THE EFFECT OF PARTIAL ROOTZONE DRYING ON THE PARTITIONING OF DRY MATTER, CARBON, NITROGEN AND INORGANIC IONS OF GRAPEVINES

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Declaration

I hereby declare that this thesis contains no material that has been accepted for the award of any other degree or diploma at any University. To the best of my knowledge and belief, no material described herein has been previously published or written by any other person, except where due reference is made in the text.

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Summary

Partial rootzone drying (PRD) is an irrigation management technique designed to reduce water use in grapevines without a decline in yield, thereby increasing water–use efficiency (measured as t/ML) (WUE). The principle of PRD is to keep part of the root system at a constant drying rate to produce soil-derived signals to above-ground plant organs to induce a physiological response. Major PRD effects include a reduced canopy size and greatly increased WUE with possible improvements in fruit quality. Although we have a good understanding of the hormonal physiology of PRD, little is known on the effect of PRD on partitioning of C, N and inorganic ions such as K. This thesis broadens our knowledge on the effects of PRD on grapevine field performance, growth and dry matter accumulation as well as its effects on physiology and biochemistry. In field experiments over 3 seasons, PRD reduced water use in grapevines without a significant decline in yield. PRD effects included reduced shoot growth and greatly increased WUE. Field-grown Cabernet Sauvignon, where the PRD grapevines were irrigated at half the control rate, and Shiraz where the PRD grapevines were irrigated at same rate as controls, confirmed that PRD is not simply an irrigation strategy that applies less water, rather it alters the way in which the plant responds to its environment, e.g. PRD alters the sensitivity of the stomatal response to atmospheric conditions and significantly influence enzymes that regulate nutrient accumulation and partitioning. PRD did not change the total amount of carbon and nitrogen on a whole plant basis. However, it caused a significant partitioning of carbon and nitrogen towards trunk, roots and fruit at the expense of shoot growth. This change in partitioning occurred as a result of altered activity of the enzymes controlling the assimilation of carbon and nitrogen. PRD significantly reduced nitrate reductase (NR) activity in grapevine leaves, which catalyses the first step in the assimilation of nitrate irrespective of the amount of water applied. The reduction in NR activity is correlated with the development of the PRD cycle and the associated reduction in stomatal conductance.

PRD also significantly altered grapevine sucrolytic enzyme activity that regulate source:sink relationships. PRD showed transient increases in leaf sucrose phosphate synthase (SPS) activity (formation of sucrose) compared to control, but significantly reduced leaf neutral invertase (sucrose cleavage) and leaf starch content in both field and potted experiments. This may indicate an increased photosynthetic capacity and a reduction in its sink strength for sucrose in favor of organs such as fruit and roots. This hypothesis was reinforced by the fact that berries

showed significantly higher levels in glucose and fructose early in the season. Berry sugar content and Brix at harvest however was unaffected. Although PRD had no significant effect on berry characteristics at harvest such as Brix and pH, it occasionally reduced per berry K^+ content and increased total amino acid concentration that may lead to positive outcomes for wine quality.

PRD-treated grapevine roots on the 'wet'- and 'drying'-sides differed greatly in enzyme activity and osmolality. PRD significantly increased osmolality in both wet and drying roots by increasing total osmolyte concentration that may facilitate the movement of water from wet to dry roots. The increases in osmolality were also associated with increased free polyamine production (spermidine and spermine) in PRD roots that may be related to increased root growth and density.

List of Abbreviations

ABA	abscisic acid
ADC	arginine decarboxylase
AI	acid invertase
GWRDC	Australian Grape and Wine Research Development Corporation
*C*i	intracellular CO_2 concentrations
CK	cytokinins
CSIRO	Commonwealth Scientific & Industrial Research Organization
°C	degrees Celsius
ET_o	evapotranspiration
FAA	free amino acid
FAN	free amino nitrogen
GDD	growing degree days
GOGAT	glutamine synthase/glutamate synthase
g_s	stomatal conductance
GS	glutamine synthase
IRGA	infrared gas analysis instrument
LA	leaf area
NADPH	nicotinamide adenine dinucleotide phosphate
NCCs	nitrogen-containing compounds
NI	neutral invertase
NR	nitrate reductase
PAR	photosynthetic active radiation
PAs	polyamines
Pn	photosynthesis
PRD	partial rootzone drying
RH	relative humidity
RuBP	ribulose- 1,5- bisphosphate
s.e.	standard error of the mean
SPS	sucrose phosphate synthase
SucSy	sucrose synthase
TDR	time domain reflectometry
TSS	total soluble solids
VSP	vertical shoot positioning
WUE	water use efficiency
Ψ_L	leaf water potential

Table of contents

List of figures

List of tables

Chapter 1: General introduction

1.1. Introduction

The grapevine is native to the warm, temperate zone between the 34° north and 49° south latitude and the optimal cultivating environment for *Vitis vinifera* requires long, warm, dry summers and cool winters while unsuited to humid summers, due to susceptibility to fungal diseases and insects, intense winter cold and areas prone to late spring and early fall frost (Winkler *et al.*, 1974). However grapes are grown outside that zone in both hemispheres. Especially in hot climatic conditions such as Australia, South Africa, Israel, western USA and South America vines rely heavily on irrigation practices and good viticultural management to establish new vineyards and maximize crop yield. Much of the labor expended in the cultivation of grapevines is a consequence of its inherent tendency to have vigorously vegetative growth under favorable soil and climatic conditions. Excessively vigorous vines could be defined as those with an excessive amount of vegetative growth relative to fruit growth. The control of excessive vegetative growth or vigor in grapevines is desirable because it leads to a reduced canopy density, better bud fruitfulness, better vine balance with decreased costs of maintenance and increased quality of fruit (Dry *et al.*, 1996). A few methods exist in modern viticulture to reduce vigor. These methods entail the use of chemical growth regulators, rootstocks, root restriction, pruning practices and reduced water supply via irrigation management. While any of these practices may reduce vigor the most useful and practical method may be reducing the water supply.

Under hot and dry climatic conditions and restricted water supply, shoot growth may be reduced with more open canopies and higher quality fruit. However, water restriction is often accompanied by a penalty in yield and may not be compensated by the higher unit value of the crop. With viticulture moving into more regions with low rainfall in the growing season and larger areas covered in vineyards, water is becoming an increasingly scarce commodity. However, it is found that with a simple reduction in irrigation to increase water use efficiency (crop/unit water)(WUE) there is a reduction in crop yield and berry weight (Matthews and Anderson, 1988; Matthews and Anderson, 1989; Goodwin and Jerie, 1992). Partial rootzone drying (PRD) is an irrigation management technique designed to reduce water use in grapevines without a decline in yield, thereby increasing WUE. The principle of PRD is to withhold water from part of the root system to produce root-derived signals to aboveground plant organs to induce a physiological response. Major PRD effects include a reduced canopy size and greatly increased WUE with possible improvements in fruit quality (Dry *et al.*, 1996; Stoll, 2000; Dry *et al.*, 2001).

1.2 Partial rootzone drying management

The evolution of plants to adapt to a wide range of environments on land led to certain structural changes in plant organs. Aerial organs are designed to prevent excessive water loss from transpiration but at the same time allow the access of CO_2 for photosynthesis (Salisbury and Ross, 1992). In most terrestrial plants, including grapevines, both leaf surfaces are covered with a cuticle that serves as an impermeable barrier to prevent loss of moisture. To enable gas exchange between photosynthetic active tissue and the atmosphere stomata exist in the lower epidermis. Stomata consist of two guard cells surrounding a stomatal pore that can be opened and closed by the guard cells (Salisbury and Ross, 1992). The only route for gas exchange between photosynthetic active tissue and the atmosphere is through the stomatal openings. The mechanism in place to control stomatal opening and closing is therefore very important because it controls water loss and photosynthesis. Understanding the processes which control stomatal aperture is important for managing plants in further expanding areas of cultivation which may involve enduring more challenging climates. Variables such as light, temperature, wind, atmospheric carbon dioxide concentration, humidity and soil water availability are found to influence the complex processes involved in stomatal aperture (Loveys *et al.*, 1998), with water availability and canopy management being important factors that we have a degree of control over.

When plants are faced with a drying soil, the first line of defence is the prompt closure of stomata to reduce excessive water loss. Research has shown that, although there is a consequent loss of turgor that leads to the closure of stomata, the first response is of a non-hydraulic, chemical nature originating in the roots (Loveys, 1984). Applying the current knowledge, a system has been developed called partial rootzone drying (PRD) where the soil of half the root system dries out slowly while the other half is kept wet by frequent irrigation (Figure 1.1). After a certain period of time the ‘wet’ and ‘dry ’ zones are alternated, allowing the former ‘wet’ zone to slowly dry while the ‘dry’ zone is irrigated (Loveys *et al.*, 1998).

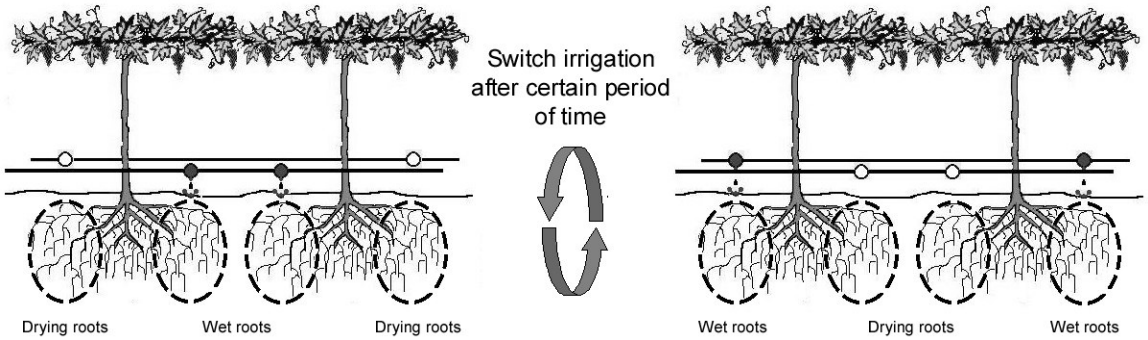

Figure 1.1 Implementation of partial root-zone drying in the field.

1.2.1 Why is alternation in wetting zones important?

The PRD system relies on hormonal signals originating from the roots in response to low soil water potentials within the 'dry' zone. Much evidence has been accumulated that drying roots are the origin of abscisic acid (ABA), which is involved in regulating stomatal aperture (Düring *et al.*, 1996; Dry *et al.*, 2000a). Normally, the closure of stomata in response to drying soil conditions serves to protect leaf tissue from excessive loss of moisture when plants are faced with low soil water conditions, thereby conserving water by reducing transpiration. In the PRD system the vine is given a false sense of water stress, because one rootzone is constantly exposed to low soil water potentials, producing ABA and sending a signal to the aboveground organs. The observed effects of ABA in aboveground organs due to PRD are a reduction in shoot growth and partial stomatal closure (Dry and Loveys, 1999). Without alternating the 'wet' and 'dry' sides, i.e. wetting only one side of the vine while the other side continues to dry out, it has been shown that stomatal conductance and shoot growth rate will start to recover after a certain period of time (Dry and Loveys, 1999). Loveys *et al*. (1998) found that this recovery correlated with a reduced production of ABA in the 'dry' roots. It was therefore suggested that a long-term effect on stomatal conductance and shoot growth in grapevines is only possible if the signal originating from the 'dry' side can be sustained. By alternating the 'wet' and 'dry' sides, it was possible to maintain a long-term response (Dry, 1997) and it became clear that a continuous chemical signal or a certain concentration of the signal is necessary to maintain a physiological response.

It has been found that the PRD system sustains such a continuous chemical signal from drying soil without a loss of leaf water potential (Dry *et al.*, 1996). Davies *et al*. (1994) concluded that stomata respond more to soil-water potential than to leaf-water potential and that shoot physiology is regulated independently of local osmotic influences, by signals originating in the roots. Evidence suggests that the hormonal control is originating from the drying roots and that abscisic acid (ABA) is involved in regulating stomatal aperture (Davies and Zhang, 1991; Düring *et al.*, 1996; Loveys *et*

al., 1998; Dry *et al.*, 2000b) and key enzymes in the carbohydrate and nitrogen assimilation pathway (Huber and Huber, 1992; Goupil *et al.*, 1998).

1.2.2 Why will a simple reduction in irrigated water not have the same effect?

Field experiments by (Stoll, 2000) showed that under conditions where water supply was the same in both PRD and control grapevines, stomatal conductance and growth of the PRD vines were restricted. However, as total water input was reduced the stomatal conductance of control vines dropped and became significantly lower than PRD, suggesting that control vines were experiencing stress whereas PRD vines were not. This may be due to deeper penetration of irrigated water in PRD treatments where water was applied to a smaller surface area or that PRD vines are just inherently better adapted/conditioned to handle a stressful soil environment. At equal but relatively low water application rates PRD outperformed control grapevines with heavier pruning weights and crop yield. It was concluded that PRD-treated vines were more tolerant of water stress and made more efficient use of available water. A simple reduction in irrigation water, the principle embodied in regulated deficit irrigation (RDI), would therefore not have the same effect as PRD since RDI is usually characterized by significant stress levels resulting in decreased leaf water potentials and crop yield due to smaller berry sizes (Smart and Coombe, 1983; Matthews and Anderson, 1988; Matthews and Anderson, 1989; Goodwin and Jerie, 1992).

1.2.3 Why is PRD of different irrigation volumes compared to control?

It seems important to address the question of the different volumes of irrigation water used in the PRD experiments. The main aims in the current commercial use of the PRD irrigation system are to firstly save water and secondly improve vine canopy architecture and influence fruit quality. There may also be secondary changes vine physiology and financial benefits by reducing management costs. It is therefore the standard practice in the commercial environment to use less water than normal when implementing the PRD system. Therefore, the treatments used in this study where PRD received less water than controls are meant to represent this commercial practice. However, it is also recognised that it is important to be able to differentiate between an effect of water volume *per se* and the way that it is applied. With this in mind, some of the experiments in the current study applied the same volume of water to both controls and PRD treatments.

However, with an equal amount of water applied to different soil surface areas, a different set of variables may arise that may be difficult to account for. Firstly, the actual speed of penetration and distribution of water within the root zone may be different in the PRD treatment compared to

control. Therefore it may be possible, especially in heavy soils like the one in the current study, that the designated “dry side” would be smaller due to lateral soil moisture movement. Secondly, the level of water saturation of the soil can be measured, but the amount of water leaching past the rootzone into deeper soil layers that would not be accessible to the plant may not be so readily measured. Constant upward movement of soil moisture into the dryer layers would then further confound the comparison between wetted soil volumes of PRD to control. This effect was seen in an experiment not included in this study where an unaccounted source of soil moisture confounded the PRD treatment. It seems therefore important to achieve the same depth of wetting and hence a difference in the volume of water applied per plant per unit time. Thirdly, higher application rates may mean greater runoff of irrigated water at the penetration site and greater evaporation rates compared to control. These points suggest that even if volumes of irrigation water are equal in the PRD and control treatments, plant available water may differ. It may therefore be very difficult to devise an appropriate control for the PRD treatments. Nevertheless, in these experiments we have assumed that when equal water volumes are applied physiological indicators such as a reduction in stomatal conductance are indicative of a PRD effect, independent of water volume effects.

1.2.4 Main focus points of the PRD research in this study

The major focus points of research into the effect of PRD on grapevine physiology are divided into three sections. Firstly, the acquisition of carbon that would include the accumulation of dry weights, sugars and starch and the role that sucrolytic enzymes play in regulating the source:sink relationship. Secondly, the assimilation of nitrogen and its partitioning into various nitrogen containing compounds and amino acids, which is highly regulated by specific enzymes. Thirdly, the accumulation and partitioning of inorganic ions in various plant organs, especially in the berries of PRD vines.

1.3 Carbon assimilation and the source:sink relationship

The sessile nature of plants requires that they must show considerable capacity to adapt to their surrounding environment, especially under adverse conditions. Ecologists have noted that reduced growth rate, a low capacity to capture resources and a high investment in reserve storage are consistently found in plants that are subjected to stressful environments (Chapin, 1991). To comprehend the development in yield we need to treat photosynthesis, translocation, growth and storage as an integrated whole since these processes are linked by numerous interactions.

1.3.1 Sources and sinks

Developing leaves in the grapevine undergo a gradual transition from sink to source where young leaves depend on imported carbohydrates until they are mature and become autotrophic. Grapevine leaves become autotrophic when they reach approximately 30-50% of their final size (Hale and Weaver, 1962) and it is believed that this transition occurs when the import of assimilates is terminated by interruption of the phloem's unloading capacity. Sink to source transitions in many species is characterized by the ability of the phloem to accumulate sugars above the osmotic threshold value and induce export by mass flow (Fellows and Geiger, 1974). Sucrose synthesis and nitrate assimilation (Figure 1.2) are major processes that occur in leaves and are generally coordinated with photosynthesis (Salisbury and Ross, 1992). Light stimulates the rate of carbon flux into sucrose and the rate of nitrate reduction for the formation of amino acids. As illustrated in Figure 1.2, both pathways depend on each other, and both pathways are regulated by each other (Lewis *et al.*, 2000; Tischner, 2000). An important cross point between the two pathways is that of phosphoenolpyruvate carboxylase (PEPCo) which delivers oxaloacetate to the citric cycle (which may be limited by the removal of oxo-glutarate for amino acid synthesis) or to aspartate synthesis. The flow of carbon has to be directed in either sugar/starch synthesis or that of organic acids for amino acid formation (Tischner, 2000).

