Sustainable Vinicultural Systems



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **NWG 1101**

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Research Organisation: **National Wine and Grape Industry Centre**

Date: **May 2013**

Table of Contents

Abstract 3
1. Executive Summary 5
2. Introduction 8
3. Project Aims and Performance Targets 9
4. Method 12
5. Results and Discussion 13
5.1 A conceptual framework for a functional-structural model of grapevine growth and development 13
Summary 13
5.1.1 Introduction 13
5.1.2 Model conceptual framework 15
5.1.3 Results 19
5.1.4 Discussion 19
5.1.6 Conclusions 20
5.2 Consequences of water stress on the impact of high temperatures on the berry ripening processes of Semillon berries and the high temperature thresholds for the ripening process in Semillon, Chardonnay and Merlot grapevines vines in controlled environments. 21
5.2.1. Introduction 21
5.2.2 Water and heat stress of Semillon vines 23
5.2.3 Temperature threshold of berry ripening in selected grapevine cultivars 29
5.2.4 Discussion 37
5.2.5 Conclusions 42
5.3 A protocol for using remotely-sensed imagery to optimise proximal sampling to forecast yield in vineyards 44
5.4 Carbohydrates and root temperature 45
5.4.1 A publication and an industry article describing an improved rapid method to analyse CHO and N, that quantifies sucrose, glucose and fructose 45
5.4.2 A rhizotron facility to assess root growth and development in response to environmental and management influences. 51
5.4.3 A publication on effects of modifying root temperature on performance of grapevines in the field. An industry article on the effects of soil temperature on grapevine fruitfulness. A scientific publication and an industry article on the effects of soil temperature on nitrogen and carbohydrate partitioning 57
Summary 57
5.5. Root-zone temperature effects on leaf gas exchange, nutrient acquisition, vegetative growth and reproductive development in Shiraz grapevines 66
5.5.1 Introduction 67
5.5.2 Materials and Methods 71
5.5.3 Results 76
5.5.4 Discussion 110
5.5.5 Conclusions 120
5.6 Regional-yield forecasting after fruit set 122
5.6.1 Introduction 122
5.6.2 Regional yield forecasts 130
6. Outcomes 131
6.1 Peer reviewed papers 132
6.2 Industry Articles 132
6.3 Conference Proceedings 132
7. Recommendations 133
8. Intellectual Property 140
9. References 140
10. Staff 159
11. Appendix 1: A protocol for regional yield forecasts 160
12. Budget Reconciliation 165

Abstract

Against a background of climate change, the experimental components of this project looked at the effects of increased soil temperature on grapevine performance and the combined effects of water stress and heat stress berry ripening. Climate change related increases in soil surface temperatures and these alterations in the root-zone environment will impact vegetative growth, final yield and fruit composition. Ripening responses to heat stress differed among *Vitis vinifera* cultivars with 40^{o}C is above the threshold for berry ripening in Chardonnay, for Semillon it was about 40^{o}C while it appears that the Merlot berry ripening threshold is much greater than 40^{o}C

A rapid and inexpensive ATR-FT-IR spectroscopy method was developed for the measurement of the free sugar component of grapevine non-structural carbohydrates (TNC). Preliminary calibrations were successfully developed for sucrose, glucose, fructose, total free sugars and TNC.

A rhizolysimeter facility with future capacity for soil temperature control and automated irrigation management was designed and constructed with joint support from GWRDC, NSW DPI, CSU and the NWGIC. This facility will allow fine control of conditions within the rootzone and non destructive measurement of root responses. This is, therefore, a valuable tool for studying root systems of grapevines, an important but hitherto poorly understood aspect of viticulture.

The project tackled the issue of better yield forecasting, which can still bedevil the industry, in two ways. First a protocol based on ground measures after fruit set was developed and tested for making regional yield forecasts accurately and inexpensively. Second, a protocol for using remotely-sensed imagery to optimise proximal sampling to forecast yield in vineyards was developed. Recommendations for future research and development to improve different types of yield forecasts are presented.

Finally, a conceptual structural-functional model of the grapevine was developed. This model is seen as an improvement on existing crop models that don't account for fruit variability or individual organ interaction and lack reliability outside that range of

conditions that they were parameterised and validated within. Potential scientific and industrial applications for the model were discussed.

1. Executive Summary

The effectiveness of ATR-FT-IR spectroscopy for the measurement of the free sugar component of grapevine non-structural carbohydrates (TNC) was assessed. Preliminary calibrations were successfully developed for sucrose, glucose, fructose, total free sugars and TNC using a sample set of 2245 samples which encompassed variability associated with 4 growing seasons, 9 growth stages, 7 varieties or rootstocks, 3 tissue types and 26 vineyards. Of these samples, scans from 520 new samples were added to a pre-existing database as part of the current project.

A rhizolysimeter facility with future capacity for soil temperature control and automated irrigation management was designed and constructed with joint support from GWRDC, NSW DPI, CSU and the NWGIC. The system consists of 12 x 780 L plastic bins suspended at ground level in two parallel concrete trenches in an outdoor bird-proof enclosure. At the time of reporting, all bins have had the water based heating / cooling system installed, instrumented with temperature sensors and TDR probes, filled with soil and planted with pre-prepared 3-year old cordon trained Shiraz grapevines. Installation of the data logging and control system, together with testing and validation will take place over the 2013/14 season.

Field studies showed that higher soil temperatures in a vineyard situation considerably alter the carbohydrate reserve dynamics in spring; particularly the roots. TNC levels are lower under warmer soil conditions. However, this mobilisation of reserves has not resulted in an advancement of development or an elevation in shoot growth in spring. Although root development and processes were not assessed in these studies, these could have likely utilised the TNC from storage instead of contributing to the vines canopy development. In contrast to the vegetative development, flower numbers was higher under warmer soil conditions in both season and shoot fruitfulness in the second season. In the second season of the study the experiment was continued through to harvest, and small-scale winemaking undertaken on three 50 kg replicates of fruit from each treatment. Analysis of these wines, as well as berry and wood samples collected during the ripening period, are still in progress. Altering soil temperature through practical means has the potential to alter the grapevine source-sink relationships and might impact on vine productivity in the longer-term. The continuation of the treatments showed also advancing grape maturation under the warmer soil conditions, with a likely impact also on grape

composition. These findings suggest that not only ambient temperature can affect berry maturation, but also the root environment can have an influence on reproductive development.

Growth cabinet studies were not able to conclusively demonstrate that water stress exacerbates the impact of a heat event on the berry development processes of Semillon vines. The water stress had a marked effect on berry size, in apparent contrast to the heat event, and this in turn resulted in a marked reduction in sugar content. The exposure of Semillon vines to the 40^{o}C threshold confirmed that berry diameter was unaffected and, therefore, appears to reinforce the conclusion that the changes in berry attributes were related to the water stress alone.

The comparison of three cultivars to high temperature exposures in growth cabinets has revealed that there are marked differences in the berry development processes to temperature. Chardonnay berries performed poorly at 40^{o}C, shrinking in size, loosing dry matter and not accumulating sugar and failed also to accumulate sugar at 35^{o}C. Thus, the berry ripening process of Chardonnay vines appeared to have an upper threshold at or below 35^{o}C. By contrast, Merlot berries alone continued to accumulate sugar at a high rate when exposed to 40^{o}C and clearly had an upper threshold above this temperature. Semillon berries accumulated sugar at a high rate at 35^{o}C but accumulated dry matter at a reasonable rate at 40^{o}C and these results suggest the upper temperature threshold for this cultivar was somewhat between 35 and 40^{o}C. These upper temperature thresholds are much higher than what might be expected from their centres of origin in France and, therefore, suggests some acclimation to the high temperatures of the Riverina must have occurred. Similarly, because the cultivars were grown in comparable conditions and still expressed differences in the ripening response to temperature, this suggests a genotypic effect was partly responsible for the differences.

Predictive mapping of within-vineyard winegrape yield, quality, and ripeness, using high spatial resolution optical remote sensing, relies upon relationships between image-derived canopy vigour metrics and fruit composition and yield components. Regular image acquisition of two contrasting vineyard sites enabled a temporal analysis of variation in these relationships. An image processing algorithm was developed to segment vineyard imagery into single grapevine objects. Various

remote-sensing vegetation indices, calculated for each grapevine object, revealed that indices sensitive to high vegetation densities performed significantly better at predicting fruit composition and yield elements than the commonly used normalized difference vegetation index. The strength and direction of correlations between canopy vigour and season-end fruit descriptors varied by phenological stage and vineyard type. The ability of optical remote sensing to successfully map within-vineyard winegrape composition and yield may vary depending upon vineyard characteristics, management, and temporal variability in overall vineyard production.

Regional yield forecasts, such as forecasts of all growers' or wineries' Chardonnay yields from the one region, can be very accurate. This is because they are made using larger samples sizes (e.g. 100 bunch counts from a range of vineyard blocks across a region, compared with 30 bunch counts from within one block) and under- and overestimates tend to cancel each other out. A protocol for making regional forecasts was developed where all of the variety blocks in one region were treated as a single large block. The protocol was tested over two consecutive seasons on Shiraz and Chardonnay with the total area of the blocks being 114 and 162.5 Ha, respectively. Across seasons and sites forecasts were out by an average of 6.21% and this was made much higher by a 14.9% difference in one year. The yields for this cultivar by season are being investigated to make sure that all the harvest intakes were accounted for. Of particular note was how useful the protocol was for predicting a 'low' yield for Shiraz in 2012. Recommendations for future research and development to improve yield forecasting are presented.

Finally, a conceptual framework for a functional-structural model of grapevine growth and development was proposed. This conceptual framework was based on an existing model, L-PEACH and a modular approach integrating different functions on a structure and carbon partitioning model. This model is seen as an improvement on existing crop models that don't account for fruit variability or individual organ interaction and lack reliability outside that range of conditions that they were parameterised and validated within. We suggested possible applications of the model and compared it with previous approaches taken before to model grapevine growth (crop models), defining the scope of these type of models.

2. Introduction

Yield instability is costing the Australian Wine Industry more than $200 million annually. Thus the prediction and stabilisation of yield potential is of utmost importance.

The fruit bearing capacity of individual shoots is the fundamental unit of grape production. Grape bearing shoots are formed over an 18 month period and subject to a range of environmental influences. Although separate impacts of key environmental elements at various developmental stages are more-or-less known from prior research, a modelling approach is the only way to understand, probe and research the complex interactions between individual environmental elements that determine shoot fruitfulness. Structural-Functional models represent state-of-the art modelling. Grapevine carbon relations and in particular carbohydrate reserves are the key drivers of grapevine performance in the face of seasonal contingencies (including inappropriate management). Furthermore, plant nutrient status has a direct effect on carbon assimilation and plant growth. In grapevines this relationship often leads to excessive growth and seasonal imbalances between fruiting and foliar growth and attendant variability in grape yield and quality. Atmospheric CO_2 is the source of plant carbohydrates and its concentration is rising. Soil N is the source of plant nitrogen and its level in viticultural systems is determined by fertilizer applications, inter-row plant species, soil water availability, soil temperature and activity of soil microbes. Both CO_2 assimilation and N uptake, like most biological processes, is temperature and water dependant. Utilization of root carbohydrate reserves is dependent on soil temperature. Australian vineyards are already facing rising global temperature and uncertain water availability.

Yield instability is estimated to cost the Australian Wine Industry more than $200 million annually (Clingeleffer et al. 2001). Thus the prediction of yield potential and its management is of utmost importance.

Global mean temperature has risen approximately 0.6^{o}C since the mid 1800s and the most recent prediction is for an increase in global mean temperature of 1.5 – 6^{o}C by the end of the present century (IPCC 2000 & 2001). Of equal concern are predicted increases in the frequency and length of extreme temperature events (Hennessey and Pittock 1995). Global warming in general and the increase in

episodic ‘high’ temperature events in particular threaten the ability of the Australian Wine Industry to meet consistent yield and quality targets. However, the lack of information on heat damage thresholds at different stages of crop development for important and emerging *Vitis vinifera* cultivars threatens adaptation strategies.

Existing process models of grapevine carbon balance and/or growth and development, do not fully account for C and/or N reserves (Holzapfel et al. 2010)

This issue is of great significance to the industry in terms of grape production efficiency, grape quality, and downstream logistical and production efficiencies.

3. Project Aims and Performance Targets

Objectives of the project were to:
Improve current industry protocols for crop estimation to improve the efficiency and accuracy of current vineyard and regional forecast from +/-15% to +/-10% and in the future to potentially +/-5%, by:

- Using aerial imagery to better stratify sampling and improve accuracy for vineyard level forecasts
- Correlating and validating aerial imagery with on-ground measurements of yield components for vineyard level forecasts
- Developing a protocol, based on on-ground measures for forecasting regional yield after budburst and after fruit set and validate this protocol for key cultivars (Shiraz, Chardonnay and Cabernet Sauvignon) in key NSW regions (Riverina, Hunter Valley and Mudgee) during the 2011/2012 season

Develop strategies for minimising the season-to-season variation in yield and quality by:

- Exploring the effects of modifying soil temperature and its subsequent effects on vine fertility, yield and fruit composition
- Understanding the carbon accumulation of grapevines at elevated temperatures, and the effects that this has on yield and berry composition
- Investigating, utilising current databases, the impacts of nitrogen and carbohydrate balance on seasonal variation in grapevine yield

Outputs the project aimed to deliver comprised:

Year 1	Output	Target Date dd/mm/yy	Activities
A	A conceptual framework for a structural-functional model of grapevine growth and development	30/06012	Collate and combine experimental outcomes and existing growth and development databases
B	A report, a scientific publication and an industry article on heat damage thresholds for important and emerging *Vitis vinifera* cultivars	30/06/12	Establish and conduct growth cabinet experiments
C	A report, a scientific publication and an industry article on the interaction between temperature and plant water status on vulnerability to heat stress for important and emerging *Vitis vinifera* cultivars	30/06/12	Establish and conduct growth cabinet experiments
D	A protocol for regional yield forecasts	30/06/12	Develop protocol and validate in the field
E	A protocol for using remotely-sensed imagery to optimise proximal sampling to forecast yield in vineyards	30/06/12	Develop protocol and validate in the field
F	A report and a scientific publication on the effects of root temperature on patterns of grapevine root growth and development and their effects on the development of yield and berry composition	30/06/12	Establish and conduct rhizotron studies

A six month extension to the project was sought and granted. The additional outputs included in the project are listed below.

Year 2	Output	Target date mm/yy	Activities
G	A publication and an industry article describing an improved rapid method to analyse CHO and N, that quantifies sucrose, glucose and fructose	31/12/2012	Develop an ATR-FT-IR calibration for the free sugar component of grapevine non-structural carbohydrate reserves. Collect, assess and calibrate (using HPLC) 300 samples across the widest possible concentration range
H	A rhizotron facility to assess root growth and development in response to environmental and management influences	31/12/2012	Develop and validate advanced techniques to assess soil temperature effects and other treatment effects on root growth, root respiration, fruit composition, vine water use efficiency and whole vine biomass accumulation.
I	A publication on effects of modifying root temperature on performance of grapevines in the field An industry article on the effects of soil temperature on grapevine fruitfulness A scientific publication and an industry article on the effects of soil temperature on nitrogen and carbohydrate partitioning	31/12/2012 30/4/2013 31/12/2012	Repeat the 'spring' field experiment on the effects of modifying root temperature on the growth, yield and carbohydrate status of grapevines Assess bud fruitfulness and vine carbohydrate status in season 2 of vines pre-treated in season 1 in the existing glasshouse experiment Analyse carbohydrate and nitrogen data from season 1
J	A comparison of regional yield forecasts made over consecutive seasons	31/12/2012	Collect season 2 of data for Griffith Chardonnay and Shiraz to further validate a protocol, incorporating rapid ground sampling techniques, for forecasting regional yield and after fruit set.
K	Revision of the protocol for regional yield forecasting based on 'end user' testing with cohorts of end users	31/12/2012	Collect and incorporate 'end user' testing of protocol for regional yield forecasting with a minimum of 2 consultants, 2 wine company viticulturists and 2 grape growers responsible for making yield forecasts

Output A is addressed in Section 5.1

Outputs B and C are addressed in Section 5.1

Output E is addressed in Section 5.3

Output F, G, H and I are addressed in Sections 5.4 and 5.5

Outputs D, J and K are addressed in Section 5.6

4. Method

The project aimed to:

1. Combine the experimental outcomes of Winegrowing Futures physiology research into a conceptual framework for a structural-functional model of grapevine growth and development that accounts for carbon, water, temperature and nitrogen interactions to better understand the drivers of seasonal yield fluctuations.
2. Utilise rhizotron and glasshouse facilities to establish an investigation into the effects of modifying soil temperature on grapevine root growth and development and their effects on the development of yield and berry composition.
3. Use high light growth cabinets to describe heat damage thresholds at flowering and during ripening and to describe responses of carbon accumulation to temperature for important and emerging *Vitis vinifera* cultivars (including Shiraz, Chardonnay and Cabernet Sauvignon).
4. Use high light growth cabinets to investigate the effects of water stress and plant water status on vulnerability to heat stress in grapevines.
5. Develop a protocol, based on on-ground measures, for forecasting regional yield for cultivars after budburst and after fruit set. Knowledge of planted area will be combined with on ground sampling informed by remote sensing. A protocol will be developed and validated for key cultivars (Shiraz, Chardonnay and Cabernet Sauvignon) in key NSW regions (Riverina, Hunter Valley and Mudgee) during the 2011/2012 season
6. Determine how remotely sensed imagery can be used to inform and optimise sampling strategies to dramatically improve yield forecasts. A protocol will be developed and validated in a range of vineyard blocks during the 2011/2012 season
7. Investigate the potential for remotely sensed imagery to detect seasonal variations in yield exclusively and in combination with on-ground measures

5. Results and Discussion

5.1 A conceptual framework for a functional-structural model of grapevine growth and development

Summary

Plant models are computer programs that simulate the growth and development of plants. Crop functional models have been successfully used in annual crops and this success led to the creation of similar models for grapevines. Crop models of grapevine present issues relating to the discontinuous and variable shape of the grapevine canopy and its perennial character making it necessary to estimate correctly carbon partitioning. Functional-structural plant models simulate the functions and structure of the plant and their interaction. They are more complex than crop models, but they present some advantages, especially when modelling complex structures and carbon partitioning. Here we used a functional structural plant model of peach tree to define the conceptual framework of a model of grapevine growth and development. The model will be implemented using L+C and C++ languages and L-studio software. A modular model with time steps of a day was proposed. The main model was structured in sub-models of weather, parameter, graphical interface, light interception, photosynthesis, respiration, storage, growth, structure, carbon partitioning, pruning response and data output that interacts between the sub-models. A list of possible applications was proposed. Finally the reasons for choosing a functional-structural plant model over crop models for grapevines were discussed.

5.1.1 Introduction

Plant models are computer programs that simulate the growth and development of plants. These models are based on existing knowledge on biophysics, plant physiology and computer programming in order to simulate plant responses using as inputs weather data and plant parameters. The first functional models known as crop models were initially designed for predicting the yield or biomass, harvest date and efficiency of fertilizers and water applications. They treated the whole canopy as a homogeneous media and the current weather conditions were the main driver. Parameters were determined based on empirical relationships obtained under

specific circumstances and so they are appropriate for the condition range in which they have been collected and validated. Crop models assume that the plants are formed of a big leaf, a big stem, a big fruit and a big root, meaning that all the organs of the same type are treated as one (Marcelis et al. 1998). This assumption simplified the models but neglected factors as fruit variability or individual organ interaction (DeJong et al. 2011a).

Subsequent development in computers capacity allowed the creation of structural models to simulate the plant structure and growth using simple algorithms (L-systems) but without considering any plant physiology (Prusinkiewicz and Lindenmayer 1990). Finally, the combination of structural and functional plant models led to the first functional-structural plant models (Godin and Sinoquet 2005; DeJong et al. 2011a). These models were more complex and they were used to simulate the growth and development of individual organs and their interactions.

Most of the models of grapevine growth have taken the initial approach of the functional crop models (Gutierrez et al. 1985; Williams et al. 1985a; Williams et al. 1985b; Wermelinger et al. 1991; Bindi et al. 1996; Bindi et al. 1997b, a; Godwin et al. 2002; Vivin et al. 2002; Nendel and Kersebaum 2004; Garcia de Cortazar-Atauri 2006; Orlandini et al. 2006; Poni et al. 2006; Lakso et al. 2008; Valdes-Gomez et al. 2009). While this approach has been useful in the past for annual crops and forest modelling, in the case of perennial plants that are heavily managed (i.e. trained, pruned and canopy managed) it presents some problems. The assumption of a homogeneous canopy is not applicable in most of the cases (except for maybe in overhead vines or dense minimal pruned vines). Carbon partitioning in grapevine crop models employed theoretical hierarchies or allometric coefficients based on field experiments (Le Roux et al. 2001; Lakso et al. 2008). In the field carbon partitioning between different organs usually changes during the year and seasonal patterns vary from year to year as well (Holzapfel et al. 2010). This means that descriptive models based on empirical relationships are usually unreliable when used out of the range of conditions in which they were parameterised (location, weather conditions, vine status and management).

Grapevine three dimensional static structure has been digitalized to model light interception (Louarn et al. 2008a; Louarn et al. 2008b) and photosynthesis at leave

and canopy level (Prieto et al. 2012). This model did not have the capacity to simulate plant growth. To our knowledge the only case of functional structural grapevine growth model is the Greenlab model (Yan et al. 2004; Pallas et al. 2008; Pallas et al. 2009; Pallas et al. 2010). It has been used to estimate the effects of carbon on plant structural growth. This stochastic model made the assumption of a common pool for all the individual organs of the same type and simulations of plants pruned to a single shoot were run for one growing season, thus avoiding the issues of the perennial nature and complex structure of the grapevine. Grapevines carbon partitioning has been mechanistically modelled at organ level in peach, L-PEACH model (Allen et al. 2005; Allen et al. 2007; Lopez et al. 2008; Da Silva et al. 2011), and kiwifruit shoots (Cieslak et al. 2011a; Cieslak et al. 2011b). One characteristic of both models is that the plant three dimensional structure is used to partition the carbon within the plant, the structure can be visualized and pruning of individual organs can be simulated. Thus, carbon partitioning is an emergent property of the model instead of being an input and the user can visualize the data and interact with the plant easily. L-PEACH model simulates the growth and development of a complex structure during several years. Our objective is to create a conceptual framework of a functional-structural model to study grapevine growth and development during several growing seasons.

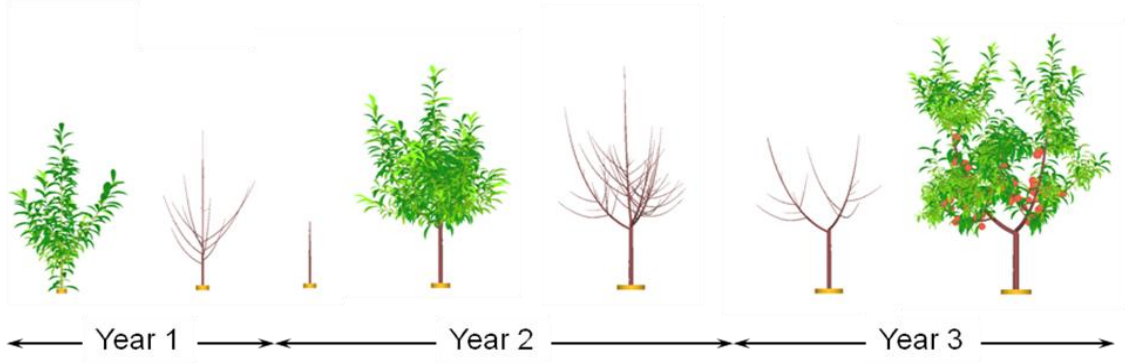


Figure 5.1.1 Simulation of a pruned peach tree over three years of growth using L-PEACH.

5.1.2 Model conceptual framework

We followed the example of L-PEACH in order to define the conceptual framework of a grapevine growth and development model. The functional-structural grapevine model was divided in several interacting sub-models (Figure 5.1.2). This modular approach facilitates the incorporation of more detailed sub-models if needed in the future and also improves the readability of the code. All the sub-models will be

written in C++ and L+C. C++ is a multipurpose programming language that is widely used mainly because it is faster than other languages and object oriented. L+C is a language based on C++ that implements the functions necessary to simulate L-systems (Prusinkiewicz et al. 2007b). L-system or Lindenmayer system is a formalism used to model plant growth and structure (Lindenmayer 1968). L-Studio software will be used for graphic visualization and interaction with the model (Prusinkiewicz 2004). We decided to use time steps of a day for the simulations because it allows enough detail in plant growth and simplifies modelling light interception and photosynthesis. Every organ (i.e. leaf, bunch, internode and bud) will be individually modelled except for the root that will be considered as one big root. We defined the following sub-models: weather data, parameters file, graphical interface, light interception, photosynthesis, respiration, storage, growth, structure, carbon partitioning, pruning response and data output.

Weather data

Weather data will include daily values of daily solar radiation and daily maximum and minimum temperatures (Lopez et al. 2010), which can be downloaded from public meteorological services. When the model will be initiated the weather data for every day will be read and incorporated in the simulation program.

Parameters file

Parameters affecting different functions, structure, management, visualization, data inputs and outputs will be defined in an independent file (Allen et al. 2005; Lopez et al. 2008) and they will be read by the model when it is initiated. A user friendly window similar to that defined in L-PEACH (Lopez et al. 2010) would be desirable.

Graphical interface

L-studio software provides a graphical interface (Prusinkiewicz 2004) that will be used for both graphical output of data and graphical input for simulating pruning (Lopez et al. 2010).

Light interception

There are several options for modelling light interception. Some of them are accurate but slow (computationally costly), for example quasi-Monte Carlo (Cieslak et al. 2008) or nested radiosity (Chelle and Andrieu 1998); others are fast but less

accurate, for example turbid medium (Monsi and Saeki 2005). As an initial approach we will use a turbid medium model as defined for L-PEACH (Lopez et al. 2008). This method is not as accurate as others but it is fast and adequate for daily time steps. In the future a model of light interception more accurate than the turbid medium and faster than quasi-Monte Carlo and nested radiosity would be desirable.

Photosynthesis

We will use the initial approach employed in L-PEACH to model photosynthesis (Allen et al. 2005). This approach is based on the observed constant photosynthetic radiation use efficiency (PhRUE) at a daily basis and the simple model proposed (Rosati and Dejong 2003; Rosati et al. 2004). Daily photosynthesis will be estimated from daily light interception for each leaf. This relationship has not been defined for grapevines and an experiment for obtaining it has been designed.

Respiration

Respiration for each organ will be modelled as a function of the specific respiration and temperature as determined by Grossman and DeJong (1994). This relationship will be established from published data and experiments.

Storage

Storage will be considered as an active sink instead of a passive process as suggested by Cannell and Dewar (1994) and applied to L-PEACH (Lopez et al. 2008). Functions for storage will be estimated in silico from the model and experiments will be designed to validate the results.

Growth

The growth of each organ will be modelled as a function of the temperature and the carbon available using published data for leaves and stems. We designed an experiment to study the berry growth as required for integration in the functional structural grapevine model.

Structure

The structure pattern of the grapevine will be modelled using L-systems (Prusinkiewicz and Lindenmayer 1990; Allen et al. 2005).

Carbon partitioning

Carbon partitioning will be simulated using an analogy to electric circuits (Allen et al. 2005). In order to transport and partition the carbon between plant structural components we will employ an implementation using L-systems (Prusinkiewicz et al. 2007a), as used in L-PEACH (Allen et al. 2005) and the kiwifruit model (Cieslak et al. 2011a).

Pruning response

Pruning will be simulated using the graphical interface and pruning response will be modelled using the concept of apical dominance (Lopez et al. 2008).

Data output

Quantitative data generated during the simulation will be recorded in several output files in comma separated values format (Allen et al. 2007). These files will be created to show data of structural carbon, soluble carbon, storage carbon, size, light interception, photosynthesis and respiration for individual organs and whole canopy every day. Separated output files will contain data related to leaves, bunches, roots, stems and canopy. The output files will be read and analysed using different programs, for example R, Matlab or Excel.

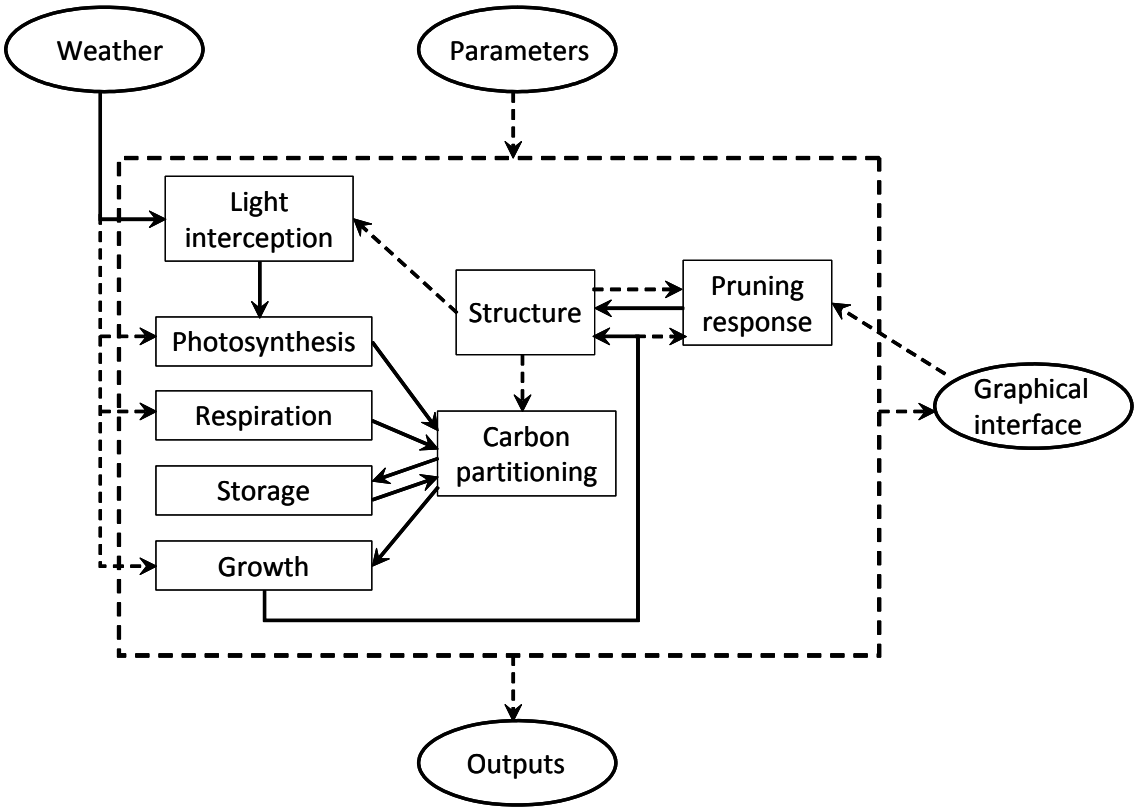


Figure 5.1.2 Simplified schematic representation of the functional-structural model of grapevine growth and development and sub-models.

5.1.3 Results

The establishment of the conceptual framework of this functional-structural model of grapevine growth and development is the first step towards the implementation of the model of a virtual grapevine. This process has already required establishing networks with other modelling groups and improving the collaborations in the NWGIC. Several experiments have been designed and they are being carried out, two examples are the study of the daily photosynthetic radiation use efficiency in grapevines and the effect of carbohydrates on the growth and composition of grapes. The design of these experiments has required new tools and techniques that can be used for further experiments in the future, expanding the scope of the research at NWGIC. The sub-models developed will further increase the capacities of the viticulture researchers. Once the first draft of the model has been completed in a subsequent phase sub-models can be improved or new sub-models can be added, allowing for more realistic and complex simulations. For example, a mechanistic biochemical and biophysical leaf gas exchange at hourly basis has been developed in L-PEACH (Auzmendi, I, R. Favreau, D. Da Silva, T. DeJong, unpublished data) and it can also be integrated in a grapevine model.

5.1.4 Discussion

We defined the conceptual framework for a functional-structural model of grapevine growth and development based on an existing model, L-PEACH. Our aim is to focus on understanding the growth of mature grapevines as grown in the field in order to improve our understanding of the grapevine responses to different management and environmental factors and their interactions. This improved understanding requires an integrated research approach considering different aspects of the grapevine growth and development, and requires the integration of different research areas: photosynthesis, fruit growth, carbon partitioning, grapevine management, computer science, modelling, etc. The final objective is defining practical outputs e.g. improving practices for adapting to climate change and controlling vine balance and yield variability. For this purpose we considered that the best example was L-PEACH. To our knowledge no similar approach has been used before in grapevine modelling. Previous grapevine models followed the crop model approaches that

have lacked reliability outside of the conditions in which they were parameterized and validated. In this sense, mechanistic functional-structural plant models are more complex and theoretical. They present the advantage of being more useful in order to understand and learn because they describe the plant in a greater detail using mechanisms instead of empirical and stochastic relations. Validation of a mechanistic functional-structural plant model with a specific set of data remains uncertain. We consider that rather than focusing on accurate predictions which are unlikely to be achieved without considering the whole plant complexity e.g. biotic factors (pest and diseases), soil complexity, phenotypic differences, etc; functional-structural plant models should focus on understanding and communicating the complexities of the grapevine growth without necessary being able to predict them, but creating practical outcomes for the wine grape growers and wine sector.

5.1.6 Conclusions

We proposed a conceptual framework for a functional-structural model of grapevine growth and development. This conceptual framework was based on an existing model, L-PEACH. This model is based on a modular approach integrating different functions on a structure and carbon partitioning model. We suggested possible applications of the model and compared it with previous approaches taken before to model grapevine growth (crop models), defining the scope of these type of models.

5.2 Consequences of water stress on the impact of high temperatures on the berry ripening processes of Semillon berries and the high temperature thresholds for the ripening process in Semillon, Chardonnay and Merlot grapevines vines in controlled environments.

5.2.1. Introduction

Summer heat events are a common occurrence in the Riverina and other parts of Australia. Heat events are defined here as a sustained period of temperatures exceeding 40oC and occurring sequentially over several days. Recent heat events have occurred in the summers of 2006 and 2009 and in both instances, the high temperatures persisted for more than 5 days (Australian Bureau of Meteorology 2011). Such heat events can occur as early as the time of flowering right through to harvest (Gladstones, 1992, Greer and Weston, 2010). Crop losses can occur when heat impacts at flowering while later heat events can slow growth of berries and impede sugar accumulation (Greer and Weston, 2010) and thus delay harvest, reduce yields and compromise berry composition. Soar et al. (2009), using chambers to increase air temperatures to about 40oC around Shiraz canopies, showed no impact of the increased temperatures on berry ripening. This suggests there are differences in cultivar variation in response to such heat events.

The effect of high temperatures on grapevines has in fact been well documented in a range of varieties. For example, Kliewer and colleagues (Kliewer, 1977, Kliewer and Lider, 1968, Matsui et al., 1986, Sepúlveda and Kliewer, 1986a, Sepúlveda et al., 1986) have extensively investigated high temperature effects on such cultivars as Emperor, Thompson Seedless, Napa Gamay, Cabernet Sauvignon, Chenin Blanc and Chardonnay. Of the many effects of high temperatures, perhaps the most important for growers and winemakers are reduced berry development and delayed ripening. As documented by Greer and Weston (2010), many studies of convective heat (mass air heating) impacts on grapevines have been conducted in controlled environments while most vineyard studies have mostly examined radiative (direct sun exposure) effects (Crippen and Morrison, 1986, Price et al., 1995, Bergqvist et al., 2001). However, it is much more difficult to compare the responses of different cultivars to high temperatures as they do not occur every summer as has been evident in Australia in the last four years. Thus, there is an advantage in screening

different cultivars to high temperatures when grown in controlled environments as the high temperatures can be dialled up as required.

There is anecdotal evidence (Webb, 2009) that water stress increases the consequences of heat stress but there is very little direct evidence. However, Shellie (2011) has shown berry diameters of Cabernet Sauvignon and Merlot vines exposed to a water deficit treatment were affected more on the western canopy side than the eastern side, suggesting an interaction with high temperatures and water deficit had occurred. There is, however, considerable evidence that water stress affects berry development. For example, Hardie and Considine (1976) have shown with Cabernet Franc vines exposed to a water deficit in the late stage of berry development had significantly smaller berries and lower soluble solids concentration in comparison with well watered controls. Although there was an effect of the timing of a water deficit, Matthews and Anderson (1989) have also shown with Cabernet Franc vines that berry expansion was impeded by the lack of water. Berry dry matter accumulation was reduced in Cabernet Sauvignon vines treated in the late stage of berry development compared with well watered controls (Basile et al., 2011). Thus, while it is well understood that water stress affects berry development, the question of the concurrent impact of a high temperature event does not appear to have been investigated.

