis in grapevine cv. Shriaz (*Vitis vinfera* L.): A histological analysis. Vitis 445:57-62.

Dry, P.R., and B.G. Coombe. 1994. Primary bud-axis necrosis of grapevines. I. Natural incidence and correlation with vigour. Vitis 33:225-230.

Field, S.K., J.P Smith, D.H. Greer, M. Meunier, B.P. Holzapfel, and N. Emery. 2008. seasonal carbohydrate, nitrogen and cytokinin dynamics and their impact on shoot and root growth. 8th International Symposium on grapevine Physiology and Biotechnology, Adelaide, Australia, November 23-28

Field, S.T., J.P. Smith, B.P.Holzapfel, R.J.N. Emery, and W.J. Hardie. 2009. Grapevine response to soil temperature: xylem cytokinins and carbohydrate reserve mobilization from budbreak to anthesis. Am. J. Enol. Vitic. 60:164-172

Glad, C., J.L. Regnard, Y. Querou, O. Brun, and J.F. Morot Gaudry. 1992. Flux and chemical composition of xylem exudates from Chardonnay grapevines: temporal evolution and effect of recut. Am. J. Enol. Vitic. 43:275-282.

Goffinet, M.C. 2004. Relation of applied crop stress to inflorescence development, shoot growth characteristics, and cane starch reserves in 'Concorde' grapevine. Acta Horticulturae 640:189-200.

Gray, J. 2002. The basis of variation in the size and composition of grape berries. PhD Thesis, University of Adelaide.

Hackett, S.D., and B.P. Holzapfel. 2002. Factors underlying restricted spring growth in vineyards. Australian Viticulture 5:76-81.

Hale, C.R., and R.J. Weaver. 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Ann. Rev. Plant. Physiol. Plant Mol. Biol. 39:355-378.

Holzapfel, B.P., and M. Treeby. 1999. Effect of nitrogen supply on Shiraz canopy development and winter wood nitrogen and starch reserves. p. 268-267. In: Blair R.J., A.N. Sas, P.F. Hayes, and P.B. Høj (eds.), Proc. Tenth Australian Wine Industry Technical Conference, Winetitles, Sydney.

Holzapfel, B., J. Smith, R. Mandel, and M. Keller. 2006. Manipulating the length and effectiveness of the post-harvest period and the impact on vine productivity of Semillon grapevines. Am. J. Enol. Vitic. 57:148-157.

Hunter, J.J., H.P. Ruffner, and C.G. Volschenk. 1995. Starch concentrations in grapevine leaves, berries and roots and the effect of canopy management. S. Afr. J. Eno. Vitic. 16:35-40.

Hunter, J.J., R. Skrivan, and H.P. Ruffner. 1994. Diurnal and seasonal physiological changes in leaves of *Vitis vinifera L.*: CO2 assimilation rates, sugar levels and sucrolytic enzyme activity. Vitis 33:189-195.

Hunter, J.J. 1998. Plant spacing implications for grafted Grapevine I. Soil Characteristics, root growth, dry matter partitioning, dry matter composition and soil utilisation. S. Afr. J. Enol. Vitic. 19:25-34.

Intrieri, C., O. Poni, O. Silvestroni, and I. Filippetti. 1992. Leaf age, leaf position and photosynthesis in potted grapevines. Adv. Hort. Sci. 6:23-27

Keller, M. 1993. Reservestoffe in Reben: Haushalt und Bedeutung. Schweiz. Z. Obst-Weinbau 129:446-452.

Keller, M., and W. Koblet. 1995a. Dry matter and leaf area partitioning, bud fertility and second season growth of *Vitis vinifera L.*: Responses to nitrogen supply and limiting irradiance. Vitis 34:77-83.

Keller, M., B. Hess, H. Schwager, H. Scharer, and W. Koblet. 1995. Carbon and nitrogen partitioning in *Vitis vinifera L.*: responses to nitrogen supply and limiting irradiance. Vitis 34:19-26.