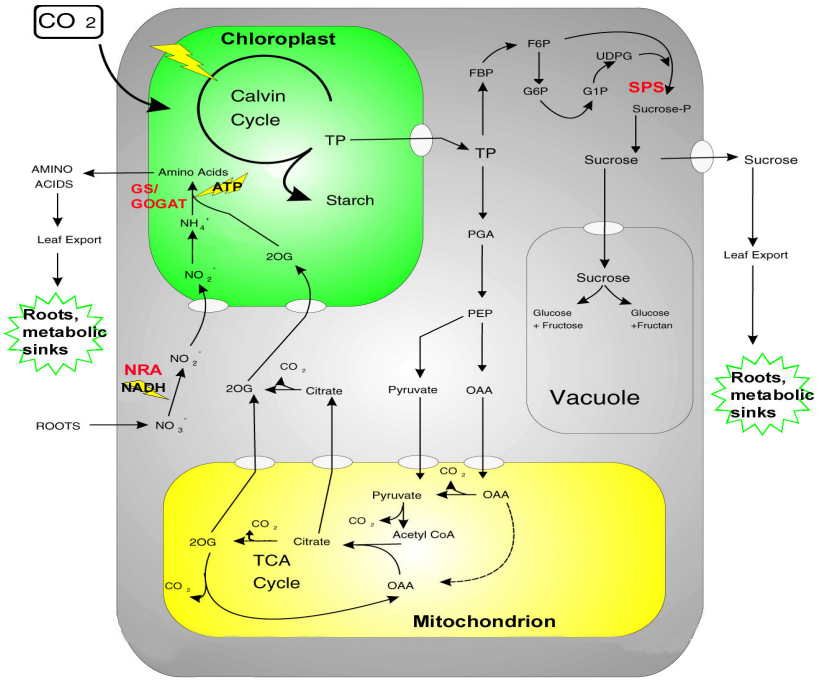

Figure 1.2 Scheme showing relationships between photosynthesis, respiration and the formation of carbohydrates and amino acids. 2-oxoglutarate (2OG), oxaloacetate (OAA), 3-phosphoglycerate (PGA), triose phosphate (TP), fructose bisphosphate (FBP), glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), uridine diphosphoglucose (UDPG) (Lewis *et al.*, 2000).

1.3.2 Phloem transport

Translocation of assimilates from photosynthetically active cells in the leaves to growing tissues, seeds and storage organs are the basis of plant performance and agricultural yield and despite other phloem solutes such as amino acids, raffinose sugars, inorganic ions and fructans, sucrose is the osmotically dominant solute in sieve tube sap. The loading, and unloading, of sucrose is the major driving force behind mass flow and its availability is determined by several metabolic pathways, especially the enzymes of sucrose synthesis (sucrose phosphate synthase of prime importance), the enzymes of sucrose hydrolysis (invertases and sucrose synthase) and the intermediate storage of starch and its mobilization (Komor, 2000). The route of assimilates is from its origin in the mesophyll cytoplasm (location of sucrose phosphate synthase) to the phloem through adjoining mesophyll cells via the symplastic or the apoplastic route. The actual phloem loading occurs against a concentration gradient and is therefore energy dependent. The driving force in grapevines is unknown, but the most likely theory is proposed by Giaquinta (1983) where proton-translocating ATP-ase induces an electrochemical potential gradient that enables the specific carrier to transfer sucrose across the membrane. Smith and Milburn (1980) suggested that phloem loading does not respond to changes in phloem sucrose concentration but rather to the decreasing turgor pressure that can be rapidly transmitted through the phloem from the sink to the source created by phloem unloading and growth of the sink.

The Munch hypothesis (Minchin *et al.*, 1993) is most widely accepted for long distance or longitudinal phloem transport based on the principle of passive flow of assimilates along a concentration gradient. The gradient is maintained by the addition of assimilates via phloem loading at the source and the unloading at the sink. The subsequent hydrostatic pressure from water entering the system at the source creates the pressure driven mass flow of solutes. There may be temporary storage depots en route between source and sink, but transfer to them does not represent leakage because they are considered to be sinks too.

The final step in phloem transport is the unloading process in sink organs and may be entirely passive and without the expenditure of energy as unloading may occur as the localized increase in permeability of the phloem membrane or by a maintained low concentration of solute outside the phloem (Ho and Barker, 1982). Depending on the sink tissue, low concentrations of solute are facilitated by chemically altering the solute or compartmenting within the sink itself. Sink unloading has been positively linked to invertase and sucrose synthase activities that would hydrolyse the sucrose to hexose sugars. In grape berries invertases convert the transported sucrose into glucose and fructose thus maintaining the sucrose concentration gradient between source and

sink. Sink regions influence the direction and magnitude of assimilate transport by removing water and solutes from the phloem and effectively steepens the osmotic gradient. According to Coombe (1989) phloem loading of grape berries is apoplastic and irreversible.

1.3.3 Source to sink relationship

Plant hormones released by the sink and by the source may influence the flow of solutes by delaying senescence or inducing meristematic growth. Sink strength may therefore be increased by auxins, gibberellins and cytokinins while ABA may increase or decrease sink strength in different plant tissues (Ho *et al.*, 1983). Endogenous ABA concentration has been linked to the triggering mechanism for the increase in solute concentrations in sinks. Parallel increases in hexoses and ABA accumulation have been found in berries (Coombe, 1989) and may be related to invertase activity. High ABA levels have been associated with high sink activity in many crops and the exogenous application to legume seed coats enhances phloem unloading (Brenner *et al.*, 1989). While the source sink gradient of sucrose maintains the import rate of storage sinks, the rate of sucrose hydrolysis or invertase activity has been reported to be the rate-limiting step in assimilate import of sinks. Events in the sink will influence the source, since phloem bridges the source with the sink organs. Experiments by Sims *et al.* (1998) demonstrated that photosynthetic acclimation and Rubisco content responded to the whole plant and not to the environment of the particular leaf. It is therefore possible that sink demand may regulate source leaf export of sucrose. The source to sink relationship in grapevines changes during the growing season where, following anthesis, the predominant sinks are the fruit and the shoot apex. After veraison the movement of solutes is directed primarily to the ripening fruit, however lateral shoot growth may be a competitive sink in vigorous vines. After ripening the source to sink status changes again towards the roots and permanent wood for storage prior to leaf senescence (Conradie, 1980).

1.3.4 Carbohydrate storage and the role of sucrolytic enzymes

Starch formation in leaves is a matter of excess - sucrose concentration in leaves is finely tuned to an upper limit in accordance to export rate (Komor, 2000) and the export rate may be finely tuned to average rates of photosynthesis (Sims *et al.*, 1998). Chatterton and Silvius (1979) showed that daily starch accumulation in leaves is also regulated in response to diurnal photosynthetic duration. The amount accumulated is adjusted to be just sufficient to support production of sucrose throughout the length of the night period. Surplus sucrose is diverted to starch that builds up in daylight when photosynthesis exceeds the combined rates of respiration and export. To achieve a steady state of sucrose synthesis, starch synthesis begins and ends gradually during the entrained light period.

Starch accumulation during stress is altered due to changes in enzyme activities in the pathway leading to starch synthesis (Vassey and Sharkey, 1989; Du *et al.*, 1998). A study of the carbohydrate status in water stressed grapevines revealed that substantial pools of sugars and starch are maintained throughout the day (Rodrigues *et al.*, 1993) with stressed leaves showing similar glucose and fructose but lower sucrose and starch concentrations as well-watered leaves. The main decrease in leaf weight was mainly due to a strong depletion of starch (up to 50% of leaf weight). Starch depletion in grapevine leaves was also noted by Düring (1984) and Quick *et al*. (1992) in response to water stress but maintained higher sucrose and fructose amounts than those found in well-watered vines. Vassey and Sharkey (1989) found that the activity of the enzyme required for sucrose synthesis, sucrose phosphate synthase (SPS), declined by 60% during mild water stress of *Phaseolus vulgaris* L. plants. Before the imposition of water stress, nearly 60% of newly fixed carbon ended up as starch while 40% ended up as sucrose. After the imposition of a mild water stress the proportion of newly fixed carbon found in starch dropped to 16%. However, the accumulation of ^{14}C into the neutral fraction, which included sucrose, was minimally affected (Vassey and Sharkey, 1989). Vassey and Sharkey (1989) concluded that the decline in extractable SPS activity was a response to the reduced rate of photosynthesis caused by stomatal closure.

Stomatal closure was also thought to reduce photosynthesis when Du *et al*. (1998) imposed increasing levels of water stress to sugarcane leaves. Even at moderate water stress SPS activity was significantly reduced and changed the sugar/starch ratio. The change in sugar contents was mainly found in elevated glucose and fructose fractions, while sucrose levels did not change under mild stress. The raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction as the leaf water potential dropped. The increases in glucose and fructose contents suggest that the activities of enzymes hydrolyzing starch and sucrose may be increased during water stress. This speculation is supported by reports that the activities of amylase (hydrolysis of starch) and invertase (breakdown of sucrose) in leaves of pigeon pea were markedly increased by water stress (Keller and Ludlow, 1993).

Lawlor (1995) states, however, that carbohydrates may accumulate under conditions of mild water stress since slight drought conditions inhibits growth and expansion of organs much more and much earlier than the inhibition of photosynthesis (Salisbury and Ross, 1992). Although photosynthesis may be reduced by stomatal closure and decreased intracellular CO_2 concentrations (Ci), a positive balance between synthesis and consumption may be maintained (Lawlor, 1995).

It could therefore also be expected that under conditions of PRD, where there is no decrease in leaf water content (Dry *et al.*, 1996), the only reduction in photosynthesis may be a result of stomatal closure and reduced *C*i. Since reduced values of *C*i lead to increased oxygenation of ribulose- 1,5-bisphosphate (RuBP) by Rubisco, photorespiration is likely to increase under moderate drought and PRD conditions. Photorespiration may be able to protect the photosynthetic apparatus against photoinhibition by sustaining photon utilization in non-assimilatory electron flow (Osmond *et al.*, 1997). The relationship between a reduction in stomatal conductance and photosynthesis is not linear (Jones, 1992). Considerable stomatal closure can occur without changes in photosynthesis, allowing plants to use less water but maintain its assimilation rate. This was also reported by Stoll (2000) that large changes in stomatal conductance had little influence on photosynthetic rates of PRD treated grapevines.

1.3.5 Biomass partitioning and water stress

Plants have a remarkable capacity to co-ordinate the growth of their organs, so there is usually a very tight balance between the biomass invested in the shoots and that invested in the roots. Whereas biomass is predominantly directed to the shoots under optimal growing conditions, the depletion of resources such as water and nutrients is known to increase allocation to the roots (Hare *et al.*, 1997). Accordingly, Amthor and McCree (1990) state that water stress tends to increase the relative allocation of carbon towards the roots and leaf production is reduced proportionally. The organ 'most limiting' is thus given priority. A simple equation indicating the carbon balance was proposed by Amthor and McCree (1990):

Carbon balance

$$dW_S/dt = Y_g (P - dW_n/dt - mW_S) - L$$

W_S = Structural biomass at a certain time

dW_S/dt = Rate of change in W_S

Y_g = Growth conversion efficiency (g^{-1}.C)

P = Rate of photosynthesis (C. d^{-1})

dW_n/dt = Rate of change in Non-structural biomass (C. d^{-1})

m = Maintenance respiration coefficient e.g. mW_S = maintenance respiration.

L = Loss of structural mass due to abscission, herbivory, etc. (C. d^{-1})

Stress on all of the components on the right hand side of the equation will change the carbon balance of the plant, and each of the components may change independently of the stress imposed.

According to the 'functional equilibrium' model of Poorter and Nagel (2000), plants increase their allocation to shoots and leaves in response to decreased aboveground resources, while decreases in belowground resources will increase allocation to roots. This may be that the plant organ most limited can explore its environment and capture more resources. Plants grown at a low nutrient supply have a more hampered growth rate than its decreased photosynthesis, as judged by the generally occurring accumulation in starch. Therefore we expect that plants experiencing an excess of photosynthates will allocate more biomass to the roots. In the case of low water availability, there would be a decreased water uptake per unit root mass, and probably a reduced nutrient uptake, as the delivery of nutrients by mass flow is hampered in dry soil (Marschner, 1995). It is therefore expected that this would increase the allocation of biomass to the roots. However, contrary to low nutrient supply, starch does not accumulate in leaves under low water supply because photosynthesis and shoot growth would be hampered to the same extent.

A reduction in vegetative growth under PRD conditions may encourage a the shift of biomass to the roots in order to explore its environment and access water and nutrients, however in grapevines the crop represent a very large sink for carbon during the ripening period that may be unchallenged by roots. Most of the dry matter accumulated by a grape crop is as solutes in the juice. According to Coombe (1989) it is useful to measure the accumulation in terms of a sink where sink strength is determined by sink size and sink activity. If fruit volume expresses sink size and sink activity by sugar concentration, sink size is by far the more important factor in commercial vineyards.

1.4 Nitrogen assimilation and water stress

Nitrogen (N) is the element most extensively taken up by higher plants from the soil environment. Knowledge about its movement, compartmentation and turnover is critical to plant physiological ecology because N availability limits plant growth and yield more than any other nutritional factor (Crawford and Glass, 1998). The absorption of NO_3^- and NH_4^+ by plants allows them to form numerous nitrogenous compounds, mainly proteins, essential to growth and metabolism. Although Miflin and Lea (1980) postulated that there exists no feedback inhibition for nitrogen uptake and nitrogen reduction in plants, the overall process of reduction of NO_3^- to NH_4^+ is an energy dependent one and summarized in Reaction 1.1 (Salisbury and Ross, 1992):

$$NO_3^- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3\ H_2O \qquad (1.1)$$

The predominant N-source for land plants is nitrate and there is evidence that nitrate assimilation can occur in either the roots or the shoots (or both) of vascular plants (Raven and Smith, 1976).

Nitrate reduction occurs in two distinct reactions catalyzed by different enzymes (Salisbury and Ross, 1992). The first reaction is catalyzed by nitrate reductase (NR). The enzyme transfers two electrons from NADH as seen in reaction 1.2:

$$NO_3^- + NADH + H^+ \xrightarrow{NR} NO_2^- + NAD^+ + H_2O \quad (1.2)$$

$$NO_2^- + 3\,H_2O + 2\,H^+ + \text{light} \longrightarrow NH_4^+ + 1.5\,O_2 + 2\,H_2O \quad (1.3)$$

The second reaction (Reaction 1.3) involves the conversion of nitrite to NH_4^+ and is catalyzed by nitrite reductase (Salisbury and Ross, 1992). Light drives the electron transport from H_2O to ferredoxin, which in turn provides the six electrons used to reduce NO_2^- to NH_4^+. The reducing substance in roots is still unknown.

1.4.1 The role of enzymes involved in nitrogen assimilation

The activity state of NR is used as a measure of the degree of phosphorylation of NR (Mackintosch *et al.*, 1995). NR is substrate inducible and it has been proposed that nitrate flux is the most important factor in the regulation of NR activity (Gojon *et al.*, 1991). However, its activity can be altered by several environmental, hormonal or metabolic factors. The metabolic state of a plant cell can exert a major impact on the regulation of the nitrate reductase (NR) activity. De Cires *et al.* (1993) found that nitrate assimilation in green barley leaves was closely coupled to and regulated by CO_2 fixation under light-dark transitions. Data from Lillo (1994) further suggest that the ratio NADH/NAD regulates nitrate reductase activity in squash leaves after a light/dark shift. It could therefore be assumed that enhanced nitrate reductase activity at adequate nitrogen levels may be associated with a higher availability of both energy and reducing power.

NR is induced by nitrate (Pouteau *et al.*, 1989; Cheng *et al.*, 1992) and repressed by glutamine or related downstream metabolites that are formed from nitrate (Hoff *et al.*, 1994). The levels of NR activity are therefore low in nitrate-deficient plants, high in nitrate replete plants, and low when downstream metabolites such as ammonium or glutamine accumulate or are supplied exogenously (Scheible *et al.*, 1992; Hoff *et al.*, 1994).