The objectives of this study, therefore, were two-fold. Firstly, the effect of a mild water deficit on the response of *Vitis vinifera* cv. Semillon in the mid to late stage of berry development to heat damage was assessed by growing the vines in controlled environment conditions and imposing a four-day, 40^{o}C heat event on the vines. Secondly, three grapevine cultivars, *V. vinifera* cv. Merlot, *V. vinifera* cv. Chardonnay and, for comparison *V. vinifera* cv. Semillon, were grown in the same controlled environment conditions but at the mid-late stage of berry development were exposed to new growing conditions that included high temperatures, namely 35 and 40^{o}C, to compare the cultivar responses to these high temperatures.

For the purposes of this report, the two projects are written separately in terms of the methods and results but the two projects are integrated together in the discussion.

5.2.2 Water and heat stress of Semillon vines

5.2.2.1 Materials and Methods

Plant material and growth conditions

A total of 15 *Vitis vinifera* cv Semillon vines were placed in a controlled environment chamber (TH6000, Thermoline Scientific, Smithfield, NSW, Australia) set at a day/night temperature of 12/8^{o}C from April 2011 and kept in these conditions for two weeks. The chamber temperatures were decreased to 10/5^{o}C for another 6 weeks to allow the vines to become dormant. The vines were all pruned to 2 bud spurs and 4 shoots were retained on each vine. From late May, the temperatures were set at 28/18^{o}C (day/night) with a 12 hour photoperiod and lighting supplied by 8 x 20W compact fluorescent lamps (Cool White, Fairway, China). In addition, the atmospheric water vapour pressures were 1.5/1.2 ± 0.2 kPa and the photon flux density was set at 750 ± 10 µmol m^{-2} s^{-1} supplied by 4 x 1kW high pressure discharge lamps (HQI-T1000, Osram, Germany) over a 12 h photoperiod. The vines were grown in these conditions for 115 days in total.

Treatment conditions

At 94 days after budbreak (DAB), half the vines were supplied with 50% of the daily water volumes supplied to control, fully irrigated vines. All these vines from 95 DAB were then exposed to a heat event where the temperature was increased to 40/28^{o}C for 4 days. Thereafter, the vines were returned to the standard growth conditions. The treatment vines had a mid day leaf water potential of -1.2 ± 0.05 MPa while the vines that were fully irrigated had a comparable leaf water potential of -0.42 ± 0.02 MPa, when both were exposed to the heat event.

Phenology

Phenology was followed using the E-L scheme of Coombe (1995) to ascertain budbreak and time of flowering. The vines were assessed at weekly intervals until flowering had occurred.

Berry attributes

Soluble solids concentration

The concentration of soluble solids was measured just before the heat treatment by selecting three berries per bunch, from the top, middle and bottom segments, from selected bunches with juice extracted from the berry with a micro syringe and measured with a micro refractometer (Eclipse, Bellingham and Stanley, UK). Thereafter, soluble solids concentration was measured at daily intervals by the same procedure on the same berries as much as practicable during the treatments at two-day intervals thereafter for a further 8 days. Soluble solids concentrations were subsequently converted to sugar concentration through a linear relationship between ^{o}Brix and sucrose concentration.

Berry diameter

The diameter of selected berries at the top, middle and bottom of each of the selected bunches on each vine and measured using a microcaliper. This enabled berry volume to be determined assuming the berry was spherical and using the appropriate mathematical formula. This determination was subsequently used along with the sugar concentration to determine sugar content of each berry, by taking into account the volume of the berry.

Gas exchange

Net photosynthesis of selected leaves was measured at a photon flux density of 1000 µmol m^{-2} s^{-1}, a temperature of 30^{o}C and CO2 concentration of 385 µmol mol^{-1}. Measurements commenced at 94 DAB and continued until 110 DAB. For each vine, two leaves were measured on each occasion.

Data analysis

All data were analysed using generalised linear models with SAS ver. 9.1.3 (SAS Institute, Cary, NC, USA) as well as using linear regression in some instances. Least-squares means and standard errors were determined and all data were analysed assuming a fully randomised design.

5.2.2.2 Results

Phenology

Budbreak occurred on 10th June and flowering occurred on the 26th of June. Veraison was not measured but occurred at about the 15th of August.

Berry diameter

The diameter of the berries in the vines selected for the control treatment averaged 11.5 mm (Figure 5.2.1) at the start of measurements and not significantly different from that in the treatment vines.

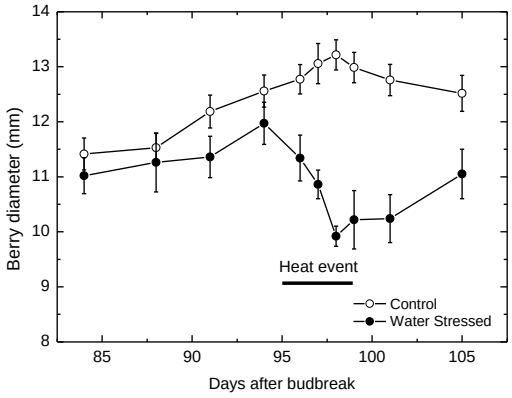


Figure 5.2.1 Changes in berry diameter (mean ± SE) during the late growing season for Semillon berries on fully irrigated and water stressed vines and exposed as indicated to a heat event comprised of a day temperature of 40°C imposed for 4 days.

Berry expansion continued to occur in vines of both treatments but there were slightly smaller berries in those vines selected for the water stress treatment although mostly, these differences were not significant.

However, once the water stress treatment was imposed at 94 DAB and the four-day heat event at 95 DAB, there was an immediate effect on berry diameter, which decreased markedly to about 9.5 mm. Thereafter, there was some increase in berry diameter after irrigation was resumed and the temperature was reduced to the growing conditions. In contrast, for those vines that were fully irrigated, berry expansion showed no effect of the heat event and these berries continued to expand at least until about 97 DAB and peaked at 13.3 mm. However, none of these changes was statistically significant; suggesting berry expansion after the heat event was at least restricted. Consequently, for both these treatments, the berry diameter

of vines treated to a concurrent water and heat stress were markedly and significantly smaller than vines treated to the heat event but well watered.

Berry ripening

There were significant differences in soluble solids concentration (Figure 5.2.2) at the start of the heat treatment between vines fully irrigated and those that had water supply reduced and actually highest in those vines with the water deficit. Despite the imposition of the heat event on vines of both treatments, the soluble solids concentration continued to rise although at a somewhat higher rate in the water stressed vines and continued to rise even when the high temperature abated. Notably, however, the berry ripening of those fully irrigated vines continued at a more or less linear rate to reach about 24^{o}Brix. By contrast, the ripening process of the berries on the water stressed vines appeared to reach a saturation point at about 99 DAB and at a concentration of 21.5^{o}Brix.

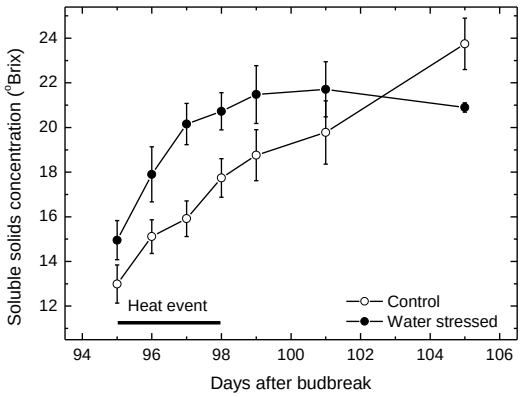


Figure 5.2.2 Changes in berry soluble solids concentration (mean ± SE) during the late growing season for Semillon berries on fully irrigated and water stressed vines and exposed as indicated to a heat event of a day temperature of 40^{o}C imposed for 4 days.

Sugar content

From the berry diameters and the soluble solids concentration, the absolute amount of sugar per berry was determined. At the start of the heat treatment, berries of both vines had identical sugar contents at 26 mg berry^{-1} (Figure 5.2.3). Through the four-day heat event, there was some increment in sugar content but initially there were no treatment differences. By the fourth day of the heat event, the sugar content of berries on water stressed vines had stopped accumulating at about 30 mg berry^{-1} and more or less stayed at this content even after irrigation was resumed and the vines returned to the growth conditions.

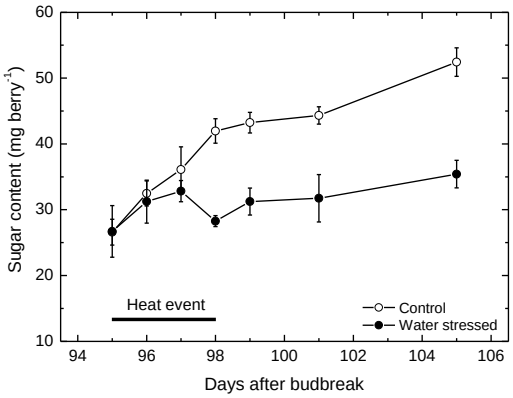


Figure 5.2.3 Changes in berry soluble solids concentration (mean ± SE) during the late growing season for Semillon berries on fully irrigated and water stressed vines and exposed as indicated to a heat event of the day temperature of 40^{o}C imposed for 4 days.

By contrast, the well watered vines showed no effect of the heat event and sugar content continued to accumulate at about a constant rate, although the accumulation slowed down thereafter but increased gradually to over 50 mg berry^{-1}.

Gas exchange

Just prior to the heat event, rates of photosynthesis differed significantly between vines selected for each treatment (Figure 5.2.4). During the heat event, these differences became greater, with the rates significantly lower in the heat and water stressed vines compared with the heat stressed vines. However after that, the photosynthetic rates continued to decline in the heat stressed vines while the rates of the water/heat stressed vines recovered somewhat. Nevertheless, in both cases, the rates remained significantly lower 10 days after the treatment than the pre-treatment rates.

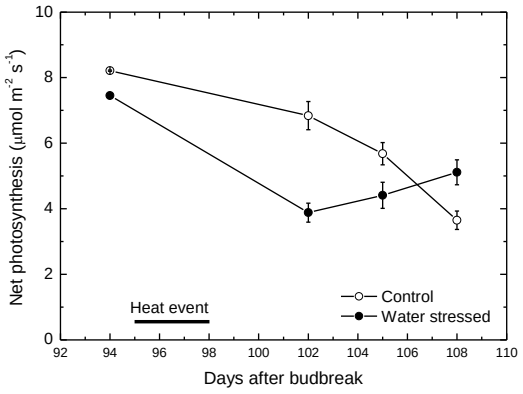


Figure 5.2.4 Changes in photosynthesis (mean ± SE) as a function of the late growing season for Semillon leaves on fully irrigated and water stressed vines and exposed as indicated to a heat event of the day temperature of 40°C imposed for 4 days.

These changes in photosynthesis were mirrored by the stomatal conductance (Figure 5.2.5) which varied after the heat event and differences between the treatments were highly significant on all occasions.

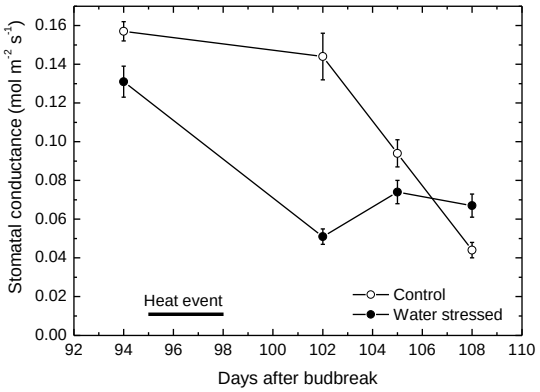


Figure 5.2.5 Changes in stomatal conductance (mean ± SE) as a function of the late growing season for Semillon leaves on fully irrigated and water stressed vines and exposed as indicated to a heat event of the day temperature of 40°C imposed for 4 days.

5.2.3 Temperature threshold of berry ripening in selected grapevine cultivars

5.2.3.1 Material and Methods

Plant material and growth conditions

In late January 2012, 16 *Vitis vinifera* cv. Chardonnay vines, 15 *V. vinifera* cv. Merlot vines and 10 *V. vinifera* cv. Semillon vines were placed in a cool room at 6^{o}C for 2 months to ensure the vines were fully senesced and had become dormant. The vines were all pruned to 2 bud spurs and 5 shoots were retained on each vine. The Chardonnay vines were kept in the cool room for an additional 14 days to enable matching of phenology with the other varieties. In late March, all the vines were transferred to two controlled environment chambers (TH6000, Thermoline Scientific, Smithfield, NSW, Australia). In each chamber, the temperature was set at 15/10^{o}C (day/night) with a 12 hour photoperiod and lighting supplied by 8 x 20W compact fluorescent lamps (Cool White, Fairway, China) to allow for acclimation to commence. Three days later, the temperature was raised to 28/18 ± 0.5^{o}C, the atmospheric water vapours were 1.5/1.2 ± 0.2 kPa and the photon flux density was set at 750 ± 10 µmol m^{-2} s^{-1} supplied by 4 x 1kW high pressure discharge lamps (HQI-T1000, Osram, Germany) over a 12 h photoperiod. The vines were grown in these conditions for 140 days in total.

Treatment conditions

At 96 DAB, the day /night temperatures were altered in each chamber to either 35/25 or 40/28 ± 0.5^{o}C but all other growth conditions as above were maintained. These treatments were imposed for four days at 40^{o}C, and 12 days at 35^{o}C.

Vine measurements

Phenology

To assess the time of budbreak and flowering, phenology of each cultivar was followed using the E-L scheme of Coombe (1995).

Shoot length and leaf count

Lengths of two shoots per vine for all vines and cultivars along with leaf counts were measured just before the temperature treatments were imposed.

Inflorescence and bunch lengths

Lengths of the bunch on two fruiting shoots of each vine and cultivar was measured at 65 DAB, when it appeared the bunches were not extending further.

Berry attributes

Soluble solids concentration

The concentration of soluble solids (SSC) was measured at the start of each treatment by detaching three berries per bunch, from the top, middle and bottom segments, from each vine and cultivar in each temperature treatment and measuring soluble solids concentration with a refractometer (PR-101, Atago, Tokyo, Japan). Thereafter, soluble solids concentration was measured at 1-3 day intervals by the same procedure until the treatments ended. Soluble solids concentrations were subsequently converted to sugar concentration through a linear relationship between ^{o}Brix and sucrose concentration (Greer and Weston, 2010).

Berry diameter and dry weight

Prior to each measurement of soluble solids concentration, the diameter of each berry used in the assay was measured using a microcaliper. This enabled berry volume to be determined assuming the berry was spherical and using the appropriate mathematical formula. This determination was subsequently used along with the sugar concentration to determine sugar content of each berry, by taking into account the volume of the berry.

At the same time, for each bunch, vine and treatment, three berries were also removed from each bunch and dried at 60^{o}C for 2 weeks in a drying oven to determine the dry weight.

Data analysis

All data were analysed using generalised linear models with SAS ver. 9.1.3 (SAS Institute, Cary, NC, USA) as well as using linear regression in some instances. Least-squares means and standard errors were determined and all data were analysed assuming a fully randomised design.

5.2.3.2 Results

Phenology

Budbreak occurred on the 7th April 2012 (EL Stage 4) for the Semillon vines and flowering (EL Stage 23) occurred 16 days after budbreak (DAB) while budbreak for the Chardonnay and Merlot vines were about a week later on the 13th and 14th of April while flowering was 26 and 28 DAB, respectively. The date for veraison was not measured.

Shoot and inflorescence growth

There were marked varietal differences in stem length (Table 5.2.1), notably with the Chardonnay vines in which the shoots had extended to well over 2 m by 95 DAB. By contrast, the shoots of Merlot and Semillon vines extended to about 1 m in the same time.

There was a significant difference ($P < 0.05$) in the number of leaves (Table 5.2.1) but Merlot and Semillon shoots both had about the same number at 21.5 ± 1.5 leaves while Chardonnay shoots had significantly more at 26 ± 1 leaves. Thus, the average internode lengths for these cultivars was 37.3 ± 2.5, 40.4 ± 2.6 and 26.3 ± 2.6 mm for Merlot, Chardonnay and Semillon, respectively, with the Semillon internodes significantly ($P < 0.05$) shorter than for the other two cultivars.

There was a highly significant effect of the cultivar ($P < 0.0001$) on the size of the bunch (Table 5.2.1). The Chardonnay inflorescences were the smallest while Merlot bunches were intermediate in length and the Semillon bunches were the largest of all.

Table 5.2.1. Differences in shoot length, leaf number and bunch length of three grapevine cultivars growing in controlled environment conditions and at day/night temperature of 28/18^{o}C and a PFD of 750 µmol m^{-2} s^{-1}.

Cultivar	Shoot length (mm)	Number of leaves	Bunch length (mm)
Merlot	992 ± 105	21 ± 1	82 ± 6
Chardonnay	2307 ± 144	26 ± 1	58 ± 2
Semillon	1024 ± 204	22 ± 2	93 ± 4

Responses of berry growth to temperature thresholds

In the mid stage of ripening, berry size during the exposure to 35^{o}C remained more or less constant (Figure 5.2.6) for all cultivars with no discernible trend. There were, however, marked and significant differences in berry size, with the Merlot berries smallest at an average of 9.7 ± 0.2 mm, with Chardonnay berries intermediate at 11.1 ± 0.1 mm and the Semillon berries largest at an average of 12.1 ± 0.1 mm. With the exception of the Semillon berries, there appeared to be no relationship between berry and bunch size.

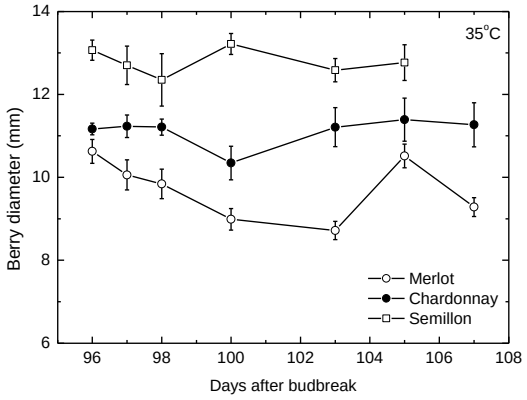


Figure 5.2.6 Changes in berry diameter (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 35^{o}C.

There was a similar pattern of little berry expansion when the vines of the different cultivars were treated at 40^{o}C (Figure 5.2.7), in that little change in berry size occurred. However, there was a significant decline in the Chardonnay berry size, which declined about 8% during the exposure. There was still a trend, though less marked, for the average berry size to increase from Merlot to Semillon (10.2 ± 0.1, 10.9 ± 0.2, 11.1 ± 0.2 mm, respectively).

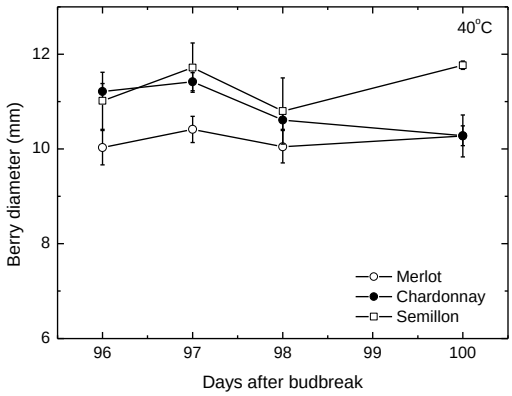


Figure 5.2.7 Changes in berry diameter (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 40°C.

The dry weight (Figure 5.2.8) of those berries of both Merlot and Chardonnay vines that were exposed to 35°C was more or less unchanged over time. By contrast, there was a significant increase in dry matter for the Semillon berries, rising significantly from 0.25 ± 0.001 to 0.34 ± 0.08 g. However, there were also significant differences in the amounts of dry weight per berry across the three cultivars, increasing from 0.107 ± 0.007 to 0.197 ± 0.004 and 0.284 ± 0.015 g berry^{-1}, for Merlot, Chardonnay and Semillon, respectively.

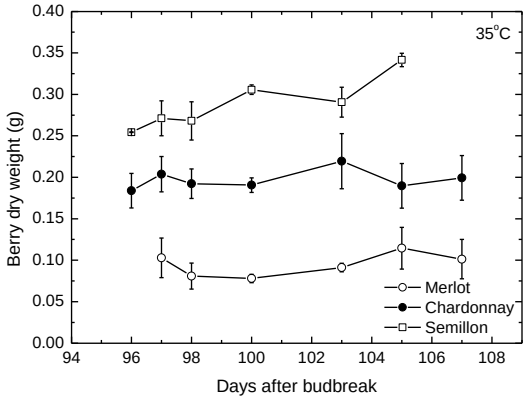


Figure 5.2.8 Changes in berry dry weight (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 35°C.

For the Merlot and Semillon vines exposed to 40°C, there was an increase in berry dry weight (Figure 5.2.9) over the time of exposure whereas for the Chardonnay vines, berry weight declined markedly and significantly. For both Merlot and Semillon, berry dry weight increased about 35% in both cases while for the

Chardonnay berries, the dry weight declined by about 30%. It was noteworthy that the dry weights of berries at the start of the treatment in the vines exposed to 40^{o}C were different from those on vines exposed to 35^{o}C and this would appear to relate more to vine to vine variability rather than to the treatments.

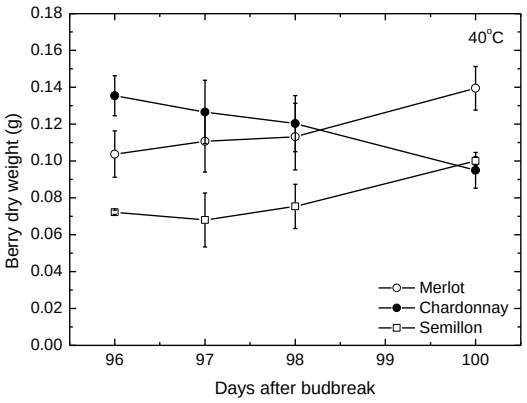


Figure 5.2.9 Changes in berry dry weight (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 40^{o}C.

Response of berry ripening to temperature thresholds

The soluble solids concentration (Figure 5.2.10) in the berries of vines of the different cultivars exposed to 35^{o}C all increased over time, though most consistently with Semillon, which appeared to increase in concentration in a linear pattern. By contrast, the Merlot berries appeared to stop ripening at about 100 DAB and did not change in concentration thereafter.

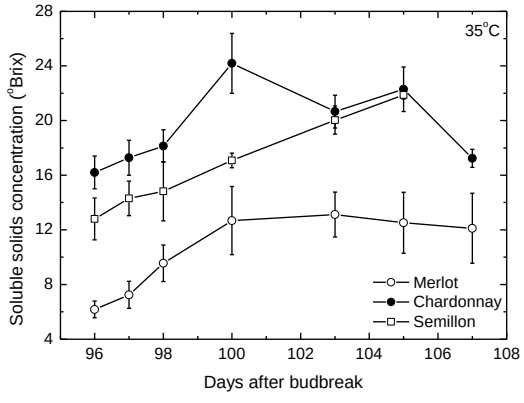


Figure 5.2.10 Changes in berry soluble solids concentration (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 35^{o}C.

For the Chardonnay vines soluble solids concentration was more variable, such that no significant change occurred from 100 to 105 DAB but a significant decrease in

concentration occurred afterwards. Notably, the concentration at 107 DAB was comparable with that at 98 DAB, thus the pattern of response to the 35^{o}C was broadly similar to the Chardonnay response. It was also evident that the concentration of soluble solids at the start of the treatment differed markedly and significantly between cultivars and probably reflected differences in phenology. Merlot berries were the least advanced with a concentration of 6.2 ± 0.6^{o}Brix, while Semillon berries were somewhat more advanced with an SSC of 12.8 ± 1.5^{o}Brix and Chardonnay vines most advanced, with an SSC of 17.2 ± 1.3^{o}Brix.

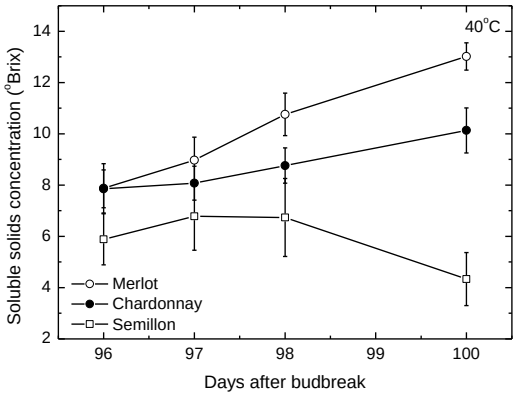


Figure 5.2.11 Changes in berry soluble solids concentration (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 40^{o}C.

There were marked differences in soluble solids concentration between the three cultivars when the vines were exposed to 40^{o}C (Figure 5.2.11). The concentration increased strongly and in a linear pattern in the Merlot berries which were the most ripe, at 13.0 ± 1.3^{o}Brix, of all the cultivars after four days at 40^{o}C.

By contrast, for the Semillon vines, the berry soluble solids concentration was initially relatively constant but then declined to 4.3 ± 0.7^{o}Brix and the least ripe. The changes in soluble solids concentration of the Chardonnay berries also followed a linear pattern, increasing significantly to 10.0 ± 0.8^{o}Brix. Thus, after the exposure to 40^{o}C, the soluble solids concentration varied significantly between all cultivars and ranged over nearly 9^{o}Brix where the difference at the start of the treatment was less than 2^{o}Brix.

Response of sugar accumulation to temperature thresholds

Sugar accumulation of berries of both Merlot and Semillon vines exposed to 35^{o}C followed a linear pattern over time (Figure 5.2.12), increasing significantly. For Merlot, the increase was from 37± 7 to 100 ± 16 mg berry^{-1}, while for the Semillon berries, the comparable increase was from 187 ± 32 to 320 ± 28 mg berry^{-1}. However, for the Chardonnay vines exposed to 35^{o}C, the sugar accumulation appear to increase slightly, at least initially but, overall, there was no gain in sugar content and over the whole treatment period averaged 181 ± 11 mg berry^{-1}.

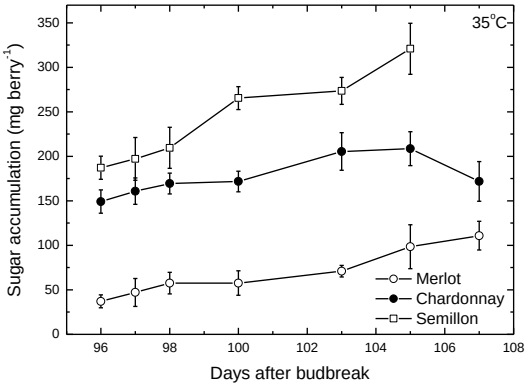


Figure 5.2.12 Changes in berry sugar content (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 35^{o}C.

For the Chardonnay vines, a similar result occurred when the vines were exposed to 40^{o}C (Figure 5.2.13), in that berry sugar content did not change over time but averaged 67 ± 2 mg berry^{-1}. It was also evident that for the Semillon vines exposed to 40^{o}C, the berry sugar content did not change significantly over the treatment time and averaged 30 ± 1 mg berry^{-1}. In contrast with these two cultivars, when Merlot vines were exposed to 40^{o}C, the sugar content increased in a linear pattern and was significantly higher after four days of treatment (59 ± 12 - 92 ± 13 mg berry^{-1}).

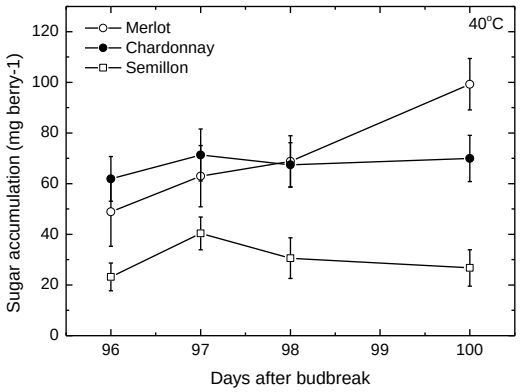


Figure 5.2.13 Changes in berry sugar content (mean ± SE) of three grapevine cultivars during the late stage of berry development and grown in controlled environment conditions at a temperature of 40°C.

5.2.4 Discussion

The results of these studies confirm that Semillon grapevine berry development is impeded by temperatures above 40°C, in keeping with the earlier study of Greer and Weston (2010). When the vines were fully irrigated, the imposition of an advective heat event over 4 days caused berry expansion to slow or even stop. This heat event, nevertheless, appeared to have no effect on the berry ripening process and an increase in the soluble solids concentration occurred in keeping with the earlier study. However, when the accumulation of sugar by the berries was considered, taking into account berry expansion and increase in soluble solids concentration, there was also a marked increase in sugar content after the heat event. This suggested, the heat event imposed on these vines did not have any persistent effect on the ripening process in the Semillon berries and was at odds with the results of Greer and Weston (2010). However, a micro-refractometer was used in the present to track changes in soluble solids concentration, which is linearly related to the destructive whole berry measurement (Weedon pers. comm.), but enabled measurements on the exact same berries. This approach was adopted because of small berry numbers in the bunches and prevented the more destructive assessment of the soluble solids concentration of a larger sample of berries. The outcome is far fewer berries were used and a particular bias may have occurred by continuous sampling of the same berries. Furthermore, berry expansion and ripening of untreated vines was not determined in the current study and this might have shown

the heat treatment did affect the berry attributes. Thus, there was uncertainty as to whether the results of the two studies really differed.

There was no doubt, however, that imposing a mild water stress on the Semillon appeared to exacerbate the growth of the berry. Berry expansion was markedly impeded, with an immediate reduction in berry size. This reduction was most likely a result of loss of turgor due to the water deficit that the vines experienced. This is consistent with the high sensitivity of the cellular expansion processes to a loss of turgor as reported in the literature (Taiz and Zeiger, 2010). There was some recovery in berry size once the temperature declined and the vines re-irrigated. This would therefore, appear to be mostly a water deficit effect rather than an effect of the heat event, given that the berries on well-irrigated vines showed no such reduction in size during the heat event. Vines without water stress (Hardie and Considine, 1976, Matthews and Anderson, 1989, Basile et al., 2011) do not show reductions in berry size in comparison with water stressed vines. More particularly, when protected from high temperatures (east facing), berries of Cabernet Sauvignon and Merlot vines exposed to water deficit conditions declined in comparison with berries on well irrigated vines on the same side (Shellie, 2011). For that study, there was a clear interaction between exposure and water deficit on berry expansion though berry temperatures were not reported, in contrast with results for Semillon. In the present study, in concert with the reduction in berry size, soluble solids concentration of the water and heat stressed berries increased significantly, at least during the actual heat event compared with the control berries but thereafter steadied. Ripening in the post-heat event period appeared to be completed as no change in concentration occurred. However, these effects were a pure artefact of the change in berry volume, as the sugar accumulation in the heat and water stressed berries stopped occurring during the heat event and did not resume after the stresses subsided.

These data, regrettably, do not assist in determining if water stress really did exacerbate the impact of a heat event on the berry ripening processes of Semillon vines. The large impact of the water deficit on berry expansion and the consequent effect of berry shrinkage on increasing soluble solids concentrations would appear to be the primary cause of the reduction in the sugar accumulation process in the heat and water stressed vines. Unfortunately, independent measurements of berry development, such as dry matter accumulation, were not undertaken to enable

resolution of the effect of water deficit from that of the heat event, given that water stress was unlikely to affect dry matter accumulation. As shown by Greer and Weston (2010), the berry ripening process of Semillon berries required approximately 10 mg g(dry weight)$^{-1}$ d^{-1} to accumulate sugar. Their study also indicated that the heat event had sustained reduction in photosynthesis over about 12 days (see also (Ferrini et al., 1995, Cartechini and Palliotti, 1995)), and the loss of ripening in heat-treated berries was due to insufficient carbon fixation. In the present study, the effects of the heat event on Semillon leaf photosynthesis were also immediately apparent but rates were significantly reduced about 4 days after the heat event and continued to decline or remained low for another 10 days.

Notably, the pattern of changes in photosynthesis was different between the heat and water and heat stressed vines with the changes more marked for the vines treated to both a water deficit and the heat event. In general terms, it was unlikely that the mild water stress, per se, had an effect on photosynthesis (Taiz and Zeiger, 2010), therefore, an interaction between heat and water stress may have occurred. Consistent with the Greer and Weston (2010) study, the stress-induced reduction in rates of photosynthesis in both present cases should have reduced supply of carbon to the berries and prevented ripening. Although the overall decline in photosynthesis of the heat stressed vines was similar to that of the heat and water stressed vines, the differences immediately after the treatments were more marked. It may have been that the rapid decline in photosynthesis and, therefore, carbon acquisition caused the ripening process of the heat and water stressed vines to stop accumulating sugar. As a corollary, the initially slower reduction in photosynthesis of the heat-treated vines was consistent with the continued ripening of the berries of these vines.

In both the earlier study (Greer and Weston, 2010) and the present study, heat-induced changes in photosynthesis were closely related to changes in stomatal conductance. Similar responses have been observed in Cardinal, Chenin Blanc and Chardonnay vines (Sepúlveda and Kliewer, 1986b). It remains uncertain why stomatal conductance would decline after the vines were heat stressed with and without water stress. Greer and Weedon (2012) have shown that the stomatal limitation for non-stressed Semillon leaves exposed to 40^{o}C averaged about 35% and non-stomatal limitations accounted for more of the impact of high temperatures

on leaf photosynthesis. It is clear that a more detailed study of the photosynthetic responses to stomatal and non-stomatal limitations during heat and water stress conditions are required to understand the effect of these treatments on leaf photosynthesis.

There were marked differences between the grapevine cultivars to the high temperature treatments during the late berry ripening stage of development. When exposed to 35^{o}C, berry expansion of all three cultivars appeared to fluctuate but remained more or less constant, with little overall change in berry size. Between the cultivars, berry size was also different and, given that all measurements were linked to their phenology from budbreak, this might suggest differences in the rate of development occurred. The cultivar Chardonnay is commonly regarded as an early maturing variety but when grown in controlled environments with the cultivar Semillon, it would appear the latter cultivar had the more rapid development. Similarly, it would appear Merlot was the slowest to develop. Of course, this assumes there were not intrinsic differences in berry size between these grapevine cultivars.

It was also evident from the initial soluble solids concentrations, that there were again differences in phenology. Chardonnay berries were the most ripe at the start of the treatment and the Merlot berries markedly less ripe and well behind in increasing soluble solids concentration. The Semillon berries were closer in ripeness to the Chardonnay berries but nevertheless, still significantly behind. These differences are in accord with Chardonnay and Semillon being early season cultivars and Merlot being a mid season cultivar. The results do imply berry size had developed independently of soluble solids accumulation. This was also apparent when comparing the increments in soluble solids with berry size. In the former case, at least two of the cultivars increased in concentration when exposed to 35oC, very strongly in Semillon berries and at least partially in the Merlot berries. Concentration of soluble solids of the Chardonnay berries was highly variable, and from about 98 DAB, there was no overall change in concentration.

It was apparent that dry matter accumulation in the berries of the different cultivars also strongly reflected the differences in berry size and/or development. However, while Chardonnay and Merlot berry dry weights did not apparently change over time,

when exposed to 35^{o}C, the Semillon berries continued to increase in dry matter content, by as much as 40%. This conforms to the increase in concentration of soluble solids that occurred in the Semillon berries.

Once again, the relative differences in size and dry weight of the different cultivars were apparent when sugar content of the berries was considered. Despite little change in berry volume, the sugar contents of both Merlot and Semillon berries increased during the exposure to 35^{o}C. The increase in sugar content of the Semillon berries appeared to be the most rapid, with a 1.7-fold increase over the 11 days of exposure. By contrast, the Merlot berries increased by nearly 3-fold in the same time so indeed, this cultivar had the most rapid ripening response. Thus, the sugar accumulation process of both cultivars was relatively rapid at 35^{o}C. For Chardonnay, by contrast, there was no change in sugar content over the 11 days and evident that the sugar accumulating process was very slow at 35^{o}C. This would suggest for the berry ripening process in Chardonnay the threshold temperature was at or about 35^{o}C.

When exposed to 40^{o}C, the berry expansion process was comparable with that at 35^{o}C in that there was little overall change in size, even though the treatment was applied for only four days. However, this was not the case for Chardonnay and over the four days, the berry size decreased significantly by nearly 10%. This suggests the Chardonnay berries were relatively more sensitive to the 40^{o}C temperature than were the other two cultivars and consistent with a 35^{o}C threshold. Indeed this was borne out when considering the dry matter accumulation of each cultivar. For the Semillon berries, there was a rise, though only weakly significant, in dry matter content but for the Merlot berries, the rise in dry matter content was highly significant. In both cases, the dry matter increased more than 30%. This contrasts with the Chardonnay berries that declined, in keeping with berry size, in dry matter content and by about 30%. Thus in terms of berry growth, there were more marked differences between the cultivars when exposed at 40 than at 35^{o}C, and Chardonnay berries were confirmed to have a threshold of 35^{o}C while the other two varieties appeared to have a threshold somewhat closer to or greater than 40^{o}C.