Koblet, W. 1969. Translocation of photosynthate in vine shoots and influence of leaf area on quantity and quality of the grapes. Wein-Wiss 24:277-319.

Koblet, W. (1977): Translocation of photosynthate in grape vines. p45-51. In: Proc. Intern. Symp. Quality Vintage, Cape Town, South Africa.

Koblet, W., M.C. Candolfi Vasconcelos, and M. Keller. 1996a. Kompensationsvermögen der Rebe unter Stress. Deutsches Weinbau Jahrbuch 47:41-46.

Korkas, E., K. Schaller, O. Löhnertz, and F. Lenz. 1994a. Die Dynamik 'nicht-stuktureller' Kohlenhydrate in Reben (*Vitis vinifera L.* cv. Riesling) im Verlauf zweier Vegetationsperioden unter dem Einfluss einer langjährig variierten Stickstoffdüngung. Teil I: Vor und während der Austriebsphase. Vitic. Enol. Sci. 49:86-95.

Korkas, E., K. Schaller, O. Löhnertz, and F. Lenz. 1994c. Die Dynamik 'nicht-stuktureller' Kohlenhydrate in Reben (*Vitis vinifera L.* cv. Riesling) im Verlauf zweier Vegetationsperioden unter dem Einfluss einer langjährig variierten Stickstoffdüngung. Teil II: Nach dem Austrieb bis zum Fruchtansatz. Vitic. Enol. Sci. 49:197-208.

Korkas, E., K. Schaller, O. Löhnertz, and F. Lenz. 1996a. Die Dynamik 'nicht-stuktureller' Kohlenhydrate in Reben (*Vitis vinifera L.* cv. Riesling) im Verlauf zweier Vegetationsperioden unter dem Einfluss einer langjährig variierten Stickstoffdüngung. Teil III: Fruchansatz bis Lesereife. Vitic. Enol. Sci. 51:44-55.

Korkas, E., K. Schaller, O. Löhnertz, and F. Lenz. 1996b. Die Dynamik 'nicht-stuktureller' Kohlenhydrate in Reben (*Vitis vinifera L.* cv. Riesling) im Verlauf zweier Vegetationsperioden unter dem Einfluss einer langjährig variierten Stickstoffdüngung. Teil IV: Nach der Lese. Vitic. Enol. Sci. 51:98-102.

Kriedemann, P.E. 1968. Photosynthesis in vine leaves as a function of light intensity, temperature and leaf age. Vitis 7:213-220.

Lakso, A. N., D.M. Eissenstat, K. Link, P. Joy, and R.M. Dunst. 2009. Crop level effects on long-term 'Concord' grape growth and biomass distribution. p83-86. In: Wolpert, J. (ed.) Proceedings of 16th International Symposium GiESCO. Davis, California, USA, July 12-16.

Lavee, S., H. Melamud, M. Ziv, and Z. Bernstein. 1981. Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard). I. Relation to vegetative vigor. Vitis 20: 8-14.

Lebon, G., G. Wojnarowiez, B. Holzapfel, F. Fontaine, N. Vaillant-Gaveau, and C. Clement. 2008. Sugars and flowering in the grapevine (*Vitis vinifera L.*). Journal of Experimental Botany 59:2565-2578.

Martin, S. 2003. Sources of seasonal variation in grapevvine yield. Workshop: Flower formation, flowering and berry set in grapevines, DPI Tatura, 22nd - 23rd May

McKenry, M.V. 1984. Grape root phenology relative to control of parasitic nematodes. Am. J. Enol. Vitic 35:206-211.

Miller, D.P., G.S. Howell, and J.A. Flore. 1997. Influence of shoot numbers and crop load on potted Chambourcin grapevines. II. whole-vine vs. single-leaf photosynthesis. Vitis 36:109-114.

Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. Biology of the grapevine. Cambridge: Cambridge University Press.

McKenry, M.V. 1984. Grape root phenology relative to control of parasitic nematodes. Am. J. Enol. Vitic. 35:206-211.