The next step in nitrogen assimilation is the conversion of ammonium to glutamine. At normal intracellular concentrations of ammonium the glutamine synthase/glutamate synthase cycle (Figure 1.3) is the usual pathway for the assimilation of ammonium (Givan, 1979; Salisbury and Ross, 1992). Ammonium is bound as glutamine in the presence of glutamic acid by the enzyme glutamine synthase (GS). GS works in conjunction with glutamate synthase (GOGAT). The substrates

involved in the GS/GOGAT cycle in addition to ammonium are α-ketoglutarate, ATP and reductants. The outputs consist of the amide glutamine or glutamic acid (or other amino acids that can effectively draw off the assimilated amino nitrogen by an aminotransferase reaction), ADP and Pi (Givan, 1979; Salisbury and Ross, 1992).

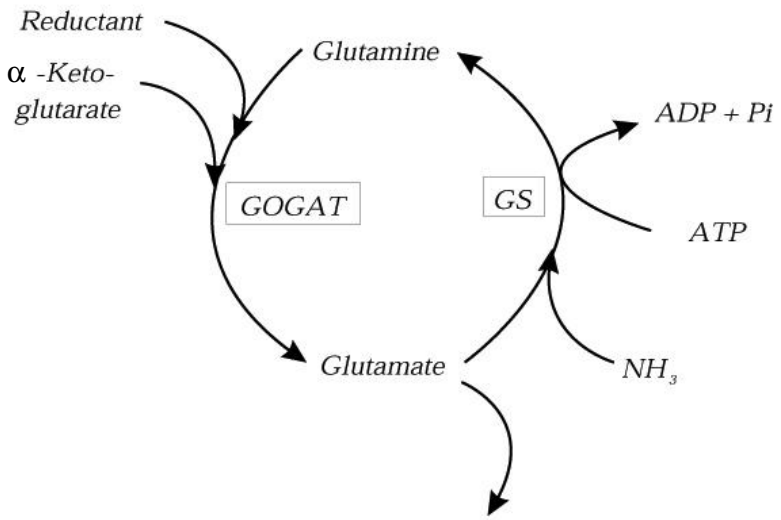

Figure 1.3 **GS/GOGAT assimilation cycle (Givan, 1979; Salisbury and Ross, 1992).**

1.4.2 Nitrogen containing compounds (NCCs)

Reviewing the literature, Rabe (1990) concluded that water stress usually causes the accumulation of nitrogen containing compounds (NCCs) in plant tissues but the nature of the stress and the plant type govern which NCCs accumulate. The NCCs that most commonly accumulate in response to water stress are proline, glycine betaine and putrescine. He further noted that a common denominator among all stress conditions is a reduction in growth rate. Rabe (1990) postulated that the lack of anabolic processes (protein synthesis and growth) and the fact that there is no feedback inhibition for nitrogen uptake and nitrogen reduction in plants leads to ammonium accumulation in plant cells (Miflin and Lea, 1980). High levels of ammonium are known to have toxic effects in plant cells (Marschner, 1986) and therefore have to be compartmentalized or sequestered into NCCs that are pH neutral and harmless to cell rheology. Unlike many other molecules and ions, ammonium is difficult to compartmentalize because it is very membrane mobile. Consequently plants are unable to use compartmentalization as a protective strategy against elevated ammonium (Roubelakis-Angelakis and Kliewer, 1992). The removal of toxic levels of ammonium therefore forces its sequestering into amides and other NCCs (Givan, 1979). Consequently, Rabe (1990) hypothesized that most, or all NCCs accumulating during environmental stress conditions serve in detoxifying the cell of ammonium (Figure 1.4).

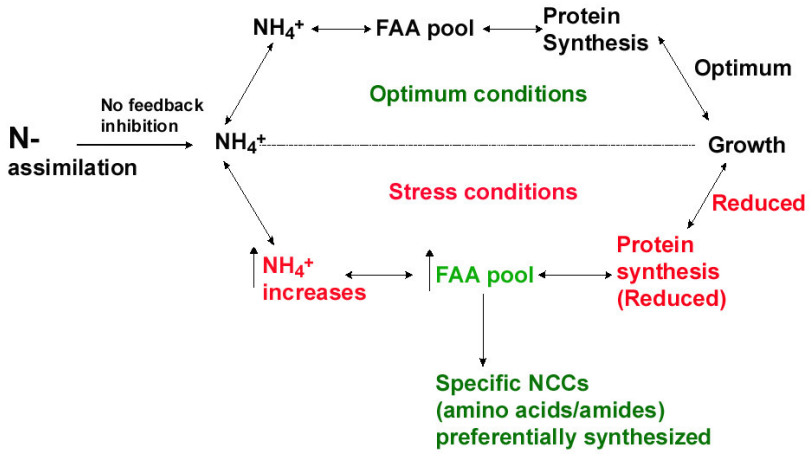

Figure 1.4 Schematic outline of detoxifying hypothesis (Rabe, 1990).

Using the proposed hypothesis of Rabe (1990) it could be postulated that under optimal conditions there would be optimal growth and protein synthesis. When plants are subjected to sub-optimal conditions, there is a reduction in protein synthesis and growth. This is supported by the fact that a reduction in cell growth, wall synthesis and protein synthesis occurs much earlier than stomatal closure, reduction in CO_2 assimilation or an influence on nitrate reductase level (Salisbury and Ross, 1992). The detoxification of the NH_4^+ concentration in plant cells then leads to a concurrent increase in the free amino acid (FAA) pool. According to Hare and Cress (1997) the synthesis of NCCs from the enlarged FAA pool may serve to reduce the excessive redox potential of a plant during stress and serve as a nitrogen and energy source when the stress is relieved.

1.4.3 Polyamines (PAs)

Historically researchers have concluded that arginine and polyamines serve as N storage compounds during stress (Rabe, 1990). However, Rabe (1990) noted that the synthesis of these compounds is expensive in terms of energy input and that the synthesis of these compounds reduces the normal rate of protein synthesis due to the diversion of energy.

PAs, especially putrescine, are well correlated with internode elongation (Tiburco *et al.*, 1993). PAs are further correlated with enhanced rooting, especially adventitious root formation. If elevated polyamine concentrations in PRD vines exist it may contribute to the knowledge of a more exploratory root system observed by Dry and Loveys (1999) and Stoll's thesis (2000). The anti-senescence effects of PA are also well documented (Tiburco *et al.*, 1993) and are not unlike the

anti-senescence effects of Ca^{2+}. Both Ca^{2+} and PA are known to attribute to the rigidification of membranes and keep the cell wall rheology intact.

A schematic illustration of polyamine biosynthesis is presented in Figure 1.5. Putrescine (Faust and Wang, 1992) may be formed directly from ornithine by ornithine decarboxylase (ODC) or indirectly from arginine by arginine decarboxylase (ADC). The respective functions of the two pathways are not clear, but in general, changes are usually noted in ODC, when cell division is affected and where elongation and non-mitotic processes are affected (like in the roots) ADC activity is usually involved (Galston and Kaur-Sawhney, 1987). Galston and Kaur-Sawhney (1990) labeled ADC as a general stress enzyme because of the general *de novo* synthesis of ADC when putrescine accumulated due to water stress.

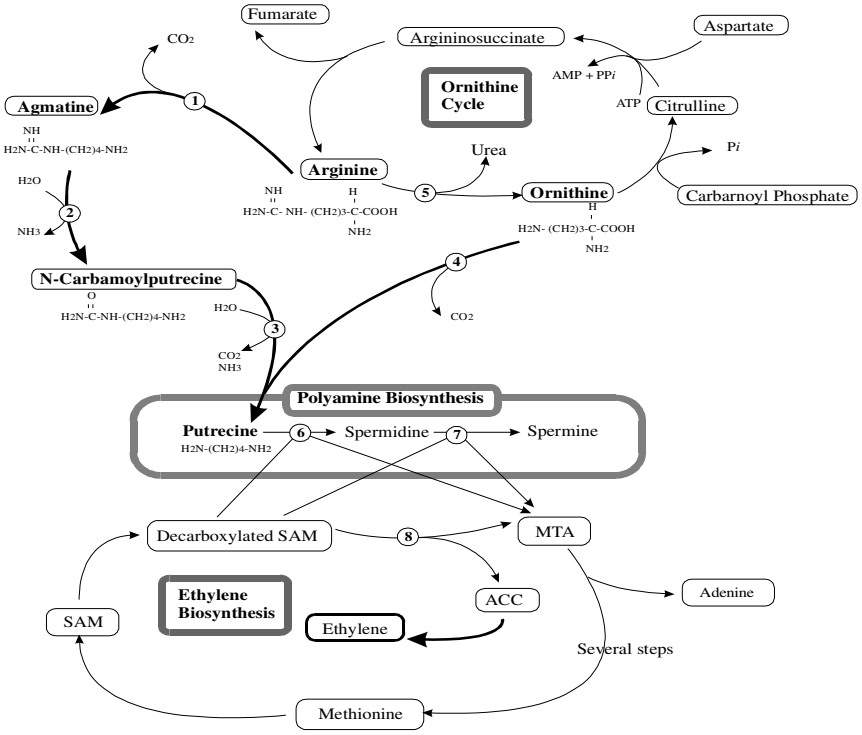

Figure 1.5 Schematic illustration of polyamine biosynthetic pathways. (1) Arginine decarboxylase (ADC); (2) Agmatine Iminohydrolase (AIH); (3) N-Carbamoylputrecine amidohydrolase; (4) S-Adenosylmethionine decarboxylase (SAMDC); (5) Arginase; (6) Spermidine synthase; (7) Spermine synthase; (8) ACC synthase (Flores, 1990).

Polyamine biosynthesis is linked to ethylene biosynthesis (Figure 1.5) by the acquisition of aminopropyl groups from ethylene precursors (Faust and Wang, 1992). Spermidine and spermine are synthesized from putrescine by addition of aminopropyl groups from decarboxylated S-adenosylmethionine (SAM). Alternatively SAM can be metabolized successfully to 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene. The link between polyamine and ethylene synthesis appears to be competitive in carnation flowers (Roberts *et al.*, 1984), but no

competition was observed in water stressed apple leaves (Wang and Steffens, 1985) or avocado fruit (Kushad *et al.*, 1988).

1.5 Function and accumulation of inorganic ions in grapevines

Uptake of water and dissolved ions is restricted to the root tips and translocated to aboveground organs via the xylem and may be remobilized depending on their mobility in the phloem. Under optimal soil conditions grapevine roots are active from early spring to late autumn, leaving a long period to absorb the required soil nutrients. Only fifteen elements are known to be absolutely necessary for normal growth and fruiting. Ten of these elements are needed in relatively large amounts, called macronutrients: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, sulphur, iron, calcium and magnesium. The other five elements, although essential, are used in relatively small amounts, called trace elements: boron, manganese, copper, zinc and molybdenum. Minerals such as potassium, calcium, sodium, sulphur, magnesium, and phosphorus can contribute significantly as osmotic components in grape berries and influence the vinification process, therefore their role is briefly discussed.

1.5.1 Potassium (K)

K plays important roles in plants that can be grouped into four categories: 1) Enzyme activation; cellular membrane transport and phloem transport; anion neutralisation and 4) osmotic potential regulation in the regulation of plant water relations (Davies and Zhang, 1991; Salisbury and Ross, 1992). Translocation of K in the xylem from roots to shoots is regulated by the capacity of the root in xylem loading and probably not by transpiration. The uptake of K may be altered by shoot demand because the shoot acts as a sink for nutrients. The accumulation of K in permanent structures can occur throughout the growing season, including the post harvest period. The amount of K in roots stays relatively the same during the growing season, but a significant amount of K that accumulates in the berries from veraison to harvest is translocated from the trunk, shoots and leaves (Conradie, 1981; Williams and Biscay, 1991). K movement occurs in both xylem and phloem but the xylem seems to be a minor route of K uptake in berries. Ollat and Gaudillere (1996) found that K uptake by Cabernet Sauvignon berries is slow before veraison and increases strongly when ripening starts in the same proportion as sink strength and phloem water influx. K is the predominant cation in sieve tube sap in many species most of the potassium in fruit is delivered by the phloem (Ziegler, 1975). Excess K in grape berries may have a negative influence on wine quality, mainly because it decreases free tartaric acid resulting in increased pH of grape juice (Mpelasoka *et al.*, 2003). In Australia, high K status is common in most vineyards that necessitate pH adjustments during the vinification process. Many factors may affect the accumulation of K by

berries and these factors include soil, plant, vine microclimate and cultural practices (for review see Mpelasoka (2003)). However, the role of amount of irrigation and root-borne ABA may play the biggest role in the PRD effect on K flux. In contrast to the ABA effect on guard cell K release, in root cells ABA reduces the K efflux from stellar cells (Roberts and Snowman, 2000). This reduction in K efflux may lead to less K in xylem sap translocated to the shoots. Furthermore, increased irrigation generally increases shoot growth (Klein *et al.*, 2000) and therefore an increase in vine and berry K may also be attributed to increased canopy density and increased shading within the canopy. PRD may have positive effects on canopy microclimate by regulating vine vigor with chemical signals (predominantly ABA) provided by drying roots without water stress. Both a reduction in shoot growth and accumulated ABA in the roots may reduce the K uptake of the vine. The effect of PRD on berry K is still unknown and this thesis may be one of the first published works on the matter. K is by far the major cation in the berry and contributes significantly as an osmolyte, especially in conditions of low sugar accumulation. However, other minerals contained in the berry such as sodium, calcium, magnesium, copper, manganese and phosphate also contribute to a lesser degree as osmotic components.

1.5.2 Phosphorus (P)

P is a macronutrient critically important to photosynthesis, phospholipids and nucleic acids. P is also indispensable in respiration and sugar and starch formation due to its part in ATP and NADPH. A deficiency in P will result in reduced growth but an excess supply would reduce nitrogen uptake (Winkler *et al.*, 1974). P is also needed by yeast during must fermentation (Markham and Byrne, 1967). Active absorption of P starts during the period after budburst and the P reserves in the roots plays a noticeable role in supplying P to new growth (Conradie, 1981). During the next period until veraison there is rapidly increased P absorption with little use of root reserves. The P content between veraison and harvest stays constant but the P contents of the fruit increases due to remobilisation from leaves. Absorption in P commences after harvest and leaves, shoots, trunks and roots show increases in P content.

1.5.3 Calcium (Ca)

Ca is an important constituent of membranes and membrane permeability keeping the cell wall rheology in-tact (Kaur-Sawney, 1991), acts as a second messenger for hormonal action via calmodulin (Giraudat *et al.*, 1994) and plays a role in the translocation of carbohydrates. After budbreak the increase in Ca content of new growth is accompanied by a decrease in root Ca. The vine shoots and leaves accumulate considerable amounts of Ca from anthesis to harvest and the bark plays a major role in storage in permanent wood. The majority of the yearly total Ca is

absorbed between budburst and veraison and a small amount during the six weeks before leaf fall. Ca accumulation in berries occurs when xylem influx is high and almost completely stops after veraison. Ca is considered to be phloem immobile and very little accumulates in berries after veraison (Conradie, 1981).

1.5.4 Magnesium (Mg)

Mg is the only mineral constituent in chlorophyll and forms part of a compound that functions as an activator for numerous enzymes (Winkler *et al.*, 1974). Mg absorption starts roughly 22 days after budburst and most is required by new growth. Absorption continues to increase until veraison when almost half of the yearly requirement is already absorbed. Bunches accumulate only small amounts during this stage, resulting in significant increased reserves of roots, shoots and leaves. In the period until harvest a smaller amount of Mg accumulates and, as in the case of Ca, very little accumulates in bunches. A significant amount of Mg is absorbed in the woody parts after harvest, however close to leaf fall leaves gain a significant amount of Mg at the expense of shoots.

1.5.5 Sodium (Na)

Na is not an essential mineral, but can be a factor in grape nutrition. In some rare cases Na may have beneficial characteristics, but in high enough concentrations can cause typical leaf burn and general vine stunting (Winkler *et al.*, 1974).

1.5.6 Sulphur (S)

S is a constituent of some amino acids, protein and vitamins and may be beneficial to growth. Sulphur is not likely to be deficient in soils suitable for grape production.

1.6 Seasonal dry matter and nutrient distribution in grapevines

Seasonal growth, carbohydrate and nutrient patterns have been studied in various cultivars and grapegrowing regions in the world (Conradie, 1980; Williams, 1987; Hanson and Howell, 1995; Bates *et al.*, 2002). General vine growth patterns indicate that between bud swell and bloom shoot growth is supported by stored carbohydrates and nutrients from the previous season as well as from newly acquired uptake in the spring. Rapid growth and berry development during the next 3 to 4 weeks after bloom prevents the replenishment of stored resources despite rapid absorption of nutrients and carbon accumulation. During budbreak and end of rapid shoot growth total root nitrogen decreases, but total plant nitrogen increases, indicating that new vine growth is supported by both stored and newly absorbed nitrogen. As shoot growth slows, fruit and wood maturation

takes place simultaneously – albeit at different rates depending on environmental factors and crop load. Total starch and starch concentration increases from 32 days after bloom to veraison in perennial tissues. From veraison to harvest, starch concentration does not change in shoots because fruit maturation may compete with cane maturation. In contrast, starch concentration increases in all other woody structures from veraison to harvest. The post-harvest period is the time to recover the stored resources because carbon assimilation and nutrient uptake is partitioned to vegetative structures. Studies with 'Chenin blanc' grapevines in South Africa indicated two periods of active fine-root growth: the first peak at anthesis and the second after harvest (Conradie, 1980).