This conclusion may not appear to have held when considering the changes in soluble solids concentration during the exposure at 40^{o}C. For Chardonnay, there

was only a slight change over the four days and well in keeping with the lack of dry matter accumulation. However, for the Semillon berries, the concentration declined markedly and perhaps at odds with the apparent increase in dry matter content. However, the results for the Merlot berries were in contrast to both other cultivars in increasing markedly in soluble solids concentration, when exposed to 40^{o}C. This result was confirmed when the changes in sugar content were assessed and for the Merlot berries only, there was a marked and significant, nearly three-fold, increase in sugar content. For the other two cultivars, the sugar content remained relatively constant over the four days of treatment. These data confirm that 40^{o}C is above the threshold for berry ripening in Chardonnay, for Semillon it was about 40^{o}C while it appears that the Merlot berry ripening threshold is much greater than 40^{o}C.

The Chardonnay vines originated in the Burgundy region of France. This has a semi-continental climate with average summer temperatures of about 20^{o}C. Merlot and Semillon originated in the Bordeaux region. Here the average summer temperatures range from 21^{o}C in Bordeaux to 25-26^{o}C in the Gironde region where Semillon is presently grown. It is not certain if the climatic origins of these grapevine cultivars affect their threshold temperatures for ripening or they have acclimated to the local Riverina conditions. Certainly, the lower threshold for the Chardonnay berries conforms to its origins in the semi-continental climate of France but the relatively high threshold would appear to reflect more the growth conditions. The same conclusion can be reached for the other cultivars, because the thresholds for berry ripening were also too high for their climatic origins. Nevertheless, because the cultivar differences in temperature thresholds for ripening were assessed in common growth conditions, the variation must have at least some genotypic origins. Further study is required to assess what contribution acclimation to the growth conditions occurred in determining these ripening thresholds.

5.2.5 Conclusions

The study was not able to conclusively demonstrate that water stress exacerbates the impact of a heat event on the berry development processes of Semillon vines. The water stress had a marked effect on berry size, in apparent contrast to the heat event, and this in turn resulted in a marked reduction in sugar content. The exposure of Semillon vines to the 40^{o}C threshold confirmed that berry diameter was unaffected

and, therefore, appears to reinforce the conclusion that the changes in berry attributes were related to the water stress alone.

The comparison of three cultivars to high temperature exposures has revealed that there are marked differences in the berry development processes to temperature. Chardonnay berries performed poorly at 40^{o}C, shrinking in size, loosing dry matter and not accumulating sugar and failed also to accumulate sugar at 35^{o}C. Thus, the berry ripening process of Chardonnay vines appeared to have an upper threshold at or below 35^{o}C. By contrast, Merlot berries alone continued to accumulate sugar at a high rate when exposed to 40^{o}C and clearly had an upper threshold above this temperature. Semillon berries accumulated sugar at a high rate at 35^{o}C but accumulated dry matter at a reasonable rate at 40^{o}C and these results suggest the upper temperature threshold for this cultivar was somewhat between 35 and 40^{o}C. These upper temperature thresholds are much higher than what might be expected from their centres of origin in France and, therefore, suggests some acclimation to the high temperatures of the Riverina must have occurred. Similarly, because the cultivars were grown in comparable conditions and still expressed differences in the ripening response to temperature, this suggests a genotypic effect was partly responsible for the differences.

5.3 A protocol for using remotely-sensed imagery to optimise proximal sampling to forecast yield in vineyards

Predictive mapping of within-vineyard winegrape yield, quality, and ripeness, using high spatial resolution optical remote sensing, relies upon relationships between image-derived canopy vigour metrics and fruit composition and yield components. Regular image acquisition of two contrasting vineyard sites enabled a temporal analysis of variation in these relationships. An image processing algorithm was developed to segment vineyard imagery into single grapevine objects. Various remote-sensing vegetation indices, calculated for each grapevine object, revealed that indices sensitive to high vegetation densities performed significantly better at predicting fruit composition and yield elements than the commonly used normalized difference vegetation index. The strength and direction of correlations between canopy vigour and season-end fruit descriptors varied by phenological stage and vineyard type. The ability of optical remote sensing to successfully map within-vineyard winegrape composition and yield may vary depending upon vineyard characteristics, management, and temporal variability in overall vineyard production.

Hall, A and Wilson, M. A. (2013) Object-based analysis of grapevine canopy relationships with winegrape composition and yield in two contrasting vineyards using multitemporal high spatial resolution optical remote sensing. International Journal of Remote Sensing 34: 1772-1797.

5.4 Carbohydrates and root temperature

5.4.1 A publication and an industry article describing an improved rapid method to analyse CHO and N, that quantifies sucrose, glucose and fructose

Summary

The effectiveness of ATR-FT-IR spectroscopy for the measurement of the free sugar component of grapevine non-structural carbohydrates (TNC) was assessed. Preliminary calibrations were successfully developed for sucrose, glucose, fructose, total free sugars and TNC using a sample set of 2245 samples which encompassed variability associated with 4 growing seasons, 9 growth stages, 7 varieties or rootstocks, 3 tissue types and 26 vineyards. Of these samples, scans from 520 new samples were added to a pre-existing database as part of the current project.

5.4.1.1 Introduction

Vibrational spectroscopy offers a rapid and low cost method for the determination of multiple analytes in intact or ground samples, and near infrared spectroscopy (NIR) in particular has become a widely accepted method for the analysis of plant or animal feed materials (e.g. Batten 1998, ISO 12099:2010). This process involves collection of NIR spectra of samples and correlating these with known analyte concentrations, and then constructing predictive models to determine the concentration of the same analytes from the spectra of other unknown samples. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) is an alternative form of infrared (IR) spectroscopy where the sample is placed in contact with a crystal or diamond sensing element, and IR absorbance information obtained after internal reflection of an IR beam from the sensing element and sample interface. The IR beam itself does not directly penetrate the sample, but spectral absorbance information can be obtained from the electric field or evanescent wave that extends beyond the sensing element. The technique has advantages over NIR spectroscopy in that absorbance bands in the mid-IR region provide more structural information about unknown compounds, samples require minimal or no preparation, and once appropriate calibrations are developed, can be used for routine analysis with minimal operator expertise and training.

Standard analytical methods for determining the carbohydrate reserve concentrations of grapevine tissues involve extraction of the soluble sugar fraction in aqueous ethanol or methanol followed by enzymatic digestion of starch. Sugar concentrations, including glucose released by starch hydrolysis, are then typically determined by enzymatic analysis (e.g. Edwards et al. 2010), liquid chromatography or colorimetric analysis (eg. Reed et al. 2004). ATR-FT-IR spectroscopy presents an alternative option for carbohydrate analysis of grapevine tissue that is accurate, rapid and of low enough cost for measurements of carbohydrate or nitrogen reserves to be used as a future vineyard management tool (Smith et al. 2011, Schmidtke et al. 2012). The availability of ATR-FT-IR spectrometers that can be readily configured for solid or liquid samples, also presents opportunities for development of methods that provide winemaking and viticultural applications for the same instrument (e.g. Edelmann 2003, Shah et al. 2010). The aim of the present study was test the effectiveness of the technique for measuring the free sugar component of grapevine wood and root tissue such that the concentration of total non-structural carbohydrates reserves can be determined in additional to starch and total nitrogen (Schmidtke et al. 2012).

5.4.1.2 Materials and methods

Sample collection

The wood, root and spur samples utilised for the study originated from an archived collection of grapevine material collected from 26 survey or research trial sites in the Riverina grape growing region of New South Wales, Australia between June 2005 and July 2009. These comprised a total of 2245 samples and spanned four seasons, nine growth stages, seven varieties or rootstocks and three tissue types on which chemical analysis had previously been undertaken (Table 5.4.1). Wood samples were collected as drillings to approximately mid depth of the trunk and cordon using a 4.8 mm drill bit. In the present study 88 of the 891 wood samples were separate cordon and trunk tissue. The remaining 803 contained an equal proportion of each combined at the time of sample collection. Root samples with a diameter range 4-10 mm were collected from around the base of the vine. Spur samples (basal two nodes) were collected after pruning, and then progressively through the season after removal of new shoots. The roots were washed after collection with phosphate free detergent and then rinsed with distilled water. All samples were then dried at 70oC

and ground to 0.5 mm using a heavy duty cutting mill (Retsch ZM2000, Haan, Germany), and then 0.12 mm with an ultra-centrifugal mill (Retsch ZM200).

Table 5.4.1 Sample numbers for ATR-FT-IR carbohydrate reserve calibration according to variety, tissue, sample collection / growth stage and season of collection. Total number = 2245.

Variety/rootstoc		Tissue type		Growth stage		Season	
Chardonna	329	spur	364	bud-break	200	2005/06	399
Riesling	456	wood	891	10-leaf	24	2006/07	572
Semillon	528	root	990	flowering	221	2007/08	794
Shiraz	817			berry pea	24	2008/09	480
Ramsey	74			veraison	248		
Ruggeri	26			mid-ripening	24		
Schwarzm	15			harvest	245		
				post-harvest	72		
				dormancy	1187		

Free soluble carbohydrates were extracted from a 20 mg sub-sample of root or wood tissue using two 1 ml aliquots of 80% ethanol incubated in a water bath at 80^{o}C for 10 min, and a third at room temperature for 10 min. Supernatants were pooled from each extraction following centrifuging at 10,000 rpm for 2 min, and the pooled fraction diluted to 10 ml with ultra-pure water. The concentration of sucrose, D fructose and D glucose were determined by commercial enzymatic assay (K-SUFRG, Megazyme International, Bray, Ireland). For starch analysis, the remaining wood sample was re-suspended in 200 µL of dimethylsulfoxide, heated at 98^{o}C for 10 min, and then 300 µL of thermostable α-amylase (29 U/ml, Megazyme International) in MOPS buffer (pH 7) was added. After incubation for a further 15 min, the samples were allowed to cool, and 400 µL of amyloglucosidase (32 U/ml, Megazyme International) in sodium acetate buffer (pH 4.5) was added. The samples were then incubated at 50^{o}C for 60 min and then centrifuged at 10,000 rpm for 2 min. The supernatant was diluted 1:6 (wood) or 1:11 (roots), and the glucose concentration determined using a glucose oxidase/peroxidase based assay (K-GLUC, Megazyme International). Samples were tested with either a Konelab Arena 20 XT or in microplate format using a Biotek µQuant microplate reader after incubation at 40°C for 30 minutes.

ATR-FT-IR calibration and validation.

Spectra were collected from a calibration and validation sample set of 2245 root and wood samples using an ALPHA ATR-FT-IR spectrometer (Bruker, Ettlingen, Germany – Figure 5.4.1). Within this sample set 520 were scanned as part of the current project, and 1725 derived from the earlier study reported by Schmidtke et al. (2012). The additional 520 samples enable the inclusion of a further four genotypes, with spur, wood and root tissues from Chardonnay, and root tissue from Ramsey, Ruggeri 140 and Schwarzmann rootstocks. Calibrations were developed using OPUS version 7 software and partial least squares regression, with samples assigned randomly to calibration or test data sets representing approximately two thirds and one third of the samples respectively. The calibrations utilized absorbance information in the region 4000-375 cm^{-1} using the first derivative of standard normal variate scaled and mean centred spectra.

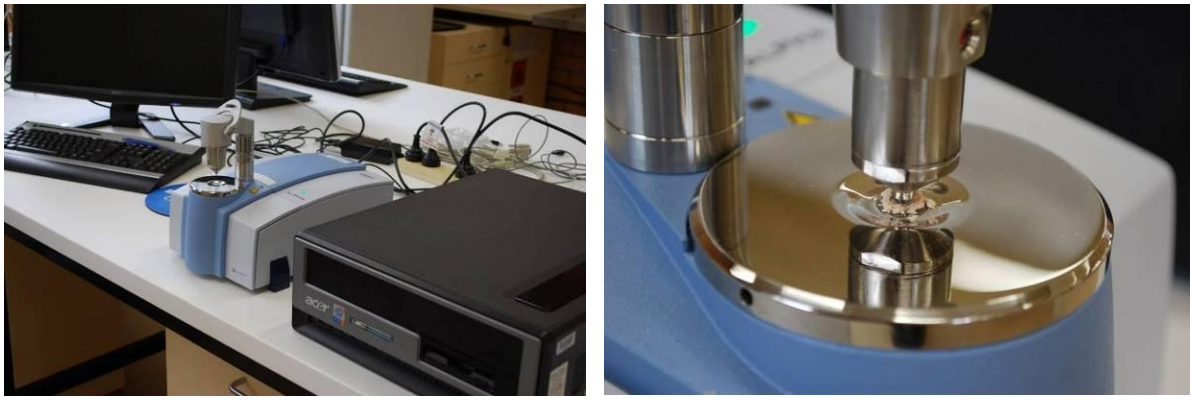


Figure 5.4.1. ATR-FT-IR spectrometer based measurement of carbohydrate reserves in dried and ground grapevine wood and root tissue.

5.4.1.3 Results and Discussion.

Predictive models were successfully produced for total free sugars and total non-structural carbohydrates, with root mean square errors of prediction (RMSEP) of 0.6 and 1.38 %DW, and R2 values of 0.93 and 0.95 respectively. Model performance for starch remained consistent with the earlier calibration with an RMSEP of 1.3 %DW and R2 of 0.96 (Schmidtke et al. 2012), and was similarly independent of genotype, tissue type and location of vineyard (Figure 5.4.2). For the individual sugars, stronger predictive models were obtained for fructose and glucose than sucrose (Table 5.4.2), with R2 values of 0.93, 0.91 and 0.79 respectively. This may in part reflect the increased error associated with the analysis of sucrose, which is determined from the difference in fructose concentrations before and after sucrose is hydrolysed by β-fructosidase to glucose and fructose. Due the large number of samples in the

calibration data set and generally good performance of calibrations based on enzymatic analysis, HPLC analysis methods were evaluated but analysis has been deferred until the completion of the new NWGIC chemistry laboratory. However, when undertaken, this would be expected to improve the accuracy of the sucrose calibration.

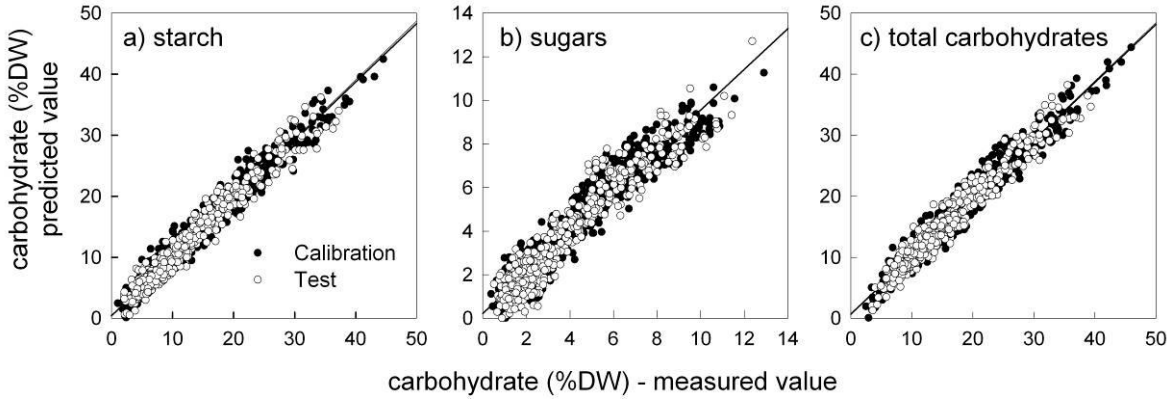


Figure 5.4.2 Measured versus predicted values for carbohydrate reserve concentration measurement of grapevine wood and root tissue by ATR-FT-IR spectroscopy

The ratio of standard error of performance to standard deviation (RPD) is a statistic commonly used to compare the effectiveness of calibration models (Fearn 2002). It relates the standard deviation of the validation population to the model error prediction, and thus provides a measure of concentration variability of a sample set to the accuracy of concentration predictions. There is general agreement that calibrations with an RPD below about 1.5 have limited application, but values between 2 and 5 are regarded as fair to well suited for screening or analysis purposes (Williams 2001, Chang et al 2001). The RPD values in the present study ranged between 2.2 for sucrose and 5.1 for starch (Table 5.4.2), and indicate that the method would be sufficiently accurate for assessing the starch, sugar and total non-structural carbohydrates for research of vineyard management applications.

Table 5.4.2 Predictive model performance for measurement of grapevine carbohydrate reserves by ATR-FT-IR spectroscopy

	data range					RMSEP %DW	Bias	RPD
	min	average	max	R^2_{cal}	R^2_{test}			
Starch	0.5	44.5	10.9	0.97	0.96	1.38	0.035	5.10
Sucrose	nd	6.0	1.3	0.80	0.79	0.45	-0.007	2.21
Glucose	nd	4.8	1.1	0.92	0.91	0.27	-0.002	3.35
Fructose	nd	4.2	0.9	0.94	0.93	0.23	0.0001	3.87
Total sugars	nd	14.7	3.3	0.94	0.93	0.65	-0.012	3.65
TNC	2.2	45.9	14.2	0.96	0.95	1.51	0.018	4.38

nd = below level of method detection.

5.4.1.4 Conclusions.

ATR-FT-IR spectroscopy is a technique suited for the rapid and low cost analysis of non-structural carbohydrate reserves in grapevine tissues. An individual analysis can completed in less than two minutes without the need for specialist laboratory skills, and aside from sample drying and grinding that would also be required for the traditional analytical methods, no other preparation is required. It has previously been shown that the method is also amenable to simultaneous measurements of total nitrogen (Schmidtke et al. 2012), and preliminary calibrations using a subset of 864 samples with matching ICP analysis indicate R2 values of between 0.92 and 0.72 for the macronutrients K, P, Mg, S and Ca (not presented). Where appropriate interpretive standards can be developed, ATR-FT-IR spectroscopy offers a tool that could allow routine measurements of carbohydrate and nutrient reserves to be incorporated into vineyard management regimes. Successful calibrations for macronutrients and several micronutrients have also been developed (Smith et al. in preparation), and with petiole nutrient range standards already available, could be a management tool ready for more immediate adoption. For research applications, ATR-FT-IR based analytical methods can offer a substantial reduction in project laboratory costs, or alternatively greatly increase the capacity for sample collection and analysis.

5.4.2 A rhizotron facility to assess root growth and development in response to environmental and management influences.

Summary

A rhizolysimeter facility with future capacity for soil temperature control and automated irrigation management was designed and constructed with joint support from GWRDC, NSW DPI, CSU and the NWGIC. The system consists of 12 x 780 L plastic bins suspended at ground level in two parallel concrete trenches in an outdoor bird-proof enclosure. At the time of reporting, all bins have had the water based heating / cooling system installed, instrumented with temperature sensors and TDR probes, filled with soil and planted with pre-prepared 3-year old cordon trained Shiraz grapevines. Installation of the data logging and control system, together with testing and validation will take place over the 2013/14 season.

5.4.2.1 Introduction.

The root system of grapevines represents 30 to 50% of the total vegetative biomass (Holzapfel et al. 2010), yet the influence of the below ground environment on root growth, vine carbon balance and fruit composition is not well understood. Soil temperature has been shown to influence the utilization of carbohydrate reserves from the root system in controlled environment studies (Field et al. 2009, Rogiers et al. 2011a), with higher soil temperatures associated with increased utilization of starch through either increased nutrient uptake, respiration or translocation to the shoot. The same response of root starch to soil temperature was confirmed with field grown vines as part of the current project, with warmer spring soils in the 2011/12 and 2012/13 season associated with decreased starch concentrations in the roots, and earlier berry ripening during summer of the 2012/13 season. During the spring of 2011/12, some effect of soil temperature was also observed on starch concentrations in the trunk, cordon and spurs suggesting that conditions experienced by the root system can influence the overall pattern of carbohydrate translocation and utilization at the whole vine level. Rogiers et al. (2011b) also reported a difference in stomatal density in response to soil temperature, suggesting that temperature of the root system not only effects carbohydrate balance of the whole vine, but may also induce physiological responses that modify the future photosynthetic and water use characteristics of the canopy.

Prolonged drought conditions have also been to shown to reduce starch storage in the roots (Smith and Holzapfel 2009), and there is evidence that the production of new fine roots can be reduced during dry seasons (Comas et al. 2005). Recent field studies with mini-rhizotron tubes at the NWGIC have confirmed a lack of fine root growth under prolonged deficit irrigation, but have also shown the responsiveness and rapid proliferation of new roots following significant re-wetting events (Mahmut Kare, unpublished). There also appears to some differences in fine root growth patterns between rootstocks, and in the hardiness or survival times of individual roots. The effect of soil moisture on the overall growth of the root system, and the costs to the plant of producing and supporting flushes of fine root growth are difficult to quantify in the field. However, these results suggest a greater need to understand how soil moisture impacts on the growth and function of the root system, and how this knowledge can be used to improve irrigation scheduling and nutrition management.

Pot studies provide precise control over growing conditions and provide a manageable approach to studying aspects of grapevine physiology and interactions between genotype and environment. However, for studies of the root system in particular, conditions and growth characteristics in small pots are not representative of field conditions. Conversely, field based studies allow research to be undertaken under normal growing conditions, but access to the root system is difficult. The heterogeneity of root distribution and the soil environment means it is also difficult to relate observations made with techniques such as soil coring, mini-rhizotron tubes, or whole vine excavations to the overall function of the root system. With the design and construction of a rhizolysimeter facility as part of the current project, the aim was to provide a experimental system that retains to capacity to maintain good environmental control over the root environment, but at the same time to do this on semi-field scale that allows good access to the root system and uses sufficiently large vines for berry compositional analysis and small scale winemaking to be undertaken.

5.4.2.2 Materials and methods.

Location

The rhizolysimeter facility was constructed inside a large newly constructed outdoor bird-proof enclosure at the NWGIC at Charles Sturt University in Wagga Wagga,

Australia (35°03’38”S, 147°21’50”E). The system utilized two purpose built parallel concrete trenches inside the enclosure with dimensions 12.2 m (l) x 1.2m (w) x 1.0 m (d), and E-W orientation.

Planting containers.

Steel frames were constructed and galvanized to suspend soil filled 780 L plastic bins with dimensions 1.16 m (w) x 1.16 m (l) x 0.78 m (d) at 1.8 m intervals along each trench. The frames were designed to allow each bin to be supported by a load cell at each corner, and to be moved between containers with the use of a small mechanical jack. A removal trellis system was installed on each frame with a fixed stainless steel rod at 1.0 m to support the cordon, and a foliage wire at 1.3 m. Each bin was filled with 7 cm depth of crushed rock (5-30 mm diameter range), a 1 cm layer of river gravel (3-10 mm diameter range) on top of the same material that packed down between the crushed rock, and then 52 cm of a commercial sandy loam garden soil mix. Sections of slotted drainage pipe were buried under the crush rock to facilitate water movement towards the drainage point at the lower end of the bin.



Figure 5.4.3 Construction of NWGIC rhizolysimeter facility between 2011 and 2013. Clockwise from top left images show: prototype frame on one of four 500 kg load cells, completed frames in position with 780 L plastic bins, layout of heating / cooling coils and mini-rhizotron tubes, the completed frames and bins prior to filling with soil, vine planting, and final addition of mulch to soil surface.

Instrumentation and temperature control

Prior to filling with soil, a layer of stainless steel weldmesh (150 mm x 150 mm x 6 mm) was suspended at a depth of 22 cm in each of the bins. A 9.5 m length of 19 mm PE irrigation line was coiled in each, and then secured to the wire mesh with plastic cable ties. Each end of the pipe exited the bin through water proof cable glands to provide the water inlet and outlet for future temperature control system. A length of 13 mm irrigation pipe with 5 inline drippers was also installed, and on filling each bin with soil, placed just below the surface. TDR probes were installed below one dripper (same position in each bin) with one halfway between the soil surface and wire mesh / 19 mm tubing layer, and a second 5 cm above the gravel layer at the base of the bin. T-type thermocouples were installed in three positions to monitor soil temperature between and below the heating / cooling coils, and an additional array of 24 installed in a grid pattern through one bin for future testing and validation. Two mini-rhizotron tubes (50mm diameter) were pre-installed prior to filling at a 40 degree angle from vertical, and provide approximately 70 cm of viewing length for subsequent root growth observations and measurement with a mini-rhizotron camera.

Four lengths of 50 mm PVC pipe with connections installed at each bin were run along each trench under the steel supporting frames to deliver cooled and heated water to each bin. The system will utilise a 20 KW recirculating water chiller and gas heating unit constructed as part of an earlier project (Field et al. 2009). Four irrigation solenoid valves will be installed with each bin, and will allow either cooled or heated water to be independently switched and circulated through the 19 mm irrigation pipe to achieve the desired soil temperature. Removable fibreglass panels were installed between each bin to allow access for installation, maintenance and additional of clear root observation panels to the sides of the bins in the future.

Soil temperature in each bin will be monitored and controlled by programmable CR1000 data logger (Campbell Scientific Australia), with thermocouple sensors connected to a AM16/32B multiplexer, and SDM-CD16AC relay control modules switching the solenoid valves for the cooling and heating water. A multiplexed MiniTrase TDR system will be used for recording the volumetric soil concentrations at two points within each bin.



Figure 5.4.4 View of NWGIC rhizolysimeter and root environment control facility after completing the construction and planting stages of the project.

5.4.2.3 Capability and applications

When fully completed, and the vines allowed several seasons for the root system to establish in each bin, the performance of the heating and cooling system will allow a soil temperature range of approximately 8 to 45^{o}C to be spanned. There would be the option to hold the soil within a set temperature range, follow a pre-determine program based on prevailing air temperatures, or track the soil temperature of the adjacent vineyards (for example vineyard temperature, and vineyard temperature +/- 5^{o}C). Irrigation will also be controlled, and the capacity to weigh individual bins and measure soil moisture content will also provide the option for irrigation treatments. Destructive harvests at the end of a study will allow whole vine biomass to be accurately determined, and with potential for the bins to be lifted in and out of the trenches, vines required for subsequent experiments could be prepared several seasons in advance.

The accompanying field study with Cabernet Sauvignon that was undertaken as part of the current project demonstrates the potential for soil temperature to independently influence vine physiology and berry development. Where mulching and management of under-vine shade may bring significant reductions in soil temperature, there appears to be immediate applications for the system in understanding how management of the root-zone might be used to improve vineyard adaptation to heat events. However, in the longer term the system could be readily expanded to include chambers to control the temperature of above ground parts of the vine.

5.4.3 A publication on effects of modifying root temperature on performance of grapevines in the field. An industry article on the effects of soil temperature on grapevine fruitfulness. A scientific publication and an industry article on the effects of soil temperature on nitrogen and carbohydrate partitioning

Summary

The carbohydrate reserve pool of the perennial structure of grapevines is determined by internal requirements for growth and the production of assimilates, through translocation, accumulation and mobilisation of carbohydrates. The processes of depletion and replenishment of reserves during the growing season are also influenced by the vineyard environment and by viticultural practices; therefore carbohydrates in the roots and the wood vary between vineyards and seasons. The mobilisation and storage of reserves can also be influenced by the soil environment, with warmer root temperatures in spring increasing the mobilisation of root reserves and the shoot biomass, with changes in soil temperatures during the flowering – fruit set period affecting fruit set in potted vines.

This study was conducted on mature, field-grown grapevines *Vitis vinifera L.* cv Cabernet Sauvignon over two growing seasons, to examine the carbohydrate reserve dynamics and growth responses to soil temperature as modified by plastic sheet and straw mulches. The surface treatments significantly influenced rootzone temperature to a depth of 30 cm or more, with the temperatures under the plastic sheet being up to 10^{o}C higher than under the plastic/straw treatment. In addition, the daily temperature fluctuations were considerably reduced dampened by the plastic/straw cover. Increases in soil temperature were accompanied by enhanced mobilisation of plant carbohydrate root reserves, with the carbohydrate reserve levels prior to bud-break being highest. These results confirm previous results from potted grapevines and highlight the significance of soil temperature in regard to seasonal carbohydrate reserve dynamics and the elevated mobilisation/utilisation of carbohydrate reserves in the roots. The implemented soil temperature regimes had minor influence on canopy growth and reproductive development, although berry maturation was enhanced by warmer soil temperatures.

These findings indicate a change of source-sink relationships through altered soil temperatures, with consequences for root processes and above ground development. This suggests that soil management practises that alter root

temperature can contribute not only to reserve dynamics, but also influence berry maturation and composition.

5.4.3.1 Introduction

The influence of soil temperature on the utilisation and replenishment of non-structural carbohydrates has been demonstrated in potted grapevines from bud-burst to veraison, with implications for biomass partitioning between major plant organs during that period (Field et al. 2009, Greer et al. 2011; Rogiers et al. 2011a). Generally, soil warming promotes utilisation of non-structural carbohydrates (TNC) in the roots, enhances shoot growth and increases flower number. In contrast, soil cooling can increase the abscission of flowers and decrease fruit set; with these effects being conditioned by the carbohydrate reserve status of the plant. In addition, differences in leaf functions were observed when different root temperatures were implemented, effecting photosynthesis and transpiration (Greer et al. 2011) and stomatal density (Rogiers et al. 2011b). However, these experiments were undertaken on relatively small grapevines and were exposed to constant soil temperatures of either 13^{o}C or 23^{o}C. A vineyard soil temperature up to veraison generally follows the seasonal increase of atmospheric temperature and also becomes progressively cooler with depth. The daily and seasonal variation is also lower with depth and is also influenced by cultural factors such as mulching (Walpole et al. 1993); other factors such as soil shading facilitated by canopy dimensions would alter the temperature dynamics of the soil.

These considerable temporal temperature fluctuations and between depth in the vineyard soil, are quite different to studies in pots under constant temperature regimes. Therefore investigating the impact of soil temperatures on carbohydrate dynamics and the consequences for vine development in the field would provide an enhanced comprehension of soil and canopy management that alter the temperature and its fluctuation of the soil. The vineyard study was undertaken to assess detailed carbohydrate reserve changes in spring and growth responses of mature grapevines to soil temperature modified by soil covers, to determine the broader implications for viticulture.

5.4.3.2 Materials and methods

Experimental site and vineyard

The own-rooted Cabernet Sauvignon grapevines utilised for the trial were located at the Charles Sturt University vineyard at Wagga Wagga (35°03'38"S, 147°21'50"E). This region is a hot and semi-arid with a mean January temperature of 24°C and average annual rainfall of 572mm. The soil was a red kandasol (Isbell 2002); with an A horizon of dark reddish brown sandy clay loam overlying, at ca 40cm a porous B horizon of massive granitic saprolite. The vines were planted in 1998, trained to a single bilateral cordon, mechanically pruned (with a hand clean up) and harvested. Vine and row spacing is 2 and 3 m respectively. The soil of the drip irrigated vineyard was maintained at field capacity during the spring periods in 2011 and 2012.

The study commenced at pre-bud break (August) and continued for two seasons, with an emphasis on the dormancy to fruit set period. In season 1, the plants were hand pruned to two bud spurs, resulting in about 30 buds per vine, shoots other than those arising from retained buds were removed within four weeks of bud-break. In the second season the vines were pruned and shoot thinned similarly, with the treatments applied at the same time, but not removed in summer in order to gain further information on the impact of soil temperature on berry development and composition.

Treatments and monitoring

Two treatments were implemented approximately one month before bud burst and were arranged in a randomised complete block design with four replicates, with each treatment plot consisting of 12 vines. All vines in a plot were used for sampling, but only the 10 centre vines were used for the measurements. One treatment used 0.2mm clear plastic, spread approximately 90-100 cm on both sides of the vines covering the soil surface above the roots. The other treatment used the same plastic cover, but in addition it was covered in a thick layer of approximately 30-40cm straw (Figure 5.4.5). The reason behind this set up was to prevent potential differences in soil moisture due to exclusion of rain by the plastic mulch. The straw was placed over clear plastic sheet laid identically to that used to raise the temperature.

Soil temperature was monitored using Thermochron™ (OnSolution, Baulkham Hills, Australia) temperature loggers programmed to record temperature every 20 minutes. Two temperature sensors were buried at 10 and 30 cm at vine 6 of each replicate,

sealed in a plastic vial and attached to the end of a wooden stick. For reference the temperature of bare soil in an adjoining non-covered area was also measured. The soil moisture was measured weekly by tensiometers gypsum blocks in representative plots at 30 and 60cm (season 1), and by a neutron probe in each plot at 10, 30, 60 and 80cm (season 2), both treatments received the same amount of water in each season.



Figure 5.4.5 Cabernet Sauvignon trial at the NWGIC (left), with plastic cover layer and the straw used for the application above the plastic (right) before bud-bust in 2012.

Sampling and measurements

Bud development was recorded every second day using a numerical developmental model, with bud burst classified when the first green tip became visible through the bud scales (Coombe 1995). The assessment of bud burst started prior to bud break on six random selected buds per plot, thereafter the growing shoots were measured and recorded at weekly intervals. The flower numbers were determined by counting all flowers from 20 inflorescences per plot prior to capfall, with two inflorescences collected from each vine of the 10 middle vines in a plot. The two inflorescences collected were taken from the same shoot, one from an upper position and one from a lower position. To determine the number of berries, bunches were collected as the inflorescences and also all berries per bunch were counted, and from the flower and berry numbers the fruit set was calculated.

Root (approximately 5mm diameter), trunk, cordon and spur samples were taken from six vines only, for the spurs only one top section of a two bud spur was used from each vine. To reduce the impact of the sampling the collection of tissues was moved one vine further in the whole 12 vine plot every week. Trunk and cordon

tissues were taken with a 5mm drill bit to a depth of approximately half of the trunk or cordon. Roots were washed in phosphate free detergent and rinsed with de-ionized water, both wood and root tissues were oven dried at 70^{o}C. Following the drying processes the samples were ground to 0.12 mm with a heavy duty cutting mill (Retsch ZM2000, Haan, Germany) and then with an ultra-centrifugal mill (Retsch ZM200). The total non-structural carbohydrates (TNC) were determined using Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR). The instrument (Bruker, Ettlingen, Germany) collects the infrared spectra of dried and powdered vine samples at ambient temperature, the calibration for TNC analysis was established by using chemometric modelling (Schmidtke et al. 2012) and allows a determination of a sample in about one minute.

5.4.3.3 Results and discussion

The soil temperatures increased during the spring period from dormancy to fruit set, with the soil temperatures being lower at the beginning of the second growing season 2-3^{o}C. The temperature fluctuations were more pronounced at the 10cm than at 30cm, the diurnal and seasonal variation was strongly reduced by the plastic/straw treatment (Figure 5.4.6). In addition, the root temperature was considerably lowered by this treatment, compared to both plastic (between 6 and 7^{o}C) and to lesser extent to the untreated vineyard (between 1 and 2^{o}C). These differences were even larger during the day, due to the small fluctuations in plastic/straw treatment. The observed temperatures were more variable in spring 2011, with the highest temperatures were observed around flowering, while the temperatures increased steadily to fruit set in spring 2011. There were only minor changes in soil moisture observed in both seasons, either measured with the tensiometers (2011) or the neutron probe (2012).

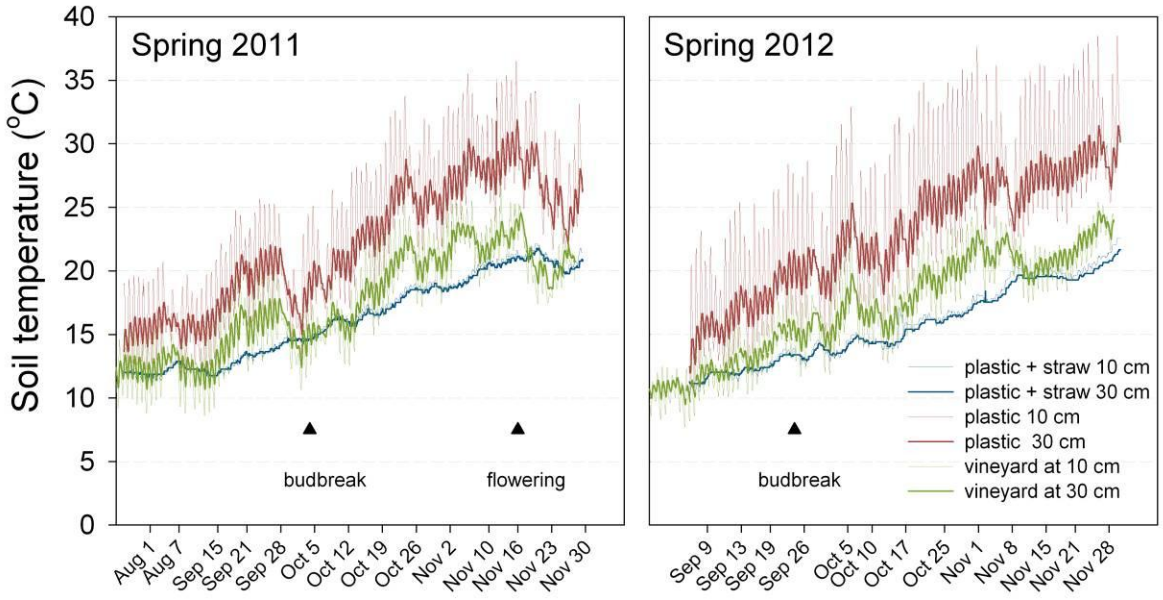


Figure 5.4.6 Soil temperature at 30 and 60cm for spring 2011 and 2012 altered by different soil cover and for the untreated vineyard.