Miller, D.P., G.S. Howell, and J.A. Flore. 1997. Influence of shoot numbers and crop load on potted Chambourcin grapevines. II. Whole-vine vs. single-leaf photosynthesis. Vitis 36:109-114.

Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. Biology of the grapevine. Cambridge: Cambridge University Press.

Petrie, P.R., N.M. Cooley, and P.R. Clingeleffer. 2004. The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera L.*) in the current and following season. Aust. J. Grape & Wine Res. 10:203-215.

Rawnsley, B., and C. Collins. 2005. Vine balance: The key to reducing primary bud necrosis in Shiraz. The Australian & New Zealand Grapegrower and Winemaker. 497a:13-14, 16-17.

Rühl, E.H., and G. Alleweldt. 1990. Effect of water stress on carbohydrate accumulation in root and stem of four different grapevine varieties. Vitic. Enol. Sci. 45:156-159.

Rühl, E.H., and P.R. Clingeleffer. 1993. Effect of minimal pruning and virus inoculation on the carbohydrate and nitrogen accumulation in Cabernet franc vines. Am. J. Enol. Vitic. 44:81-85

Sánchez, L.A., and N.K. Dokoozlian. 2004. New clues for unravelling the mystery of bud fruitfulness in table grapes. San Joaquin Valley Table Grape Seminar, Visalia, USA, February 18:1-20.

Scholefield, P.B., T.F. Neales, and P. May. 1978. Carbon balance of the Sultana vine (*Vitis vinifera L.*) and the effects of autumn defoliation by harvest-pruning. Australian Journal of Plant Physiology 5:561-570.

Skinner, P.W., and M.A. Mathews. 1989. Reproductive development in grape (*Vitis vinifera* L.) under phosphorus limited conditions. Scientia Horticulturae 38:49-60

Smith, J.P. 2004. Investigations into the mechanisms underlying grapevine rootstock effects on scion growth and yield. PhD Thesis, Charles Sturt University, Wagga Wagga.

Smith, J.P., and B. Holzapfel. 2003. The post-harvest period, carbohydrate reserves and vine productivity. The Australian & New Zealand Grapegrower & Winemaker:31-34.

Smith, J.P., and B. Holzapfel. 2009. Cumulative response of grapevine (*Vitis vinifera L.*) to late season perturbation of carbohydrate reserve status. Am. J. Enol. Vitic. 60:(in press).

Sommer, K.J., and P.R. Clingeleffer. 1996. Vine canopy development and carbohydrate partitioning as influenced by pruning. p. 123–127. In: C.S. Stockley, A.N. Sas, R.S. Johnstone, and T.H. Lee (eds.), Proc. Ninth Australian Wine Industry Technical Conference, Winetitles, Adelaide.

Staudt, G. 1982. Pollenkeimung und Pollenschlauchwachstum in vivo bei Vitis und die Abhängigkeit von der Temperature. Vitis 21, 205-216

Uys, D.C., and C.J. Orffer. 1983. Changes in carbohydrates in nursery-grown grapevine rootstocks. S. Afr. J. Enol. Vitic. 194:13-19.

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

Williams, P.J., C. Wieslawa, L. Francis, J. Gray, P. Iland, and B.G. Coombe. 1995. Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. J. Agric. Food Chem. 43:121-128.

Williams, L.E. 1996. Grape. In Photoassimilate distribution in plants and crops. Source-Sink Relationships, eds. E. Zamski and A. Schaffer, 851-881. New York: Marcel Dekker.

Williams, C.M., N.A. Maier, and L. Bartlett. 2004. Effect of molybdenum foliar sprays on yield, berry size, seed formation, petiole nutrient composition of ‘Merlot’ grapevines. Journal of Plant Nutrition 27:1891-1916.

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

Williams, L.E. 1996. Grape. In Photoassimilate distribution in plants and crops. Source-Sink Relationships, eds. E. Zamski and A. Schaffer, 851-881. New York: Marcel Dekker.