1.7 Importance of vigor for plant nutrition

Once a vineyard is established the grower is primarily concerned with obtaining consistent large crops of good quality fruit. The capacity of a vine to produce fruit depends on the production of wood. Therefore, to produce heavily over a long period (i.e. sustainable viticulture), a vine must be capable not only of maturing a crop each year, but also maturing a good growth of wood (Winkler *et al.*, 1974). To explain the importance of vigor for plant nutrition (Winkler *et al.*, 1974) it is important to define the two terms: vigor and capacity. *Vigor* is the quality or condition that expresses the rapid growth within the parts of the plant and refers to the rate of growth. *Capacity* on the other hand refers to the quantity of action in respect to total growth and crop of which the plant is capable. A young vine may show great vigor in the qualitative sense, but has much lower capacity to grow and fruit in the quantitative sense than a mature grapevine. Grapevines are prolific producers of bunches and therefore have an abundant crop potential. However, the fruit buds develop only to the primordia of the inflorescences in the year in which they are differentiated. Therefore it is possible to regulate or eliminate the crop even before the flowers are formed. All conditions being equal the largest factor affecting fruit set and growth would be in the capacity of the grapevine. Conversely, severe pruning and high cropping levels can easily depress vine capacity (Winkler *et al.*, 1974).

1.8 Importance of water use in plant nutrition and the carbon and nitrogen ratio

Shoot growth rate early in the growth season is a sensitive indicator of the availability of soil moisture. Persistently low soil available moisture may lead to reduced shoot growth and characteristic signs of water stress unlike that of wilting leaves. The osmotic potentials of berries are higher than leaves between veraison and harvest, indicating a preference of water movement to the berries under stressed conditions. Older leaves turns yellow and dry around the edges, curl up and fall off. This is mainly due to induced nitrogen and magnesium deficiency (Kuretani, 1968). The excessive loss of leaves may lead to reduced maturation of wood and fruit and ultimately

reduce vine capacity to crop the next year (van Zyl, 1981). Remaining leaves will also be smaller and total leaf area reduced. Irrigation however can greatly boost vegetative growth and enhance berry size. Excessive irrigation, however, may increase the ratio of yield to pruning weight and delay maturity (Smart and Coombe, 1983). Bad irrigation management has the potential to over-crop the vine and create a dense canopy, resulting in a loss of fruit quality and a waste of water.

Under adequate water and mineral conditions a number of conditions may be indicated in carbon and nitrogen ratios that are directly related to fruiting and vegetative growth (Winkler *et al.*, 1974):

a) Moderate carbohydrates and very high nitrogen content is normally associated with strong vegetative growth and little or no fruit bud formation. This may be typical in young vines or vines grown in very fertile soils with high moisture. Grapevines have characteristic large leaves, long internodes, late growth and poor wood maturity.
b) High carbohydrates and moderate nitrogen content is normally associated with moderate vegetative growth with abundant fruit bud formation. Typically found in areas with moderate soil fertility and moisture. Grapevines have normal size leaves, internodes and early wood maturation.
c) Very high carbohydrates and low nitrogen content is normally associated with poor vegetative growth and limited fruit bud formation. This may be typical of grapevines grown in poor soils deficient in nitrogen. Grapevine leaves are small and yellow green with shoots that are mature but have short internodes.

Most importantly the carbon and nitrogen ratio indicate the need to modify cultural practices to bring vegetative growth and fruiting more nearly in balance. PRD has the effect of reducing vegetative growth on fertile soils and may be a successful cultural practice to bring vigorous grapevines in balance.

1.9 General research hypothesis

With the progression of viticulture as a science, irrigation practices have evolved with the frequent aim of developing systems using water more effectively. Improvement of conventional ways of whole surface irrigation (flood, furrow and over-head sprinklers) to modern micro-irrigation has decreased the waste of water and fertilizers as well as labor. Over irrigation not only wastes water, but may cause excessive vigor that have negative implications for fruit quality, disease control and vine balance. However, vineyards and mature trees converted from sprinkler irrigation, with large wetting surfaces, to drip irrigation are often associated with a reduction in shoot growth earlier in the season. This may be possible to explain in terms of partial drying of the root system. There is a wealth of evidence that plants have the ability to sense drying soil in contact with their roots and to communicate with aboveground organs to regulate its growth and physiology (Davies and Zhang,

1991). Much work has been done to evaluate PRD in grapevines for commercial application (Loveys *et al.*, 2000; Stoll *et al.*, 2000a; Dry *et al.*, 2001) and to elucidate the hormonal influences on stomatal closure (Dry *et al.*, 2000a; Stoll *et al.*, 2000b). However, no information exists on the effect of PRD and its hormonal signals, ABA in particular, on grapevine assimilation and partitioning of carbon and nitrogen.

The general hypotheses to be tested in this study are that:

'Partial drying of the root system of grapevines will change the partitioning of dry matter, carbon, nitrogen and inorganic ions of grapevines away from shoot growth, towards the permanent structure and fruit by affecting the enzyme activity associated with growth.'

Chapter 2: General materials and methods

2.1 Sites and conditions

All experiments were conducted on potted or field-grown grapevines (*Vitis vinifera* L.) between November 2000 and November 2003. Potted vines were grown in open shade houses (Cabernet Sauvignon) or in temperature controlled greenhouses (Cabernet Sauvignon) at the Waite campus of the University of Adelaide. All potted plants were on own roots. Potting mixes varied with experimental design. Potted grapevines used for PRD had a split-root system and were grown in two pots and sizes varied with experimental design. In all PRD experiments irrigation water was applied either to one side of the root system at any time (PRD; Figure 2.1 A) or to both sides (control; Figure 2.1 B).

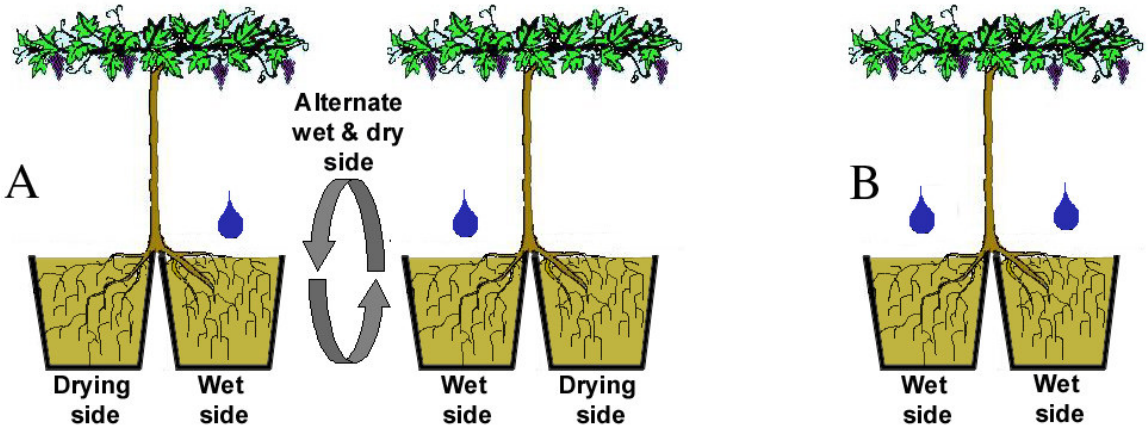

Figure 2.1 Implementation of PRD irrigation set up in pots: A)PRD: water withheld from one side; B) control: water on both sides.

PRD field experiments were conducted in the Coombe and Alverstoke vineyards at the Waite campus of the University of Adelaide and the SARDI experimental vineyard at the Nuriootpa Research Station (Barossa Valley, South Australia).

Cabernet Sauvignon vines were planted in 1997 in the Alverstoke vineyard, Waite Campus of the University of Adelaide. The vines were planted in a trench (sandy soil, 28 m long, 1.5 m wide and 1.5 m deep) with roots divided by a plastic membrane. The original soil was replaced by a sandy soil (Appendix A) that enabled easier access to the root system for collecting root samples. The vine and row spacing was 1.5 m and 3.5 m respectively and trained to VSP trellis system. Vines were irrigated with drip emitters 0.4 m away from the planting row, either on both sides of the membrane at 1 L/h (control) or only on one side at any time at 2 L/h (PRD). The consequence is that all vines

received the same amount of water throughout the season. Soil measurements conducted with gypsum blocks during the 2000/1 season and with the Diviner 2000®, (Sentek, Adelaide, South Australia) during the following two seasons indicated no free draining of water further than the rooting zone (70cm). The drip lines were positioned 40 cm from the trunk.

Cabernet Sauvignon and Shiraz vines in the Coombe vineyard were planted in 1992 at the Waite Campus of the University of Adelaide. The vineyard had a vertical shoot positioning (VSP) trellis system and was spur pruned according to the measured mass of pruning weight (30nodes/kg). Details of pruning will be provided in Chapter 4. A drip irrigation system was installed in the planting line and drippers were situated 0.4 m from the trunk of each vine on either side. Shiraz: PRD and Control vines received the same amount of water (2 L/h). Cabernet Sauvignon: PRD received half the amount of water (2 L/h) as control (4 L/h). Irrigation management was done manually during the 2000 season, but was replaced by a Galcon 7001D computerized irrigation control unit (Plasflo, Adelaide, South Australia) in 2001 and 2003. When necessary, additional applications of water were done to ameliorate the soil moisture content to soil water capacity. This was only done when the normal scheduled irrigations failed to increase the soil water volume to this level. Amount and timing of additional irrigations and frequency of switches in the wetting pattern were determined by evaluating observed rates of soil drying and summed soil water contents (more detail on irrigation in Chapter 4). On average, irrigation was done 3 times a week for 3 h, pulsed with 1.5 h in the morning (06:00) and 1.5 h in the late afternoon (17:00) The soil type is classified as 'Dr2.23 Hard Pedal Red Duplex' with 8% clay content at 0-110 mm and 60% clay content at 300-690 mm (Litchfield, 1951) that is well suited for viticulture (Appendix A).

The sites located at the Waite campus were on a relatively sheltered, gentle slope with northwest aspect. Dry and Smart (1988) classified the region as 'hot, moderately maritime, arid sunny and not humid'. The mean daily maximum temperature in January and February for Adelaide (latitude 34.97 S; longitude 138.63 E; elevation 115m) is 27.7 °C and 27.5 °C respectively, while the mean daily minimum temperatures are 16.2 °C and 16.5 °C. On average the mean daily temperatures (9:00 am) in January and February for Adelaide are 22 °C and 21.7 °C respectively while the mean maximum for summer is 28.2 °C and the minimum is 16.5 °C. The mean annual rainfall is 585 mm of which 30% (182 mm) falls between September and March inclusively (Meteorology, 2004).

Shiraz vines grown under PRD at Nuriootpa were subjected to three pruning levels as well as to PRD treatments. The vineyard had a single wire trellis system and was spur pruned. A drip irrigation system was installed in the planting line 0.4 m from the trunk of each vine on either side.

PRD vines received half the amount of water of control vines. Vines were subjected to two irrigation treatments (PRD and Control at 2 L/h) and three pruning levels (30, 60 and 120 nodes/vine).

The differences in elevation and climate between experimental sites are shown in Table 2.1. The Waite campus data was compiled using some data from the Bureau of Meteorology (2004). Although the average temperatures of the sites are comparable, the annual radiation and rainfall is different. The Barossa has slightly more annual evaporation and lower rainfall with more sunshine hours per day during the growing season than the Waite Campus. This indicates that the experimental site in Nuriootpa is slightly more arid than the Waite Campus.

Table 2.1 Climatic data for the experimental sites located in South Australia.

	Adelaide, Waite Campus [a]	Nuriootpa [b]
Elevation (m)	115	274
Mean Jan. temp. (°C)	22.0	21.2
Continentality (Mean Jan – Jul; °C)	11.1	12.4
Annual Rainfall (mm)	621	502
Growing season rainfall (mm)	190	163
Growing season rain days	36	38
Growing season average evaporation (mm)	1101	1274
Average relative humidity (%; 9:00 am) for Jan	49	52
Sunshine days (Hrs of sunshine per day; Oct-Mar)	8.3	9.0

[a] Bureau of Meteorology (2004).

[b] Dry and Coombe (2004).

All replicates at the Waite campus consisted of 3 vines; the centre vine used as the population and two buffer vines, one on either side, not used for sampling. In the field-grown split rooted Alverstoke vineyard the number of replicates per treatment was four and in the Coombe vineyard there were seven replicates. The five replicates per treatment at Nuriootpa consisted of 4 vines; the two centre vines used as the population and two buffer vines, on either side, not used for sampling.

Weeds were controlled under vines either by using herbicides (Roundup®, Monsanto, USA) or manual pulling in the Alverstoke and potted experiments.

2.2 Production of split-root vines

Split rooted vines were propagated from thick cuttings (*Vitis vinifera* L. cv. Cabernet Sauvignon) taken from the Coombe vineyard. Cuttings (0.35-0.45 m long) were selected in the winter and callused in a heat bed (25°C) until budbreak was observed. Cuttings with well-developed root systems were selected and the base split for 0.1-0.15 m towards the tip with a band saw. The split-rooted vines were then planted so that the root system was divided into separate pots with standard potting mix (Table 2.2).

Table 2.2 Standard potting mixture

Golden grove sand. Sterilized at 100°C for 30min	Peat moss	Calcium Hydroxide	Calcium Carbonate	Nitrophoska 12-5-14
1200 L	750 L	1.1 kg	2 kg	2 kg

Potted grapevines were kept well watered in a shade for at least one year before used for experiments. In the winter prior to their use in experiments, the potted vines were cut back to two node spurs. Older split-rooted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) propagated by Manfred Stoll (2000) were also used during this study.

2.3 Soil moisture measurements

Soil moisture in the Alverstoke vineyard was measured with EnviroSCAN® (Sentek, Adelaide, South Australia) probes. Capacitance sensors were installed at depths of 0.1 m, 0.2 m, 0.3 m, 0.4 m, 0.5 m, 0.7 m and 1 m on either side of the vine row at a distance of 0.4 m from the vine trunk. Volumetric soil water content was calculated by measurement of the electrical capacitance of the soil at each depth and was expressed as the soil water content (in mm) and recorded on a solar powered automatic logger. The soil water content is a function of the frequency of the electric field created by each sensor through the EnviroSCAN® access tube. Installation of the access tubes is a proven technique (Sentek, Adelaide, South Australia) that allows for the minimal soil disturbance to preserve the soil integrity and water penetration characteristics. One probe on either side for each treatment was installed to monitor the soil water contents.

Soil moisture in the Coombe vineyard was initially measured using gypsum blocks (Measurement Engineering Ltd., Adelaide, South Australia). Gypsum blocks were installed in 2000 using a 24 mm soil auger to depths of 0.4 m, 0.6 m, 0.8 m and 1 m in a 15 cm circular pattern around the dripper, 0.4 m away from the trunk. The holes were filled to near the top with active gel Bentonite and the rest of the way with the original soil. One gypsum block was installed at every depth in every

treatment and in both cultivars. One more gypsum block was installed at every depth for both cultivars in the PRD opposite side to measure the alternation in wetting pattern. Soil suction (kPa) was measured and recorded by a hand held soil moisture logger (Trian Electronics Pty Ltd, Melbourne, Victoria) by clipping the gypsum block leads to the logger leads.

In 2002 a Diviner 2000®, (Sentek, Adelaide, South Australia) was used to measure soil water content in the Coombe vineyard. Installation of the access tubes is a proven technique (Sentek, Adelaide, South Australia) that allows for the minimal soil disturbance to preserve the soil integrity and water penetration characteristics. One access tube was installed 0.4 m away from the vine trunk underneath the dripper in every treatment and in both cultivars (Cabernet Sauvignon and Shiraz) to monitor the soil water contents. One more access tube was installed for both cultivars in the PRD opposite side to measure the alternation in wetting pattern. Volumetric soil water content was measured at every 0.1 m depths from 0 to 1m with a portable capacitance probe on either side of the vine row at a distance of 0.4 m from the vine trunk. Volumetric soil water content was calculated by measurement of the electrical capacitance of the soil at each depth and was expressed as the soil water content (in mm) and recorded on a portable automatic logger connected to the capacitance probe. The soil water content is a function of the frequency of the electric field created through the access tube at each soil depth as the capacitance probe moves down the access tube.