The TNC dynamics and concentrations were higher and more pronounced in spring 2011 compared to spring 2012, where only minor changes in wood concentrations were present. However, generally the decline in TNC in the spring period was most pronounced in the one-year old wood (spurs) and roots, indicating a strong mobilisation and the resource requirements for the new shoot growth and root growth. The concentrations in the spurs declined from over 12% to about 4% DW at fruit set and root reserves declined from over 16% to about 5% DW in spring 2011. The concentration reduction in cordon and trunk was somewhat similar, but only about half of the other parts. The decline in root TNC was similar in spring 2012, declining from about 11% to less than 5%. The higher soil temperatures resulted in a decline in TNC in the roots in both years, but an elevation in the upper parts of the vine, particularly in the spurs was only present in 2012. This is similar to the observations in pots, where higher soil temperatures reduced the concentrations and amounts of TNC (Field et al. 2009, Rogiers et al. 2011). These findings suggest that vineyard soil temperature can impact on carbohydrate reserve mobilisation, with consequences for the development and growth in spring in a field situation.

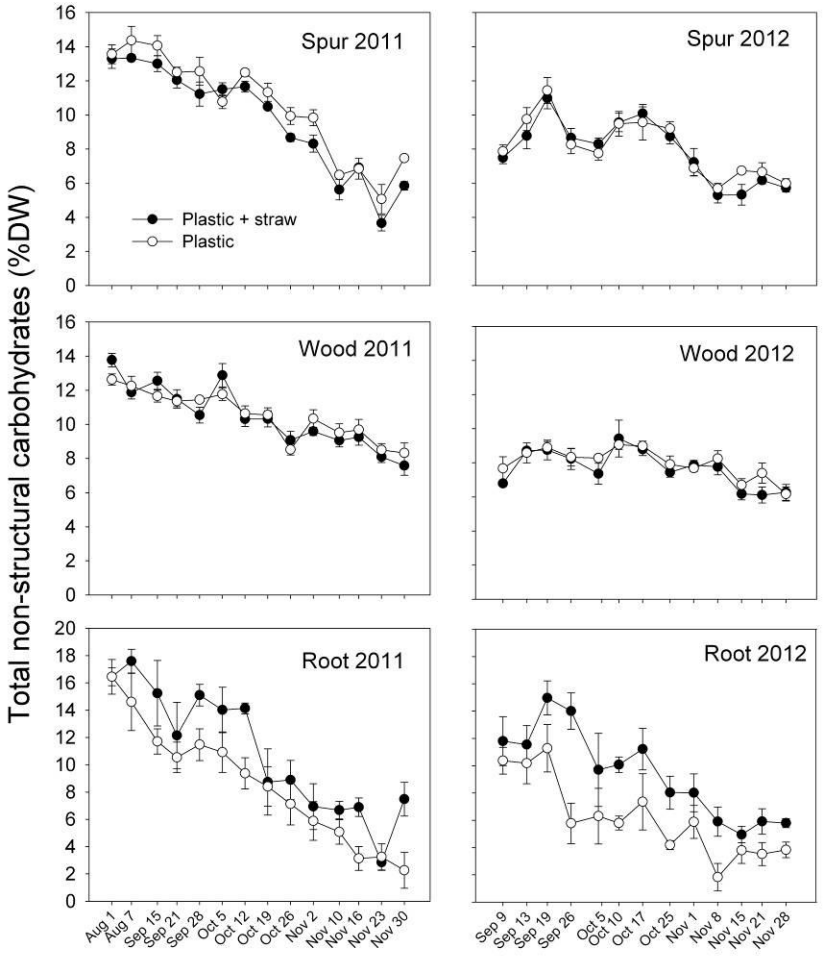


Figure 5.4.7 Carbohydrate concentration in different parts of Cabernet Sauvignon vines (spurs, wood, roots) as influenced by different soil temperatures in spring 2011 and 2012

The season had no major influenced on bud-burst, shoot length at flowering or other times (data not presented) and flower numbers, but berry number per bunch were lower in the second season, consequently also the fruit set. These differences could be due to lower soil temperatures after flowering in 2011 compared to spring in the following growing season. Generally, the impact of the altered soil temperature on vegetative and reproductive development was not very strong, indicating the considerable buffer capacity of the perennial structure of grapevines (Holzapfel et al. 2010). The time of bud burst appeared to be unaffected, with flower numbers higher in the cooler treatment in both seasons, fruitfulness was elevated in the cooler treatment in the second season. However, the differences in shoot lengths at flowering were not consistent and relatively small, in the first season shoots were slightly shorter in vines that received the cool treatment and the reverse was present in the second season. This minor response is in contrast to an earlier pot study,

which showed an increase in annual growth due to higher root temperature (Field et al. 2009). However, the root activity and growth was not assessed in this study, and also the nutrient uptake could have been likely enhanced under the warmer soil conditions, contributing to the decline in root TNC described above.

Table 5.4.3 Time of bud-burst, shoot length (flowering), flowers per inflorescence, berries per bunch, fruit set and bunches per shoot in Cabernet Sauvignon vines exposed to two different root temperatures.

	2011/12				2012/13			
	Plastic		Plastic+Straw		Plastic		Plastic+Straw	
Bud-burst (EL stage)	3.4	0.09	3.3	0.09	2.0	0.13	2.3	0.09
Shoot length (cm)	54.7	3.78	58.3	2.73	53.6	2.70	50.6	5.39
Flowers/inflorescence	371.0	2.20	389.0	1.93	372.3	8.76	413.8	9.10
Berries/bunch	111.2	7.16	100.1	2.06	93.1	9.82	94.9	8.47
Fruit set (%)	27.0	0.33	28.8	2.27	25.0	2.72	22.9	1.66
Bunches/shoot	1.35	0.16	1.35	0.12	1.34	0.10	1.54	0.12

E-L stage 3/10/11 and 24/9/12; shoot length 2/11/11 and 2/11/12; SE of the means in small type

5.4.3.4 Conclusions

The study has shown that higher soil temperatures in a vineyard situation considerably alter the carbohydrate reserve dynamics in spring; particularly the root TNC levels are lower under warmer soil conditions. However, this mobilisation of reserves has not resulted in an advancement of development or an elevation in shoot growth in spring. Although root development and processes were not assessed in this study, these could have likely utilised the TNC from storage instead of contributing to the vines canopy development. In contrast to the vegetative development, flower numbers was higher under warmer soil conditions in both season and shoot fruitfulness in the second season. In the second season of the study the experiment was continued through to harvest, and small-scale winemaking undertaken on three 50 kg replicates of fruit from each treatment. Analysis of these wines, as well as berry and wood samples collected during the ripening period, are still in progress.

The work shows that altering soil temperature through practical means has the potential to alter the grapevine source-sink relationships and might impact on vine productivity in the longer-term. The continuation of the treatments showed also advancing grape maturation under the warmer soil conditions, with a likely impact also on grape composition. These findings suggest that not only ambient temperature can affect berry maturation, but also the root environment can have an influence on reproductive development.

5.5. Root-zone temperature effects on leaf gas exchange, nutrient acquisition, vegetative growth and reproductive development in Shiraz grapevines

Summary

Springtime root-zone warming activates root carbohydrate reserve mobilisation, a critical source of carbon for early canopy and reproductive development in grapevines following winter dormancy. Seasonal variability in soil temperatures during spring may result in heterogeneity in root growth, nutrient uptake, canopy development, leaf gas exchange and fruit set with consequences on berry composition at harvest. We monitored below- and above-ground plant growth, fruitfulness, flowering and ripening of berries in Shiraz grapevines (*Vitis vinifera* L.) exposed to three root-zone temperatures from budburst to fruit set in spring.

Elevated root-zone temperature resulted in higher daytime stomatal conductance, transpiration and net assimilation rates across a range of leaf-to-air vapour pressure deficits, air temperatures and light levels. Intrinsic water use efficiency was, however, lowest in those plants with warm root-zones. CO_2 response curves of foliar gas exchange indicated that the maximum rate of electron transport and the maximum rate of Rubisco activity did not differ between the root-zone treatments, therefore it was likely that the lower photosynthesis in cool root-zones was predominantly the result of a stomatal limitation. Night-time stomatal conductance and transpiration rates were also greater for plants grown in warm root-zones. One week after the discontinuation of the temperature treatments, gas exchange was similar between the plants, indicating a reversible physiological response to soil temperature.

Warm root-zone temperatures stimulated new root growth and nutrient acquisition. The summed length of these new roots was double after the two month root-zone warming treatment and branching of these new roots was also stimulated. Conversely, root-zone cooling inhibited new root growth and branching. The macronutrients K, Ca and Mg were elevated in leaf petioles as a result of root-zone warming, as were the micronutrients B and Mn. Roots, trunk, cordons and spurs all contained similar levels of N on a dry weight basis at fruit set however shoot N concentrations were nearly double that of the woody components and leaf N concentrations were 6-fold higher. Primary and lateral leaf N concentrations were

highest in those vines exposed to root-zone warming. Roots of vines exposed to the cooling treatment declined in N content between dormancy and fruit set, however there was no change between these two developmental periods for vines exposed to the other ambient or warm treatments. The fruit represented another N sink and concentrations at harvest were highest for those vines that had received the root-zone warming.

Warm root-zone temperatures after budburst hastened root starch mobilisation, leaf expansion, net positive carbon assimilation, onset of flowering and fruit set, berry enlargement and the onset of veraison. At harvest, berry pH as well as fresh and dry weights were higher for vines that had been exposed to warm root-zones while berry titratable acids were lower. Total soluble solids and anthocyanins were also higher on a berry basis however the concentrations were not different among the treatments. Vine bunch number (bud fruitfulness) and flowering date in the second season was not affected by root-zone temperatures in the previous season.

Because root-zone temperature is an environmental driver of both below- and above ground growth, nutrient uptake and leaf gas exchange models predicting yield and berry composition must incorporate this critical parameter, especially in light of global increases in air and soil temperatures.

5.5.1 Introduction

Vitis vinifera berry development is dependent on a number of environmental and physiological factors such as air temperature, light and plant water status (Hardie and Considine 1976, Crippen and Morrison, 1986, Dokoozlian and Kliewer 1996, Bergqvist et al. 2009). Root-zone temperature effects on berry growth and maturation have not yet received much attention despite its considerable impact on both above- and below- ground plant growth and development (Lopushinsky and Kaufmann 1984, Cai and Dang 2002, Dawes et al. 2011, Wu et al. 2012). Root-zone temperature has an influence on root metabolism (Huang et al. 2005), root growth (Kasper and Bland 1992), nutrient uptake (Radin 1990) and water absorption (BassiriRad et al. 1991), all of which are critical for optimal whole plant functioning and reproductive development. Expected increases in global air temperatures, including the night period (Vose et al. 2005), will also likely have consequences on

soil surface temperature patterns (van Gestel et al., 2011) and this has the potential to affect plant productivity.

The development of new vegetative and reproductive structures in perennial deciduous crops following winter dormancy is particularly sensitive to the temperature of the soil in early spring (Greer et al. 2006, Rogiers et al. 2011a). In grapevines, root pressure during early spring is responsible for the transport of water, nutrients, newly mobilised carbohydrates and plant signals (Skene 1967, Field et al. 2009) through the xylem to the developing buds containing both leaf and inflorescence primordia. These primordia differentiate and expand over the coming weeks to form fully functional leaves and inflorescences, each bearing up to several hundred flowers. The ovaries undergo pollination and fertilization but the process is highly variable with successful fruit set ranging anywhere from 15 to 60% (May 2004). Air temperature (Keller et al. 2010), availability of photoassimilates (Caspari et al. 1998, Lebon et al. 2008) and plant carbohydrate status (Zapata el al. 2004) are significant factors affecting fruit set. More specifically, it has been shown that competition for photoassimilate between simultaneous root growth, shoot growth and carbohydrate reserve restoration all impinge on successful fruit setting in Chardonnay (Rogiers et al. 2011b). Considering the importance of root-zone temperature on early vegetative growth and carbohydrate reserve mobilisation (Field et al. 2009), this abiotic factor may also play a role during the critical periods of flower formation and ovule fertilisation. Since root-zone temperature is dependent on soil type, depth, aspect, slope and extent of inter-row ground cover by vegetation this environmental parameter may explain some of the within-vineyard variability that is often observed in fruit set, yield and composition (Bramley and Hamilton 2004, Trought 1997). Variable fruit composition generally decreases the value of the crop and is undesirable because it makes grading more complicated and results in an inconsistent product (Bramley and Hamilton 2004).

Berry development and ripening during late spring and summer may also be subject to early springtime root-zone temperature. Since root-zone warming can hasten root reserve mobilisation, canopy development and net positive carbon assimilation (Field et al. 2009, Rogiers et al. 2011b) this may alter source-sink relations within the plant later in the season with consequences for berry growth and ripening as well as final fruit size and composition. For instance, warm root-zones resulted in heavier fruit in

canola (Kleier et al. 1998) and increased yield in tomatoes (Gosselin and Trudel 1984, Hurd and Graves 1985) and oranges (Cary and Weerts 1978). There are few reports of root-zone temperature effects on grape composition with the exception of a set of controlled environment studies carried out in the mid- to late 1970s (Kliewer 1975, Zelleke and Kliewer 1979) on Cabernet Sauvignon highlighting lower acidity in berries grown in warm root-zones. Most other root-zone studies concentrate on grapevines grown in hydroponics. For example, in Muscat of Alexandria berries various composition parameters were altered with root-zone temperatures but this was dependent on when the treatments were applied (Kubota and Shimamura 1982). Mulching trials sometimes report soil temperature and there have been links between soil temperature and composition (Oke et al. 2007, Chan et al. 2010), however soil water retention is also improved with the use of mulch and it is difficult to separate out these factors.

Leaf gas exchange is particularly responsive to the root-zone environment. While there are many studies characterising the consequences of soil moisture on leaf gas exchange, soil temperature has also been found to affect stomatal conductance and photosynthesis in perennials (Cai and Dang, 2002; Wan et al., 2004; Wu et al., 2012) and annuals (He and Lee, 2001; He et al., 2001, Zhang et al., 2008). Root-zone temperature can restrict photosynthesis through alterations in photosynthetic reactions (Cai and Dang, 2002; Zhou et al., 2004; Erice et al., 2006) or changes in stomatal conductance (Wan et al., 2004). For instance, boreal and alpine plants are often exposed to low soil temperature stress and in conifer species the maximum rate of carboxylation (Vc,max) and the maximum rate of electron transport (Jmax) increased with soil temperature up to an optimum and then declined (Cai and Dang, 2002). Conversely, it was found that when *Populus tremuloides* was exposed to cool soil net assimilation was limited by stomatal conductance (Wan et al., 1999). As a consequence, it was suggested that similar root-to-shoot signalling pathways may operate in plants subjected to drought and low root temperature (Wan et al., 2004). As such, it was found that roots of Cucurbitaceae species can regulate stomatal behaviour in response to root-zone temperature by modifying delivery of ABA to shoots and leaves (Zhang et al., 2008).

Those environmental parameters regulating stomatal conductance and transpiration during the day may also exert some control during the night. Stomata can remain

partially open during the night and, though the function of night-time transpiration is yet to be resolved, this can result in substantial night-time transpiration if the vapour pressure difference between the leaf and air (VPD) is high. In some species and varieties, nocturnal transpiration can even cause disequilibrium in plant water status (Caird et al., 2007; Dawson et al., 2007; Fisher et al., 2007; Kavanagh et al., 2007). For instance, *Vitis vinifera* Grenache, an isohydric grapevine variety, has low g both during the day and night. This is in contrast to Semillon, an anisohydric variety, with comparatively high g throughout the diurnal cycle and as a result incomplete plant rehydration prior to dawn especially during warm, windy nights (Rogiers et al., 2009). Aside from inherent genetic factors, nocturnal g appears to be influenced by abiotic factors such as VPD, wind, atmospheric CO2 concentration and soil moisture (Ludwig et al., 2006; Howard and Donovan, 2007; Zeppel et al., 2010; Zeppel et al., 2012). Another key environmental factor which may regulate nocturnal g is root-zone temperature. To date the consequences of root-zone temperature on nocturnal stomatal conductance, to our knowledge, are unknown. It is therefore difficult to incorporate soil temperature into crop production models because there is little information available on how soil temperature affects daytime and especially night-time foliar gas exchange.

We assessed the foliar gas exchange response of Shiraz, an anisohydric grapevine variety (Schultz, 2003) originating from temperate Bordeaux, France and grown widely in warm climates across southern Australia and Argentina. Shiraz is grown across a broad range of latitudes, elevations and topographic conditions that expose this variety to a wide range in root-zone temperatures. Slope orientation and the extent of ground cover in the inter-row area by cover crops or weeds will also affect the temperature of the soil. Other factors such as rain patterns, irrigation and soil type influence rooting depth, and therefore root-zone temperature, and this will also affect plant response.

The objective of this work was to clarify the role of early season root-zone temperature on flowering, berry development and final composition. Using well-watered Shiraz grapevines, we assessed reproductive development in response to cool, ambient and warm root-zone temperatures applied over a two month period from budburst to fruit set. Here we show that root-zone temperature in spring can have consequences on carbohydrate reserve mobilisation, root growth, nitrogen

uptake, canopy and inflorescence development as well as final berry size and composition.

5.5.2 Materials and Methods

Root-zone temperature treatments

Dormant six-year-old own-rooted Shiraz grapevines (*Vitis vinifera*, L., clone 1654, were removed from their pots and replanted in 40 L containers fitted with a heat exchange system. Ten weeks prior to bud-burst, the plants were pruned to two spurs, each carrying two buds. The root-zone temperature treatments (n=20 per treatment) were applied using 1.1 m long and 13 mm diameter polyethylene tubing coiled between the root mass of the plant. This tubing was attached to 50 mm pipe connected to a recirculating water system (UPS 32-80 N 180, Grundfos, Bjerringbro, Denmark) and one of three temperature-controlled 1200L water tanks. The sides and bottom of the pots were insulated with 10 mm dense foam, allowing space for the drainage holes. The three root-zone temperature treatments were applied for two months, from the onset of budburst (modified E-L stage 4, Coombe 1995) to the termination of fruit-set (stages 27-29). The plants were irrigated daily and excess water was allowed to drain from the bottom of the pot. Beginning 25 days after budburst plants were fertilised with 200 mL tap water containing 1 mL nutrient concentrate (Megamix Plus, equivalent to N:P:K 130:100:150 mg plant^{-1}) every five days until veraison, then every 10 days thereafter.

Environmental monitoring

Every vine had a temperature probe (DS18B20, Maxim Integrated Products, Sunnyvale, CA) inserted into the centre of the root mass and these were logged at 30 minute intervals (Mini Trase data logger, Soil Moisture Equipment Corporation, Santa Barbara, CA). The root-zone temperatures were allowed to fluctuate diurnally, with the ambient treatment mimicking the nearby vineyard under-vine soil at a depth of 15 cm. Maximum root-zone temperatures over the 62 day treatment period averaged 16.9 ± 0.3 °C for the cool, 22.6 ± 0.5 °C for the ambient and 24.8 ± 0.4 °C for the warm treatment, while minimum root-zone temperatures averaged 13.3 ± 0.3 °C for the cool, 17.8 ± 0.5 °C for the ambient and 22.6 ± 0.2 °C for the warm treatment.

Using a TDR soil moisture probe (Trase, Soil Moisture Equipment Corporation, Santa Barbara, CA) placed in the centre of the root mass (n=9 per treatment), soil moisture was logged half-hourly over the 63 day treatment period and averaged 21.9 ± 1.3% for the cool, 21.5 ± 1.4% for the ambient and 21.1 ± 1.4% for the warm treatment. Growing degree day (GDD_{soil}) calculations for each shoot from the date of average budburst per vine were based on actual temperature of the soil and a 10 °C temperature threshold (Amerine and Winkler 1944, Williams et al. 1985).

A weather station, equipped with an air temperature probe (Intercap HMP50, Vaisala, Hawthorn, VIC, Australia) and a quantum sensor (Apogee, SQ-110, Logan UT) logging at half hourly intervals, was placed at canopy height within the trial. Air temperature ranged between an average minimum of 10.3 °C and maximum of 28.5 °C while daily maximum PAR averaged 1200 µmol m^{-2} s^{-1}.

Gas exchange

Instantaneous net assimilation, stomatal conductance, transpiration and leaf intercellular CO_2 concentrations were measured using a portable photosynthesis system (LI-6400, LI-COR Biosciences Inc., Lincoln NE, USA). Midday measurements were carried out weekly from budburst to fruit set. An artificial red-blue light source attached to the chamber head was used to illuminate the leaf at 1500 µmol m^{-2} s^{-1} PAR. The CO_2 concentration of the incoming air was maintained at 400 µmol mol^{-1} and the block temperature was set at 25 °C. On each shoot (18 plants per treatment with four shoots per plant), the leaf opposite the basal inflorescence (most often located at the fourth or fifth node from the base) was measured. Nocturnal measurements (nine plants per treatment) were carried out every two weeks on the same leaves prior to dawn with the chamber light turned off, incoming CO_2 concentration set at 400 µmol mol^{-1} and the block temperature set to 18 °C. Time integrated values of transpiration were calculated based on a normal distribution of *E* (Rogiers et al., 2009) over a 15 hour light period and constant *E* during the 9 hour dark period. Light, temperature and VPD response curves were carried out at midday, 43-50 days after the onset of the root-zone temperature treatments, on four to six plants per treatment. VPD was not controlled in the temperature or light response curves.

A/C_i responses to root-zone temperatures

Measurements of photosynthetic responses to cool and warm root-zone temperatures were carried out 50 days after onset of treatments on four plants per treatment, targeting fully expanded leaves opposite the basal inflorescence. The A/C_i response was measured according to Ainsworth et al., (2002). Photosynthesis was initially measured at 400 µmol mol^{-1} CO_2 under saturating light (1500 µmol mol^{-1} s^{-1}) and a block temperature of 25 ºC, until A was steady-state. The CO_2 concentration surrounding the leaf (C_a) was then reduced to 50 µmol mol^{-1} over nine steps with A and C_i recorded as soon as C_a was stable. C_a was then returned to 400 µmol mol^{-1} and increased over eight steps to 2000 µmol mol^{-1}. Each A/C_i curve was processed to obtain J_{max}, the maximum rate of electron transport, $V_{c,max}$, the *in vivo* maximum rates of Rubisco activity and R_d, the day respiration, according to the method of Greer and Weedon (2012) using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Canopy characteristics

Leaf expansion was determined non-destructively by measuring leaf length twice weekly on each leaf opposite the basal tendril in the three-node repeating leaf/tendril arrangement (T_e of Gerrath and Posluszny 2007, Figure 3), resulting in measurements on approximately one-third of all leaves. Total plant leaf area was determined at fruit set and harvest destructively with a leaf area meter (LI-COR Biosciences Inc., Lincoln, Nebraska).

Leaf chlorophyll was assessed non-destructively with a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan) twice weekly from 30 days after budburst to fruit set. The meter readings were converted to chlorophyll content using a calibration curve derived from paired meter and spectrophotometer readings. The spectrophotometer readings were converted to an estimate of chlorophyll content following the method of Steele et al. (2008). Measurements were carried out on the leaf opposite the basal inflorescence and all leaves opposite the basal tendril in the three-node repeating metamer pattern that occurs along *V. vinifera* shoots. Stomatal density was determined on the same leaves. Nail polish was applied to the leaf and allowed to dry for 10 minutes. Clear adhesive tape was used to peel off the nail polish and then mounted on a microscope slide. Stomata were viewed at three positions of standard area (1 mm^2) in the lower right quadrant of each leaf, next to the mid-rib on the

abaxial surface using a bright field microscope (Provis AX70, Olympus America, Melville NY).

Flower number and fruit set

Flower number and fruit set were determined by enclosing each inflorescence within a nylon mesh bag. The flower caps and aborted flowers were counted at the termination of fruit set. Fruit set was regarded as the stage when abortion of unfertilised flowers and unexpanded ovaries had ceased and nascent ovaries had initiated swelling. Fruit set was determined as the number of nascent berries as a proportion of the number of flowers. At harvest, the number of undeveloped berries was assessed as those that had not undergone a colour change or softening.

Berry diameter and composition

Berry diameter was measured with digital callipers on 6 plants x 4 infructescences per plant x 8 berries per infructescence. Veraison was assessed visually on all infructescences as first signs of colour change.

At harvest the infructescences were cut from the vine at the junction with the shoot. The samples were placed on ice and left in a cool room at 4 °C overnight. The berries were cut from the rachis, counted, weighed and stored at -20 °C until processing. While frozen, 15 berries were chosen at random from each infructescence, weighed and freeze dried (Gamma 1-16 LSC, Christ, Germany) to a constant weight. The remaining berries were thawed at 4 °C, homogenised (UltraTurrax T25, Janke & Kunkel, IKA Labortechnik, Germany) at room temperature, then centrifuged at 720 g for 10 min. Total soluble solids were assessed on the supernatant using a digital hand-held refractometer at 25 °C (ATAGO, Tokyo, Japan). Berry sugar content was estimated by multiplying the total soluble solids reading with average berry fresh weight. pH and titratable acidity were determined on 5 ml of supernatant using an autotitrator (TitraLab 80, ABU901 AutoBurette and SAC80 Sample Changer, Radiometer, Copenhagen). Anthocyanins were extracted from 1 g of homogenate in 10 mL of 50% ethanol at pH 2. After centrifugation at 3000 rpm for 10 min, 1 mL of supernatant was added to 9 mL of HCl and incubated for 3 hours at room temperature. Absorbance was read at 280 and 520 nm.

Starch and nitrogen

Starch and nitrogen were assessed on plant components at three time points during the season: immediately prior to the onset of the temperature treatments, at the termination of fruit set and at berry maturity. At each time point six plants per treatment were removed from their containers and divided into the following components: roots, below-ground trunk, above-ground trunk, cordons, spurs, shoots, leaves, infructescence rachis and berries. The roots were washed three times with the last rinse in de-ionized water containing 0.1% detergent (Decon 90). The plant components were dried at 60 °C, weighed and ground to 0.12 mm with a centrifugal mill (Retsch ZM200, Hann, North Rhine-Westphalia, Germany). The large woody samples were pre-ground to 5 mm with a heavy cutting mill (Retsch SM2000). For nitrogen, 40 mg of sample was combusted with a VarioMAX combustion analyser (Elementar, Hanau, Germany).

For starch, a 20 mg sub-sample of each tissue was weighed, and soluble sugars extracted twice with 1 ml of 80% (v/v) ethanol at 80^{o}C for 10 min, and a single 1 ml wash of 80% (v/v) 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 with commercial enzyme assays (Megazyme International, Bray, Ireland). For starch analysis, the remaining wood sample was re-suspended in 200 μL of dimethylsulfoxide and heated at 98^{o}C for 10 minutes. The remainder of the analysis was then performed using commercial starch and glucose assay kits (Megazyme International, Bray, Ireland). Briefly, 300 μL of thermostable ∝-amylase in MOPS buffer was added, mixed, and incubated for 15 min in a 98^{o}C water-bath. After cooling, 400 μL of amyloglucosidase in sodium acetate buffer was added and incubated at 50^{o}C for 60 min. The samples were mixed at 20 min intervals, and then centrifuged at 8,000 g for 2 min. Supernatant from root samples was diluted 1:11, and trunk and shoot samples 1:6 in ultrapure water. Glucose concentration of the diluted samples was then determined colorimetrically at 510 nm against glucose standards and the amount of starch in the original 20 mg sample calculated.

Petiole nutrients

Leaf petioles opposite the basal infructescence were removed at harvest (four per plant), washed, dried and ground as above, then sent to a commercial laboratory

(Waite Analytical Services, University of Adelaide) for analysis by inductively coupled plasma optical emission spectrometry (ICPOES, Zarcinas and Cartwright 1983).

Root growth

At fruit set six plants per treatment were removed from their containers. The soil was gently shaken from the roots and digital images were acquired of the entire root system. New root growth was assessed by counting and measuring the length of white roots as a proportion of the total root area using AnalySis LS Research (V 1.3.2, Olympus Soft Imaging Solutions)

5.5.3 Results

Effects of root-zone temperature on leaf gas exchange

Nocturnal gas exchange

Nocturnal leaf stomatal conductance, g_n, ($P<0.001$) and transpiration, E_n, ($P<0.001$) responded to root-zone temperature (Table 5.5.1). In the cool root-zone treatment, g_n was 14.5 % lower than the ambient treatment and 20.7 % less than the warm treatment. Similarly, compared with the ambient and warm root-zone treatments, E_n was 13.8 % and 19.8 % less, respectively, in the cool treatment. This decline was apparent without any treatment differences in soil moisture ($P=0.105$) which averaged at 20.9 % at the time of measurement. A linear regression of g_n with soil moisture at the time of the gas exchange measurements ($P<0.01$) explained only 4 % of the variance in the data while a regression of this parameter with root-zone temperature ($P<0.002$) accounted for 3 % of the variance. Further detailed examination of these two environmental parameters, however, showed that vines exposed to high root-zone temperatures combined with high soil moistures had highest g_n and E_n values while those exposed to low temperatures and low moisture responded with low g_n and E_n (Figure 5.5.1). The combined factors of leaf age, leaf temperature, soil moisture and soil temperature accounted for 31 % of the variance in g_n ($P<0.001$) and 58 % of the variance in E_n ($P<0.002$).

Leaf respiration ($-A$) was not affected ($P=0.20$) by root-zone temperatures and averaged 0.49 µmol CO_2 $m^{-2}s^{-1}$ (Table 5.5.1). Internal CO_2 concentrations (C_i), were higher by about 20 µmol mol^{-1} in cool root-zone temperatures compared with the

other treatments (P<0.001), however this was apparently not linked to g_n because a linear regression of C_i with g_n was not significant (P=0.15).

Midday gas exchange

As leaves matured over a twenty day period of rapid shoot growth, midday stomatal conductance (g_d) increased (P<0.001) and had doubled by the time full lamina expansion had taken place. This occurred in all three treatments within one month after budburst (Figure 5.5.2). On each measuring date, g_d was substantially less in the cool root-zone as compared to the two other treatments. As the season progressed, air temperatures rose, the soil temperatures of the ambient and the warm treatments converged, and so did leaf g_d of plants grown in those treatments.

Table 5.5.1 Nocturnal leaf *A* (µmol CO_2 $m^{-2}s^{-1}$), g_n (mol H_2O $m^{-2}s^{-1}$), E_n (mmol H_2O $m^{-2}s^{-1}$) and C_i (µmol mol^{-1}) of grapevines treated with cool, ambient or warm root-zone temperatures. Values are means of three sampling dates over a 60 day treatment period (n=9 on each sampling date). Root-zone temperatures and soil moistures represent the means at the time the gas exchange measurements were made.

Treatment	root-zone temperature (°C)	soil moisture (%)	*A*	g_n	E_n	C_i
cool	14.8 a	21.2 a	-0.53 a	0.0314 a	0.275 a	455.2 a
ambient	20.8 b	20.9 a	-0.45 a	0.0367 b	0.319 b	437.8 b
warm	24.2 c	19.7 a	-0.48 a	0.0396 b	0.343 b	435.5 b
LSD	0.2	1.8	0.11	0.0034	0.025	11
F pr <	0.001	0.179	0.199	0.001	0.001	0.001

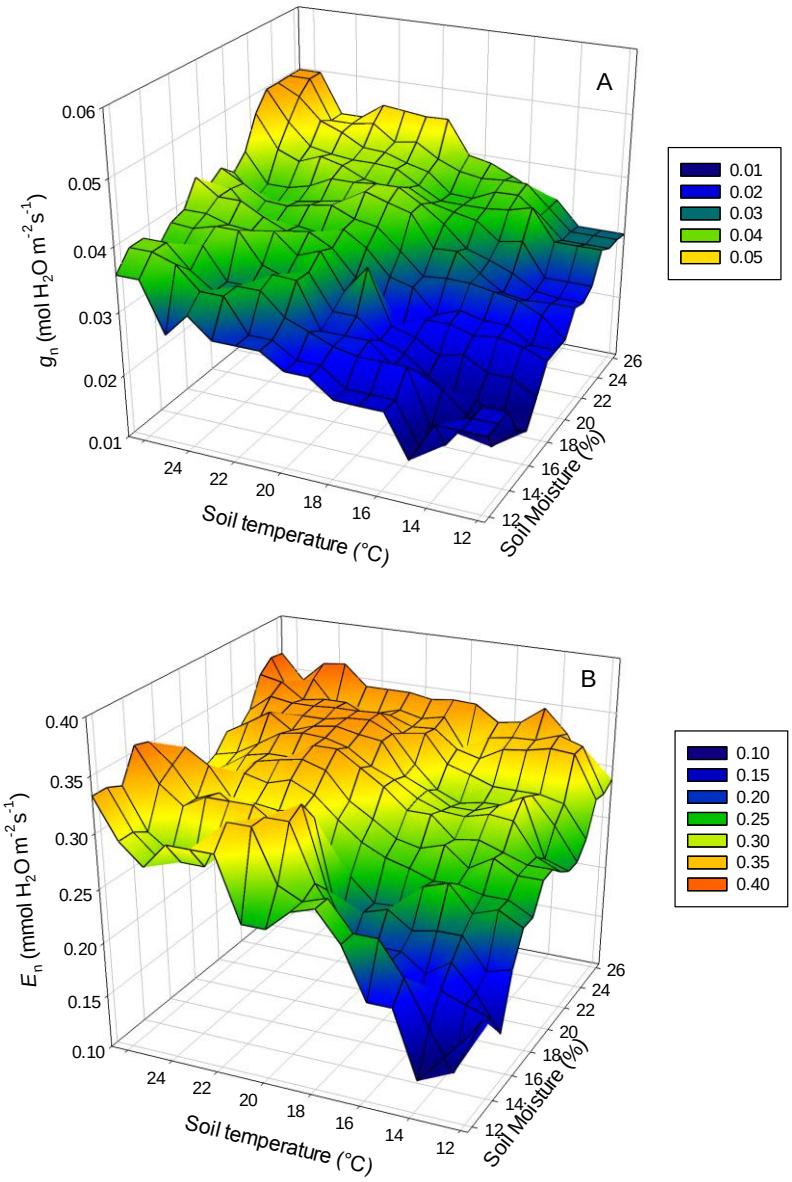


Figure 5.5.1. Nocturnal leaf (A) g_n and (B) E_n as a function of root-zone temperature and soil moisture. Shiraz plants were treated with cool, ambient or warm root-zone temperatures over a 60 day period from budburst to fruitset. Smoothed data represents three sampling dates (n=9 on each sampling date).

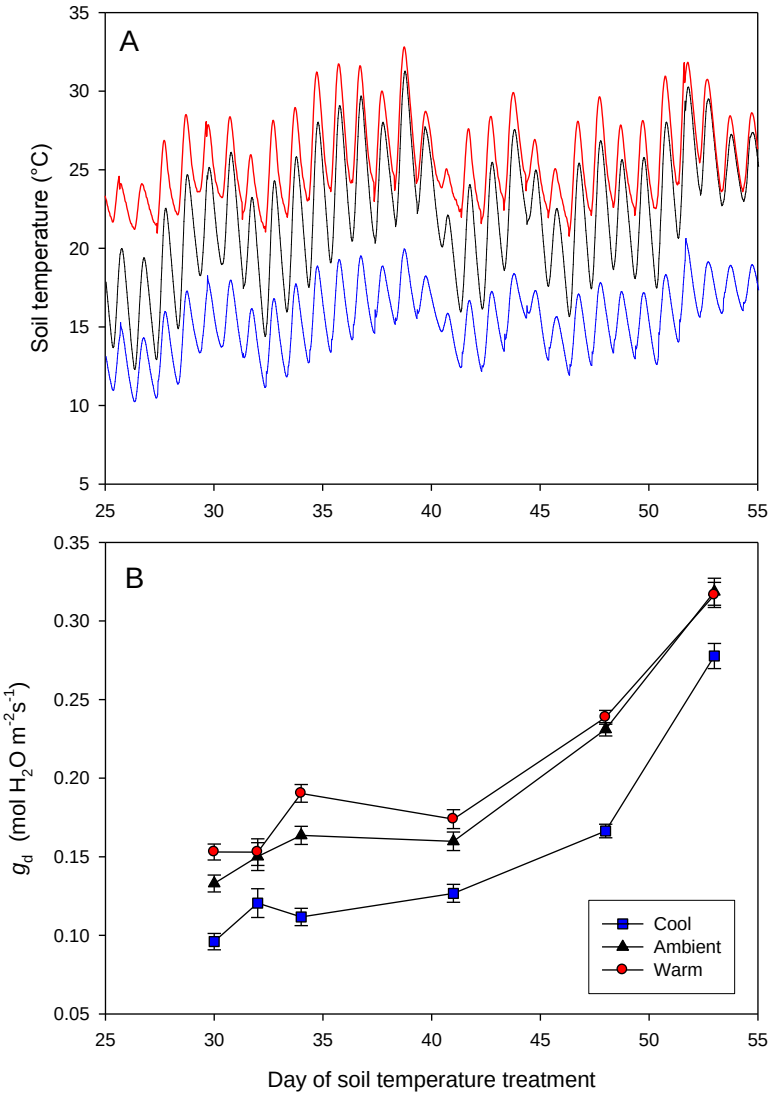


Figure 5.5.2 (A) Average root-zone temperature of the cool, ambient and warm treatments (n=18), and (B) midday leaf stomatal conductance (g_d) in response to cool, ambient and warm root-zone temperatures applied over a 60 day period from budburst to fruit set in Shiraz vines. The first measurements were made when leaves opposite the basal inflorescence had obtained an approximate area of 10 cm^2.