Winkler, A., and W. Williams. 1945. Starch and sugars in *Vitis vinifera*. Plant Physiol. 20:412-432.

Weyand, K.M., and H.R. Schultz. 2006. Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in cool climate. Am. J. Enol. Vitic. 57:172-182.

Yang, Y.S., Y. Hori, and R. Ogata. 1980. Studies on retranslocation of accumulated assimilates in 'Delaware' grapevines. II. Retranslocation of assimilates accumulated during the previous growing season. Tohoku J. Agric. Res. 31:109-119.

Yang, Y-S., and Y. Hori. 1979. Studies on retranslocation of accumulated assimilates in “Delaware” grapevines. I. Retranslocation of C-assimilates in the following spring are C_{14} feeding in summer and autumn. Tohoku J. Agric. Res. 30:43-56.

Zapata, C., E. Deléens, S. Chaillou, and C. Magné. 2004. Mobilisation and distribution of starch and total N in two grapevine cultivars differing in their susceptibility to shedding. Functional Plant Biology 31:1127-1135.

APPENDIX 3 PROJECT STAFF AND ACKNOWLEDGEMENTS

The project had five chief investigators at the start of the work in mid 2005 with responsibilities to the different sections as indicated in this report. Jason Smith was present from the start of the project, however his appointment to the project began July 2006 and he conducted and directed the majority of the work of the survey and management section. John Gray was responsible for the microscopy studies but left the project in December 2007, similarly Michel Meunier was meant to look after the winemaking and departed the project in December 2007. Therefore the project leader's commitment, Bruno Holzapfel, was increased in early 2008 and Leo Quirk was added to the project providing significant input into the survey section and to the extension activities. Emily Rouse undertook the experimental winemaking in both seasons. In addition, the technical support input from Rob Lamont was increased due to the departure of two chief investigators. These changes ensured that the in-kind contributions were met as outlined in the original proposal. Suzy Rogiers was responsible for fruit-set/carbohydrate study and methodology development for mobilisation studies. The other main technical support was provided by the project appointments of Angela Wilton (September to February 2006), Edwina Blackney (March to October 2007) and Kirstina Lamont (October 2007 to June 2009), There was a number of other staff involved in the project from National Wine and Grape Industry Centre, including Peter Carey, Campbell Meeks, Col Lamont, Ned Sharkey, Shayne Hackett, Helen Pan, Markus Müller, Stewart Field, Michel Meunier and Chris Western. The authors also thank Jeff Dance, Geoff McCorkelle and Anthony Pitt from McWilliams Wines, Kerry DeGaris from Constellation Wines, Andrew Drumm from the CSU winery and all growers involved with the research from the Riverina and SW slope regions for their ongoing assistance with this work. The authors greatly appreciate the statistical help provided by Beverley Orchard for the analysis of the large data set collected during the carbohydrate reserve survey.

APPENDIX 4 OTHER RELEVANT MATERIAL

Regional carbohydrate and N dynamics

The field trials for monitoring seasonal changes in carbohydrate and N concentrations were conducted in Shiraz vineyards in the Riverina and Canberra grape growing regions. In the Riverina (34ºS, 146ºE) the mean January temperature (MJT) is 23.8ºC and the average annual rainfall is 406mm. The elevation of the Riverina vineyard is 125m, with vines (SA1654) planted in 1997. These were mechanically pruned, the row width was 3.6m and the vines were planted 2.7m apart. The Shiraz vineyard (1654) in the Canberra region was planted in 1999 and the elevation is 600m. The regions (35ºS, 148ºE) mean January temperature (MJT) is 20.4ºC and the average annual rainfall is 653mm. The row spacing was 2.8m and between vines 1.8m, the vines were drip irrigated and spur pruned. Both vineyards were drip irrigated and mechanically harvested.