Soil matrix potential was measured in potted experiments using a portable tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA). The unit consisted of a thermally isolated tube with a porous ceramic tip on the one end and a "null knob" sealing cap and vacuum gauge on the other. When it is filled with water and the ceramic tip inserted into the soil, water can move in and out of the probe through the connecting pores in the tip. When faced with drying soil this creates a vacuum inside the probe that registers as soil suction on the gauge. Soil suction was measured in centibars as instructed by the manufacturer.

Soil moisture was also measured in potted experiments by the Model 6050X1 Trase system (Soil moisture equipment corp., California, USA) that uses Time Domain reflectometry (TDR) to measure instantaneously the volumetric water content of the soil. Two metal Waveguides (15 cm) are inserted into the drying soil and the speed with which an electro-magnetic pulse of energy travels down the parallel transmission line is measured. The speed it travels is inversely correlated to the dielectric constant of the material in contact. Because the dielectric constant of water differs greatly from the other constituents in the soil, the speed of travel of an electromagnetic pulse will depend on the water content of the soil. The Trase system also has the advantage that with the

waveguides in the soil it can take soil measurements automatically at any interval and for any length of time.

2.4 Gas exchange measurements

Stomatal conductance of leaves was measured (in units of $mmolm^{-2}s^{-1}$) using a portable AP4 diffusion porometer (DELTA-T Devices Ltd, Cambridge, UK). The porometer works on the basis of measuring the time it takes for the leaf to release enough vapor to change the relative humidity in the measuring chamber to reach a predetermined level. The time is then compared to calibration curves obtained before measurements started with a calibration plate of known conductances. Calibration curves were only accepted if the degree of error was indicated less than 5% (determined by the instrument software) and if the light reading was higher than 300 (measured by an inbuilt photoreceptor on the measuring unit. Between each measurement a desiccant dried the air in the chamber to reduce the relative humidity and hence the errors in measurement. When measurements differing by less than 1% were presented two or three times in succession, the unit indicated this with an audible sound and the measurement was then accepted and saved. No less than 4 and no more than 10 readings per leaf were done to let the system equilibrate and minimize influences of measurement on leaf stomatal aperture respectively. The instrument measures stomatal conductance only on one side of the leaf surface, so the leaf was positioned with the abaxial side towards the measuring unit. Measurements was made on mainly cloudless days between 10am and noon or 2pm and 4pm on mature, sun exposed leaves on the outside of the canopy.

2.5 Leaf gas exchange and photosynthesis

Assimilation of carbon dioxide and stomatal conductance were measured using a LICOR open photosynthesis system (Li 6400, Lincoln, Nebraska, USA) with an infrared gas analysis instrument (IRGA). This instrument measures differential or absolute changes caused by leaf gas exchange and calculates photosynthesis. The open system design allows a constant airflow through the measuring chamber and minimizes the effect of the measurement on leaf gas exchange. To minimize the effect of the measurement chamber on leaf photosynthesis the same photosynthetic active radiation (PAR), CO_2 concentration and relative humidity (RH) of ambient air (microclimate) had to be maintained during measurements. An internal light source provided ambient light intensity (between 400 and 800 $\mu molm^{-2}s^{-1}$ PAR) that was pre-determined by the apparatus's inbuilt photoreceptor. Chamber CO_2 concentration was controlled by the unit by firstly scrubbing all the CO_2 from the incoming air by passing it through soda lime and mixing in a known concentration from a CO_2 cartridge ($10cm^3$ pure CO_2, ISI soda charger, Vienna, Austria). The concentration of the CO_2 (400 to 410 $\mu mol/mol$ CO_2) was equivalent to ambient air CO_2 concentration. Chamber RH

was kept at ambient air levels (8 to 12 mmol H_2O mol^{-1}) by manually controlling the amount of air that passed through a desiccant (Drierite, anhydrous $CaSO_4$). Because PRD had significantly lower stomatal conductances, the unit had to be recalibrated to simulate ambient RH when switching between treatments. The leaves were clamped in the leaf chamber (6 cm^2) and the flow of air was set to 500 $\mu mols^{-1}$. Before every measurement the instrument was allowed to stabilize as determined by real time monitoring within the system. The unit used the difference between inlet and outlet air concentrations of CO_2 and H_2O to measure gas exchange. Photosynthesis was measured in $\mu molm^{-2}s^{-1}$ and stomatal conductance in $molm^{-2}s^{-1}$.

2.6 Xylem sap collection

Xylem sap was collected during the ripening season using a collection apparatus coupled to a vacuum pump (Figure 2.2). A 1.5 mL Eppendorf vial was placed inside to collect the sample. After cutting the shoot above the third node it was cinctured close to the end with a scalpel and the phloem removed. The stripped end was inserted into the suction apparatus for a tight fit and the vacuum pump switched on for 30 to 60 seconds. Xylem sap (200 to 1000 µL) extracted from the shoot would drop into the collection vial. After extraction the vacuum would be released, collection vial retrieved and snap frozen in liquid nitrogen.

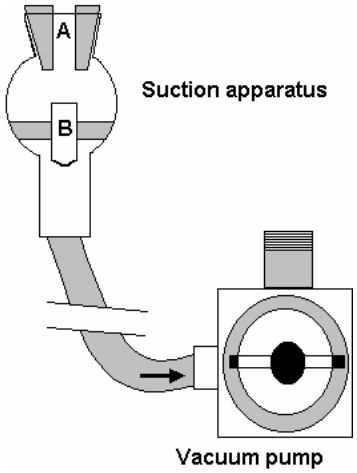

Figure 2.2 Xylem collection apparatus. A. Shoot insertion point. B. Collection vial.

Bleeding sap was collected during spring when budbreak was observed in experimental vines. Where active bleeding was observed on freshly cut spurs a 4-5 cm PVC tube was attached to the shoot with a 5 mL Eppendorf pipette tip that was cut short. Exuding xylem sap was collected in 10 mL centrifuge tubes with screw caps. The PVC tubes fitted in a hole drilled in the tube screw cap. After 30 min enough xylem sap was obtained and the collection tubes were recapped and frozen at -20°C for further analyses.

2.7 Plant organ sampling

2.7.1 Root sampling

Roots sampled from potted vines were immediately washed, snap frozen in liquid nitrogen and stored at -40°C for further analyses.

2.7.2 Leaf sampling

Leaves were sampled at the same time during the day when stomatal conductances were measured on cloudless days, avoiding times of alternation in wetting zones. Basal leaves were fully mature, sun-exposed and located between the third and fifth nodes. Apical leaves were fully sun exposed and located within the last five nodes. Five leaves per test vine were collected and immediately frozen in liquid nitrogen and stored at either -20°C or -40°C for further analyses.

2.7.3 Shoot sampling

Shoots were sampled at the same time as leaves. Two samples of three nodes and internodes above the basal two nodes were sampled in every test vine. Samples were immediately frozen in liquid nitrogen and stored at -20°C for further analyses.

2.7.4 Fruit sampling and measurements

Berries were sampled once a week from the beginning of veraison until harvest. Berry samples of 50 were randomly chosen from the test vine at different positions within the bunch and within the canopy. An equal amount of berries were chosen from the top, middle and bottom part of each bunch and equally from bunches inside the canopy and on the periphery. When fruit was more mature (higher that 21°Brix) and at harvest, sample size increased to 100 and 300 berries respectively. Berries collected were stored in plastic sampling vials and plastic bags at -20°C. Before freezing berry mass was determined by calculating the mean of 50 to 100 berries depending on sample size on an electronic balance. Smaller samples (20 to 30 berries) were then used for total soluble solid analyses (TSS) and pH. Berries were crushed and pressed in a citrus press and the juice transferred to centrifuge vials (10 mL) and centrifuged for 5 min at 1500g. 50 µL of the supernatant was then used for the measurement of TSS (°Brix) using a digital refractometer (BRX242, Erma Inc., Tokyo, Japan) that was zeroed using distilled water. The pH was measured using a standard pH meter (Activon 110. Thornleigh, NSW).

At harvest the number of bunches and total weight from each test vine was collected. Weight of the rachis was ignored. The mean bunch weight was calculated by dividing the final fruit weight by bunch number. A 300 berry sample at harvest was used to analyze fruit characteristics and composition at harvest. The mean berry number at harvest was calculated by dividing the mean bunch weight by mean berry weight at harvest. After the determination of berry weight, TSS and pH of a 100 berry sample the rest of the sample was frozen at -20 °C for later analysis of sugars, amino acids, K and minerals, polyamines and total C and N contents.

2.8 Soluble sugars analysis

Sugar analysis was conducted as described by Naidu (1998). Leaves, shoots, roots and berries were frozen in liquid nitrogen and lyophilized. Leaves and roots were powdered using a mortar and pestle while shoots and berries was powdered using a commercial coffee grinder, with all metal cup and blade. Lyophilized tissue was placed in a chilled mortar and 5 mL/g of ice-cold methanol:chloroform:water (MCW; 60:25:15) added. The mortar was held in ice and the contents completely homogenized. 5 µmol of D-sorbitol was added as internal standard and the contents poured into a 10 mL plastic centrifuge tube. The pestle and mortar was washed with equal amounts of distilled water and added to the homogenate. This water addition also broke the MCW emulsion. The tubes were then centrifuged at 10,000 g for 10 min at 4°C. The clear upper methanol-water (MW) phase was removed and dried in a rotary evaporator (Speed Vac®, Savant SC11A) connected to a refrigerated condensation trap. After being re-dissolved in 500 µL of water the osmolytes were passed through a SepPak C_{18} cartridge (Waters Corporation) and injected into a High Pressure Liquid Chromatography system (Hewlett Packard LC1100) passing through a Waters Sugar-Pak I HPLC column maintained at 80°C. Column eluate passed into a diode array detector scanning every second from 190 to 400 nm at an interval of 1.2 nm. Optimum absorbency was attained at 192 nm. Standards of soluble sugars (sucrose, glucose, fructose) and other osmolytes (alanine betaine, glycine betaine, hydroxy-N-methyl-proline, methyl proline and proline) were analyzed in the same way to generate standard curves over a 10-fold concentration range. The mobile phase was bacteria free water containing 50 mg/L Ca-EDTA. To ensure that the mobile phase was gas free it was passed through an in-line degasser. Flow rate was maintained at 0.6 mL/min. The mobile phase is deionised, degassed (vacuum filtration through a Millipore HA 0.45 mm filter) and bacteria free Millipore water containing 5 mg/L Ca-EDTA. Prior to the initial use and after running about 200 samples, the column was reconditioned by passing 1 L of 500 mg/L Ca-EDTA solution. The column was then washed with the mobile phase (5 mg/L EDTA) until the base line was stable.

2.9 Amino acid analysis

Amino acid analyses were as described by Hernandez-orte (1997). Tissue samples were kept frozen by liquid nitrogen whilst being ground to a fine powder in a domestic coffee grinder. A sub-sample of 0.5 g was accurately weighed and 20 µL 0.1 M α-amino butyric acid was added as internal standard to be used for amino acid extraction by HPLC. Roughly 300 mg of PVP was added and free amino acids were extracted from the powdered tissue in 3 mL of 4:4:2 (v/v/v) methanol:chloroform:water on a rotating wheel at room temperature for 10 min. Samples were then centrifuged at 10000 g for 10 min and 1 mL of the supernatant was dried in a rotary evaporator (Speed Vac®, Savant SC11A) connected to a refrigerated condensation trap. The sample was then re-dissolved in 1 mL distilled water and passed through a SepPak C_{18} cartridge (Waters Corporation). After 100 µL of coupling buffer was added the samples were dried in the rotary evaporator again. Coupling buffer consisted of a mixture of acetonitrile:ethanol:triethylamine:water (10:5:2:3). After the sample was dry it was re-dissolved in 100 µL of coupling buffer and 5 µL of Phenylisothiocyanate (PITC) was added to derivatise the amino acids. The mixture was kept at 25°C for 20 min and dried again. When the samples were dry it was dissolved in solvent A and ready to be analyzed in the High Pressure Liquid Chromatography system (Hewlett Packard LC1100). Solvent A consisted of a 50 mM ammonium acetate buffer (pH 6.5) and solvent B consisted of 100 mM acetate buffer (pH 6.5) in acetonitrile:water adjusted with acetic acid. Separation was carried out using a 250 x 4 mm Exsil ODS C_{18} column (SGE, Adelab, Adelaide, South Australia) filled with silica spheres with a particle size of 3 µm. The inline pre-insert had the same characteristics. PITC-amino acids were separated using the following linear gradient: 0 to 45 min, the gradient goes from 0% to 70% of solvent B. It is kept at 70% for one minute, reaching 100% by 48 min. The entire run lasted for 60 min with the stabilizing time included. Column temperature was 50°C and detections were done at 254 nm.

2.10 Free polyamine analyses

Free polyamine analysis was done as described by Flores and Galston (1982). Crude extracts was prepared from tissue extracted in cold 5% $HClO_4$ at a ratio of about 100 mg fresh weight/mL $HClO_4$ for 1 hour in an ice bath. After centrifugation at 48,000 g for 20 min the supernatant fraction was used for polyamine analyses by HPLC. Samples stored at -20°C will remain stable for at least 2 months (Flores and Galston, 1982). Extracts were analyzed by HPLC according to the benzoylation procedure modified by Flores and Galston (1982). One mL of 2N NaOH was mixed with 250–500 µL of $HClO_4$ extract. After the addition of 10 µl benzoyl chloride, vortexing for 10 sec, and incubation for 20 min at room temperature, 2 mL saturated NaCl was added. Benzoyl-polyamines

were then extracted in 2 mL diethyl ether (anhydrous grade; Baker). After centrifugation at 1500 for 5 min, 1 mL of the ether phase was collected and evaporated to dryness under a stream of nitrogen and re-dissolved in 100 µL methanol (Baker; HPLC grade). Standards of putrescine, spermine, spermidine and cadaverine were treated in the same way with up to 50 nmol of each polyamine in the reaction mixture. Stored benzoylated samples are stable at -20°C for several months. Separation, identification and quantification were done with a High Pressure Liquid Chromatography system (Hewlett Packard LC1100) equipped with a 250 x 4 mm Exsil ODS C_{18} column (SGE, Adelab, Adelaide, South Australia) filled with silica spheres with a particle size of 3 µm. Benzoyl-polyamines are eluded with 64% methanol at a flow rate of 1 mL/min and detected at 254 nm.

2.11 Inorganic mineral analyses

Potassium (K), calcium (Ca), magnesium (Mg), Phosphorus (P) and sulphur (S) analyses were done on plant tissue that was dried in a forced air oven for at least 2 weeks at 60°C. All plant tissues were powdered in a commercial coffee grinder and samples of 0.25 g were weighed into open top 75 mL digestion tubes for plant digest (Zarcinas and Cartwright, 1983; Zarcinas *et al.*, 1983). A standard wheat sample of known mineral content and a blank sample were placed within the samples on a regular interval of 30 samples. After the addition of 3 mL concentrated Nitric acid (HNO_3) the digestion tubes were placed in a digestion block and an automatic digestion sequence initiated. The digestion sequence increased the block temperature in steps to 70°C for 60 min, 90°C for 180 min, 100°C for 30 min, 110°C for 30 min, 120°C for 30 min and 125°C for 210 min. The digest typically ran overnight and by the morning the samples had cooled. It was important that the sample had boiled down to approximately 1 mL before further analysis (to reduce the amount of acid in the final volume). The digested samples were then diluted to 20 mL with distilled water and vortexed for 10 sec. Root samples that may have contained silica were filtered through Whatman no. 42 filter paper. The supernatants were then drawn off into an ICP for optical emission spectrometry (CSIRO Div. Soils).

2.12 Starch analysis

Leaf and shoot samples (500 mg) were washed with 5 mL 80% ethanol and centrifuged at 3000 g for 10 min. The supernatant was decanted and the pellet rewashed. The pellet retained was oven-dried (60°C) and after the addition of 2 mL 0.5N NaOH samples were boiled for 5 min. The samples were then neutralized with 0.5 N acetic acid to a pH of 6.5 and centrifuged at 3000 g for 10 min. The supernatant was taken and 100 units of α-amylase (in MOPS buffer, pH 6.5) added and incubated for 1 h at 30 °C in a water bath. The pH was then adjusted to 5.1 with Na-acetate buffer, 100 units of aminoglucosidase added and incubated for 1 h at 30 °C. The solution was cooled to

room temperature and 50 µL taken to be analyzed for glucose. Glucose is analyzed by the addition of 950 µL GOPOD reagent and incubated for 30 min at 30 °C.

GOPOD reagent consisted of a mixture of 3.45 g $Na_2HPO_4 \cdot H_2O$, 1.6 g $NaH_2PO_4 \cdot H_2O$, 2350 U Glucose oxidase, 375 U Peroxidase and 125 mg ABTS in 250 mL distilled water. The samples were then transferred to spectrophotometer cuvettes, diluted with 1 mL distilled water and the absorption read at 436 nm. Standard absorption curves were created using cornstarch and the same method. The measured absorption in samples was then compared to standard absorption curves and the amount of starch calculated in the original sample.