Rates of CO_2 assimilation (*A*) were highest in the warm and lowest in the cool root-zone temperatures (P<0.001), each diverging by about 5 % from the ambient treatment (Table 5.5.2). This was not the result of earlier budburst in the warm or later budburst in the cool treatment because budburst occurred at roughly the same time for all the treatments (P=0.37). Leaf development after budburst may have proceeded at a greater rate in the warm root-zone treatment, however even though leaves of plants grown in this treatment were larger (Table 5.5.4), lamina length and *A* were not correlated (P=0.822). Higher *A* was also not due to higher chlorophyll levels in warm plants because chlorophyll levels in the leaves from nodes 2 to 6 over this period were not different from each other in the cool and warm treatments (Table 5.5.3). Soil moisture can also not explain these differences because, at the time gas exchange was measured, this parameter varied only slightly between the treatments with lower levels in the warm treatment (22.8 ± 0.5 % for cool, 22.6 ± 0.5 % for ambient and 20.6 ± 0.5 % for warm) which would result in stomatal closure and decline in *A* rather than an increase.

Table 5.5.2. Midday leaf *A* (µmol CO_2 $m^{-2}s^{-1}$), g_d (mol H_2O $m^{-2}s^{-1}$), E_d (mmol H_2O $m^{-2}s^{-1}$), A/g_d (µmol CO_2 mol^{-1} H_2O), A/E_d (µmol CO_2 $mmol^{-1}$ H_2O) and C_i (µmol CO_2 mol^{-1} air) of grapevines treated with cool, ambient or warm root-zone temperatures. Values are means of seven sampling dates over a 60 day treatment period (n=18 on each sampling date). Root-zone temperatures represent the means at the time the gas exchange measurements were made.

Treatment	root-zone temperature (°C)	*A*	g_d	E_d	A/g_d	A/E_d	C_i
cool	16.7 a	8.83 a	0.155 a	2.70 a	62.0 a	3.74 a	262.3 a
ambient	23.1 b	9.17 b	0.198 b	3.26 b	50.5 b	3.24 b	280.0 b
warm	26.5 c	9.60 c	0.201 b	3.25 b	53.8 c	3.35 b	275.4 b
LSD	0.3	0.30	0.006	0.09	2.7	0.13	5.0
F pr <	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 5.5.3. Leaf chlorophyll concentrations and leaf photosynthetic characteristics derived from intercellular CO_2 response curves of mature leaves that emerged during the cool or warm rootzone treatments (n=4). J_{max} is the maximum rate of electron transport, $V_{c,max}$ is the *in vivo* maximum rates of Rubisco activity and R_d is the day respiration rate. Chlorophyll concentrations represent averages of nodes 2 to 6 of weekly measurements during the temperature treatments (n=18 plants, 4 shooter per plant).

Treatment	Chlorophyll (mg m^{-2})	J_{max} (µmol m^{-2} s^{-1})	$V_{c,max}$ (µmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})
cool	424.4	91.0 a	34.8 a	0.29 a
warm	424.9	97.1 a	33 a	0.58 a
LSD	2.56	17.4	9.15	0.45
F pr <	0.756	0.442	0.655	0.18

Table 5.5.4 Stomatal density of leaves that emerged during the root-zone temperature treatments and of those leaves that emerged after the cessation of the treatments. Data during the treatments are the means of leaves up to and including node 6, where gas exchange measurements were made (n=5 plants, 4 shoots per plant). Data of leaves that emerged after the treatments were discontinued are of leaves up to node 27.

	Stomatal density (stomata mm^{-2})		Lamina length (mm)	
Treatment	During treatments	Following treatments	During treatments	Following treatments
cool	127.93 a	127.04 a	83.6 a	77.2 a
ambient	123.15 ab	120.08 a	87.0 b	79.5 b
warm	116.67 b	129.70 a	90.6 c	81.8 c
LSD	8.26	9.76	3.0	1.4
F pr <	0.018	0.127	0.001	0.001

Despite no differences in soil moisture between the cool and the ambient treatments, midday g_d (P<0.001) and E_d (P<0.001) were lower by 22 and 17 %, respectively, in the cool treatment (Table 5.5.2). While there were no differences, in g_d or E_d between the ambient and warm treatments, regressions of g_d and E_d with root-zone temperature were highly (P<0.001) significant (Figure 5.5.3). During a nine hour period of darkness, total E_n amounted to 160 ml H_2O m^{-2} leaf area in the cool, 186 ml H_2O m^{-2} in the ambient and 200 ml H_2O m^{-2} in the warm treatment. During the 15 hour light period, total E_d amounted to 1312 ml H_2O m^{-2} in the cool, 1584 ml H_2O m^{-2} in the ambient and 1580 ml H_2O m^{-2} in the warm treatment. Daily E_n as a proportion of E_d varied by only 1% between the treatments (12.2% for the cool treatment, 11.7% for the ambient treatment and 12.7% for the warm treatment).

Plants grown with cool root-zones had 15 % greater transpiration efficiency (*A/E*) (P<0.001) and 23 % greater intrinsic transpiration efficiency (*A/g*) (P<0.001) than plants grown in ambient soil (Table 5.5.2). Those grown in the warm soil also had somewhat higher *A/g* (6 %) but this may have been an artefact of the slightly reduced soil moistures in this treatment. While there were no differences in *A/E* between the ambient and warm treatments (Table 5.5.2) the regression of *A/E* with soil temperature was significant due to the strong cool treatment effect (Figure 5.5.3). Similar to g_d, midday leaf C_i was somewhat less (7 %) in the cool treatment, indicating depleted CO_2 concentrations and a stomatal limitation to photosynthesis.

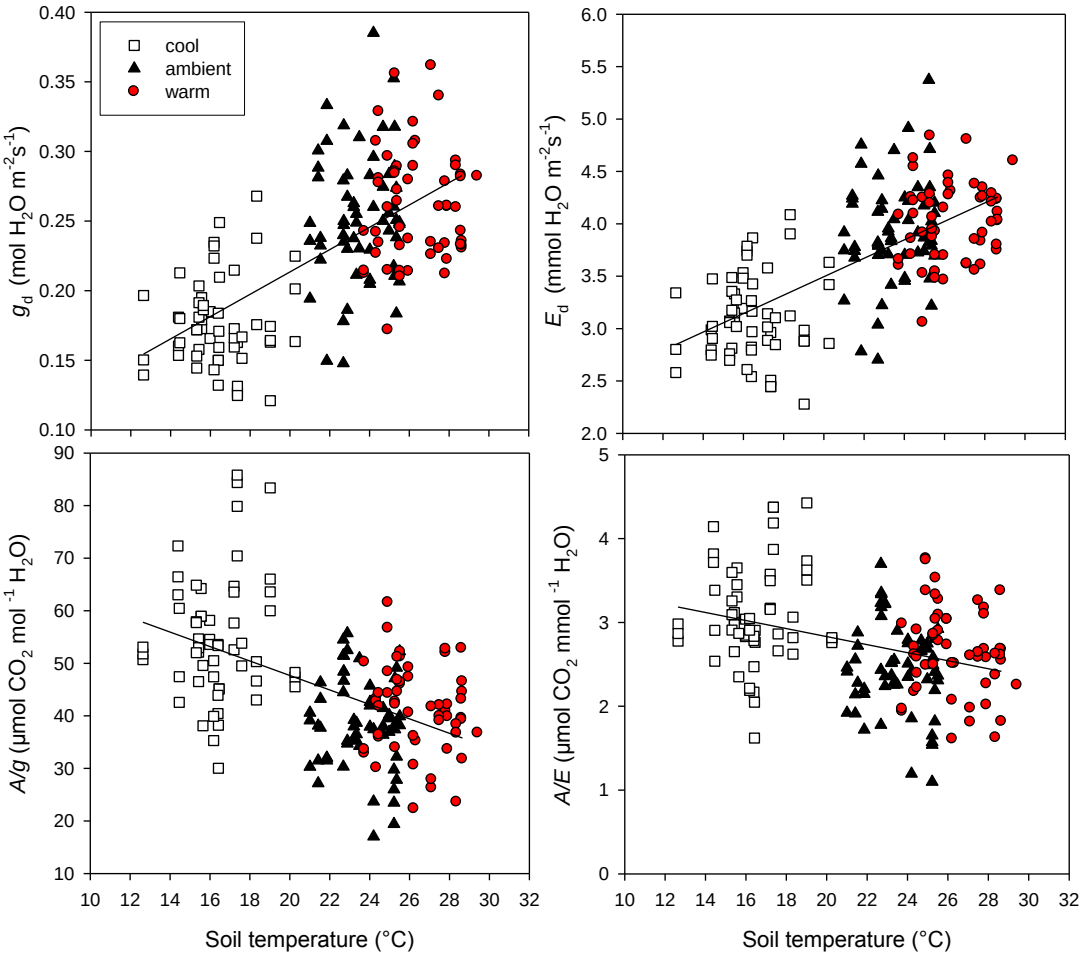


Figure 3. Midday leaf g_d, E_d, *A/g* and *A/E* as a function of root-zone temperature 48 days after the onset of treatments. Soil moisture was not different between the treatments (F pr = 0.72) and averaged 22.6 ± 1.4 % at the time of measurement. F pr of linear regression for *g* =0.001, *E*= 0.001, *A/g*= 0.001, *A/E*= 0.001; variance accounted for in *g*=0.47, *E*=0.49, *A/g*= 0.34, *A/E*= 0.19.

Light, temperature and VPD response curves

Air temperature, light and VPD response curves (Figures 5.4.4, 5.5.5 and 5.5.6) provide more extensive data of how leaf gas exchange was affected by root-zone temperatures. In both cool and warm root-zones, stomata were progressively more closed across an air temperature range of 18 to 38°C (Figure 5.5.4). VPD was not controlled over this temperature range and E_d increased linearly ($P<0.001$) with air temperature in both root-zone temperature treatments. However A did not change with this environmental parameter in either the cool ($P=0.38$) or warm ($P=0.73$) root-zones. C_i followed similar trends to that of g_d with lower values in the cool root-zones.

There were similar trends in g_d and E_d with increasing VPD (Figure 5.5.5). While g_d declined, E_d increased linearly across the VPD range of 1 to 4 kPa. Increases in E_d in the warm root-zones were double (slope =1.9 mmol H_2O $m^{-2}s^{-1}$ kPa^{-1}) the rate of that in the cool root-zones (slope =0.86 mmol H_2O $m^{-2}s^{-1}$ kPa^{-1}) ($P<0.001$). Despite stomatal closure, A increased slightly with VPD in both root-zone treatments. C_i was lower in the cool root-zones and responded in a curvilinear manner to VPD, unlike in the warm root-zone treatment.

As with the air temperature and VPD curves, light response curves showed good separation between the root-zone treatments with higher values in the warm treatment as compared with the cool treatment (Figure 5.5.6). Both g_d and E_d increases were curvilinear and did not plateau until the highest PAR value tested (2000 µmol m^{-2} s^{-1}). A increased steeply in response to rising PAR and leaves from the warm root-zone reached a 15 % higher plateau at 1000 µmol m^{-2} s^{-1} than in the cool root-zone. Conversely, C_i values declined with increasing PAR and above 1500 µmol m^{-2} s^{-1} C_i settled at 300 µmol mol^{-1} in the warm and 260 µmol mol^{-1} in the cool root-zone treatment

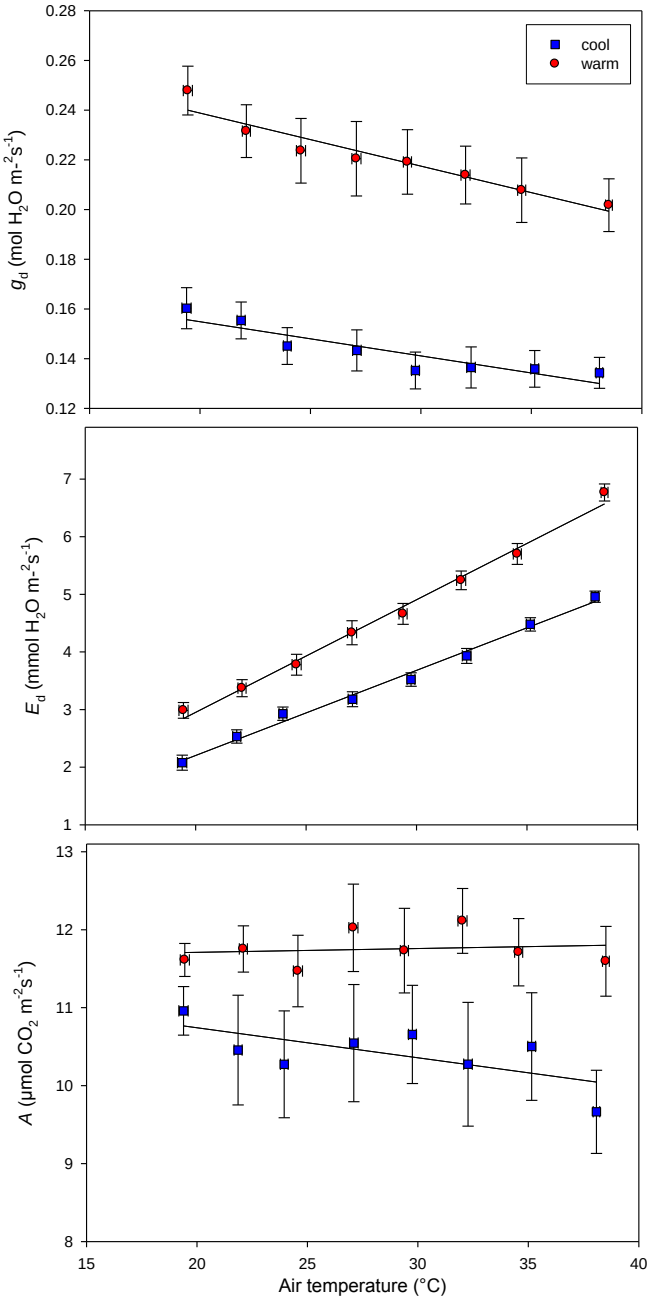


Figure 5.5.4 Midday leaf g_d, E_d, A and C_i in response to air temperature (n=4) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was 26.9 ± 1.3 °C and 15.0 ± 1.0 °C in the cool treatment (P<0.001). Soil moisture was not different between the treatments (F pr =0.72) and averaged at 17.6 ± 0.6 %. The intercepts and slopes of the regressions for the cool and warm cohorts are different from each other for g_d (P<0.001), E_d (P<0.001), A (P<0.001) and C_i (P<0.001).

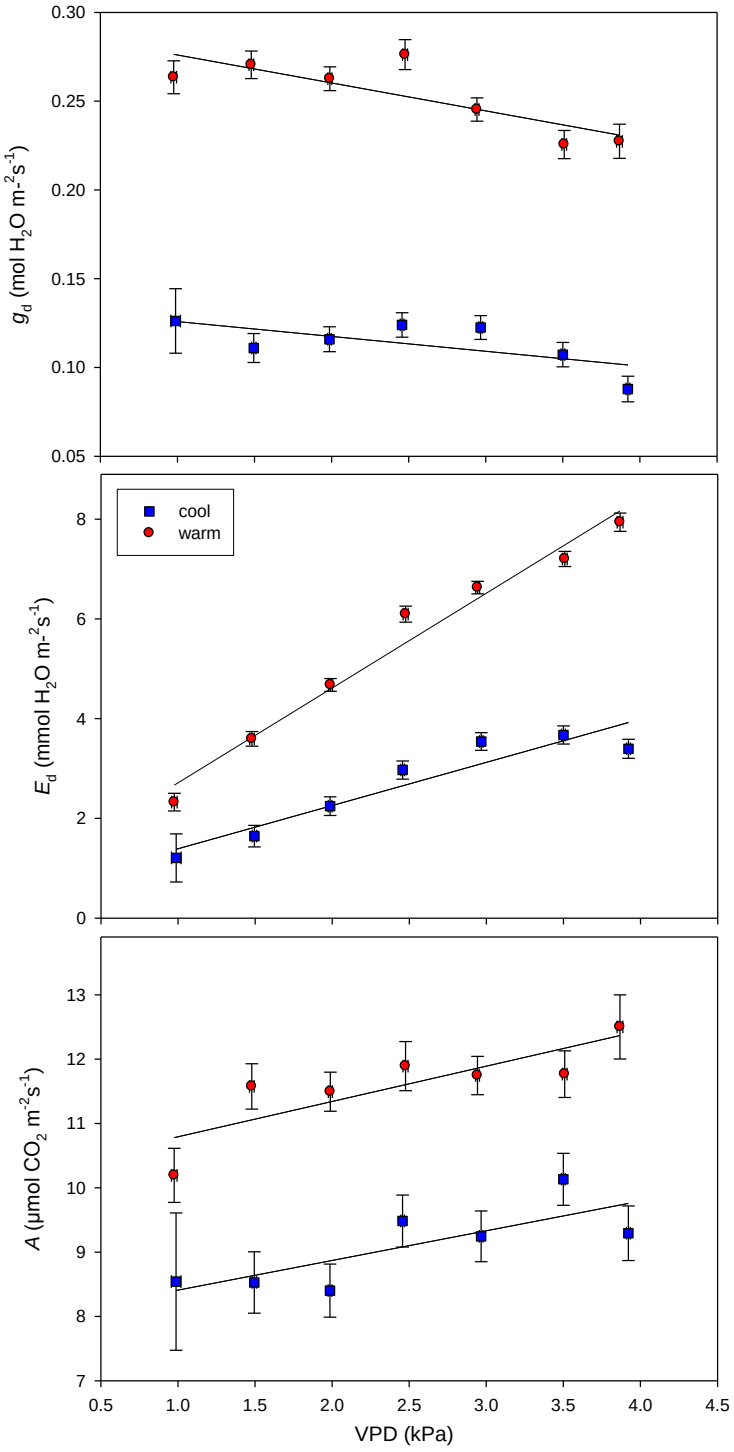


Figure 5.5.5 Midday leaf g_d, E_d, A and C_i in response to VPD (n=4) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was 20.3 ± 0.9°C and 13.3 ± 0.7°C in the cool treatment (P<0.001). Soil moisture was not different between the treatments (F pr =0.50) and averaged at 16.8 ± 0.5 %.

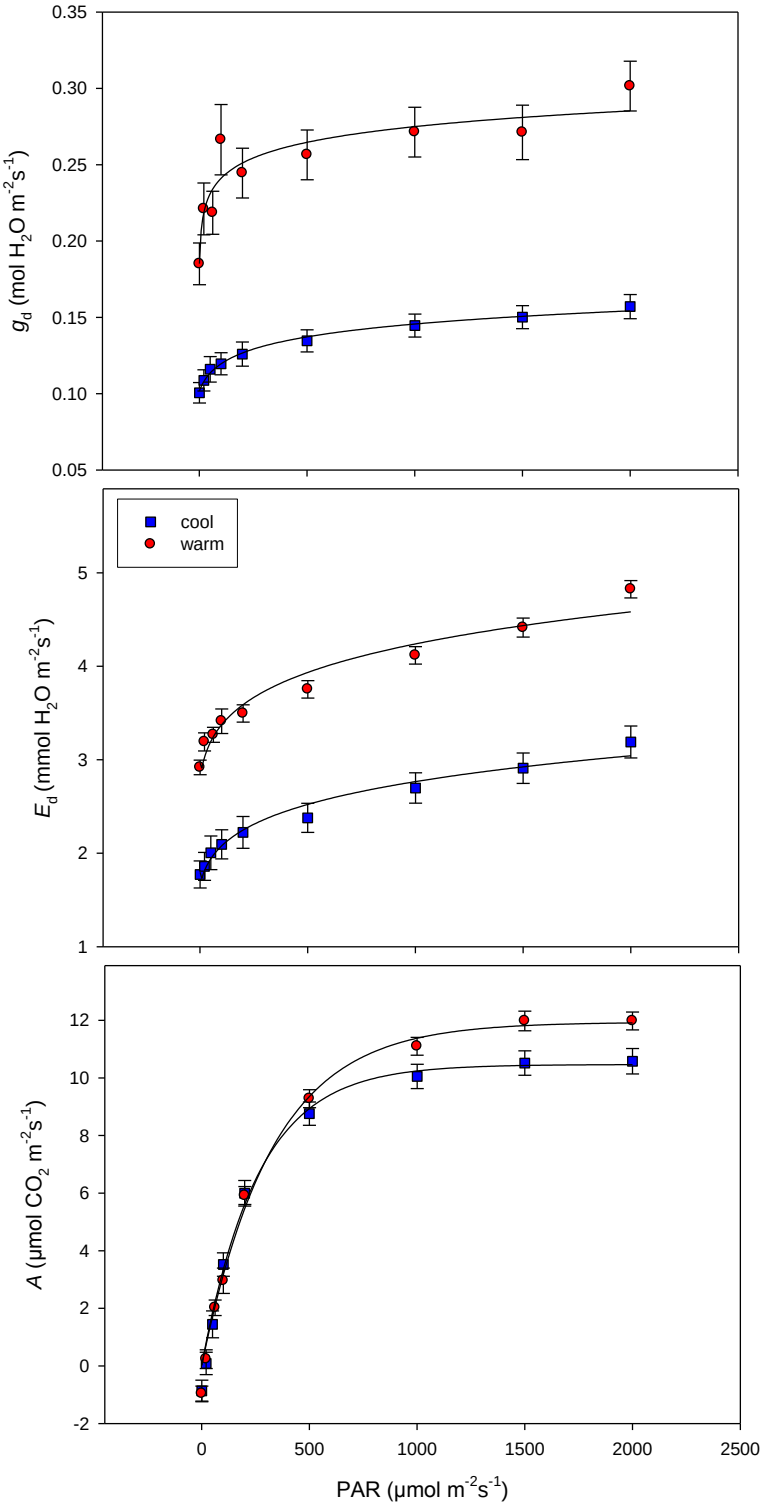


Figure 5.5.6 Midday leaf g_d, E_d, A and C_i in response to PAR (n=6) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was 24.2 ± 1.1°C and 13.3 ± 0.7°C in the cool treatment (P<0.001). Soil moisture was not different between the treatments (F pr =0.63) and averaged at 17.6 ± 0.9 %.

Leaf and photosynthetic characteristics

A/C$_i$ curves in response to root-zone temperatures are presented in Figure 5.5.7. Further analyses of these curves indicate there were no differences in J_{max} (P = 0.44) or $V_{c,max}$ (P = 0.65) between the treatments and they averaged 94.1 ± 7.5 and 33.0 ± 4.0 µmol m^{-2} s^{-1}, respectively (Table 5.5.3). Daytime dark respiration was also not different between the treatments (P = 0.18) and averaged 0.4 ± 0.2 µmol m^{-2} s^{-1}. Furthermore, leaf chlorophyll concentrations did not differ (P = 0.75) and averaged 424.6 ± 1.3 mg m^{-2}.

One leaf characteristic that did differ between the treatments was stomatal density. Of those leaves that emerged during the treatments, stomatal density was 10 % (P<0.05) higher in the cool treatment as compared to the warm treatment (Table 5.5.4). Despite 8 % larger leaves in the warm treatment as compared to the cool treatment (Table 5.5.4), there was no significant relationship between stomatal density and leaf area (F pr = 0.10 for a data set with combined treatments), therefore other factors aside from leaf area appear to be involved. There were no differences in stomatal density of those leaves that emerged after the discontinuation of the treatments and they averaged 125.6 ± 5 stomata mm^{-2}. Note, however, that stomatal density of newly emerging leaves of plants grown in the warm treatment increased by 11 % after that treatment had been removed. As averaged over 60 days following the termination of the treatments, leaf length remained 6 % larger in the warm treatment than the cool treatment (P<0.001).

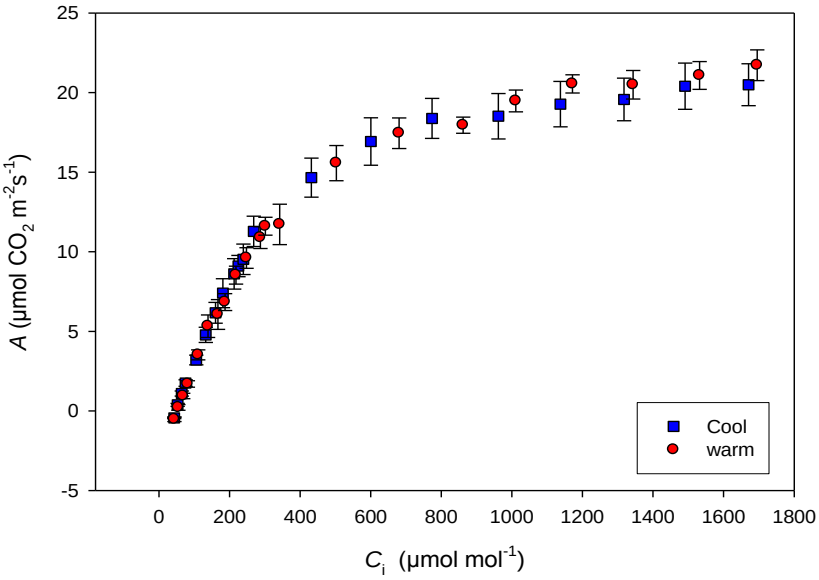


Figure 5.5.7 *A*/C_i curves of grapevines grown in warm or cool soil (n=4). At the time of measurement, root-zone temperature of the warm treatment was 25.9 ± 0.8°C and 15.1 ± 1.3°C in the cool treatment (P<0.001). Soil moisture was not different between the treatments (F pr =0.12) and averaged at 18.0 ± 0.5 %.

Gas exchange after the termination of the treatments

After the discontinuation of the treatments the midday root-zone temperatures averaged at 20.6 ± 0.3 °C and varied by less than 4 % (Table 5.5.5). Despite equal amounts of water applied, soil moisture was slightly (3 %) higher in those plants that received the cool treatment as compared to the warm treatment. This is probably because the cool plants had less total leaf area (Table 5.5.4) and therefore less whole plant transpiration and lower rates of soil water utilisation. Leaf *A*, g_d and E_d were no longer different between plants that had previously received the three treatments.

Table 5.5.5 Midday leaf *A* (µmol CO_2 $m^{-2}s^{-1}$), g_d (mol H_2O $m^{-2}s^{-1}$), E_d (mmol H_2O $m^{-2}s^{-1}$) and C_i (µmol mol^{-1}) of grapevines after root-zone temperature treatments were discontinued. Values are means of three sampling dates over a 54 day period after the discontinuation of the treatments (n=12 on each sampling date). Root-zone temperatures and soil moistures represent the means at the time the gas exchange measurements were made.

Treatment	root-zone temperature °C	soil moisture %	*A*	g_d	E_d	C_i
cool	21.0 a	23.6 a	10.3 a	0.223 a	3.70 a	286.2 a
ambient	20.2 b	20.1 b	10.2 a	0.225 a	3.74 a	288.9 ab
warm	20.7 a	20.0 c	10.5 a	0.218 a	3.66 a	283.0 b
LSD	0.3	2.1	0.4	0.008	0.14	4.5
F pr <	0.007	0.001	0.314	0.703	0.573	0.038

Effects of root-zone temperature on carbohydrate partitioning

Starch

At the termination of dormancy, starch concentrations ($P < 0.001$) in the roots were double (28.4 ± 1.4%) those of the below-ground (14.0 ± 0.9%) and above-ground trunk (12.7 ± 0.5%). The starch concentrations declined distally from the roots and were 9.3 ± 0.5% in the cordons and 7.9 ± 0.5% in the spurs (Figure 5.5.8). Root-zone temperature between budburst and fruit set had a significant impact on root starch concentrations. Specifically, starch concentrations declined by 3% in the cool, 12% in the ambient and 15% in the warm treatment ($P < 0.001$) (Figure 5.5.8). Moreover, root starch concentrations were linearly correlated ($P < 0.001$, $r^2 = 0.79$) with GDD_{soil} at fruit set (Figure 5.5.9). Starch concentrations of the below-ground trunk were also high in the cool treatment at fruit set relative to the ambient and warm root-zone treatments (Figure 5.5.8). Shoots and leaves contained a relatively small amount of starch (less than 0.5%) at fruit set with no treatment differences ($P = 0.07$ for shoots and $P = 0.27$ for leaves).

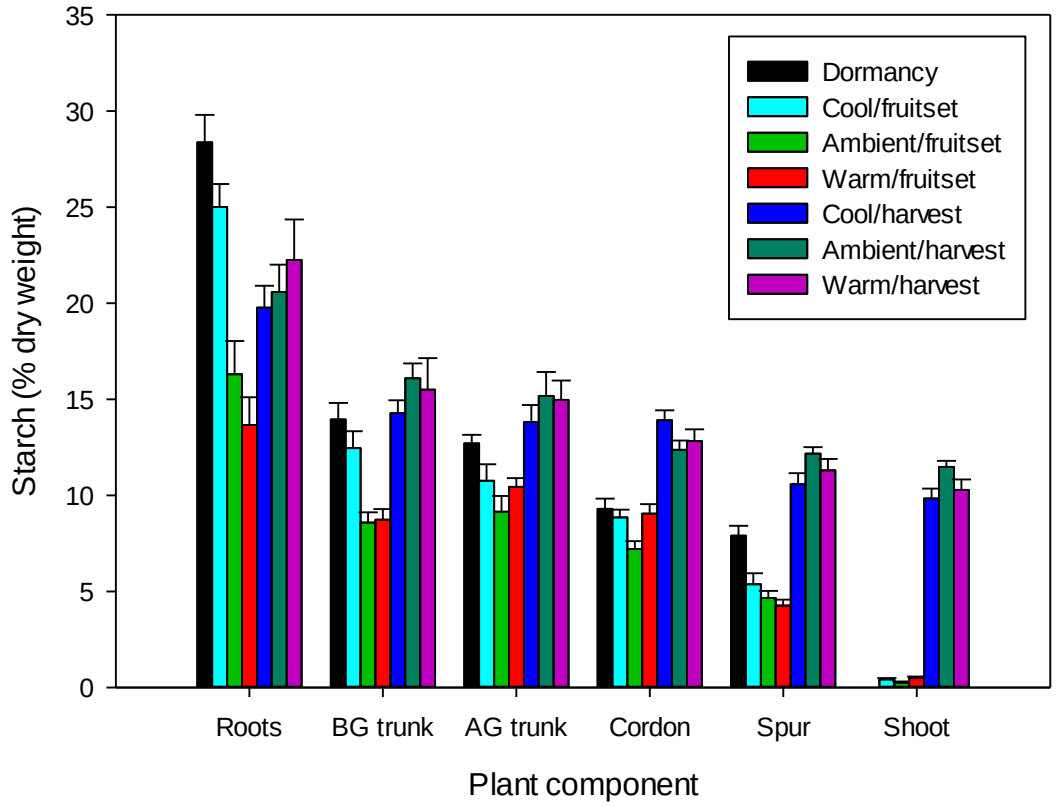


Figure 5.5.8. Starch concentrations in Shiraz vine components at dormancy, fruit set and harvest in response to cool, ambient or warm root-zone temperature treatments applied from budburst to fruit set (mean ± s.e., n=6 plants). The vine component effect was significant at dormancy (P<0.001), fruit set (P<0.001) and harvest (P<0.001). The temperature effect was significant only at fruit set (P<0.001). The soil temperature treatment x vine component interaction was significant at fruit set (P<0.001) but not at harvest (F=0.192). BG trunk refers to below-ground trunk. AG trunk refers to above-ground trunk.

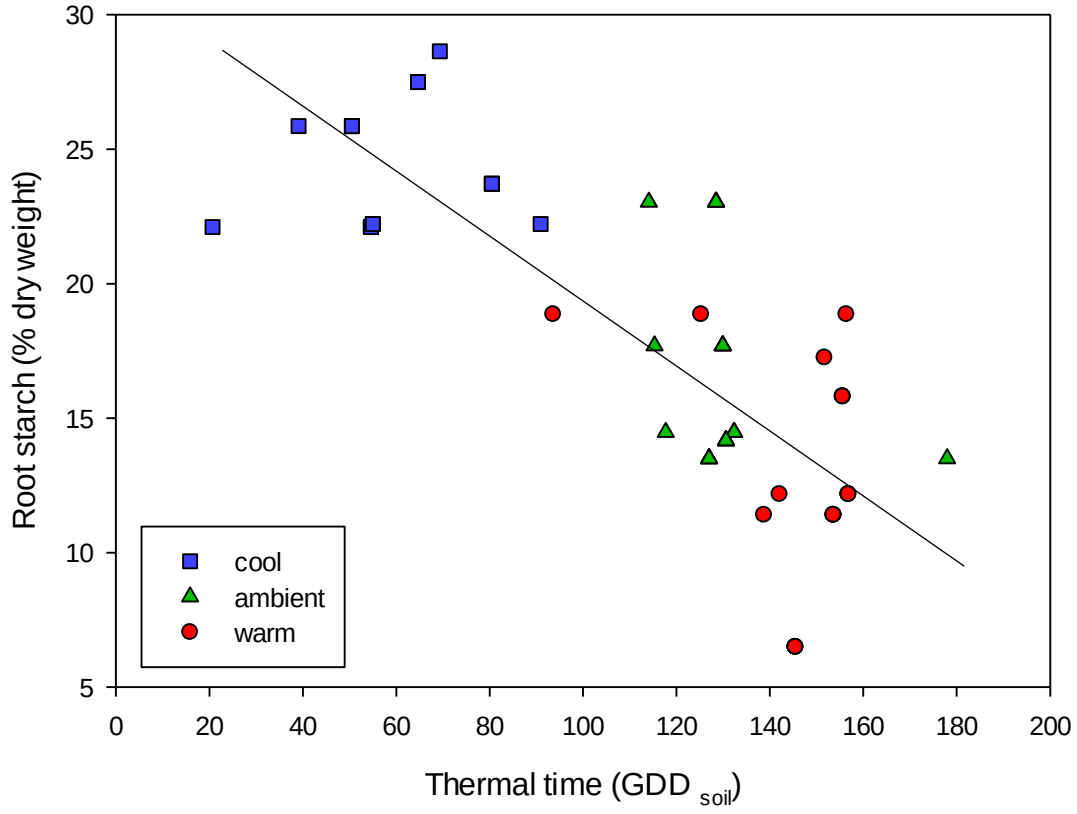


Figure 5.5.9. Shiraz root starch concentrations at fruit set as a function of GDD_{soil} ($P < 0.001$, $r^2 = 0.79$). Each data point represents the GDD_{soil} of an individual shoot calculated from a base soil temperature of 10°C (n=6 plants). The cool, ambient and warm rootzone temperature treatments were applied from budburst to fruit set.

Similar to starch concentrations, total starch was highest within the root component of the plant (Table 5.5.6). At fruit set, the roots that had received the cool treatment contained twice the amount of starch (66 g) as the ambient or warm treatments ($P < 0.001$). Similar differences were observed in the below-ground trunk. The shoots and leaves combined only accounted for 3 to 7% of the total vine starch. Starch content of the entire plant was highest for the cool treated vines (88 g compared to 50 g for the cool treatment, $P < 0.001$).

Table 5.5.6 Starch content (g) of Shiraz grapevine components at fruit set following 62 days of cool, ambient or warm root-zone temperature treatments (n=6 plants).

Root-zone temperature	Roots	Below-ground trunk	Above-ground trunk	Cordons	Spurs	Shoots	Leaves	Plant total
				Starch (g)				
cool	65.8 a	9.89 a	7.32 a	4.14 a	0.60 a	<0.2	2.46 a	90.2 a
ambient	32.7 b	6.56 b	6.05 a	2.56 b	0.52 a	<0.2	3.11 a	51.5 b
warm	30.4 b	6.33 b	5.90 a	4.88 a	0.52 a	<0.2	3.77 a	51.8 b
LSD	9.5	2.63	2.15	1.54	0.28	-	1.38	10.9
F pr	0.001	0.022	0.31	0.014	0.806	-	0.143	0.001

By harvest and berry maturity, starch concentrations had increased in all of the woody plant components with the exception of the cool treated roots which declined by 5% (Figure 5.5.8). Between fruit set and harvest the shoots had the greatest increase in starch concentrations, attaining 10.5% (Figure 5.5.8). When compared with the other plant components, however, roots still maintained the highest starch concentrations at approximately 21% of dry weight. This was followed with the below-ground trunk at 15.3%, 14.7% in the above-ground trunk, 13.0% in the cordon and 11.3% in the spur (Figure 5.5.8). The leaves only contained 0.3 % of starch by dry weight. At harvest, starch concentrations of the various plant components were not affected by the earlier temperature treatments (P = 0.192). Similarly, average whole plant total starch was 81.5 g and was no longer different as a result of the previous temperature treatments (P = 0.497).