The sampling of root and wood was conducted in three plots of 10 vines during the growing season in monthly intervals, starting with bud-burst and concluding with dormantly in the 2006/07 growing season. The date for bud-burst was September 14 and the last sampling was conducted on 19 June in the Riverina, the dates for Canberra were 26 September and 3 July respectively. To reduce the impact on the roots, only five vines in each plot was sampled at each of these dates. Roots of a diameter of approximately 5mm were obtained and wood samples by collecting the drillings from the trunk and cordon. After drying and grinding the tissues the starch and soluble sugars were analysed using an enzymatic method and the N was determined with an N analyser. Further details on the sampling of the tissues and the consequential analysis can be found in earlier sections of the report. This work shows the seasonal dynamic for root and wood tissues in two regions for starch, soluble sugars and total N (figure A4.1).

Microscopic methodology and studies

Shoots were sampled at both Shiraz vineyards over the growing season at the same time wood and root samples were collected for microscopic studies of the bud and inflorescence premodia development. Five shoots from each plot were taken and the shoot was separated into its bud/node section and fixed with FPA50 for microscopic assessment. The second bud from three shoots was dehydrated in alcohol and then embedded in resin.

The FPA was first replaced by a solution of 60% ethanol. The buds were then placed in 70% ethanol, followed by 80% ethanol and finally 90% ethanol. The samples were then infiltrated with Historesin to fix the samples for sectioning. The Historesin (Leica Microsystems) was made up by mixing 50ml basic resin with one bag of Historesin Activator. The samples were infilled with pure resin for 2 weeks, the resin being refreshed after one week. The samples were finally embedded with fresh Historesin in gelatine capsules, with the polymerisation being overnight at 60° in an oven.

The embedded buds were sectioned with a microtome using a steel blade (Leica Microsystems). For the light microscopy used in this study, the sections were cut to 5µm thickness (Fully automated rotary microtome, Leica Microsystems RM2265). The sections were stained with Toulidine blue 0.1% pH4.4, this enhanced the structure of the images. With this stain the xylem appeared dark blue, the phloem light blue and the celluloid cells pink. The initial imaging was done on the Olympus A052 microscope. Analysis of these images was completed using the AnalySIS FIVE Life Science, Soft Imaging Sytem (Olympus). Images for this publication (Figure A4.2) were taken on the Leica M216 FA Flourescence Stereomicroscope. Buds assessed around fruit set had always inflorescence premodia, while a month prior to that developmental stage their presence was variable.