2.13 Enzyme assays

2.13.1 Sucrose Synthase (SucSy) activity

Enzymes were extracted with a modified crude enzyme extraction method from (Claussen *et al.*, 1985). During the whole procedure all samples were kept in ice when not used. An average of 1 g fresh weight sample (powdered in liquid N) was extracted in 5 mL ice-cold extraction medium and 0.3 g PVP (berry tissue only). The extraction medium consisted of 0.1 M Tris HCL, 0.1 M Cysteine, 10 mM MgSO4, 3% w/v Carbowax 4000 and 2.5 mM DTT. The slurry was then filtrated through 1 layer of Miracloth and centrifuged at 2°C for 10 min at 50000 g. Thereafter 400 µL of the supernatant was desalted on a Sephadex G25 column (1 x 1.5 cm) equilibrated with 5 mM MOPS buffer (pH 7.4) and 0.01% BSA (Helmerhorst and Stokes, 1980). Enzyme assays were immediate conducted after extraction. To create a 'zero' sample, 150 µL of the crude enzyme extract was boiled for 4 min.

Sucrose synthase (SucSy) activity was assayed using 50 µL of the desalted enzyme extract and adding 200 µL reaction mixture (Claussen *et al.*, 1985). The final reaction mixture contained 30 µM Tris HCL (pH 8.7), 3 µM Fructose and 5 µM MgSO4 in both active enzyme and zero samples. To initiate the reaction 50 µL of 1 µM UDPGlucose was added to the active samples or 50 µL H_2O in the zero samples. At this time 200 µL 0.2 M NaOH was added to the zero samples. All samples were then incubated in a water bath for 60 min at 30°C. At the end of 60 min the reaction in the active enzyme samples was stopped by the addition of 200 µL 0.2 M NaOH. All samples were then closed and boiled for 10 min to destroy Fruc-6-P. After cooling, 100 µL was taken to determine the formation of sucrose by adding 250 µL 0.1% Resorcinol in 95% ethanol and 750 µL 30% HCL. The mixture was incubated for 8 min at 80°C. After cooling for 40 min the A_{520} was measured and compared to standard absorption curves of sucrose content.

2.13.2 Invertase activity

Soluble invertase activity was assayed on the same filtered and desalted crude extract for SucSy analysis, using the modified method described by Zhu *et al*. (1997). Acid invertase (AI, pH 4.5) activity was assayed using 25 µL of the desalted enzyme extract and adding 25 µL 1 M Na-acetate (pH 4.5). After the addition of 50 µL 120 mM Sucrose the mixture was incubated for 60 min at 37°C. Zero samples were done in the same manner as active enzyme samples. Adding 35 µL 2.5M Tris base and boiling the capped samples for 3 min stopped the reaction. Before the measurement of the amount of glucose formed the sample was dissolved by adding 1 mL H_2O.

Neutral invertase (NI, pH 7.5) activity was assayed using 25 µL of the desalted enzyme extract and adding 25 µL 1 M Na-acetate (pH 7.5). After the addition of 50 µL 120 mM Sucrose the mixture was incubated for 60 min at 37°C. Zero samples were done in the same manner as active enzyme samples. Boiling the capped samples for 3 min stopped the reaction. Before the measurement of the amount of glucose formed the sample was dissolved by adding 1 mL H_2O.

The amount of glucose formed was measured using 25 µL of the sample and 450 µL of GOPOD reagent. GOPOD reagent consisted of dissolving 3.45g $Na_2HPO_4 \cdot 2H_2O$, 1.6g $Na_2HPO_4 \cdot H_2O$, 2350 U Glucose oxidase, 375 U Peroxidase and 125mg ABTS in 250 mL distilled water. The mixture was incubated at 30°C for 30 min in a water bath, dissolved with 500 µL distilled water and the A_{436} was measured and compared to standard absorption curves of glucose content.

2.14 Total carbon and nitrogen analyses by dry combustion

Carbon and nitrogen analyses were done by Penny Day (Soil and Land Systems, Roseworthy Campus, University of Adelaide) using the method described in Rayment and Higginson (1992). All plant material was dried in a forced air oven at 60°C for at least 2 weeks and ground to at least 2 mm sieve size. A LECO CN2000 with a furnace temperature of 1200 °C was used.

2.15 Automatic weather station

Meteorological data were recorded at 15 min intervals with a solar powered automatic weather station (Measurement Engineering Ltd., Adelaide, South Australia) located in the Alverstoke vineyard, 400m from the Coombe vineyard. Data recorded included relative humidity (RH), rainfall, air temperature, solar-radiation and wind speed.

2.16 Growing degree days

Growing degree-days were calculated using the method of (Williams *et al.*, 1985). MS Excel© worksheets were used to subtract 10°C (minimum threshold) from each of the 15 min logging events of average air temperature. If the result was positive the number was divided by 96 (15 min = 1/96 of a day), and then all of the 96 values were summed at the end of each 24 h period. Growing degree days were then expressed as a cumulative value starting from budbreak until harvest.

2.17 Evapotranspiration (ET_o)

Reference crop evapotranspiration (ET_o) was estimated on a daily basis using average data inserted into the Penmann-Monteith evapotranspiration equation. ET_o was calculated using an Excel® calculator provided by Measurement Engineering Australia (Grayson *et al.*, 2000) that can be used in conjunction with their Magpie® software for weather stations.

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma\frac{900}{T+273}U_2(e_a - e_d)}{\Delta + \gamma(1 + 0.34U_2)}$$

where...
ETo = reference crop evapotranspiration [mm/day]
Rn = net radiation at crop surface [MJ/m^2/day]
G = soil heat flux [MJ/m^2/day]
T = average daytime temperature [degC]
U2 = average daytime wind speed measured at 2m height [m/sec]
(ea-ed) = vapor pressure deficit [kPa]
delta = slope of vapor pressure curve [kPa/degC]
gamma = psychometric constant (= 0.66) [kPa/degC]
900 = conversion factor

2.18 Statistical analyses

Statistical analyses were done using the Microsoft® Excel 2000 Data Analyses Toolpack. Results were analyzed using ANOVA and student T-tests to determine which groups were significantly different. Significance values are indicated by the P-values. Correlation and factorial analyses were performed using the SAS System (SAS Institute Inc., Cary, North Carolina, USA).

Chapter 3: Summary of weather conditions 2000-2004

Based on average monthly temperatures over the ripening period for January to March (Table 3.1) the season of 2000/1 was the warmest of the three years and the 2001/2 season the coolest. Due to instrument failure the 2002/3 season is missing some values, however some data was substituted using measurements made in Kent Town (Adelaide, South Australia, 34.92 S lat., 138.62 E long., 48m elev.) obtained from the Bureau of Meteorology (Meteorology, 2004). Unfortunately, only the rainfall data for the months of October, November and December during the 2002/3 season was available. The difference between seasons was evident in measured average maximum and minimum (Appendix B) temperatures. The 2000/1 and 2002/3 seasons had much higher maximum and minimum monthly temperatures from October onwards as well as average solar radiation (Appendix B). This greatly increased the accumulation of growing degree days (GDD) in the 2000/1 season compared to the 2001/2 season (Figure 3.1) from about 50 days after bud-burst onwards. There was a near linear increase in GDD after this time and by day 80 the accumulation rate increased even further in the 2000/1 season. By 180 days after bud-burst (around harvest time) about 434 more GDD had accumulated in 2000/1 compared to 2001/3.

Table 3.1 Growing season monthly average temperature (°C) 2000-2003 of the Waite campus. Due to instrument failure the 2002/3 season is missing some values.

	2000/1	2001/2	2002/3
September	14.6	15.5	14.2
October	15.7	13.7	16.3
November	17.9	16.7	20.3
December	21.7	17.4	22.0
January	25.4	20.9	25.3
February	25.1	20.4	22.4
March	19.8	19.9	18.3
Average Jan - Mar	23.4	20.4	22
Average Sept - Mar	20	17.8	19.8

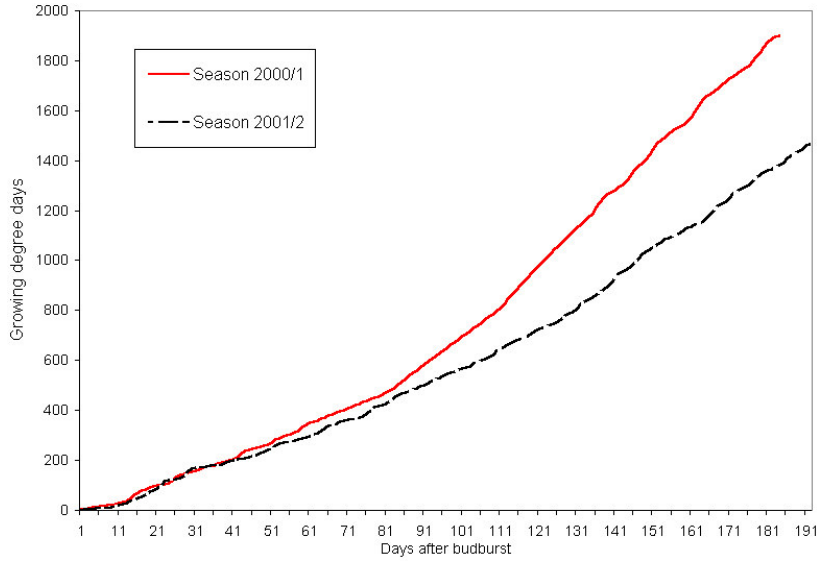

Figure 3.1 Cumulative growing degree-days after budburst for two growing seasons of the Waite campus.

Not only was 2000/1 the hottest but also the driest based on average monthly humidity (Appendix B). The 2000/1 season had lower average humidity than the next two seasons during the majority of the growing season. The warmer conditions during the 2000/1 season compared to the following seasons are reflected in data for monthly evaporation (Table 3.2). The 2000/1 season had much higher monthly ET_o than the following season; 46% higher in total. However, 2000/1 also had the highest precipitation of the three seasons (Table 3.3) during the growing period although most of it fell in March after the grapes were harvested. In comparison, most of the rain in the 2001/2 and 2002/3 seasons fell early in the growing season. All three seasons were characterized by low seasonal rain during the ripening period (middle January to early March). In comparison to the historical average, all of the three seasons were extremely dry with less than 50% of average precipitation in 2001/2 and 2002/3 and 60% in 2000/1.

Table 3.2 Growing season monthly ET_o(mm) of the Waite campus. Due to instrument failure the 2002/3 season is missing some values.

	2000/01	2001/02	2002/03
September	29.3	23.0	29.2
October	56.2	30.1	35.2
November	85.0	73.5	82.0
December	135.3	95.8	110.1
January	125.4	100.9	108.6
February	122.1	80.3	85.9
March	127.2	55.3	75.9
Total	680.3	465.8	526.9

Table 3.3 PRD period monthly effective rainfall (mm) 2000-2003 of the Waite campus (Effective rain is classified as precipitation more than 2 mm).

	2000/1	2001/2	2002/3	Long term avg.
October	15	35.6	22.4	51
November	9.2	23.2	31	33
December	13.4	7	9	28
January	18.6	8.8	13	22
February	8	0	0	22
March	45.2	12.2	7.2	26
Total	109.4	86.8	81.2	182

Chapter 4: Effects of partial rootzone drying on water use efficiency and the reduction of shoot growth and canopy density

4.1 Introduction

In low rainfall conditions such as in much of Australia, South Africa, Israel, western USA and South America, the cultivation of wine grapes rely heavily on irrigation practices and good viticultural management to establish new vineyards and maximise crop yield. Grapevine productivity and carbon assimilation is influenced greatly by its canopy architecture and density (Smart *et al.*, 1990). Densely shaded canopies caused by excessive vigour may result in the depression of fruitfulness by reducing inflorescence initiation, set and berry growth (Winkler *et al.*, 1974). In turn, the reduction in fruit weight may stimulate vegetative growth due to changes in assimilate partitioning. The imbalance in growth is detrimental to the canopy architecture and may have negative effects on wine quality (Jackson, 1986; Smart *et al.*, 1990). Therefore, much of the labour expended in the cultivation of grapevines is to maintain a proper balance of desirable vigour without diminishing the crop. A few methods exist in modern viticulture to attain the desirable vigor. These methods entail the use of chemical growth regulators, rootstocks, root restriction, pruning practices and reduced water supply via irrigation management. While any of these practices may reduce vigor the most useful and practical method is reducing the water supply.

With the ever-increasing demand of urbanization and industrialization on a finite water resource and the constant increase of hectares of planted grapevines, water is becoming an increasingly scarce and expensive commodity. Increasing a vineyard's water use efficiency (WUE), the amount of dry matter produced per unit of water applied, seems important to decrease costs of production and to sustain viticulture in areas of low rainfall. An efficient way to increase WUE in grape production is by the use of partial rootzone drying (PRD), designed to decrease the irrigated water without penalizing crop yield.

Much of the previous PRD work was done by applying considerably less water compared to control irrigation. This raised the question if the PRD effect is independent of the amount of irrigation water applied or would a simple reduction in irrigated water have the same effect. It was already shown in field experiments by Stoll (2000) that at low application rates of the same amount of irrigation water, control vines were experiencing stress whereas PRD vines were not. PRD outperformed control grapevines with heavier pruning weights and crop yield. It was concluded that PRD-treated vines were

more tolerant of water stress and made more efficient use of available water. Similarly, de la Hera Orts *et al.* (2002) have also shown that PRD can significantly improve the WUE in grapevines that received the same amount of irrigated water as control vines by producing a higher yield per volume of water.

The experiments described in this chapter were conducted to test the hypothesis that *PRD increases water use efficiency and reduces stomatal conductance, shoot growth and canopy density irrespective of the amount of irrigation water applied.*

4.2 Materials and methods

PRD field experiments were conducted in the Coombe and Alverstoke vineyards at the Waite campus of the University of Adelaide and the SARDI experimental vineyard at the Nuriootpa Research Station (Barossa Valley, South Australia) as described in Section 2.1.

4.2.1 Field experiments where PRD received half the amount of control irrigation

a) Mature Shiraz vines were used that grew at the Nuriootpa Research Station (Barossa Valley, South Australia) and were irrigated with a 50:50 mixture of mains and bore water. Bore water has a higher salinity than mains and would be unsuited to be used on its own. Therefore a mixture of mains and bore water was used to reduce the salt content of the irrigated water. The vines were trained to a single wire trellis system and allowed to sprawl. Drip emitters (2 L/h) were installed in the planting line 0.4 m from the trunk of each vine on either side. PRD vines received half the amount of water of control vines. Experimental plots consisted of four vines where the centre two vines were used for analysis and the two border vines as buffers. Experimental design consisted of a total randomised treatment layout spanning over three rows (Appendix C). Treatments consisted of two irrigations, PRD and control, and three pruning levels (30, 60 and 120 nodes/vine) with 5 replications of each treatment.

b) Cabernet Sauvignon vines in the Coombe vineyard were trained to a vertical shoot positioning (VSP) trellis system and in all of the three seasons were positioned manually between the foliage wires three weeks after flowering. Roughly five and nine weeks after flowering all vines were mechanically trimmed and netted for bird protection with the onset of veraison. Cabernet Sauvignon vines were irrigated with 2L/h drip emitters 0.3 m from the trunk. PRD received water on only one side at any

Chapter 4: Effects of partial rootzone drying on water use efficiency and the reduction of shoot growth and canopy density

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concluded that PRD-treated vines were more tolerant of water stress and made more efficient use of available water. Similarly, de la Hera Orts *et al.* (2002) have also shown that PRD can significantly improve the WUE in grapevines that received the same amount of irrigated water as control vines by producing a higher yield per volume of water.

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veraison. Cabernet Sauvignon vines were irrigated with 2L/h drip emitters 0.3 m from the trunk. PRD received water on only one side at any given time while water was withheld from the other side. Control vines received water on both sides amounting to 4 L/h. No means of soil moisture measurement was available in the Coombe vineyard in 2001, so the amount of water to be used was predetermined from an average commercial water usage for viticulture in this climatic region (Stoll, 2000). However, in 2001/2 and 2002/3 the soil moisture was measured using a Diviner 2000®, (Sentek, Adelaide, South Australia) as described in Section 2.3. Irrigation management was done manually during the 2000/1 season, but was replaced by a Galcon 7001D computerized irrigation control unit (Plasflo, Adelaide, South Australia) in 2001/2 and 2002/3. When necessary, additional applications of water were done to ameliorate the soil moisture content to soil water capacity. This was only done when the normal scheduled irrigations failed to increase the soil water volume to this level. On average, irrigation was done 3 times a week for 3 h, pulsed with 1.5 h in the morning (06:00) and 1.5 h in the late afternoon (17:00). Experimental design consisted of a randomized block design with two treatments, control and PRD irrigation, and 7 replicates within one row. Each plot consisted of three grapevines and data were only collected from the centre grapevine thereby leaving 2 buffer grapevines between each treatment. Grapevines were pruned to leave 30 nodes/kg winter pruning weight and bunch thinning was done every season before flowering; aiming for 60 bunches per grapevine.