Effects of root-zone temperature on nutrient uptake and nitrogen partitioning

At fruit set leaf petiole K, Ca, Mg, Na, B and Mn concentrations were highest in the warm root-zone treatments (Table 5.5.7). By the time the berries were fully mature petiole K, Mg, Na and Zn were still higher in the warm root-zone (Table 5.5.7). There were no root-zone temperature effects on petiole Al, Cu, Fe and P concentrations. Between fruit set and harvest K, Zn, Ca, Mg, Na and Mn increased by 14, 37, 38, 47, 138% and 9.7-fold (P<0.001 for each element)

Table 5.5.7 Grapevine leaf petiole nutrient concentrations (mg kg^{-1} dwt) as a function of cool, ambient and warm root-zone temperature treatments applied for 63 days from budburst to fruit set.

Root-zone temperature	Fruit set						
	K	Ca	Mg	Na	B	Mn	Zn
cool	27833 a	10983 a	6550 a	1670 a	30.9 a	17.8 a	88 a
ambient	34167 b	11483 a	4933 b	1442 b	33.6 b	28.3 b	87 a
warm	39833 c	12867 b	4383 b	1025 b	36.7 c	35.4 b	72 a
LSD	2805	1369	1091	399	2.1	8.2	36
F pr	0.001	0.031	0.003	0.014	0.001	0.003	0.559
	Harvest						
cool	35000 a	16150 a	8700 a	3467 a	31.7 a	302 a	125.8 a
ambient	38000 b	16467 a	7750 b	3500 a	31.9 a	252 ab	115.1 ab
warm	43000 c	16233 a	6850 c	2917 b	30.9 a	233 b	99.6 b
LSD	1874	1978	880	503	2.4	59	19.6
F pr	0.005	0.934	0.003	0.047	0.69	0.063	0.04

While there were no root-zone temperature effects on P, S and Cu concentrations in leaf petioles, these elements increased between fruit set and the fully ripe stage of development by 61, 13 and 164% (Table 5.5.8). Al an Fe did not change over time and averaged at 10.6 and 26.7 mg kg^{-1} dwt.

Table 5.5.8: Grapevine leaf petiole nutrient concentrations (mg kg^{-1} dwt) as a function of berry developmental stage.

Develomental stage	P	S	Cu
Fruit set	10506 a	1757 a	87 a
Harvest	16922 b	1986 b	230 b
F pr	0.001	0.009	0.001

N concentrations of the woody components during dormancy varied between 0.33 and 0.38% (Table 5.5.9). They were the lower in the above-ground trunk as compared with the other components.

Table 5.5.9 Nitrogen concentrations of the woody components during dormancy

Vine component	N (% dry weight)
Roots	0.360 ± 0.034
BG trunk	0.368 ± 0.039
AG trunk	0.328 ± 0.019
Cordon	0.370 ± 0.026
Spur	0.383 ± 0.033

At fruit set, N was highest in the leaves, averaging at 2.0%, and below 0.4% in all the woody components including the roots ($P<0.001$) (Figure 5.5.10). As meaned across the vine components N was lower in the cooled plants (0.96%) with little difference between the ambient (1.02 and 1.04%) and warm plants ($P<0.001$). Nitrogen was lowest in the cool treatment for the primary (1.82 ± 0.03) and lateral leaves (1.77 ± 0.05) with little differences between the ambient and warm treatments (1.96 ± 0.07 to 2.0 ± 0.05%). There was also a strong correlation between N concentrations of the primary and lateral leaves (Figure 5.5.11) as well as that of the above ground and below ground portions of the trunk (Figure 5.5.12).

The change in N concentrations of the woody components between dormancy and fruit set was dependent on the tissue. In all the treatments N levels of the cordon and spurs declined by 2-4% between budburst and fruit set. Root-zone cooling also resulted in a 4-5% decline of N concentrations within the roots and the below ground trunk during this period (Table 5.5.9 and Figure 5.5.10).

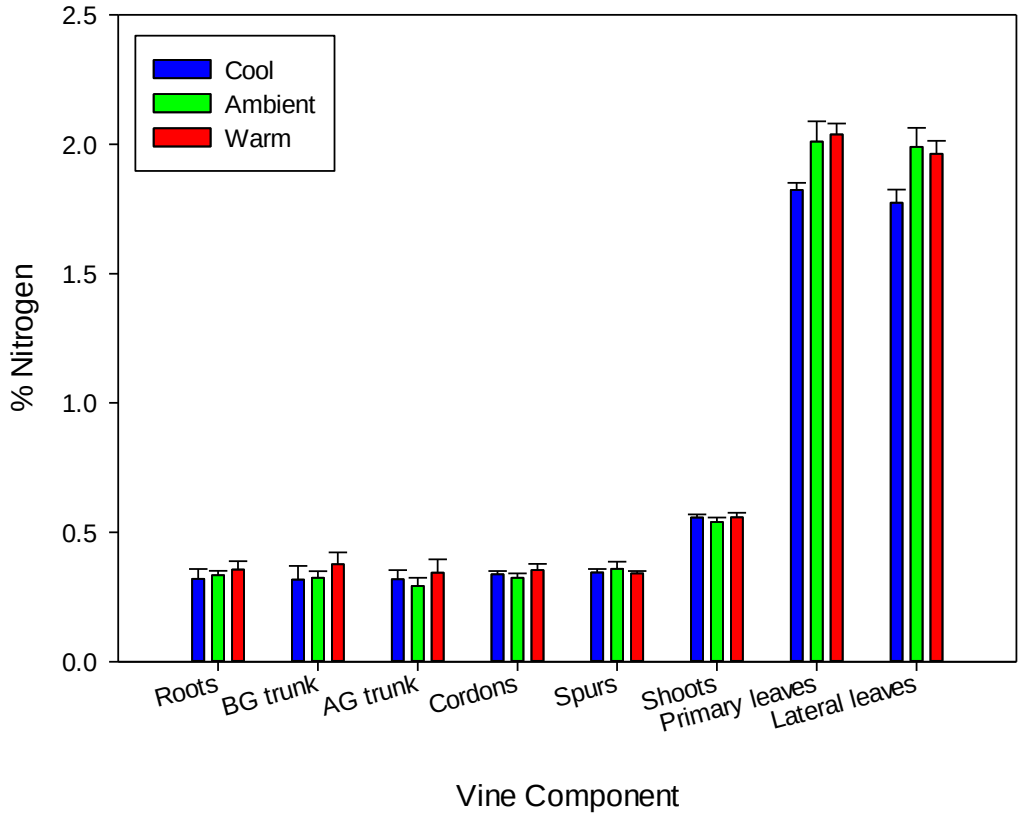


Figure 5.5.10 Nitrogen concentration in above and below ground components of Shiraz vines at fruit set in response to cool, ambient or warm root-zone conditions. The treatments were applied over a 62-day period from budburst to fruit set (n=6 plants). The vine component by temperature interaction was significant (P<0.05)

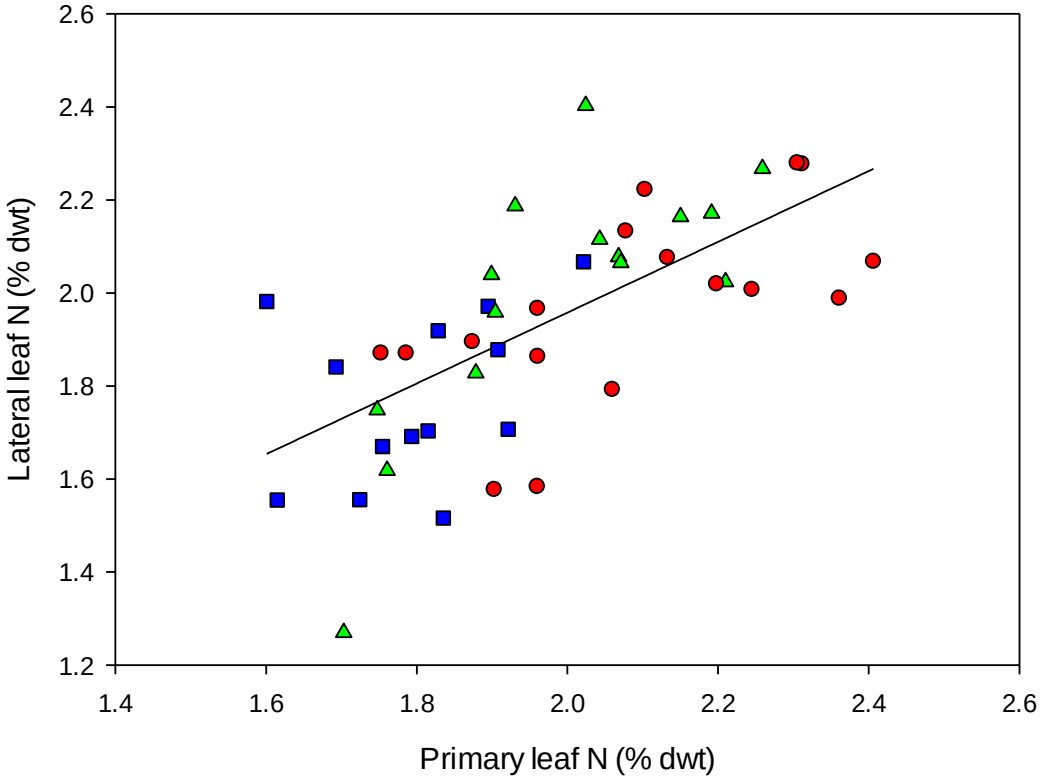


Figure 5.5.11 Lateral leaf nitrogen correlated with primary leaf nitrogen at fruit set (R^2=0.46, P<0.001).

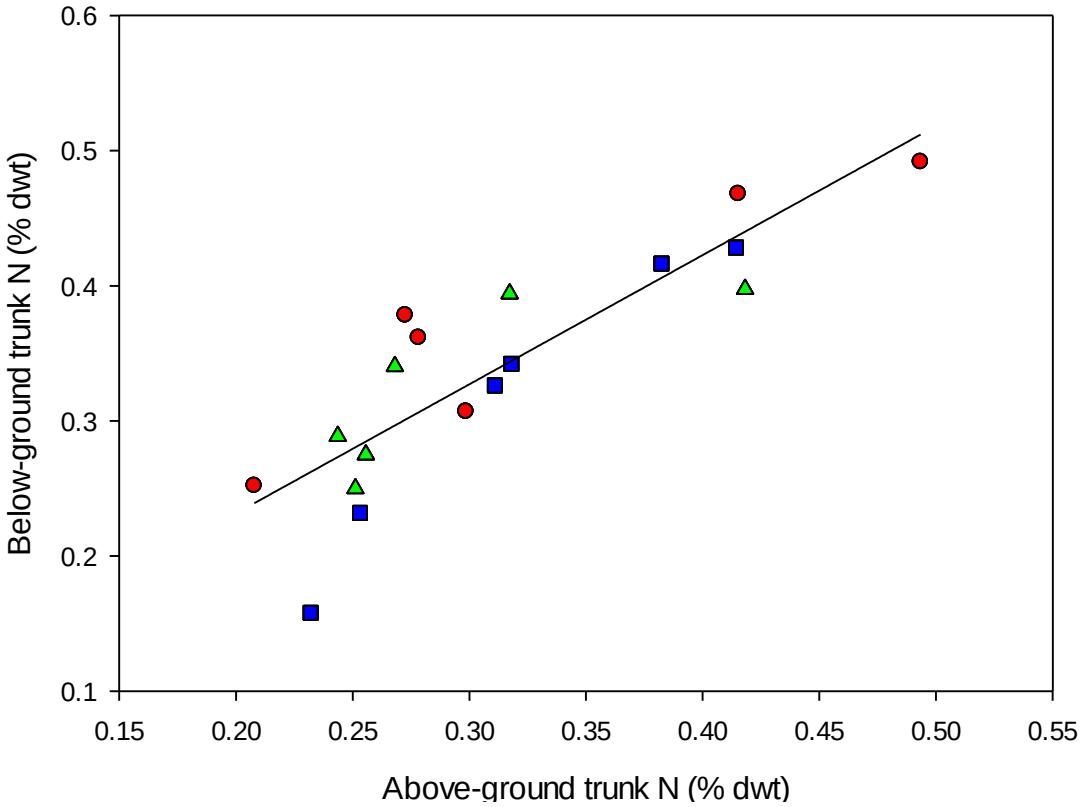


Figure 5.5.12 Correlation of above-ground trunk and below ground trunk N concentration at the fruit set stage of development (R^2=0.75, P<0.001).

Effects of root-zone temperature on grapevine vegetative growth

At the termination of the root-zone temperature treatments following fruit set, plant canopies had developed to a greater extent in those vines that had received the warm treatment. Despite no difference in leaf number on the primary shoots (approx 14-15), average dry weight of the primary leaves and shoots of vines grown in the warm treatment was nearly double that of the cooled vines, and average leaf size was greater by 41% (Table 5.5.10). At fruit set total leaf area of the primary shoots was greater by 56%, lateral shoot leaf area was double and the resultant total leaf area per plant was greater by 63%. At harvest, nearly three months after the cessation of the treatments, primary shoot dry weight was still greater by 61% despite no differences in primary shoot leaf area. Lateral shoot leaf area and total plant leaf area were also greater by nearly 50% and 18%, respectively, in the warm treatment as compared to the cool treatment, however differences in primary shoot leaf area were no longer apparent.

Table 5.5.10 Leaf characteristics and leaf area of Shiraz plants in response to root-zone temperature. The treatments were applied over a 62 day period from budburst to fruit set and the canopy assessments were carried out on a subset of vines that were destroyed at fruit set and harvest (n=6 plants).

Root-zone temperature	Fruit set						
	Primary leaf number/shoot	Primary leaf size (cm^2 $leaf^{-1}$)	Primary leaf dwt (g $shoot^{-1}$)	Primary shoot dwt (g $shoot^{-1}$)	Primary leaf area/plant (cm^2)	Lateral leaf area/plant (cm^2)	Total leaf area/plant (cm^2)
cool	13.6 a	59.3 a	3.89 a	3.2 a	2811 a	548 a	3359 a
ambient	14.8 a	66.4 ab	4.42 ab	3.8 a	3479 a	1051 b	4467 b
warm	15.1 a	83.6 b	5.56 b	6.3 b	4386 b	1110 b	5493 c
LSD	2.4	13.8	1.29	1.6	717	406	972
F pr	0.441	0.02	0.029	0.001	0.002	0.019	0.002
Root-zone temperature	Harvest						
cool	19.2 a	59.8 a	8.15 a	4.7 a	4297 a	1527 a	5801 a
ambient	19.0 a	57.0 a	7.91 a	5.6 b	4076 a	2153 b	6292 a
warm	20.9 a	59.9 a	8.36 a	7.6 c	4608 a	2273 b	6892 b
LSD	3.7	12.3	2.40 a	0.8	867	573	533
F pr	0.523	0.901	0.929	0.001	0.422	0.025	0.004

Effects of root-zone temperature on root growth

New fine root growth was stimulated by warm root-zones (Figure 5.5.13). The number of root segments per total root area was 19% greater in the ambient and 113% greater in the warm treatment relative to the cool treatment. Summed new fine root length was 112% greater in the ambient and 291% greater in the warm treatment relative to the cool treatment. Branching of these new fine roots was also responsive to root-zone temperature with 0.5 ± 0.07, 1.0 ± 0.08 and 1.7 ± 0.26 branches cm^{-2} for the cool, ambient and warm root-zone treatments, respectively (Figure 5.5.14).

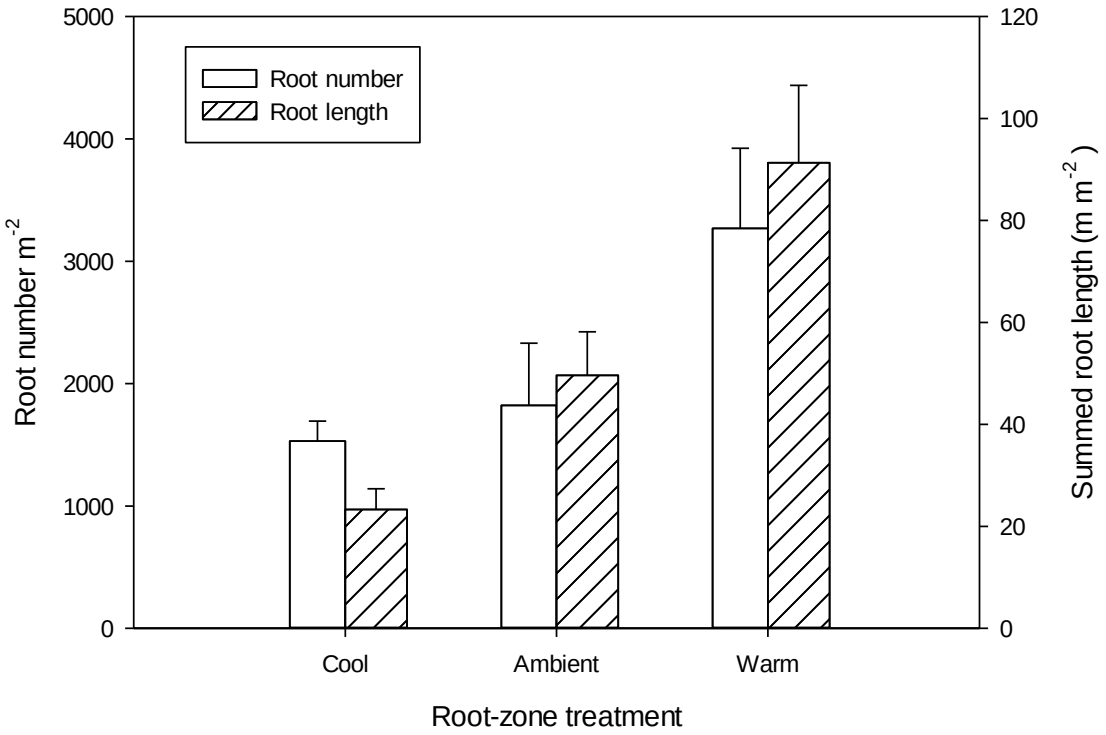


Figure 13. Fine new root growth as a function of root-zone treatments. Root number (P<0.05) and summed root length (P<0.01) are based on per m^2 of total root area.

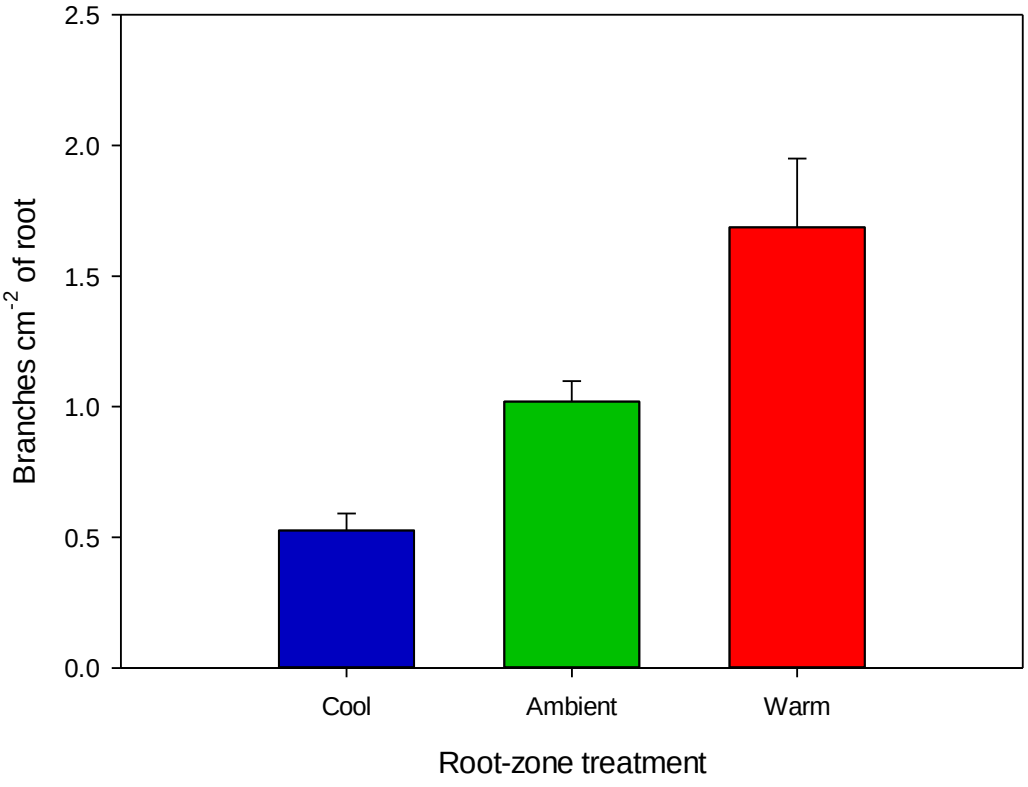


Figure 5.5.14 Fine root branching as a function of root-zone temperature (P<0.001)

Effects of root-zone temperature on capfall and fruit set

Flower opening, marked by the dehiscence of the calyptra from the flower apex, began approximately 48 days after the initiation of the treatments and was completed within 10 days (Figure 5.5.15). On day 50, capfall was 13% in the cool, 22% in the ambient and 31% in the warm treatment ($P < 0.001$). Three days later capfall had reached 92% in the warm and ambient treatments, but inflorescences of the cool treatments were only at 82% capfall and required a further six days to reach this point.

Flower number and fruit set were not affected by root-zone temperature in the concurrent season. Inflorescences carried on average 170-190 flowers and while flower number was lower in the warm treated vines this was not significant (Table 5.5.11). There was also no significant linear relationship between GDD_{soil} and flower number. Fruit set ranged from 51 to 58% across the treatments (Table 5.5.11). Infructescence berry number was linearly correlated to flower number ($P < 0.001$, $r^2 = 0.54$, Figure 16) and was 24% higher in the cool treatment as compared with the warm treatment ($P < 0.02$), but a larger proportion of these (32% as compared to 26%) did not develop to full maturity (Table 5.5.11). Infructescence berry number and percentage fruit set were not correlated to starch concentrations (%) or content (g) in any of the roots, trunk, cordon, spur, shoots, leaves, rachis or berry.

The position of the inflorescence on the shoot was a significant factor on flower number and fruit set (Table 5.5.11). Those inflorescences in the basal position carried on average 45% more flowers that those in the distal position (nodes T_e and T_u of Gerrath and Posluszny 2007, respectively). Fruit set was 23% higher in the distal position but infructescence total berry number was still 41% higher in the basal bunches. The number of fully developed berries was 10% greater in the basal bunches as compared with the proximal bunches. Shoot position on the spur also had a significant effect on flower and berry number (Table 5.5.11). Flower number and total berry number were greater by 53% and 36% in the proximal shoots while developed berry number was greater by 40%.

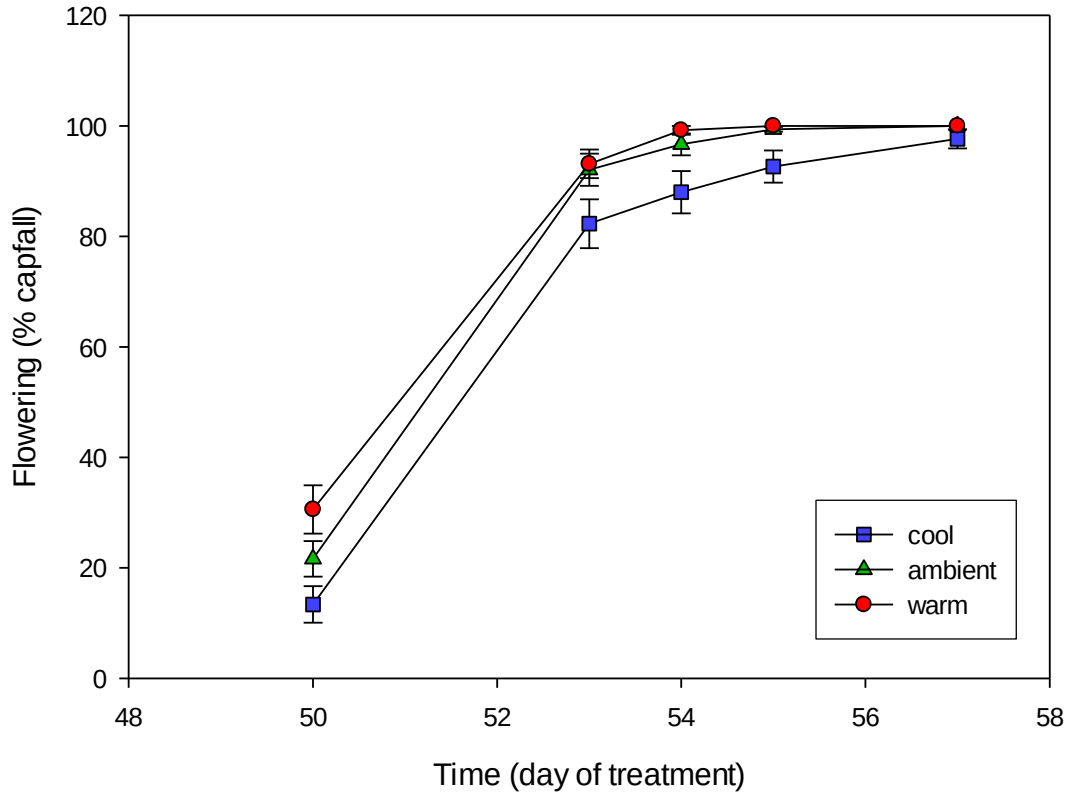


Figure 5.5.15 Onset of flowering expressed as percentage capfall in Shiraz as a function of cool, ambient and warm root-zone temperature treatments applied for 62 days from budburst to fruit set (mean ± s.e., n=18 plants).

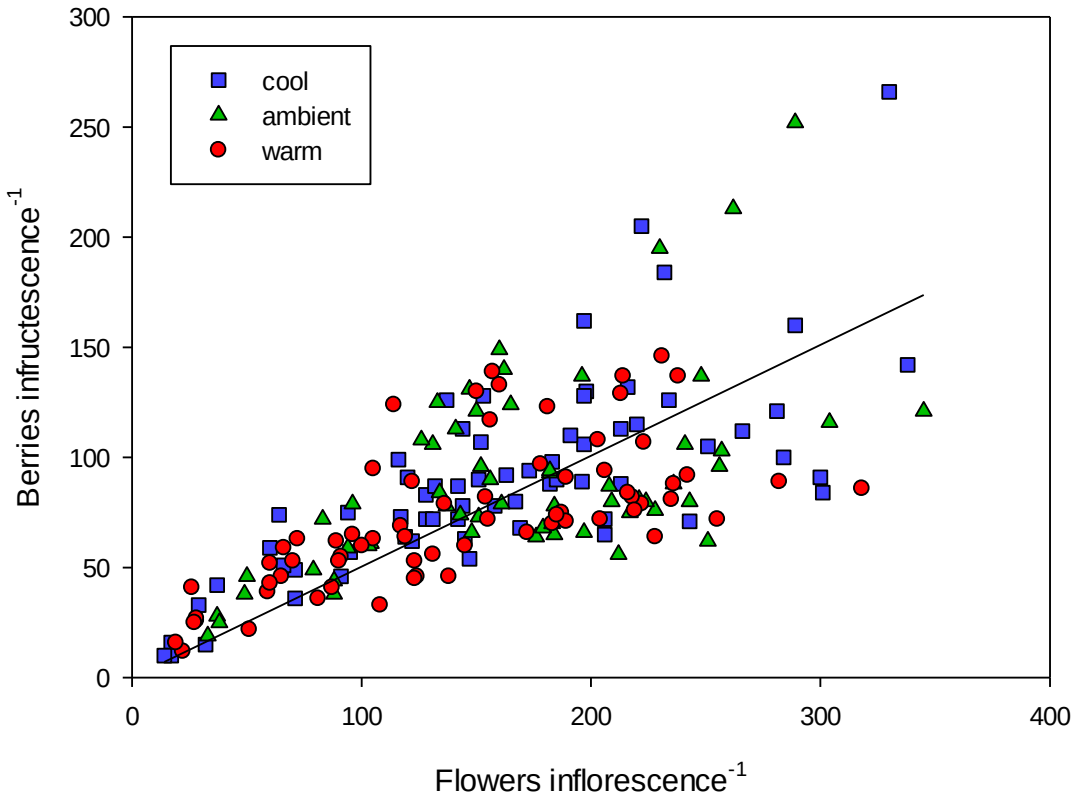


Figure 5.5.16 (A) Infructescence berry number as a function of flower number (n=18 plants, *P* < 0.001, r^2 = 0.54). Cool, ambient and warm root-zone temperature treatments were applied for 62 days from budburst to fruit set. Each data point represents an individual infructescence. There were no treatment differences in the slopes of the individual regressions therefore a global curve was fitted to the data.

Table 5.5.11 Flower number, fruit set and berry number of Shiraz vines that were exposed to three root-zone temperatures from budburst to fruit set in the concurrent season (n=12 plants).

Main effect	Inflorescence flower number	Fruit set (%)	Infructescence berry number	Undeveloped berry number	Developed berry number
			Root-zone temperature		
cool	191.5 a	54.3 a	80.3 a	31.7 a	48.6 a
ambient	184.6 a	58.2 a	65.9 a	24.4 b	41.5 a
warm	172.6 a	51.3 a	63.6 b	19.3 c	44.2 a
LSD	33.9	9.5	10.7	5.4	6.7
F pr =	0.396	0.368	0.015	0.001	0.372
			Inflorescence position on shoot		
basal	203.4 a	50.8 a	74.5 a	28.3 a	46.2 a
distal	140.7 b	62.5 b	62.0 b	19.7 b	42.2 a
LSD	29.3	8.1	9.3	4.7	5.8
F pr =	0.001	0.004	0.002	0.001	0.07
			Shoot position on spur		
basal	138.2 a	57.8 a	57.1 a	21.4 a	35.8 a
distal	211.2 b	52.5 a	77.7 b	27.5 b	50.0 a
LSD	28.2	8.1	9.1	4.6	5.7
F pr =	0.001	0.277	0.001	0.008	0.001

Effects of root-zone temperature on berry development and composition

Similar to the commencement of flowering, the onset of veraison occurred earlier in those berries grown on vines in the warm treatment (Figure 5.5.17). Berries on vines from the cool treatment were last to undergo veraison, lagging by approximately three or five days compared to the ambient or warm root-zone treatments. Across the experiment, the onset of veraison spanned from 110 to 126 days after the treatments were initiated, and was complete within 16 days.

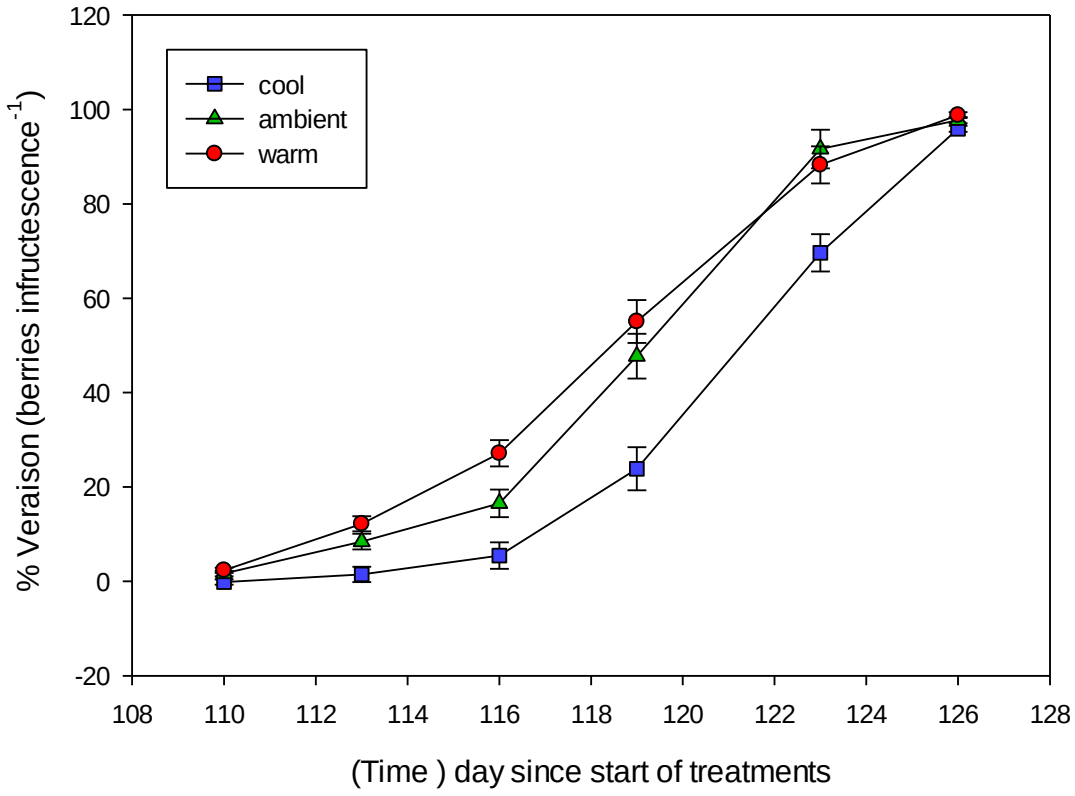


Figure 5.5.17 Onset of veraison in Shiraz following root-zone temperature pre-treatments from budburst to fruit set (mean ± s.e., n=12 plants). Temperature treatments were applied for 62 days and veraison commenced 48 days after treatment termination.

Measured 22 and 49 days following the termination of the root-zone temperature treatments, berry diameter was a function of the previous root-zone temperatures applied until fruit set (Figure 5.5.18). During the pre-veraison and veraison stages of development berries from vines grown in the ambient root-zone were 2-3% greater in width than those grown in the cool root-zone and these were 5-6% more narrow than the berries from the warm treatment ($P < 0.001$). At harvest, berries from vines grown in the warm root-zone treatment were still larger with a 9% higher fresh weight and a 13% greater dry weight as compared to the ambient treatment (Table 5.5.12). At harvest, berries from the cool treatment were no longer different from the ambient treatment in terms of size.

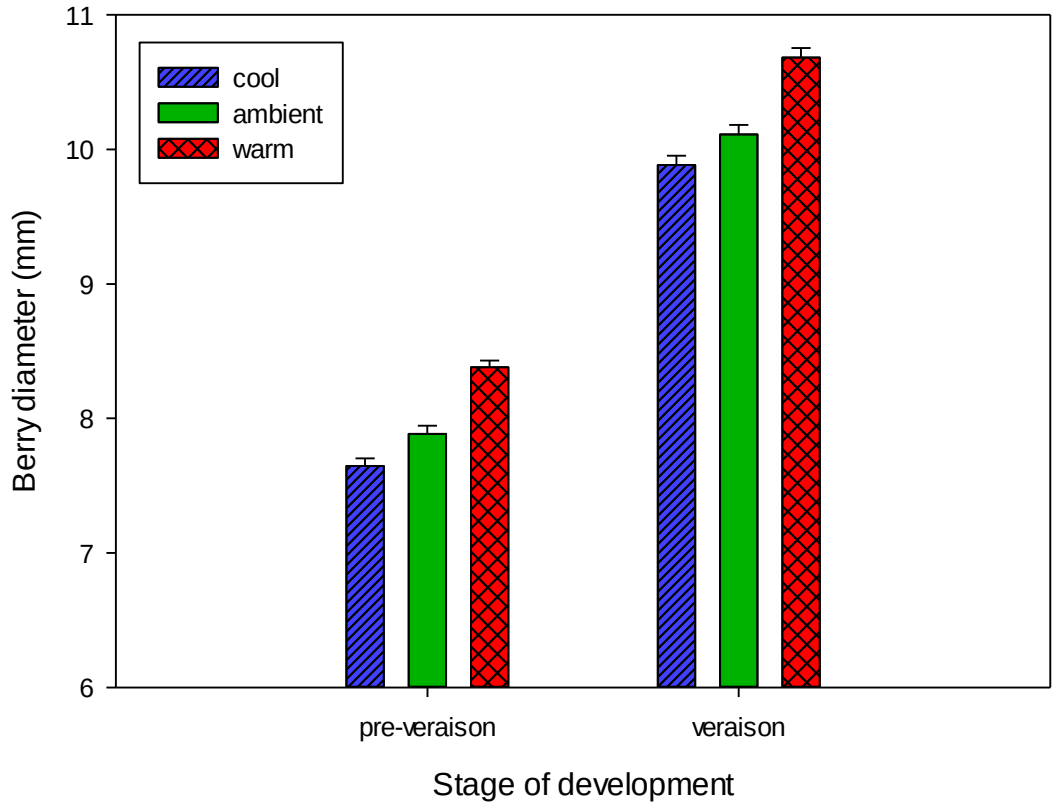


Figure 5.5.18 Berry diameter in Shiraz at the pre-veraison and veraison stages of development. Measurements were carried out 22 (pre-veraison, P<0.001) and 49 (veraison, P<0.001) days following the termination of the root-zone temperature pre-treatments. The root-zone temperature treatments were applied 62 days from budburst to fruit set and veraison commenced 48 days after treatment termination (mean ± s.e., n=6 plants).