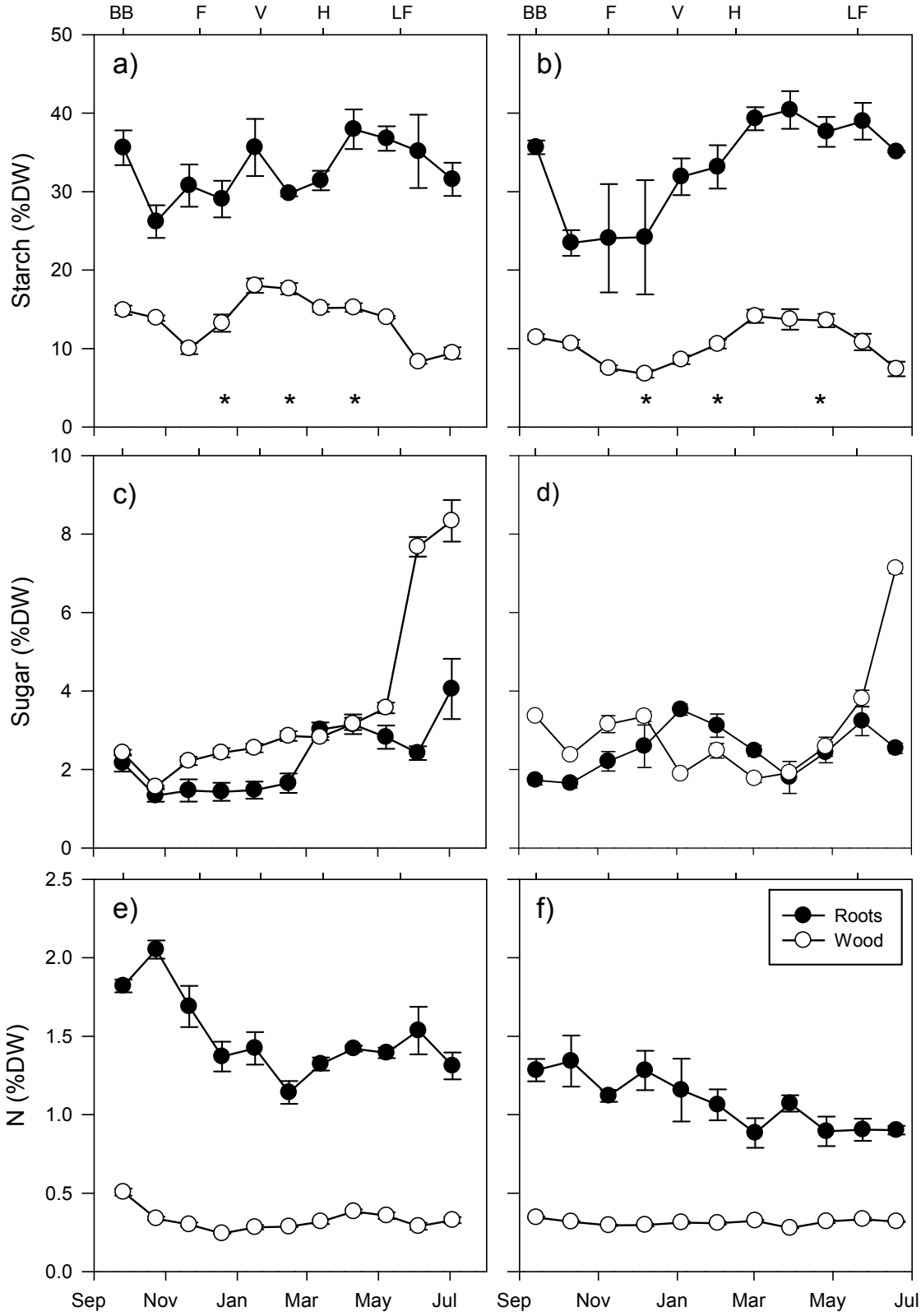


Figure A4.1 Seasonal concentration changes in root and wood tissues for starch (a, b), for sugars (c, d) and nitrogen (e, f) for Canberra (left) and Griffith (right) for the 2006/07 growing season in Shiraz. Bud-burst (BB), flowering (F), veraison (V), harvest (H) and leaf-fall (LF) indicated for both locations.