4.2.2 Field experiments where PRD received the same amount of water as control.

a) Shiraz vines were used that grew right next to the row of Cabernet Sauvignon in the Coombe vineyard. Both cultivars were planted in 1992 and the climatic and soil conditions between the two cultivars were regarded to be the same during the three years of the experiment. Pruning, training and vineyard management were also done in the same manner as above. The only difference was in the amount of irrigation water applied in addition to annual rainfall. Shiraz vines were irrigated with 2 L/h and 4 L/h drip emitters 0.3 m from the trunk. PRD received 4 L/h of irrigation water on only one side at any given time while water was withheld from the other side. Control vines received water on both sides amounting to 4 L/h. Irrigation of Shiraz was applied and monitored similarly to Cabernet Sauvignon above. Experimental design consisted of a randomized treatment layout in one row with two treatments, PRD and control, and 7 replicates.

b) Cabernet Sauvignon vines in the Alverstoke vineyard that were planted in 1997 in a trench filled with a sandy soil and with roots divided by a plastic membrane. The vines were trained to VSP trellis system and allowed to sprawl. Experimental design consisted of randomised treatment plots within one row. There were 6 replicates of three treatments (Control, PRD and girdled). Girdling was done shortly after set, before irrigation started, by removing ± 3 mm strip of bark from the trunk with a double-bladed knife. Vines were irrigated with drip emitters, either on both sides of the membrane at 1 L/h (control) or only on one side at any time at 2 L/h (PRD). The consequence is that all vines received the same amount of irrigation water throughout the growing season. The flow rates of the drip emitters were checked before irrigation started and in the middle of the growing season. Drip emitters deviating more than 20% from specification were replaced. The irrigation was scheduled according to soil moisture measurements made by EnviroSCAN® (Sentek, Adelaide, South Australia) probes as described in Section 2.3. PRD irrigation started when significant soil drying was observed in the soil moisture content curve produced by the EnvironSCAN® system. In order to maintain an adequate water supply to both control and PRD grapevines the summed soil water content of top 700 mm in the 'wet' zone was never allowed to fall below between 60 and 70 mm soil water content, referred to as refill point 1 (Figures 4.1 and 4.2) in both PRD and control vines. When refill point 1 was reached, the vines were irrigated until field capacity was reached roughly at 150 mm. This point was identified by the change of the very steep slope of the drying curve after re-watering at the top of the graph, indicating a slowed leaching rate. The PRD cycle was achieved by switching the wetting zones as soon as the summed soil water content in the 'dry' zone reached refill point 2. Refill point 2 is an arbitrary value between 45 and 50 mm where the slope of the graph of the soil water content in the 'dry' zone flattens to indicate a low rate of soil water extraction. As shown in Figure 4.2, the 'wet' zone of the PRD system was irrigated when the soil water content reached refill point 1. Refill point 1 corresponded roughly to the refill point calculated in a normal irrigation regime and therefore the 'wet' zone constituted a normal irrigation regime. The length of one PRD cycle varied between 10 to 15 days depending on weather conditions and seasonal growth. During the first irrigation of each new PRD cycle 20% to 30% more water was applied compared to other irrigations during a cycle to replenish the former 'dry' side. The amount of water applied was measured by a water flow meter placed in each irrigation line.

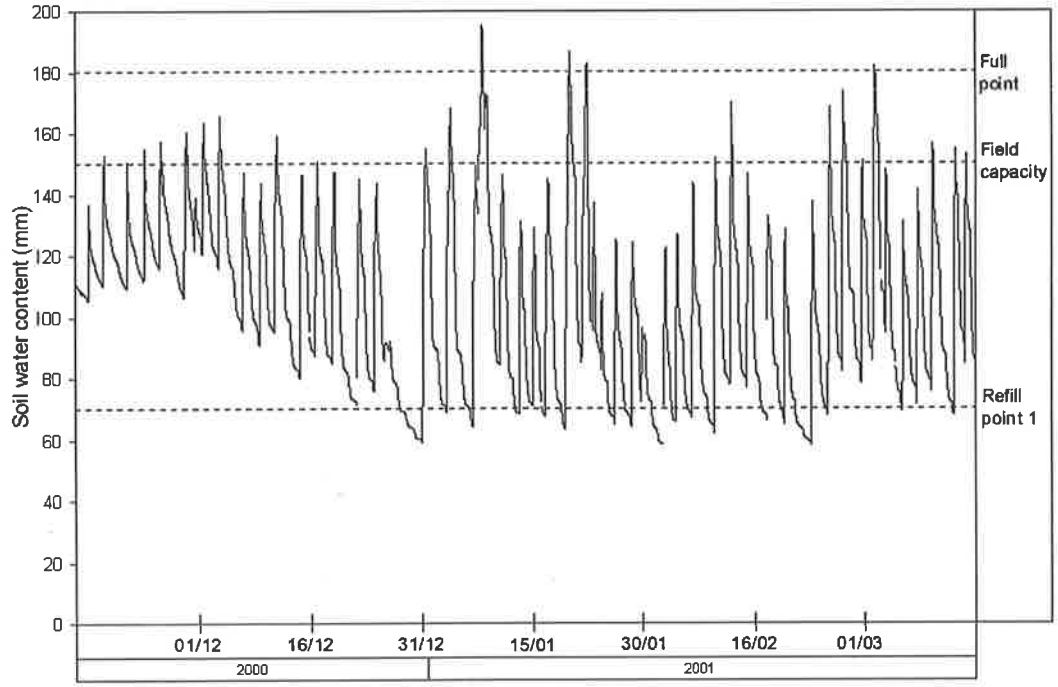


Figure 4.1 Soil water content (mm) of control irrigation of Cabernet Sauvignon in the Alverstoke vineyard measured at 0–700 mm depth by EnviroSCAN® during the 2000/01 season.

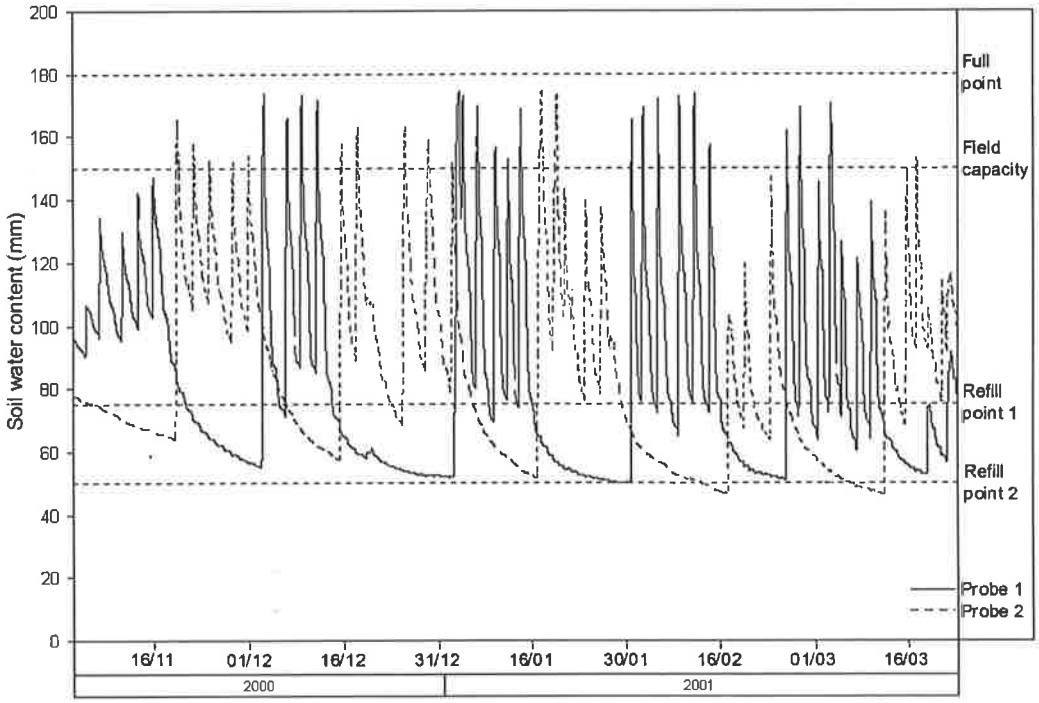


Figure 4.2 Soil water content (mm) of PRD irrigation of Cabernet Sauvignon in the Alverstoke vineyard measured at 0–700 mm depth by EnviroSCAN® during the 2000/01 season. (Two lines represent the data collected from different sides of the PRD vine)

4.2.3 Pot-grown Cabernet Sauvignon

Three-year old split-rooted Cabernet Sauvignon grapevines were used that were grown in two 3 L pots filled with a standard potting mix (Section 2.2). The bottom of the pots were cut out and replaced with plastic mesh to allow roots to grow through into two second 3 L pots filled with vermiculite. This allowed easy access to harvest clean and new roots. The split-rooted grapevines were grown in a temperature-controlled greenhouse at the CSIRO Horticultural unit (Waite campus, Adelaide). The vines were allowed have 5 shoots and to bear 3 bunches. In all PRD experiments irrigation water was applied either to one side of the root system at any time (PRD) or to both sides (Control) as described in Section 2.1. Soil moisture was measured in potted experiments by the Model 6050X1 Trase system (Soil moisture equipment corp., California, USA) that uses Time Domain reflectometry (TDR) to measure instantaneously the volumetric water content of the soil (Section 2.3).

4.2.4 Measurement of yield and harvest parameters

Methods of harvesting fruit and measuring total soluble solids (TSS; °Brix) and pH are described in Section 2.7.4.

4.2.5 Stomatal conductance

Stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day.

4.2.6 Leaf area and canopy measurements

Leaf area (m^2) in the Nuriootpa Shiraz and Alverstoke Cabernet Sauvignon was assessed after a subset of five whole shoots was harvested from buffer vines. Main shoots were randomly chosen within the canopy that had 12 or more internodes. The shoot lengths and the lengths of all leaf midrib were then recorded. Using the leaf midrib lengths, the leaf area for every leaf was then calculated using a leaf length: leaf area formula specific to Shiraz and Cabernet Sauvignon vines; $y = 0.798x^2 + 3.63x$ (acquired from Assoc. Prof. Peter Dry). The leaf areas were then summed to produce the total leaf area per shoot. Plotting the total leaf area to shoot length for all harvested shoots per treatment produced an equation to calculate leaf area using any known shoot lengths. To calculate mean shoot length of experimental vines, five shoots

chosen at random were measured per plot to produce the mean shoot length per treatment. After the shoot count the average canopy size was then calculated by multiplying the shoot number per vine with average leaf area per shoot for any given length.

Light penetration in the Coombe vineyard was determined with a ceptometer (DELTA-T Devices, Cambridge, UK) just before harvest during the 2002 season. The ceptometer was inserted perpendicular to the planting line within the bunch zone through the upwardly positioned shoots. Three readings were taken on every 'test' vine at locations evenly distributed along the vine cordon. The ceptometer was configured to average readings from all sensors.

4.2.7 Shoot growth rate

Shoot measurements started when PRD irrigation commenced at the end of November until active shoot growth stopped in the beginning of January. Shoot growth rate was measured by selecting a reference node at five to seven nodes below the shoot tip. It was labeled and the distance between the reference node and the shoot tip was measured at intervals of seven days. Shoot growth rate (cm/day) was calculated as the average increase in shoot length since the previous measurement. When a shoot stopped growing, that shoot was discarded from the pool and measurements continued on only the remaining shoots. Therefore shoot growth rate was representative of actively growing shoots. In some cases, shoots were replaced after the shoot tip was damaged by wind or machinery.

4.2.8 Pruning weights

Coombe Cabernet Sauvignon and Shiraz vines were spur pruned during winter dormancy according to mass pruning weight of the 'test' vine. After the shoots ('canes') were cut to two node spurs they were counted and weighed on site with a hand-held scale. The average shoot weight was calculated by dividing the total pruning weight by the number of shoots. The remaining nodes per vine were then adjusted to a ratio of 30 nodes per kg pruning weight by thinning out the remaining spurs per vine. The fruit weight to pruning weight ratio was then calculated by dividing the total crop yield by the total amount of pruning weight.

Dry weights of summer hedging were calculated after the clippings were collected directly after mechanical hedging. Fresh weights of shoots per plot were recorded and divided by the number of vines per plot. The total fresh weight per vine was then multiplied by a factor of 0.21 to convert to total dry weight per vine. Winter pruning weights were multiplied by a factor of 0.45

to convert fresh weight to dry weight (Values were acquired from Assoc. Prof Peter Dry that were derived from his earlier work).

4.2.9 Leaf and stem water potentials

Leaf water potential was measured on fully matured leaves and removed with a single cut across the petiole with a razor blade. To minimize transpiration during the procedure, the leaves were wrapped in a polyethylene bag shortly before removal. Leaf water potential was measured by placing the leaf, wrapped in the bag, into a pressure bomb (Scholander *et al.*, 1965) attached to a nitrogen gas cylinder. The time elapsed from severing the petiole until placing the leaf in the chamber did not exceed 10 seconds. Pressure was increased slowly until exudation of xylem sap from the cut end of the petiole was observed under a magnifying glass. Stem water potentials were measured in a similar way to leaf water potentials except that leaves were covered with plastic bags impenetrable to light for at least 6 hours. The plastic bags were black on the inside and white on the outside and kept folded at the petiole by a paper clip until the leaf was removed for measurement.

4.3 Results

4.3.1 Effects of PRD on shoot growth

To determine the effect of PRD on vegetative growth, the shoot growth rate was monitored in the field experiments during the 2000/1 and 2001/2 growing seasons. Shoot growth measurements began when irrigation started between full bloom and set and continued on a weekly basis until shoot growth slowed to a very low rate. Growth rate is reported as an average increment in shoot length (cm/day) measured on a weekly basis and as an 'accumulated' shoot length (cm) i.e. the summed shoot length since measurements began.

Coombe Cabernet Sauvignon and Shiraz

The effect of PRD on Cabernet Sauvignon and Shiraz main and lateral shoot growth in the Coombe vineyard during 2000/1 is shown in Figure 4.3 and Figure 4.4 respectively. PRD in Cabernet Sauvignon significantly decreased shoot growth rate in the 2000/1 growing season (Figure 4.3) when irrigated with half the amount of water as the control, amounting to a 34% decrease in accumulated main shoot length and a 74% decrease in accumulated lateral shoot length. On average the PRD shoot growth rate (cm/day) in Cabernet Sauvignon was reduced by

34% and 57% for main and lateral shoots respectively compared to control. Although not significant (Figure 4.4), Shiraz PRD grapevines receiving the same amount of water as control grapevines showed a 20% decrease in accumulated main shoot length and a 33% decrease in accumulated lateral shoot length compared to control. On average, the Shiraz PRD shoot growth rate (cm/day) was reduced by 18% and 16% for main and lateral shoots respectively compared to control.

The effect of PRD on Cabernet Sauvignon and Shiraz main and lateral shoot growth during 2001/2 is shown in Figure 4.5 and Figure 4.6 respectively. PRD decreased the accumulated shoot length of Cabernet Sauvignon by 14% in main shoots and 24% in lateral shoots compared to control. On average, the PRD shoot growth rate (cm/day) of Cabernet Sauvignon in 2001/2 was reduced by 12% and 22% in main and lateral shoots respectively compared to control. Shiraz PRD grapevines that received the same amount of water as control grapevines showed no significant decrease in accumulated main shoot length ($P=0.608$) or in accumulated lateral shoot length ($P=0.286$) compared to control. On average, the Shiraz PRD shoot growth rate (cm/day) was reduced by 8% and 19% for main and lateral shoots respectively compared to control.