The number of fully developed berries within an infructescence was not different between the root-zone treatments (Table 5.5.11), however as infructescence berry number increased there was a greater number of undeveloped berries (P < 0.001, r^2=0.81, Figure 5.5.19A). Rachis dry weight at harvest was more closely related to the number of post-veraison berries (Figure 5.5.19B, r^2 = 0.74) as compared with the total berry number within an infructescence (r^2 = 0.61). Final yield per plant was not different between the temperature treatments and averaged at 199 ± 16 g fresh weight (P = 0.22) and 48 ± 4 g dry weight (P = 0.28).

At harvest, berry total soluble solids concentrations (°Brix) were not different due to the previous root-zone temperatures however sugar per berry (mg) was 13% higher in the warm treatment as compared with the ambient treatment (Table 12). While

anthocyanins on a fresh weight basis were not different between the treatments, anthocyanin content per berry was 14% higher in the warm treated plants as compared with the cool treated plants (Table 5.5.12). Infructescence position on the shoot also had a significant effect with greater anthocyanins on a fresh weight basis in the basal bunches as compared with the distal bunches (*P* = 0.03, 1.01 mg vs 0.93 mg).

Table 5.5.12 Berry size, sugar and anthocyanin concentrations as a function of cool, ambient and warm root-zone temperature treatments applied from budburst to fruit set (n=12 plants).

Root-zone temperature	Berry fresh weight (g)	Berry dry weight (g)	TSS (°Brix)	Sugar (mg berry^{-1})	Anthocyanins (mg g fwt^{-1})	Anthocyanins (mg berry^{-1})
cool	1.01 ab	0.239 a	19.6 a	198 a	1.00 a	0.90 a
ambient	0.98 a	0.228 a	19.2 a	193 a	1.00 a	0.99 b
warm	1.08 b	0.258 b	20.1 a	219 b	0.96 a	1.03 b
LSD	0.08	0.02	0.9	19	0.08	0.09
F pr <	0.013	0.004	0.106	0.006	0.459	0.042

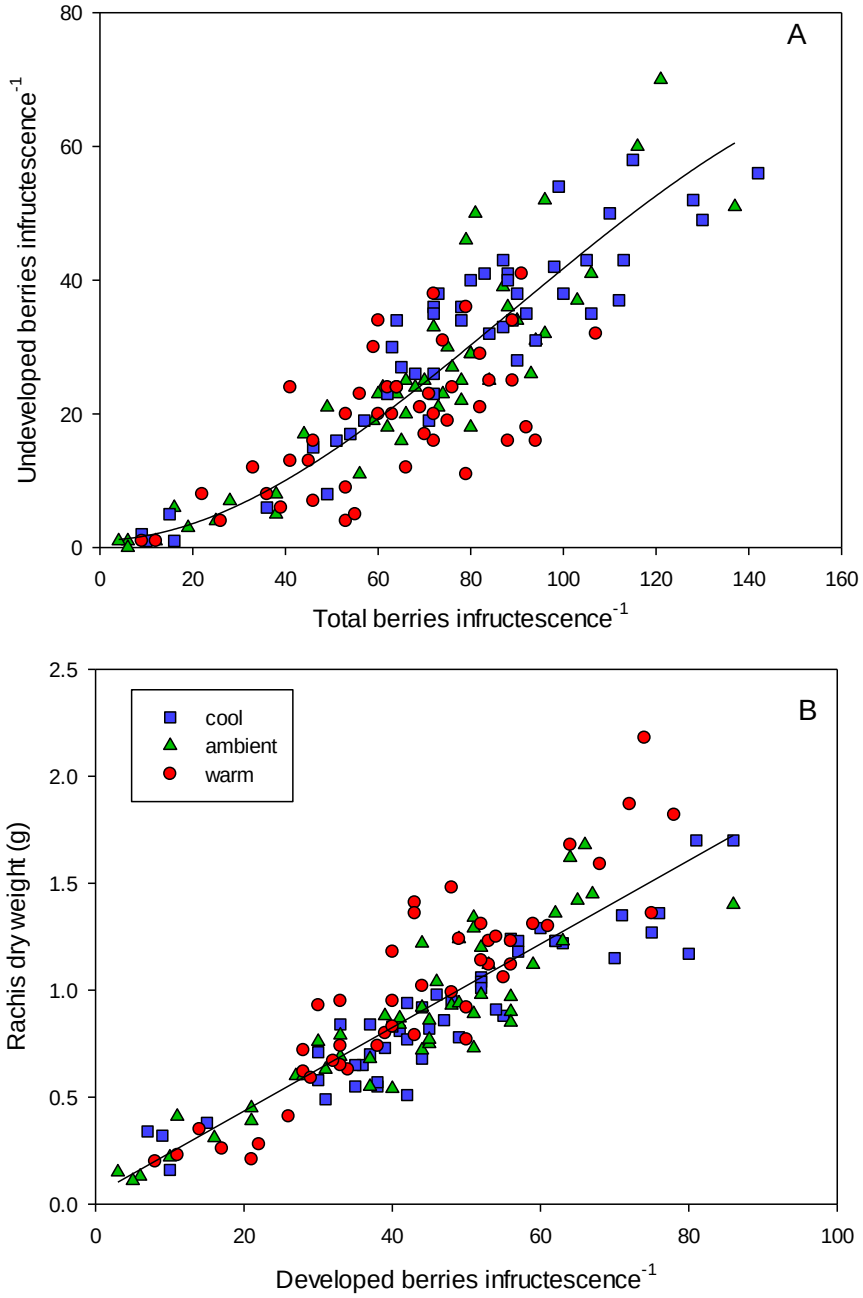


Figure 5.5.19 (A) number of undeveloped berries as a function of total berry number ($P < 0.001$, $r^2 = 0.81$). (B) Rachis dry weight as a function of the number of fully developed berries ($P < 0.001$, $r^2 = 0.81$). Cool, ambient and warm root-zone temperature treatments were applied from budburst to fruit set. Each data point represents an individual infructescence (n=12 plants). There were no treatment differences in the slopes of the individual regressions therefore global curves were fitted to the data.

Berry N concentrations were lower in the cool treatment as compared with the other treatments at 0.33% compared with 0.39% (Table 5.5.13). Plants from the warm root-zone treatment also developed berries that were less acidic (Table 13). pH was higher by approximately 0.1 units while titratable acidity was lower by 0.3 g L^{-1} in those berries from the warm root-zones compared with the cool treatment. Berry pH and anthocyanin content were linearly correlated with titratable acidity (Figures 5.5.20A and 5.5.20B).

Table 5.5.13 Berry N, pH, titratable acidity and petiole K concentrations at harvest as a function of cool, ambient and warm root-zone temperature treatments applied from budburst to fruit set (n=12 plants).

Root-zone temperature	Berry N (% dwt)	Berry pH	Berry titratable acidity (g L^{-1})	Petiole K (g kg dwt^{-1})
cool	0.333 a	3.70 a	4.41 a	35.0 a
ambient	0.399 b	3.74 a	4.36 a	38.0 a
warm	0.393 b	3.82 b	4.06 b	43.0 b
LSD	0.041	0.06	0.287	4.1
F pr =	0.003	0.001	0.045	0.005

Vine K status

Petiole analyses of samples taken at harvest indicate that leaves accumulated 23% more K (*P* = 0.005) in vines with warmed roots as compared to vines with cooled roots (Table 5.5.13). Berry pH was also correlated to leaf petiole K concentrations (*P* = 0.002) however only 21% of the variance was accounted for (data not shown).

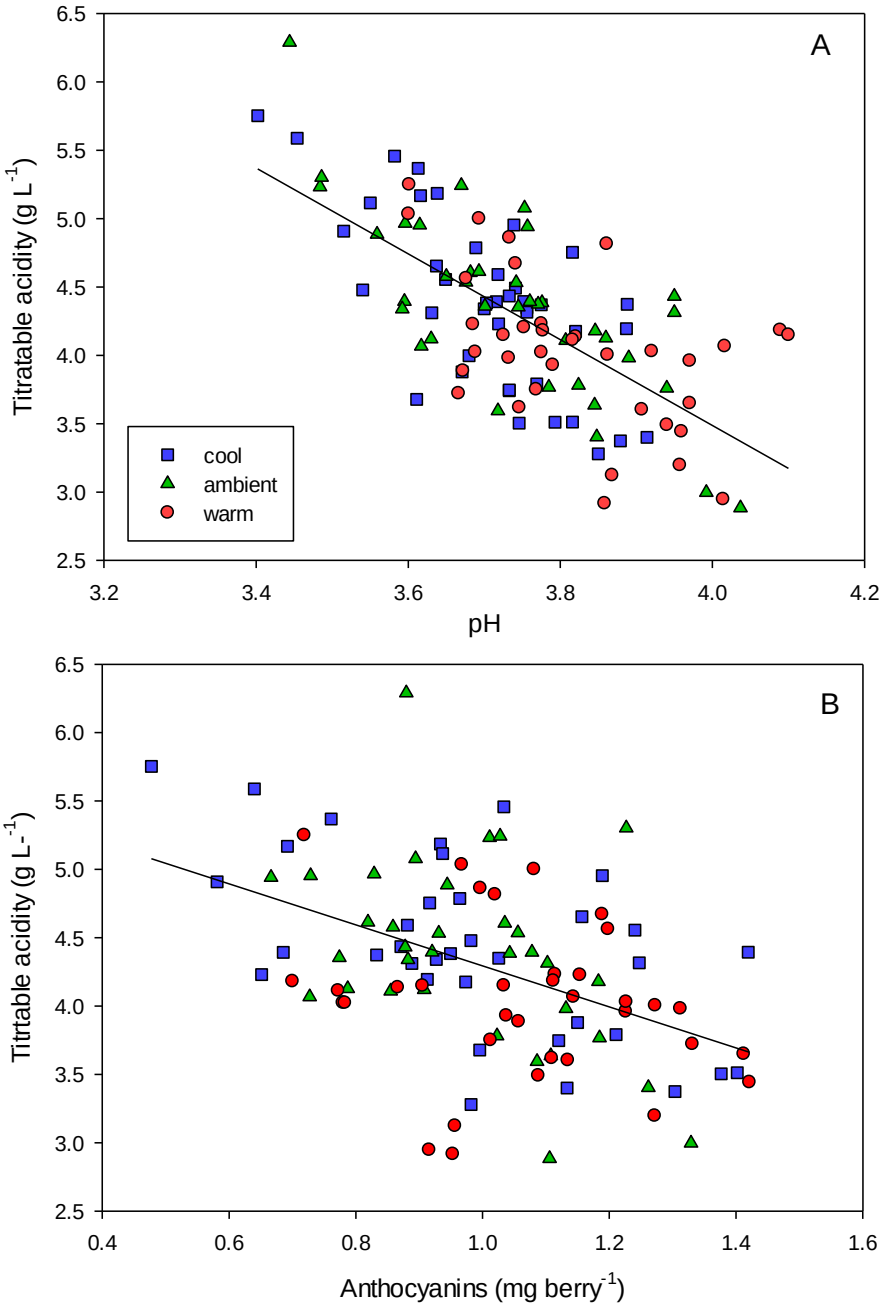


Figure 5.5.20 (A) Relationship of titratable acidity with (A) pH ($P < 0.001$, $r^2 = 0.47$) and (B) anthocyanins ($P < 0.001$, $r^2 = 0.20$). Cool, ambient and warm root-zone temperature treatments were applied from budburst to fruit set. Each data point represents an individual infructescence (n=12 plants). There were no treatment differences in the slopes of the individual regressions therefore global curves were fitted to the data.

Root-zone temperature effects on bud fruitfulness

Table 5.5.14 Plant inflorescence number in the second season following cool, ambient and warm root-zone temperature treatments applied from budburst to fruit set in the first season (n=6 plants).

Root-zone temperature	Inflorescences per plant
cool	2.7
ambient	2.7
warm	2.0
LSD	2.4
F pr =	0.78

Root-zone temperature during the bunch initiation period did not have an effect on bud fruitfulness in the following season. Plants exposed to cool or ambient root-zones bore 16 inflorescences across the six vines of each treatment but only 12 inflorescences in the warm treatment. This equates to 2.7 inflorescences per plant for the first two treatments and 2.0 inflorescences per plant for the warm treatment (Table 5.5.14). These differences were not, however, significant. Flowering date in the season following the root-zone treatments was also not affected and averaged on 10/11/2012 ± 0.6 in the cool and ambient treatments and 9/11/2012 ± 0.5 in the warm treatment.

5.5.4 Discussion

Root-zone temperature effects on leaf gas exchange

Nocturnal stomatal conductance

Our study confirms that daytime stomatal conductance of grapevines, like many other species, responds to root-zone temperature. Our present findings extend this observation to nocturnal stomatal conductance. There is very little experimental work on the interaction of root-zone temperatures with nocturnal gas exchange and we believe this is one of the first reports to characterise this. Cool root-zones resulted in a sustained suppression in *g* and *E* both during the day and night and leaves transpired nearly 20% less than those grown in warm root-zones . Aside from

the stomatal limitation evident in our data, others have shown that low E can be the result of reductions in both soil and plant hydraulic conductance (Kramer and Boyer, 1995; Fennell and Markhart, 1998; Berndt et al., 1999) possibly through an increase in water viscosity (Muhsin and Zwiazek, 2002). After the discontinuation of the treatments, differences in leaf g_d and E_d were no longer apparent. This signifies that the root-zone temperature effect on gas exchange was not due to a permanent anatomical change but rather a transient physiological effect, perhaps relayed through signals targeted at the stomata. Another study on aspen has demonstrated that cool root-zone temperatures can reduce g through pH, ion and ABA signals transported in the xylem sap (Wan et al., 2004). It would be expected, therefore, that these signals persist throughout the diurnal cycle, including the night. The role of such signals was not addressed in this study, however, and further work is required to verify their contribution to g_d and g_n in grapevines.

The resultant reduction in g_n of plants growing in cool root-zones may thus be an after-effect of daytime changes, or it may offer a physiological advantage such as hastened plant rehydration through concomitant decreases in E_n. Nocturnal transpiration, assessed in this study under mild spring-time conditions, was generally about 12 % of E_d. This is similar to other species grown in wetter environments (Caird et al., 2007; Dawson et al., 2007) and less than the 40 to 75 % found in desert species (Ogle et al., 2012). Substantial night-time transpiration as a consequence of elevated g_n in warm night-time root-zones may prevent complete plant rehydration prior to dawn (Kavanagh et al., 2007), resulting in greater water stress during the day as evaporative demand increases. Because our plants were well watered in all treatments with soil moistures not dropping below 20 %, soil moisture was not a stressor. In grapevines, wilting tendrils at the shoot tips are the first signs of water stress (Winkler et al., 1974) and we did not observe this in any of our plants. In a field situation, however, under the absence of irrigation or under reduced water allocations, plants may experience daytime water stress due to elevated night-time soil temperatures, especially in warm, dry climates. It follows then that daily minimum soil temperature may be just as critical as daily maximum or average soil temperature in determining plant water relations.

Absence of acclimation

One of most significant findings of this study was that stomatal conductance remained low in the cool root-zones throughout the two-month treatment period, with apparently no temperature acclimation. Sustained reduction in *g* may be required over weeks if plants are to prevent dehydration, especially with rising midday and nocturnal VPD driving *E* as the season progresses.

Other factors controlling nocturnal stomatal conductance

Because nocturnal gas exchange has not yet received the same depth of attention as daytime gas exchange, far less is known about the plasticity of g_n to environmental factors. We found that aside from root-zone temperature, soil moisture was another variable that impinged on nocturnal stomatal conductance. Nocturnal stomatal conductance was found to decline with drying soil (Cavender-Bares et al*.,* 2007; Howard and Donovan, 2007) and increasing VPD (Barbour and Buckley, 2007) in other studies. VPD did not explain any of the variance in g_n in our Shiraz plants but this may be because the leaf chamber temperature was held constant and the resultant VPD range was fairly narrow.

In the first few weeks after budburst g_n increased with leaf age, mimicking trends in g_d. Leaf maturity thus appears to be another variable controlling g_n. When leaves first emerge there is a progressive increase in g_d as stomata mature and the stomatal apertures expand (England and Attiwill, 2011) and we reason that a similar developmental effect persists on g_n during the night. We did not measure g_n during the latter half of the season but aging and senescing leaves often have lower g_d and thus likely g_n as well, but this is yet to be verified. Other factors that might affect g_n and E_n include developmental stage of the plant and plant nutrient status (Ludwig et al., 2006).

Root-zone temperature regulates photosynthesis

Rates of CO_2 assimilation were highest in the warm and lowest in the cool root-zones, and this was apparent across a wide range in PAR, VPD and air temperatures. The decline in C_i values under cool root-zone temperatures points to a stomatal limitation and therefore a curtailed CO_2 supply to photosynthesis under these conditions. There were no apparent limitations at the chloroplast level since chlorophyll content and both J_{max} and $V_{c,max}$ did not differ between the treatments. This

indicates that the low photosynthesis of the cool root-zones was not due to Rubisco or RuBP limitations. Others (Cai and Dang, 2002) found that root-zone temperature had an effect on both J_{max} and $V_{c,max}$ in conifer species however a much wider temperature range (from 5 to 35 °C) was employed. These parameters had a maximum at around 25 °C, declining at supra-optimal temperatures. Similar to our grapevines, dark respiration was not affected by root-zone temperature in any of the four species tested.

The key point is that soil temperature did influence *A*, and furthermore *A* was more sensitive to soil temperature than to an air temperature range of 20 to 35 °C. Similarly, photosynthesis of red spruce saplings grown in a cold temperature region was limited more by minimum soil temperature than minimum air temperature, especially in spring (Schwarz et al*.,* 1997). If this is the case for grapevines grown in cool regions, it would be advantageous for the plant to direct root growth to a depth where diurnal temperature fluctuations are dampened. When field grapevines are drip irrigated, however, most of the roots can be found in the top layers (Stevens and Douglas, 1994) and therefore they are exposed to significant fluctuations in temperature. Natural daily cycling in temperature is often overlooked in root-zone studies (Pregitzer et al*.,* 2000), however in order to mimic field conditions, we allowed soil temperature to fluctuate diurnally by approximately 5º C. Diurnal soil temperature fluctuations would also be relevant to newly established seedlings with small root systems as well as shallow rooted species growing on impenetrable soils.

Transpiration efficiency responds to root-zone temperature

Despite greater *A* in warm root-zones, both *A/g* and *A/E* were inversely proportional to root-zone temperature. In other words, leaves of plants grown in the warmer root-zones lost greater amounts of water per unit of carbon gained. This is similar to the drop in transpiration efficiency of grapevine varieties grown in high VPD (Düring*,* 1987; Pou et al*.,* 2008) or ample soil moisture (Cueves et al*.,* 2006). There is wide varietal diversity in transpiration efficiency of grapevines (Schultz, 1996; Bota et al*.,* 2001; Gibberd et al*.,* 2001; Rogiers et al*.,* 2009). While the adoption of irrigation technology that confers water savings has improved water use efficiency at the vineyard level, further gains can be made by planting inherently efficient varieties (Condon et al*.,* 2004). Varietal selection therefore must not only take into

consideration VPD and soil moisture responses, but as shown here, soil temperature as well.

Stomatal density responds to root-zone temperature

Stomatal density of concurrently formed leaves was lowest in the warm root-zone treatment and highest in the cool treatment. It would seem intuitive that the high stomatal densities of the cold treatment might lead to higher g_d and g_n, but as is apparent here, there was no such relationship. The low *g* characteristic of the cool root-zone treatment is therefore not due to low stomatal density and must be related to stomatal aperture only.

In a previous study on Chardonnay grapevines it was found that root-zone temperature and atmospheric carbon dioxide both impinged on stomatal density (Rogiers et al*.,* 2011*a*). Because this leaf parameter was closely and inversely correlated with starch concentration in roots and trunks we suggested that the carbohydrate reserve status of the plant may be an important endogenous determinant of stomatal density. Depleted starch reserves, elicited by several weeks of high metabolism in the warm root-zones, would require replenishment and indeed we find that, in the current study, stomatal density of newly emerging leaves of grapevines grown in warm soil increased after the treatment had been removed.

Root-zone temperature effects on carbohydrate partitioning

Our study confirms that warm root-zone temperature during spring stimulates root reserve mobilisation, supports canopy development and hastens net positive leaf assimilation. At the onset of the experiment, most of the starch reserves were sequestered in the roots of the plants. Soil warming resulted in greater depletion of reserves from this plant component as well as the below-ground trunk. The strong linear relationship of GDD_{soil} with root starch is likely the direct result of increased temperature dependent metabolic activity (Atkin et al. 2000). Our observations are therefore consistent with the mobilisation of below-ground carbohydrate reserves for the release of both energy and carbon skeletons to support canopy development through more rapid leaf emergence and the development of larger leaves. Earlier leaf development also resulted in earlier net positive assimilation per unit of leaf area and this remained higher throughout the warm temperature treatment.

At the time of berry harvest, some 80 days later, the roots of plants grown in the warm and ambient root-zone treatments had partially replenished their carbohydrate reserves while the trunk, cordon and spurs had completely regained these reserves, thus indicating that these other woody plant components are also strong sinks for carbohydrates. Conversely, root reserves declined in the cool treatment from fruit set to harvest after modest mobilisation between budburst and fruit set. It appears that in this treatment the root reserves may have served as a carbohydrate source after fruit set, rather than a sink, and it is likely that carbon was diverted to complete canopy development and facilitate ripening.

Root-zone temperature effects on nutrient uptake and nitrogen partitioning

Nutrient uptake by plants is dependent on a number of biotic and abiotic factors including root-zone temperature (Radin 1990). The most vigorous periods of root N uptake in grapevines occurs after the cessation of rapid shoot growth and after harvest (Van Zyl 1984). Our results indicate that root-zone warming can augment the accumulation of a number of macro- and microelements in grapevine leaves between budburst and fruit set. The elevated N concentrations in the leaves but not the woody components points to preferential allocation to the new vegetative growth. In all the treatments N levels of the cordon and spurs declined between budburst and fruit set, indicating that these above-ground woody vine components behaved as a N reserve source. Root-zone cooling also resulted in a decline of N concentrations within the roots and the below ground trunk between dormancy and fruit set, indicating that in these vines the N was drawn from this reserve pool as well and not replenished. Conversely, root-zone warming did not result in a decline, indicating that (1) either this pool was not a source of mobilised N for new vegetative growth early in spring or (2) if it was a source that it had been replenished by fruit set. We did not sample during the initial period of rapid shoot growth when any mobilisation of N reserves would have been most evident. Therefore further refined work is required to address this issue of N reserve mobilisation from the woody components after dormancy.

Root-zone temperature effects on root growth

Early season root growth was stimulated by root-zone warming. It is likely that carbon availability was increased in the warm root-zones due to greater starch mobilisation and this provided energy and carbon skeletons to drive new root growth. Temperature dependent metabolic reactions important for root growth would also have a direct impact on cell division and cell expansion. Root growth is most evident between bloom and veraison period and after harvest (Conradie 1980; Löhnertz et al. 1989). Our results indicate that the seasonal variability in the amount and timing of root growth (Comas et al., 2005) is likely partially determined by root-zone temperature.

Root-zone temperature effects on capfall and fruit set

Warm root-zones after budburst accelerated flower development and brought forward the onset of cap-fall. These observations indicate that the concurrent strong canopy growth that was characteristic of this treatment did not compete for carbon to the detriment of reproductive development. Hastened inflorescence development in the warm root-zone treatment was not a function of fewer inflorescences. This is because bud fruitfulness was likely determined in the previous season (Boss and Thomas 2000), prior to the application of the root-zone temperature treatments, and therefore the number of inflorescences per vine was similar. As in our Shiraz vines, warm root-zones hastened flowering in Cabernet Sauvignon berries by up to one week when vines were grown at a soil temperature of 25-30°C compared to 12°C (Kliewer 1975). It is likely that the carbon reserves helped fuel the process of inflorescence emergence and expansion until the leaves at the inflorescence zone were sufficiently mature to commence exporting photoassimilate to these organs.

Despite earlier inflorescence emergence, flower number and percentage fruit set were not increased by the warm root-zone temperature. Fruit set does not draw on carbohydrate reserves but is highly dependent on concurrent photoassimilation (Keller and Koblet 1994, Caspari et al. 1998, Rogiers et al. 2011*b*). The earlier maturing leaves, greater leaf area and higher assimilation rates of the warm treatment likely resulted in higher net vine carbon assimilation yet this did not augment the number of ovaries that were successfully fertilised. Since reserve restoration occurs at around fruit set (Mullins et al. 1992; Rogiers et al. 2011*b*) it may compete with the fertilisation process, especially in those plants that were exposed

to the warm root-zones. Root growth, as indicated by visual assessment during the destructive harvest at fruit set, was also stimulated in warm soils and is often considered to compete with reproductive activities (Hansen, 1977) but as shown here, not to the extent of causing a reduction in percentage fruit set. Cool root-zones can reduce uptake of those nutrients, such as Mo and Zn, critical for fruit setting, (Christensen 1980, Williams et al. 2004) but these did not appear to be in short enough supply to cause a reduction in fruit setting in our cooled vines.

In a previous experiment on Chardonnay, flower number was increased in plants exposed to warm soil from budburst to the onset of capfall but percentage fruit set was lower (Rogiers et al. 2011*b*). This could be a varietal effect since the Chardonnay inflorescences held on average fewer flowers and flower maturation occurs earlier than in Shiraz. Furthermore, in our experiment individual flowers may have already been predetermined by the time the treatments were initiated (approximately one week later than in the experiment on Chardonnay). Cool root-zones can result in flower death in grapes (Shimamura and Kubota 1978) as can high root-zone temperatures in oranges (Cary and Weerts 1977) but it appears that our warm and cool treatments were not severe enough to cause greater than normal flower abscission compared to the ambient treatment.

Root-zone temperature effects on berry development and composition

Warm root-zone temperature during spring hastened berry expansion and the onset of ripening. Earlier flowering and fertilisation in the warm root-zones led to earlier berry expansion so that at the pre-veraison stage berries were generally larger than those berries grown on vines that had received the cool treatment. Berry enlargement is mostly due to cell expansion but the treatments were applied until shortly after fruit set, during a phase of active cell division (Considine and Knox 1981). The larger berries of the warm treatment may thus be the combined result of a greater number of cells as well as bigger cells.

Veraison was brought forward in berries of vines exposed to warm soil and this was likely the result of the earlier flowering and greater photosynthetic capacity of these vines. In grapes, the onset of pericarp ripening occurs when seeds have reached full size (Ristic and Iland 2005) and the accumulation of seed reserves slows (Cadot et al. 2006). Seed development is thus highly dependent on photoassimilate and, upon

reaching maturity, signals may be released to instigate berry ripening in preparation for seed dispersal by birds.

Warm root-zone temperature during spring resulted in infructescences with fewer undeveloped berries, greater final berry size and lower berry acidity at harvest. These attributes, along with earlier flowering and veraison, point to a faster rate of phenological development in vines with warmed roots. Fewer undeveloped berries per infructescence may also be related to smaller infructescence size; there were fewer berries amongst which to distribute the carbon supply and therefore each berry had a greater capacity to undergo normal development.

Berry pH and titratable acidity were inversely related in our Shiraz grapes and this has been shown previously in a number of other varieties (Boulton 1980). An increase in berry pH after root-zone warming from budburst to fruit set may be linked to greater vine nutrient uptake. High grape juice pH has been correlated to high K concentrations in the juice (Somers 1975) and, as indicated by petiole analyses, there was greater K uptake by warmed vines. It has been shown in Shiraz that the pH of grape juice decreased as K^+ concentrations increased, resulting in higher pH (Walker and Blackmore 2012). A decline in berry titratable acidity after root-zone warming from budburst to fruit set may be a consequence of the dilution of acids in the larger berries. Alternatively, the greater decline of tartaric acid relative to malic acid that accompanies ripening can reduce titratable acidity because malic acid is a weaker acid (Gong et al. 2010) and perhaps there was more tartaric degradation in the warm root-zone treatment. Cabernet Sauvignon grapes grown at 25°C from budburst to harvest also had lower total acidity but it was malic acid that was lower compared to berries grown on plants exposed to 12°C (Zelleke and Kliewer 1979). High air temperatures can result in a greater decline in malic acid due to greater respiration (Coombe 1987). Berry temperature was not measured in this study but it is very unlikely that root-zone temperature would have influenced this parameter.

Grape berry anthocyanin development is mainly driven by light and air temperature (Kliewer 1970, Buttrose et al. 1971, Dokoozlian and Kliewer 1996) but, in Shiraz, can also be negatively correlated to high crop load (Wolf et al. 2008). The lack of treatment effects on anthocyanin concentrations suggests that differences in canopy size were not great enough at the onset of ripening to impinge on anthocyanin

development. On a per berry basis anthocyanin content was greater in berries grown on plants that had received the warm treatment because these berries were larger. The inverse relationship between anthocyanin content per berry and titratable acidity highlights that riper berries had more colour.

There was no effect of the earlier root-zone temperature treatments on berry total soluble solid concentrations however sugar per berry was higher in warm treated vines. Again, this is likely because these berries were larger. Warm soils have been reported to lower total soluble solids in two orange varieties (Cary and Weerts 1978). Conversely, soil temperature at a depth of 10 cm was highly correlated to total soluble solids concentrations in Chardonnay vines (Zorer et al. 2005) and Cabernet Sauvignon grapes grown at 25°C throughout the entire season had higher total soluble solids than at 12°C (Zelleke and Kliewer 1979). Kyoho grapes grown in hydroponics at 30°C also had bigger fruit with more colour and sugar content than vines grown at 20°C or 25°C during ripening (Yasuda and Azukizawa 2000). In all the above studies the temperature treatments were applied throughout the ripening period, while in our study they were applied only during flowering and fruit setting. The absence of temperature effects on berry sugar concentrations in our vines may also be the result of the diversion of photoassimilates to carbohydrate reserve restoration or possibly to the greater lateral shoot growth that was evident in the vines grown in the warm treatment (Hunter 2000).

Lower N concentrations were evident in those berries which had received the cool treatment and this is likely indicative of lower ammonium ions, amino acids and/or peptide levels. While not examined here, the species of amino acid and peptide molecules may also differ as a result of the treatments and this will have implications for yeast activity during the wine making process (Bell and Henschke 2005). Further research profiling these berry constituents in response to root-zone temperature is warranted based on these preliminary findings.

Root-zone temperature effects on bud fruitfulness

Root-zone temperature between budburst and fruit set did not impact on bud fruitfulness in the following season. It was hypothesised that root-zone warming would stimulate carbohydrate mobilisation, promote earlier vegetative development and photoassimilation. This extra carbon supply could then be transferred to the bud

directly or as a signal and support inflorescence initiation, increasing bunch number in the following season. The higher photoassimilation and carbohydrate levels of those vines exposed to root-zone warming did not appear, however, to stimulate bunch initiation. Conversely, these vines had slightly lower bud fruitfulness, but this was not statistically significant. The lack of treatment effects in this study was associated with overall low bud fruitfulness in the second season. Bud fruitfulness is susceptible to air temperature and light and perhaps these above-ground conditions were not optimal during the bunch initiation period of the first season. The ability of these other abiotic factors to over-ride root-zone temperature implies that root-zone temperature may not be a critical aspect of bunch initiation.

5.5.5 Conclusions

Warm root-zones during spring resulted in greater root carbohydrate reserve mobilisation and hastened canopy development, effectively altering the source-sink relations of the plant through the remainder of the season. Warm root-zones produced larger berries with greater sugar and anthocyanin content and lower acidity. Conversely, cool root-zones delayed canopy and inflorescence development as well as the onset of veraison, and berries were smaller at harvest time. These results indicate that root-zone temperature is a strong driver of both vegetative and reproductive phenological development and is thus an environmental factor that can contribute to variability in berry composition and yield across a vineyard.

Warm root-zones during spring also stimulated new root growth and resulted in greater nutrient uptake. Leaf N concentrations were elevated as a result of the root-zone warming and cooled roots were not able to replenish those N reserves that were mobilised prior to fruit set.

We report that night time transpiration of anisohydric grapevines can be a small (12%) but significant part of daily transpiration. Similar to daytime stomatal conductance, night-time stomatal conductance was responsive to root-zone temperature and thus those regulatory systems that are operative during the day also persist during the night. Removal of the cool or warm treatments brought *g* back to ambient levels, indicating the presence of a system that is able to react to environmental conditions. The limitation in photosynthesis induced by cool root-zones was the result of a stomatal limitation rather than limitations at the chloroplast

level and accompanying increases in stomatal density brought on by the cool root-zones were not able to offset this stomatal limitation. Intrinsic transpiration efficiency declined with increasing root-zone temperature. Therefore it is likely that carbon assimilation will occur at the expense of a high water loss when anisohydric grapevines are grown in warm climates with warm soils.

The results of this study are relevant to viticulture in the foreseeable future since global warming will result in increases in soil surface temperatures. These alterations in the root-zone environment will therefore impinge on both vegetative growth, final yield and fruit quality.

5.6 Regional-yield forecasting after fruit set

5.6.1 Introduction

Although yield forecasts based on bunch counts in spring are common, many wineries base their intake planning on forecasts provided by growers a month or two before harvest. By this time, fruit set is complete, and there is an opportunity to use the measure of berries per bunch to improve the prediction of bunch weight at harvest. A lot of berries fall off in the first few weeks after flowering, so bunches are not usually collected, and berries not counted, until at least a month after flowering. Although they can be counted at any time until harvest, the timing will be governed by the need to meet winery deadlines. If correct procedures are used, growers can expect an accuracy of about ±15% to 20% (mean absolute difference) on a block basis.

This article first describes best-practice yield forecasting at the block level based on berry counts after fruit set. It also discusses how to minimise the time required to make these forecasts. Finally, a method is proposed for making accurate regional forecasts.

When to make a forecast

Experience has shown that the earliest time to count berries is about 4 weeks after fruit set (Clingeleffer 2001), when the berries are pea-sized (Figure 5.6.1). Counting at this time allows time for the inevitable loss of some of the berries in the weeks following fruit set.



Figure 5.6.1 Pea-sized grape berries

Using the correct formula to calculate yield at the block level

For post-set berry counts the formula for calculating the amount of fruit that makes its way from the vineyard to the winery is:

Vines (or vine segments)/patch

×

bunches/vine (or vine segment)

×

berries/bunch

×

berry weight (at harvest)

×

harvest efficiency

=

yield/patch.

The number of vines or vine segments per patch is known. The number of bunches per vine (or vine segment) can be taken from an earlier inflorescence count forecast. However, if no inflorescence count forecast has been made before, then you will need to estimate bunches per vine (or vine segment) in the same way that an inflorescence count forecast is made, and taking into account the same considerations. For information on how to make best-practice inflorescence counts, see presentation and fact sheet and resources *Yield Forecasting* (Dunn 2010), at http://www.gwrdc.com.au/webdata/resources/files/GWR_066_Yield_Forecasting_Fact_Sheet_FINAL.pdf. This fact sheet provides information on:

- measuring at the right time
- know your block's dimensions
- avoiding bias
- ensuring adequate and optimum sampling
- using the correct formulae
- evaluating for improvement

The number of berries per bunch can be estimated from a subsample of bunches, and weight per berry at harvest will need to be predicted. Typical harvest efficiencies for different harvesting methods can be found in Dunn (2010).

Sampling considerations

The range of typical coefficients of variation shows that a sample of 60 bunches is usually adequate for estimating the number of berries per bunch at a 95% confidence level and percentage error of 10% (Clingeleffer 2001). After you have counted the number of berries per bunch and entered the data, it is easy to use software to determine whether the sampling has been adequate. Examples of such software are forecaster version 7 (http://www.gwrdc.com.au) or purpose-built crop-forecasting software (http://www.fairport.com.au/index.asp).

Selecting bunches in the vineyard

Because we are estimating an average number of berries per bunch from a population of bunches across the vineyard, it is important that sampling is random and representative. A 'bunch' is defined as a stalk with one or more berries on it.

This definition is used because it allows the true mean of the population to be estimated, is consistent, and removes any subjectivity. It is very important not to overlook small bunches.

'Random sampling' means that sampling locations are randomly selected before entering the vineyard. The types of software mentioned above select random locations (vines or vine spaces) within vineyard blocks and random sampling positions at these locations.

Because the variation in bunch size is much greater within a vine than from vine to vine, it is more efficient to sample a greater number of bunches (say six) from fewer locations (say 10) than it is to sample each bunch from a separate location (60 individual sampling locations). To select six bunches from the one location, locate the randomly selected vine and sampling position, identify the nearest bunch (Figure 5.6.2), and then select it and the next five bunches in one direction horizontally. This procedure forces you to take the next five closest bunches and ensures that smaller and larger bunches are not overlooked.