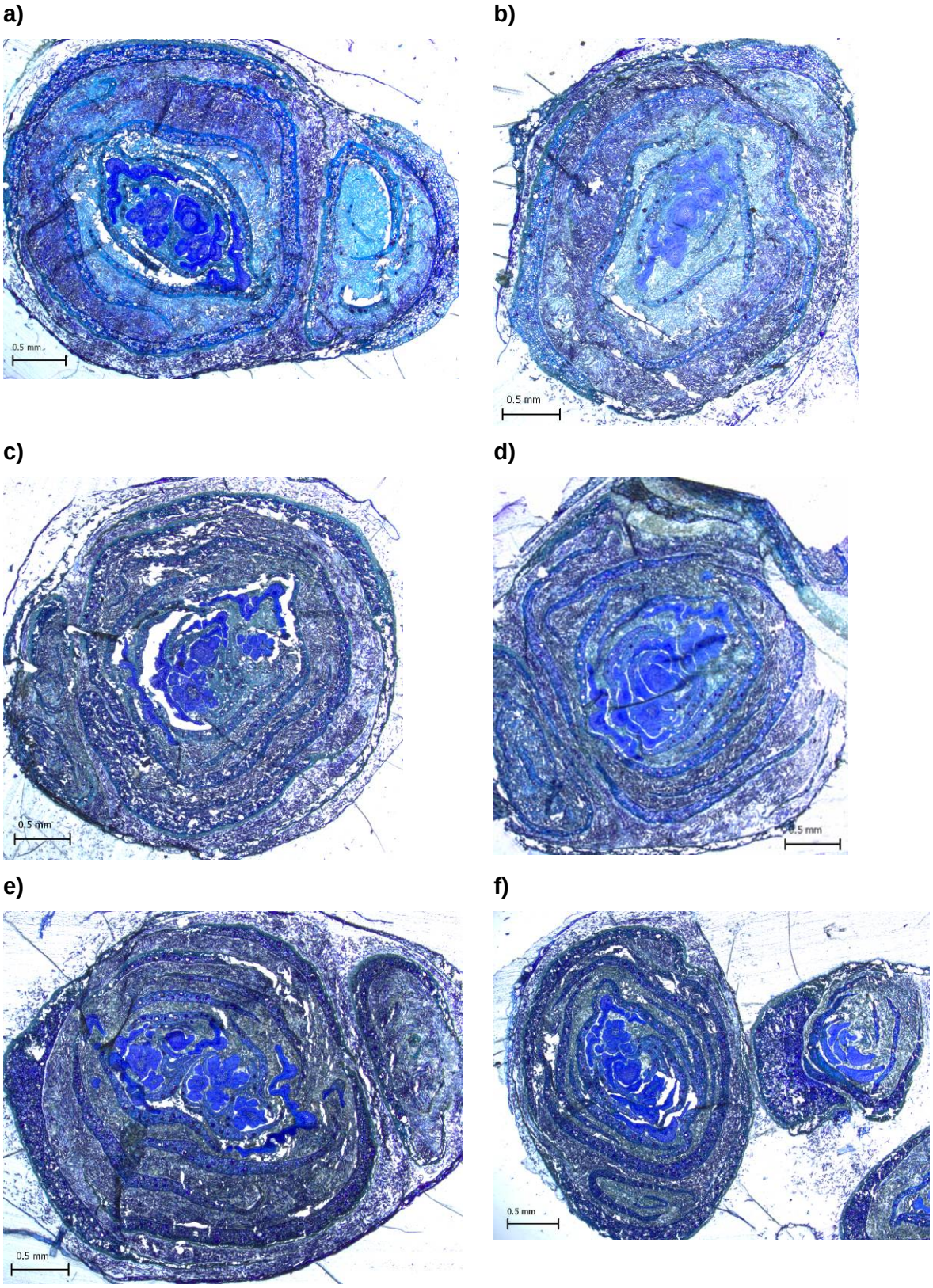


Figure A4.2 Microscopic images of buds with inflorescence premodia at different times around fruit-set (a, b), during maturation (c, d) and in the post-harvest period (e, f) for Canberra (left) and Griffith (right) for the 2006/07 growing season in Shiraz. The stars on figure A4.1a and b indicate the time when the buds were taken for these images.

Canopy light and temperature monitoring system

In the two Shiraz vineyards a canopy environment monitoring system was installed prior to bud burst in three plots of ten vines. This methodology was assessed for determining the influence of light and temperature on the bud development in relation to fruitfulness. The system consisted of light sensors and thermocouples; these were connected via multiplexer to a data logger placed in the row in the centre of a plot. The temperature was recorded at the central location of the vine plot, while the light was also recorded in two outer vines of the plot. The light sensors were fixed on a metal rod at 10, 35, 60 and 85cm vertically from cordon, while the temperature sensors were located at buds at 10 and 35 cm from the cordon and glowed to the lower two buds. The temperatures of buds and light in the canopy were continuously recorded every five minutes. The installation and sensors, together with the light and temperature fluctuation is shown in figure A4.3.