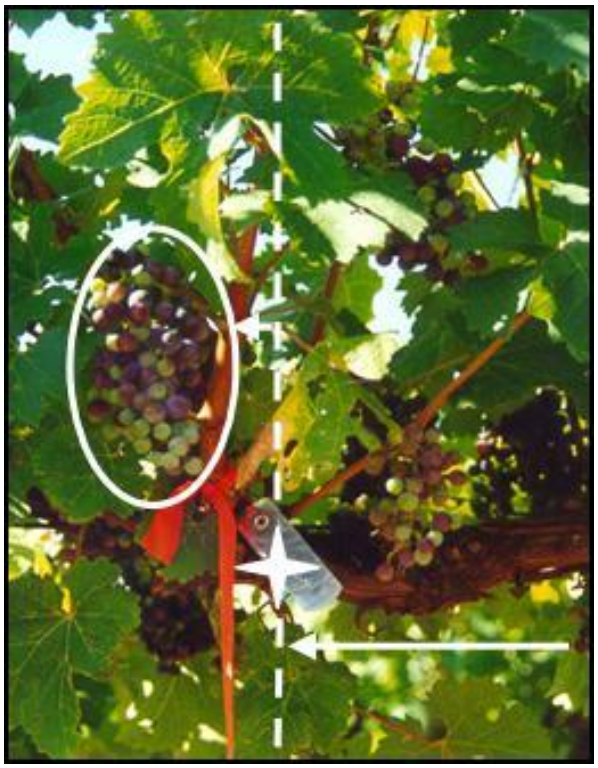


Figure 5.6.2 Select the bunch that has the base of its stalk nearest to the imaginary line or plane at a randomly selected sampling position (designated by the star) within a randomly selected vine. When removing bunches, snip or pinch them off at the base of the stalk.

Removing bunches

It is best to remove bunches and count berries back in the shed, laboratory or office. When removing bunches, snip or pinch them off at the base of the stalk. Bunches in plastic bags may ‘cook’ in direct sunlight, so it is best to keep them shaded and cool, using an insulated cooler box if necessary. Bunches in sealed bags can be stored in a refrigerator for a week or two. If they need to be stored for longer periods, they can be frozen.

Counting berries

There can be up to three types of berry on any bunch (Figure 5.6.3). These are: normal berries (‘hens’); smaller ‘chicks’ (which at veraison are about 3 to 4 mm in diameter and average about 0.2 g; they usually do not have a viable seed and stay green for longer than the hens); and ‘shot berries’ (or hard green ovaries), which have a diameter of about 1 mm and are tiny, green and hard. ‘Shot’ berries and ‘chickens’ tend to fall off before harvest, so they shouldn’t be counted.

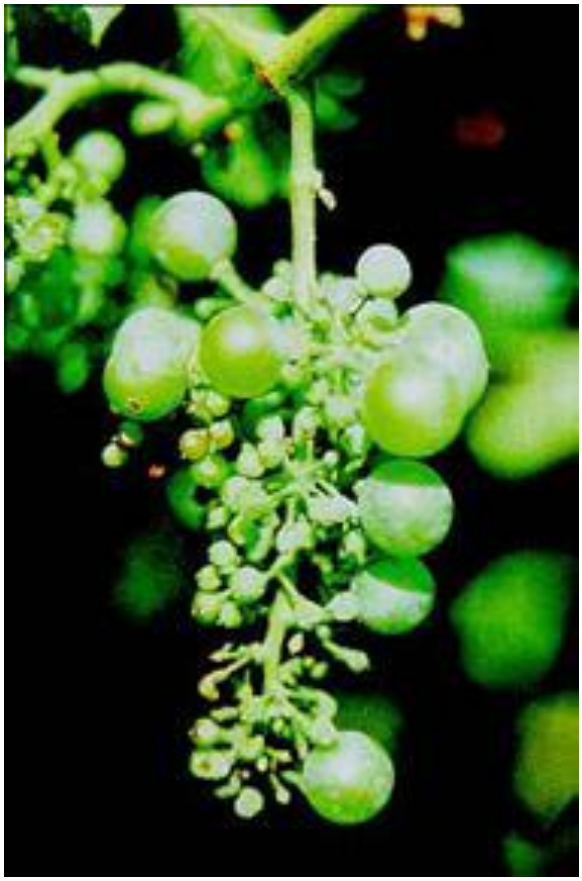


Figure 5.6.3 A bunch after fruit set, showing normal berries (hens), smaller berries (chickens) and ‘shot berries’ (live green ovaries). Photo: Steve Martin, DPI Victoria

Estimating bunch weight

Mechanised harvesting tends to remove only berries, so the bunch stems (rachises) are left behind and are not included in an estimate of bunch weight. You can therefore estimate bunch weight by simply multiplying the average number of berries per bunch by a prediction of berry weight at harvest, (usually around 1g. depending on the cultivar and the trellising type).

Hand-harvested fruit includes the rachis, which accounts for about 5% of the final bunch weight, so your estimate of bunch weight will need to incorporate this extra weight.

Estimating harvest bunch weight: a new method

Counting all the berries on many bunches is time-consuming and tedious and is a barrier to the adoption of measurement-based forecasting, so here we propose a revised, rapid method. The method is based on the knowledge that about 60 bunches are required to estimate average bunch weight but only 100 berries are needed to estimate average berry weight at a 95% confidence level and percentage error of 10% (Clingeleffer 2001). Theoretically, if a representative and random sample of about 100 berries could be collected from 60 bunches, then both average berry weight and average bunch weight could be relatively quickly estimated and then used to predict bunch weight at harvest. We therefore propose the following method.

Collect the 100 bunches in the field and weigh them to estimate post-set bunch weight (BuWt1). From 10 of these bunches, remove 20 berries, starting at the base. This subsample from the base of the bunch should be reasonably representative of the whole bunch (Dr Luis Sanchez, personal communications). The 200 berries collected should provide an acceptable estimate of average post-set berry weight (BeWt1). It may help to use a counting frame (Figure 5.6.4). The rachis weight a month after set is about 15% of the bunch weight (Dr Michael Trought, personal communication), but this drops to about 5% at harvest, indicating that the rachis gains little weight between the post-set period and harvest (Huglin and Schneider 1998; Ribéreau-Gayon et al. 1998). We can then use a prediction of berry weight at harvest to predict bunch weight at harvest, if we assume that berry weight at harvest (BeWth) is predictable and relatively stable from one season to the next.



Figure 5.6.4 A counting frame used to count 100 berries. Photo: Dr Luis Sanchez

For mechanised harvesting the rachises are generally left on the vine, so the weight of the berries per bunch after fruit set is (BuWt1 × 0.85) and the weight of the berries per bunch at harvest can be predicted as follows:

$$\text{BuWth (berries only)} = (\text{BuWt1} \times 0.85) \times (\text{BeWth})/(\text{BeWt1})$$

For hand-harvested system you will need to incorporate the weight of the rachis:

$$\text{BuWth (berries and rachises)} = (\text{BuWt1} \times 0.85) \times (\text{BeWth})/(\text{BeWt1}) + (\text{BuWt1} \times 0.15)$$

Predicting veraison bunch weight

The berry growth curve is double sigmoidal; Figure 5.6.5 shows actual bunch-weight-gain curves for two cultivars. It is theoretically possible that these curves can be used to predict bunch weight at harvest. Simply put, a measurement of weight per bunch at the onset of veraison can be multiplied by a factor (e.g. 1.8 for Cabernet Franc and 1.6 for Cabernet Sauvignon) to predict final bunch weight. The onset of veraison is the time when the first berries begin to soften and colour before the rapid importation of sugar. At this stage most of the berries won't have reached veraison

and will be growing more slowly than at earlier or later stages. This is a good time to measure, because it is easily observed in the vineyard and any variation in timing will have less impact on the measurement.

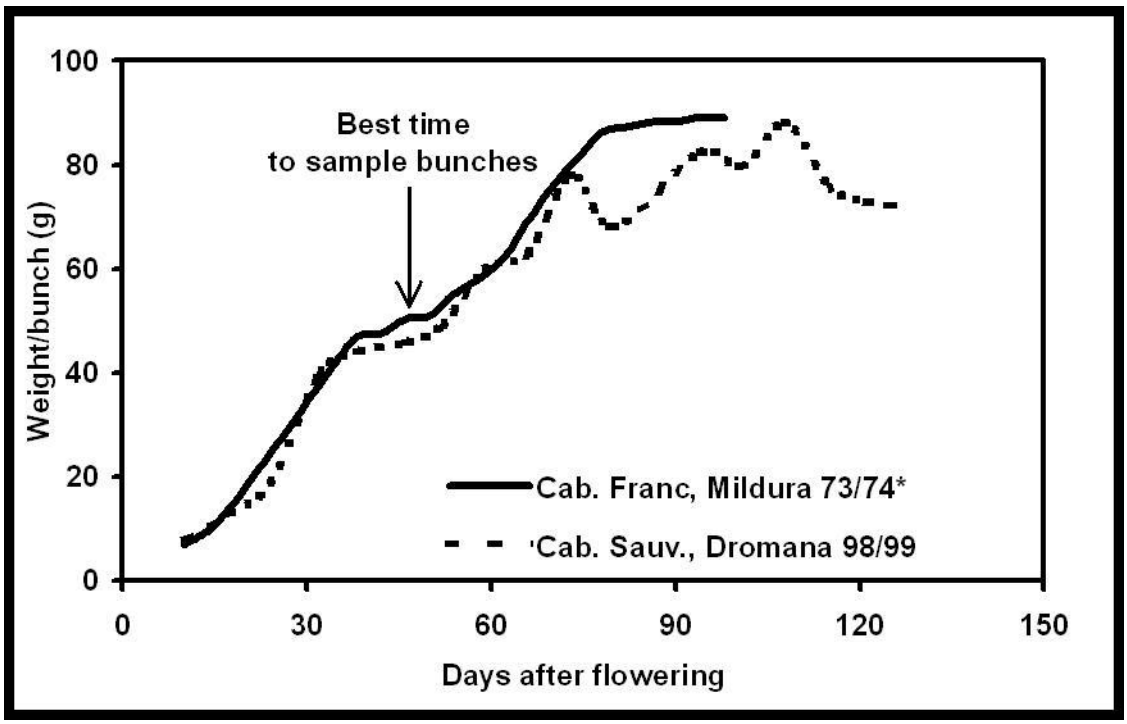


Figure 5.6.5 Bunch growth curves for Cabernet Franc and Cabernet Sauvignon. The arrow represents veraison.

Nevertheless, studies have shown that the factor mentioned above can vary considerably among and within cultivars from one season to the next. The technique is not stable enough to reliably predict bunch weight at harvest.

Other bunch-weight predictors

Using an approach similar to that used to predict veraison bunch weight, bunch weight predictions based on the relationship between bunch weight gain and the accumulation of growing degree days have been studied. However, the relationships are costly to obtain and are not stable across cultivars or regions (Dr Nick Dokoozlian, personal communication).

5.6.2 Regional yield forecasts

Regional yield forecasts, such as forecasts of all growers' or wineries' Chardonnay yields from the one region, can be very accurate. This is because they are made using larger samples sizes (e.g. 100 bunch counts from a range of vineyard blocks across a region, compared with 30 bunch counts from within one block) and under- and overestimates tend to cancel each other out. Early results have shown that regional forecasts made after fruit set can be accurate to within ±5%.

The simplest way to make a regional forecast is to treat all of the variety blocks in one region as a single large block. You need to know the number of linear metres of fruiting wire used on each block and in the entire collection of blocks. This will allow you to use the existing software mentioned above to sample representatively from across vineyards. If there are many vineyard blocks, then you will not need to take samples from all vineyard blocks. The approach to bunch counting and sampling bunches remains the same as for an individual block, as does the estimation of bunch weight at harvest.

A protocol was developed (Appendix 1) and tested on company Shiraz, Chardonnay and Cabernet Sauvignon vineyards in Griffith and Hilltops in the 2011/12 and 2012/13 season. Comparisons of predicted versus actual are presented below (Table 5.6.1)

Table 5.6.1 Comparison of forecasts made after set with actual yields in 2012 and 2013

Season	Location	Cultivar	Fruiting wire (m)	Bu/m	BeWt1 (g)	BuWt1 (g)	BuWth* (g)	Yield (t) pred.	Yield (t) actual	% diff.
2012	Griffith	Chardonnay	442266.9	63.104	0.701	70.421	102.5**	2860	2961	3.5%
2012	Griffith	Shiraz	310355.8	43.833	0.545	42.038	65.6**	892	923	3.5%
2013	Griffith	Chardonnay	424410.9**	60.797	0.620	64.362	97.1**	2504	2435	-2.8%
2013	Griffith	Shiraz	310355.8	55.321	0.521	60.448	88.8**	1524	1296	-14.9%

*predicted bunch weight at harvest

**Berry weight assumed to be 1.2 and 1.1g for Chardonnay and Shiraz, respectively in 2012 and 1.1 and 1g for Chardonnay and Shiraz, respectively in 2013 as 2012 was a relatively wet year

End user testing of protocol for regional yield forecasting with 2 consultants, 2 wine company viticulturists and 2 grape growers is being completed. However, preliminary feedback can be found in Appendix 1.

6. Outcomes

Year 1	Output	Progress
A	A conceptual framework for a structural-functional model of grapevine growth and development	Completed
B	A report, a scientific publication and an industry article on heat damage thresholds for important and emerging *Vitis vinifera* cultivars	Experiments completed and written up, publication in preparation
C	A report, a scientific publication and an industry article on the interaction between temperature and plant water status on vulnerability to heat stress for important and emerging *Vitis vinifera* cultivars	Completed
D	A protocol for regional yield forecasts	Completed
E	A protocol for using remotely-sensed imagery to optimise proximal sampling to forecast yield in vineyards	Completed
F	A report and a scientific publication on the effects of root temperature on patterns of grapevine root growth and development and their effects on the development of yield and berry composition	Completed

Year 2	Output	Activities
G	A publication and an industry article describing an improved rapid method to analyse CHO and N, that quantifies sucrose, glucose and fructose	Completed
H	A rhizotron facility to assess root growth and development in response to environmental and management influences	Completed
I	A publication on effects of modifying root temperature on performance of grapevines in the field An industry article on the effects of soil temperature on grapevine fruitfulness A scientific publication and an industry article on the effects of soil temperature on nitrogen and carbohydrate partitioning	Completed
J	A comparison of regional yield forecasts made over consecutive seasons	Completed
K	Revision of the protocol for regional yield forecasting based on ‘end user’ testing with cohorts of end users	Collecting final ‘end user’ feedback

6.1 Peer reviewed papers

Hall, A and Wilson, M. A. 2013 Object-based analysis of grapevine canopy relationships with winegrape composition and yield in two contrasting vineyards using multitemporal high spatial resolution optical remote sensing. International Journal of Remote Sensing **34**: 1772-1797.

Rogiers SY, Clarke SJ. 2013. Nocturnal and daytime stomatal conductance respond to root-zone temperature in 'Shiraz' grapevines. Annals of Botany **111**: 433-444.

Rogiers SY, Clarke SJ. Stomatal behaviour, vegetative growth and infructescence development of Shiraz grapevines subjected to partial root-zone cooling. AoB Plants (in review).

Rogiers SY, Clarke SJ, Schmidtke LM. 2013. Elevated root-zone temperature hastens vegetative and reproductive development in Shiraz grapevines. Australian Journal of Grape and Wine Research (in review).

Schmidtke, L.M., J.P. Smith, M.C. Müller, and B. Holzapfel. 2012. Rapid Monitoring of grapevine reserves using ATR-FT-IR and chemometrics. Analytica Chimica Acta **732**:16-25.

Smith, J.P., Schmidtke, L.M, Müller, M.C., and Holzapfel, B.P. Measurement of macronutrient and micronutrient concentrations in grapevine petioles by ATR-FT-IR spectroscopy. (in preparation).

6.2 Industry Articles

Holzapfel, B.P. and Smith, J.P. 2013 Influence of management strategies on root and wood nitrogen reserves. Grapevine Management Guide 2012-2013, pp 12-14.

Dunn, G.M. 2013 Block- and regional-yield forecasting after fruit set. Grapevine Management Guide 2012-2013, pp 15-18.

6.3 Conference Proceedings

Auzmendi, I. 2013. Integration of a mechanistic biochemical and biophysical leaf gas exchange model in L-PEACH, 7th International Conference on Functional-Structural Plant Models, Saariselkä, Finland, June 9th to 14th 2013

Smith J, Quirk L, Müller M, Schmidtke L, Holzapfel B 2011. Variation in starch and nitrogen reserve storage by grapevines and measurement by ATR-FT-IR spectroscopy. Proceedings of the 17th GiESCO Symposium, August 29-September 2, Asti-Alba, Italy, pp. 263-266.

7. Recommendations

ATR-FT-IR spectroscopy methods for analysis of grapevine tissue

An ATR-FT-IR spectroscopy method for the rapid and relatively inexpensive measurement of the free sugar component of grapevine non-structural carbohydrates (TNC) was assessed. Preliminary calibrations were successfully developed for sucrose, glucose, fructose, total free sugars and TNC. The method has the potential to be further developed for the rapid and inexpensive analysis macro and micro nutrients in grapevine tissue.

Rhizolysimeter facility

A rhizolysimeter facility with future capacity for soil temperature control and automated irrigation management was designed and constructed with joint support from GWRDC, NSW DPI, CSU and the NWGIC. When fully completed, and the vines allowed several seasons for the root system to establish in each bin, the performance of the heating and cooling system will allow a soil temperature range of approximately 8 to 45^{o}C to be spanned. There would be the option to hold the soil within a set temperature range, follow a pre-determine program based on prevailing air temperatures, or track the soil temperature of the adjacent vineyards (for example vineyard temperature, and vineyard temperature +/- 5^{o}C). Irrigation will also be controlled, and the capacity to weigh individual bins and measure soil moisture content will also provide the option for irrigation treatments. Destructive harvests at the end of a study will allow whole vine biomass to be accurately determined, and with potential for the bins to be lifted in and out of the trenches, vines required for subsequent experiments could be prepared several seasons in advance.

Effects of soil warming on grapevine performance

Field studies showed that altering soil temperature through practical means has the potential to alter the grapevine source-sink relationships and might impact on vine productivity in the longer-term. The continuation of the treatments showed also advancing grape maturation under the warmer soil conditions, with a likely impact also on grape composition. These findings suggest that not only ambient temperature can affect berry maturation, but also the root environment can have an influence on reproductive development. While glasshouse trials showed that warm root-zones during spring resulted in greater root carbohydrate reserve mobilisation

and hastened canopy development, effectively altering the source-sink relations of the plant through the remainder of the season. Warm root-zones produced larger berries with greater sugar and anthocyanin content and lower acidity. Conversely, cool root-zones delayed canopy and inflorescence development as well as the onset of veraison, and berries were smaller at harvest time. These results indicate that root-zone temperature is a strong driver of both vegetative and reproductive phenological development and is thus an environmental factor that can contribute to variability in berry composition and yield across a vineyard. Global warming will result in increases in soil surface temperatures and these alterations in the root-zone environment will therefore impinge on vegetative growth, final yield and fruit quality. Further work should focus on quantifying and managing the adverse affects of increased soil temperature on grapevine performance.

Heat stress studies

The study was not able to conclusively demonstrate that water stress exacerbates the impact of a heat event on the berry development processes, at least for cv Semillon. This is a puzzling finding warrants further study given that timely irrigation is considered to be the main defence against heat stress damage. Water stress had a marked effect on berry size, in apparent contrast to the heat event, and this in turn resulted in a marked reduction in sugar content. The exposure of Semillon vines to the 40^{o}C threshold confirmed that berry diameter was unaffected and, therefore, appears to reinforce the conclusion that, at these temperatures, the changes in berry attributes were related to the water stress alone.

The comparison of three cultivars to high temperature exposures has revealed that there are marked differences in the berry development processes to temperature. Chardonnay berries performed poorly at 40^{o}C, shrinking in size, loosing dry matter and not accumulating sugar and failed also to accumulate sugar at 35^{o}C. Thus, the berry ripening process of Chardonnay vines appeared to have an upper threshold at or below 35^{o}C. By contrast, Merlot berries alone continued to accumulate sugar at a high rate when exposed to 40^{o}C and clearly had an upper threshold above this temperature. Semillon berries accumulated sugar at a high rate at 35^{o}C but accumulated dry matter at a reasonable rate at 40^{o}C and these results suggest the upper temperature threshold for this cultivar was somewhat between 35 and 40^{o}C. These upper temperature thresholds are much higher than what might be expected

from their centres of origin in France and, therefore, suggests some acclimation to the high temperatures of the Riverina may have occurred.

This preliminary study needs to be expanded to include more cultivars and incorporate a study of responses to heat stress at different phenological stages. A quantitative understanding of the effects of cultivar and phenology on heat stress damage thresholds is critical baseline information for informing the development of management options and guidelines.

Yield forecasting

Regional forecasts

Regional forecasts, i.e. in-season forecasts of the production of cultivars within regions, are useful to both wineries and growers. A protocol was developed for improved regional forecasting, based on field and laboratory measurements made after fruit set. Future efforts should focus on wider testing of the protocol across regions, seasons and cultivars and strategies to promote adoption. However, data collection is likely to remain a barrier to adoption and, therefore, future research should focus on potential new forecasting techniques including the use of image analysis, remote sensing and whether 'sentinel' or 'bell-weather' vineyard blocks can be identified and used to improve regional yield prediction.

Aside from more rapid ways to estimate bunch numbers per vine, improvements to the regional forecasting protocol would include:

- Improvements to the software to more easily amalgamate blocks and facilitate representative sampling. This would require minor modifications to existing software.
- 'Web-based' availability of the software and guidelines.
- Improved sampling guidelines based on further testing of the protocol across cultivars, seasons and viticultural regions.

Better block level forecasts

Although regional forecasts are extremely useful for intake planning and fruit purchasing decisions, wineries and growers would arguably gain more substantial benefits from more accurate block level forecasts. At present, and using existing best practice measurement based tools, the best that can be expected of a post fruit set

forecast is around +/- 10-15% accuracy. While the best that can be expected of a block level forecast made in spring and based on counting inflorescences is +/- 20% (Dunn and Martin 2003). Although these figures would lead to substantially improved forecasting above industry practice, this level of accuracy is an impediment to more widespread adoption of better yield forecasting techniques. Coupled with this is the view that the field based measurements are time consuming and measurement is labour intensive despite the considerable effort that has gone into optimising field measurements to reduce sampling and measurement. Therefore, accuracy needs to be improved whilst at the same time reducing effort and cost.

There are a number of options that could be usefully explored:

- More rapid proximal assessment of yield components in real-time based on image analysis and processing. Techniques would need to be able to differentiate foliage from inflorescences or bunches at different phonological stages and account for inflorescences or bunches being obscured by foliage or other inflorescences or bunches.
- Remote sensing of canopies, within a season, can explain much of the variation in yield spatially (see below). This is not surprising as canopy size/vigour is related to yield, i.e. bigger vines tend to have larger yields. The use of 'drones' is becoming more commonplace and would reduce the costs of having to 'fly' blocks and, thereby, allow cheaper acquisition of multiple images across the season. However, any method will have to account for season to season variability, where similar sized vines may carry different fruit loads from one season to the next. This would suggest an in-season calibration step needs to be developed.
- The use of remote sensing to zone within blocks can lead to improved sampling and, therefore, accuracy. However, these benefits need to be weighed against the costs of having to construct maps to define zones and sample from these zones.

Use of remote sensing

An image processing algorithm was developed to segment vineyard imagery into single grapevine objects. Various remote-sensing vegetation indices, calculated for each grapevine object, revealed that indices sensitive to high vegetation densities performed significantly better at predicting fruit composition and yield elements than

the commonly used normalized difference vegetation index. The strength and direction of correlations between canopy vigour and season-end fruit descriptors varied by phenological stage and vineyard type. The ability of optical remote sensing to successfully map within-vineyard winegrape composition and yield may vary depending upon vineyard characteristics, management, and temporal variability in overall vineyard production.

Other options

- Pollen counting is an interesting technique that has been tried in countries outside Australia including Spain and Portugal. The approach integrates the number of flowers and conditions during flowering. However, to our knowledge it is not possible to separate cultivars, individual blocks or vineyards.
- Veraison multipliers are based on weighing bunches at veraison and multiplying these weights by a factor to predict bunch weight at harvest and, therefore yield. Unfortunately, multiplication factors can vary wildly and unpredictably from one season to the next.
- Although we know that yield in grapevines is strongly related to weather, we lack a quantitative understanding of these relationships and this precludes the development of weather-based models at present.

A functional-structural model of the grapevine

Following the schemes used by Prusinkiewicz (2004) and Room et al. (1996), we identified some possible applications of a functional-structural grapevine model:

A. Advancement of science:

- Vine biology and physiology: exploration of hypotheses relating genetics or physiology to grapevine structure, plant vigour or crop load. Integration of knowledge from different areas. Research gap detection, definition of new hypotheses and design of new experiments.
- Grapevine ecology and phylloclimate: exploration of the competition for the space and interactions at the level of single shoots, roots and individual plants. Exploration of the relationship between grapevine structure and berry phylloclimate, for example improving our understanding on heat damage and shading in berry growth and quality.

B. Construction of practical models, in which grapevine structure is essential:

- Grapevine variety and clone selection, breeding and genetic engineering: specification of ideotypes identifying the effect of rootstocks, varieties or clones on grapevine structure, stress tolerance, quality and quantity of grapes.
- Viticulture: identification of planting strategies (spacing or row orientation) and optimal treatments (training, pruning, leaf removal in the fruit zone, trimming, application of chemicals, temperature and shade manipulations, adaptations to climate change) for grapevines.
- *In silico* experiments: time saving respect to real time experiments, applying treatments in the virtual grapevines and obtaining results in hours instead of years. Simulations can be validated especially when compared to experiments under controlled environment conditions. Simulations of carbon partitioning could help to explain results of experiments that otherwise are difficult to interpret due to the high number of factors and interactions.
- Visual representation: creation of 3D models of grapevines for inclusions in books or documents, and videos for teaching or training students and farmers.

C. Construction of practical models, in which grapevine structure is not of primary importance, but the inclusion of structure may improve the predictive value of the models:

- Management of pathogens: improved understanding of disease dynamics through simulation of pathogen deposition and growth in the microclimates produced by growing grapevines.
- Management of pests: improved definition of action thresholds through the simulation of interactions between grapevine structure, pesticide deposition, insect movement and feeding, and compensatory growth of grapevines.
- Creation of independent sub-models: stand-alone modules can be used in a functional-structural grapevine model or separately, for example light interception or gas exchange models.

D. Educational applications: Explanation and illustration of processes in grapevines, and the relationships between the grapevine and its environment, for teaching and training purposes.

Results obtained with L-PEACH and commented on previously by DeJong et al. (2011b) illustrate real applications of a functional-structural plant model:

- The development of the fruit sub-model led to study the source and sink limitations during the fruit development period (Pavel and DeJong 1993; Grossman and DeJong 1995a, b) and improved the recommendations for timing of fruit thinning in commercial peach production. Later the same model was used for studying the effect of nitrogen deficiency and water stress in fruit size (Berman and DeJong 1996; Saenz et al. 1997). A discovery was the possibility of using the temperature during the first month after flowering to predict the harvest date (Ben Mimoun and DeJong 1999) and harvest size (Lopez and DeJong 2007; Lopez et al. 2007). Using these models a web-based decision support tool was created to alert growers during seasons in which fruit size could be too small so they could thin the fruit earlier to compensate the fruit size (Lopez and DeJong 2008).
- The development of the shoot growth model motivated the study of the shoot-length growth under different crop loads (Grossman and DeJong 1994, 1995c), temperature and water stress (Berman and DeJong 1997b) and effect of the fruit on the same stem growth (Berman and DeJong 1997a). It was also important for explaining the growth on different size-controlling rootstocks (Basile et al. 2003; Solari et al. 2006b, a) and following this research a new simple technique was discovered to select for vigour control capacity in new peach rootstocks (Tombesi et al. 2010; Tombesi et al. 2011).
- The development of the photosynthesis model induced the study of the daily photosynthetic radiation use efficiency of individual leaves (Rosati and Dejong 2003) that led to the proposal of a simple method to estimate whole canopy photosynthesis on a daily basis (Rosati et al. 2004).
- The development of the carbon partitioning model has produced some advances in modelling techniques, for example using L-systems for simulating transport using an electrical analogy (Prusinkiewicz et al. 2007a).

8. Intellectual Property

Not applicable

9. References

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10. Staff

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11. Appendix 1: A protocol for regional yield forecasts

(Modified after Martin, Dunstone and Dunn (2003); How to forecast wine grape deliveries using Grape Forecaster excel workbook version 7 http://www.gwrdc.com.au/wp-content/uploads/2012/09/2003-10-FS-Forecast-Grape-Deliveries.pdf)

Preparing Sampling Forms
This protocol can be implemented anytime after berries reach pea size (E-L stage 31)

Enter block data into excel spreadsheets for every block containing the cultivar being assessed using after How to forecast wine grape deliveries Using Grape Forecaster Excel workbook version 7 (can be found here: http://www.gwrdc.com.au/resource_categories/yieldharvest/page/2/)

Calculate total area of all blocks and total linear meters.

Select sampling locations for blocks in proportion to their percentage of total area. For instance if cultivar block is 3 hectares and there are 30 hectares of this cultivar in the vineyard or region then 10% of the samples (e.g. 7 out of 70 bunch counts) need to be collected from this block. This will mean that not all blocks may need to be sampled. Make sure that a total of 70 locations are selected

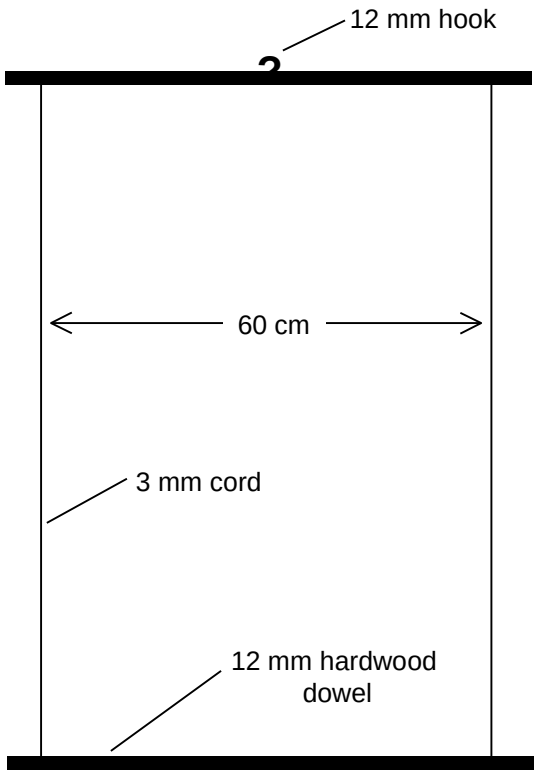
Print out sampling sheets

Sampling in the Field
When you have set up sampling forms you can take them into blocks and use them to find where to count. You can then count the number of bunches in each segment and record the counts on the form.

Equipment

- Bunch Counting form set up for the blocks being sampled
- Clipboard
- Measuring tape (in centimetres)
- Segment frame or ladder
- Pencil

Example of a segment frame



Procedure

For each segment:

1. Look up the block, row number and the vine-space number in the row. Go to the vine.

If you lose count of the vine number as you go down a row, don't worry too much. The main thing is to avoid human bias.

If you land on a missing or weak vine, still go to the trunk or where it would have been.

2. Look up the bearer number and distance to measure from the vine trunk. Take note of where this spot is.

3. Hang the segment frame over the canopy so that its centre is in line with the spot, at right angles to the row direction.

It doesn't matter if the segment overlaps into the next vine.

4. **Remove** and count all the bunches in the segment.

Take your time. Work systematically along cordons or canes, looking at each shoot as you go. Scan each shoot from the base up. Try not to miss bunches.

A ***bunch*** is a stalk with **one or more berries** on it.

Only count bunches that have **the base of the stalk** inside the segment.

Usually there will only be 0, 1 or 2 bunches on a shoot, but there can be 3 or more. If you find a tendril as you scan upwards you can stop looking at that shoot, because there will be no more bunches above it.

Count all bunches on all bearers at any position in the canopy within each segment.

5. Record the count in the 'Bunches in segment' column on the Bunch counting form.

If there are no bunches in the segment, record the bunch count on the form as a zero. This could occur due to a missing vine or bare wire.

6. For 15 randomly selected segments collect 10 bunches and place into individual freezer bags. The recommended method for collecting the 10 bunches is to select the bunch nearest to the left edge of the segment and working toward the right collect that bunch and the next 9 bunches.

Estimating bunches/segment and checking the sampling
When you've returned to the computer after counting bunches in a block, you can enter the data recorded on the bunch counting form into the workbook. From this data the workbook will calculate an estimate of mean bunches/segment and how many more segments would need to be counted if the amount of doubt exceeds your tolerance. This bunch number can then be expressed as bunches per linear metre.

Bu/m

Measuring bunch and berry weight in the Laboratory
Collect the 150 bunches in the field and weigh them to estimate average post-set bunch weight

(BuWt1)

From 10 of these bunches, remove 20 berries, starting at the base. This subsample from the base of the bunch should be reasonably representative of the whole bunch (Dr Luis Sanchez, personal communications). The 200 berries collected should provide an acceptable estimate of average post-set berry weight

(BeWt1)

It may help to use a counting frame (below). The rachis weight a month after set is about 15% of the bunch weight (Dr Michael Trought, personal communication), but this drops to about 5% at harvest, indicating that the rachis gains little weight

between the post-set period and harvest (Huglin and Schneider 1998; Ribéreau-Gayon et al. 1998). We can then use a prediction of berry weight at harvest to predict bunch weight at harvest, if we assume that berry weight at harvest

(BeWth)

is predictable and relatively stable from one season to the next.



A counting frame used to count 100 berries. Photo: Dr Luis Sanchez

For mechanised harvesting the rachises are generally left on the vine, so the weight of the berries per bunch after fruit set is (BuWt1 $\times$ 0.85) and the weight of the berries per bunch at harvest can be predicted as follows:

$$\text{BuWth (berries only)} = (\text{BuWt1} \times 0.85) \times (\text{BeWth})/(\text{BeWt1})$$

For hand-harvested system you will need to incorporate the weight of the rachis:

$$\text{BuWth (berries and rachises)} = (\text{BuWt1} \times 0.85) \times (\text{BeWth})/(\text{BeWt1}) + (\text{BuWt1} \times 0.15)$$

Yield is then simply a function of:

Yield = Bu/m x BuWth x total linear metres x harvest efficiency

Results

Time taken for various tasks

1. *Preparing sampling forms*: 2 hours per cultivar. This could be reduced to minutes with modified software that amalgamates vineyard blocks
2. *Field sampling*: A crew of technicians was used with some being on foot and some being in vineyard vehicles. Translated to a full time equivalent field sampling averaged between 11 and 16 minutes per Ha. This would be

expected to drop substantially if vehicles were used exclusively as the majority of the time was spent walking between sampling locations.

3. *Laboratory measures*: 90 minutes
4. *Data entry and calculations*: 30 minutes

Comparison of forecasts made after set with actual yields in 2012 and 2013

Season	Location	Cultivar	Fruiting wire (m)	Bu/m	BeWt1 (g)	BuWt1 (g)	BuWth* (g)	Yield (t) pred.	Yield (t) actual	% diff.
2012	Griffith	Chardonnay	442266.9	63.104	0.701	70.421	102.5**	2860	2961	3.5%
2012	Griffith	Shiraz	310355.8	43.833	0.545	42.038	65.6**	892	923	3.5%
2013	Griffith	Chardonnay	424410.9**	60.797	0.620	64.362	97.1**	2504	2435	-2.8%
2013	Griffith	Shiraz	310355.8	55.321	0.521	60.448	88.8**	1524	1296	-14.9%

*predicted bunch weight at harvest

**Berry weight assumed to be 1.2 and 1.1g for Chardonnay and Shiraz, respectively in 2012 and 1.1 and 1g for Chardonnay and Shiraz, respectively in 2013 as 2012 was a relatively wet year

Feedback from potential end users

We are still awaiting complete feedback from end users. However, preliminary feedback is:

- Software to put blocks together is needed to replace cumbersome spreadsheet approach for each block
- Web-based software would be desirable
- 'Dropping' the small amount of fruit in the field is not seen as an issue
- Flexibility of making forecast anytime after pea-size (E-L stage 31) is useful
- Earlier forecasts (after budburst) still useful
- Not everyone has historical records of berry weight at harvest and these are needed for the protocol
- Would like to be involved in more widespread testing
- Need access to laboratory with electronic balance
- Berry counting cradle is essential

12. Budget Reconciliation

Please use the End of Project Financial Statement, downloadable from the GWRDC website at www.gwrdc.com.au