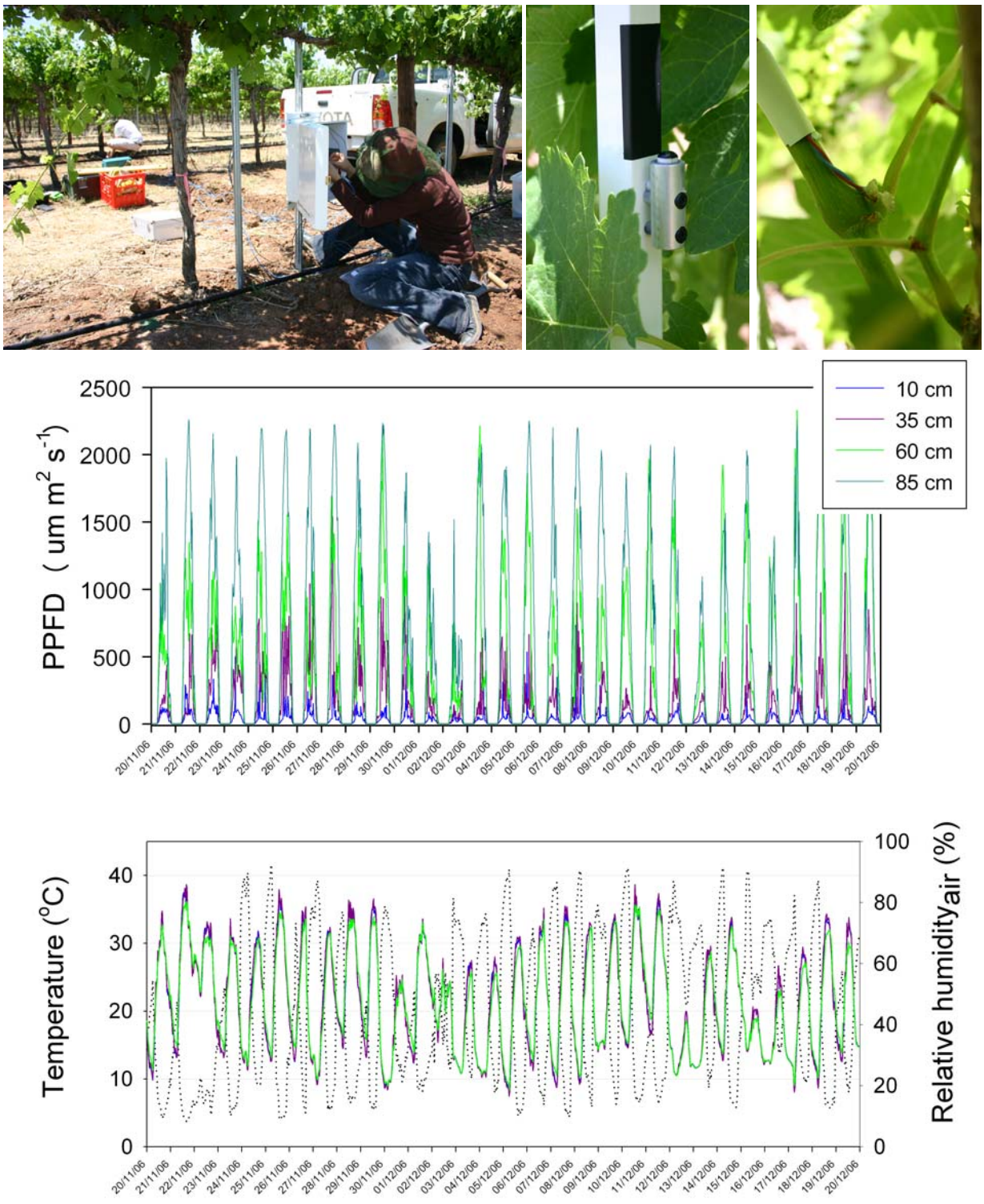


Figure 1. Installation of canopy environment monitoring system for recording light intensity and bud temperature (upper images), and sample data from the Canberra site at flowering in 2006 showing light intensity at four heights in the canopy, and corresponding temperatures of the lower tow buds.