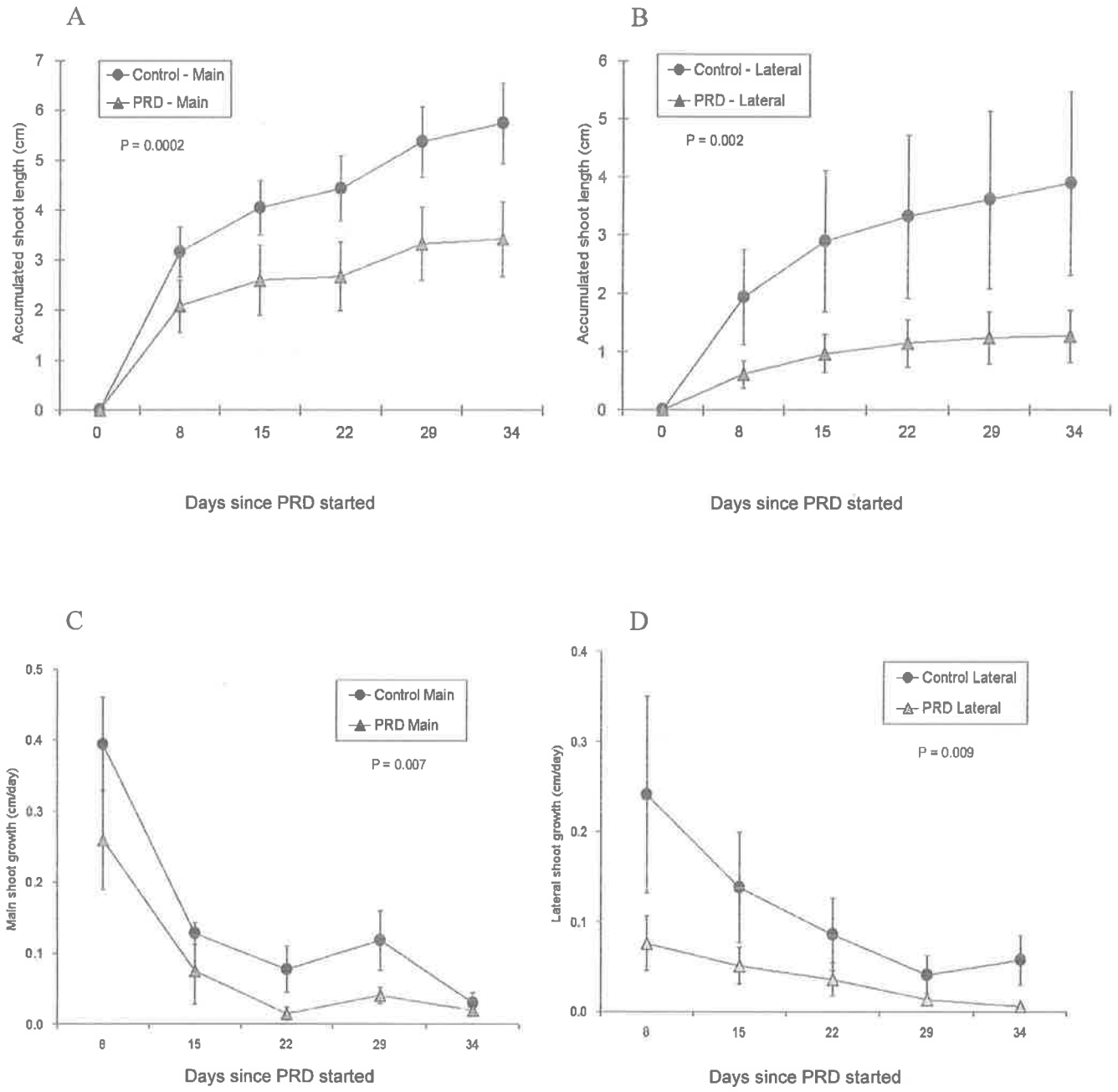


Figure 4.3 PRD effect on shoot growth in Waite Cabernet Sauvignon during the 2000/1 season. **(A+B)** accumulated and **(C+D)** daily shoot growth rates of actively growing main and lateral shoots. PRD received half the amount of water as control. (means n=7; average of 5 measurements per plot; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

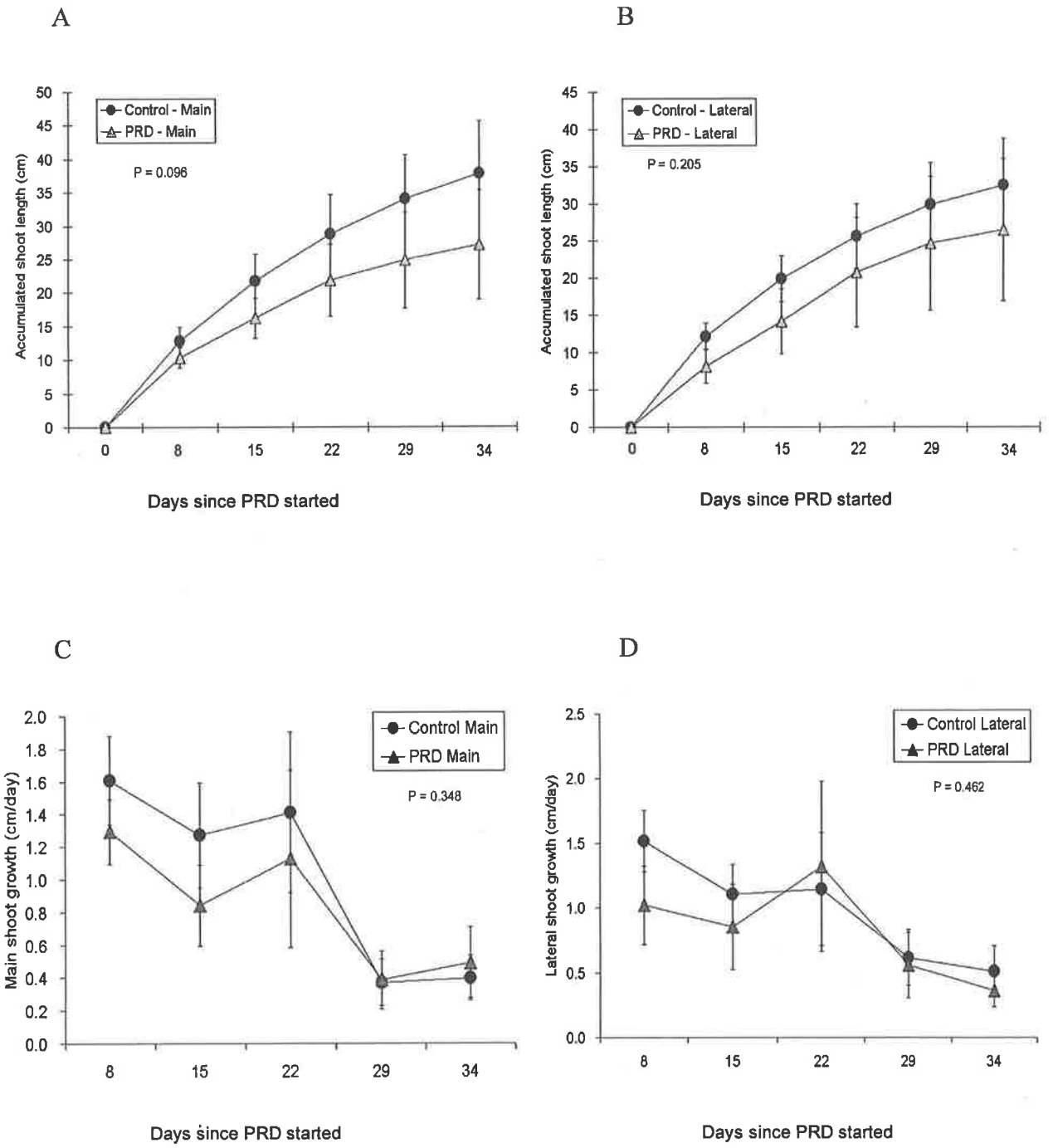


Figure 4.4 PRD effect on shoot growth in Waite Shiraz during the 2000/1 season. **(A+B)** accumulated and **(C+D)** daily shoot growth rates of actively growing main and lateral shoots. PRD received the same amount of water as control. (means n=7; average of 5 measurements per plot; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

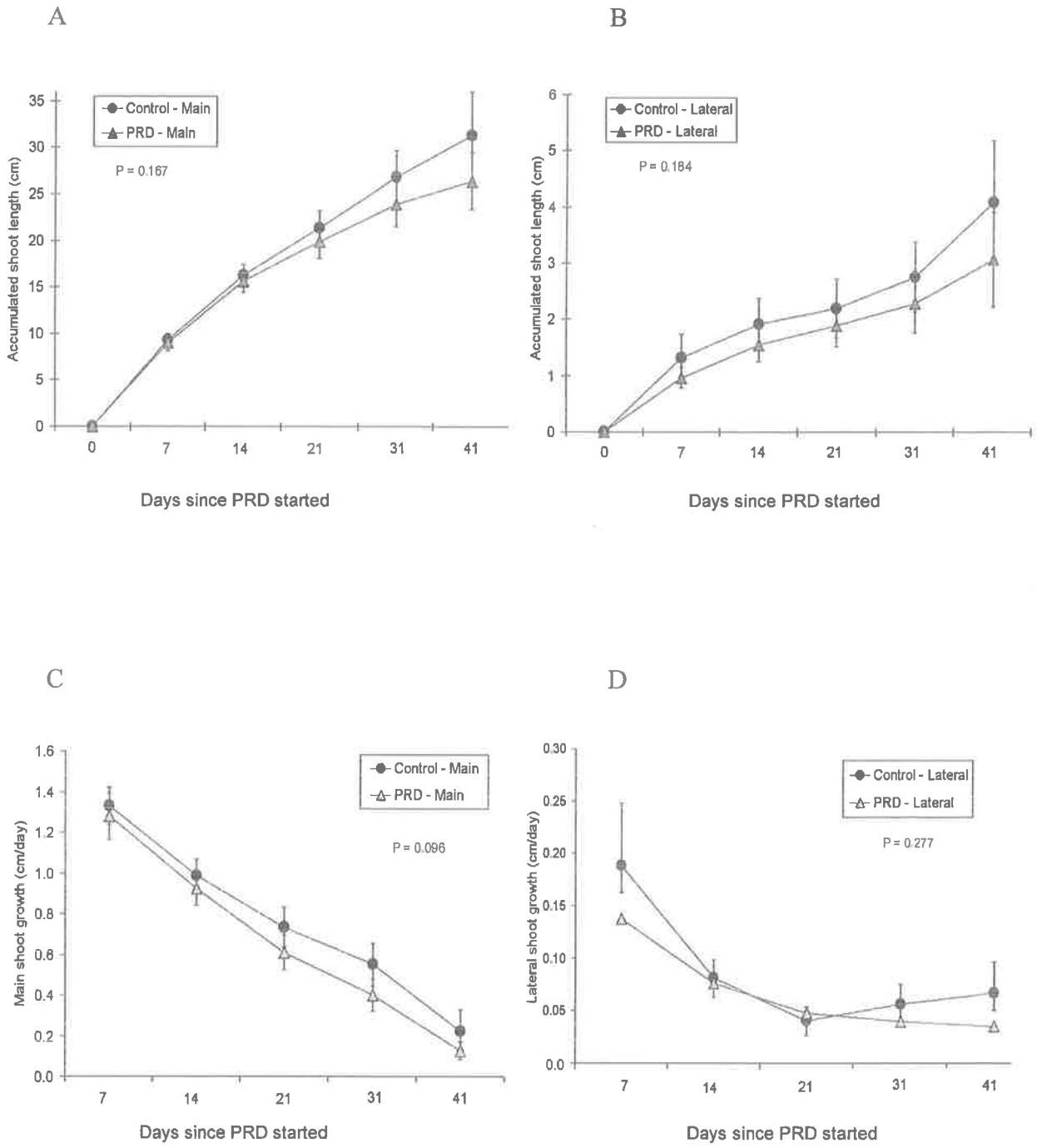


Figure 4.5 PRD effect on shoot growth in Waite Cabernet Sauvignon during the 2001/2 season. **(A+B)** accumulated and **(C+D)** daily shoot growth rates of actively growing main and lateral shoots. PRD received half the amount of water as control. (means n=7; average of 5 measurements per plot; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

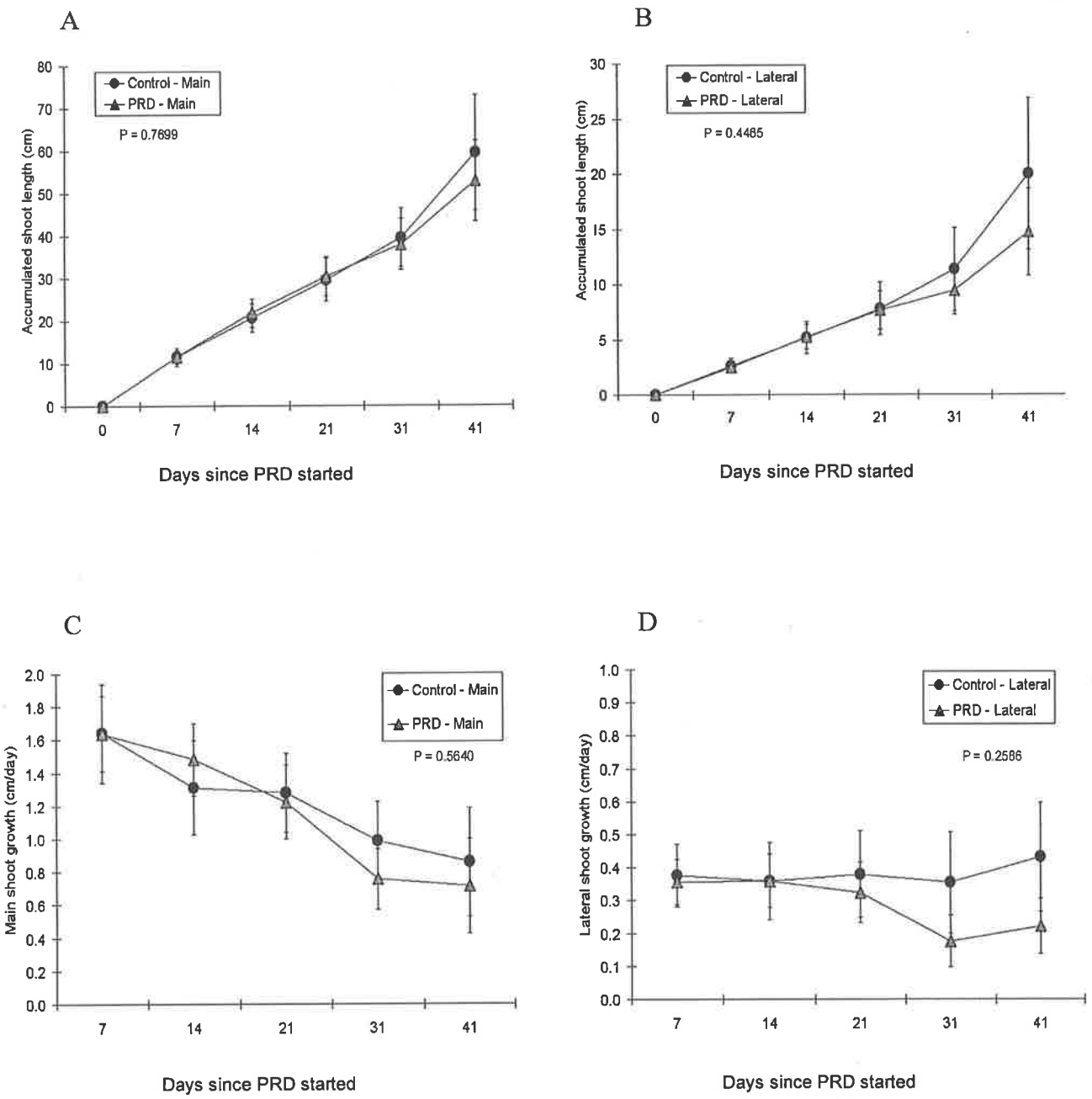
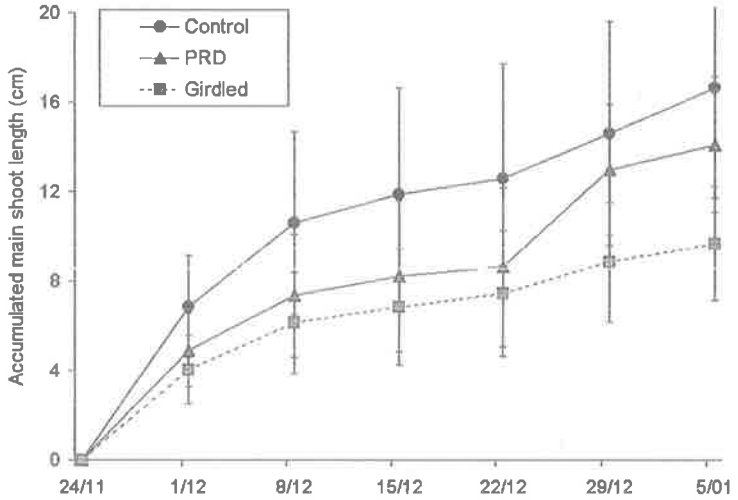


Figure 4.6 PRD effect on shoot growth in Waite Shiraz during the 2001/2 season. **(A+B)** accumulated and **(C+D)** daily shoot growth rates of actively growing main and lateral shoots. PRD received the same amount of water as control (means n=7; average of 5 measurements per plot; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

Alverstoke Cabernet Sauvignon

The effect of PRD and girdling on Alverstoke Cabernet Sauvignon grapevines is shown in Figure 4.7. PRD and girdled vines received the same amount of irrigation as control. Both PRD and girdling reduced the accumulated shoot length by 15% and 42% respectively, compared to control. On average the main shoot growth rate (cm/day) in PRD and girdled Cabernet Sauvignon was reduced by 14% and 32% respectively, compared to control. However, the differences were not large enough to be statistically significant.

A



B

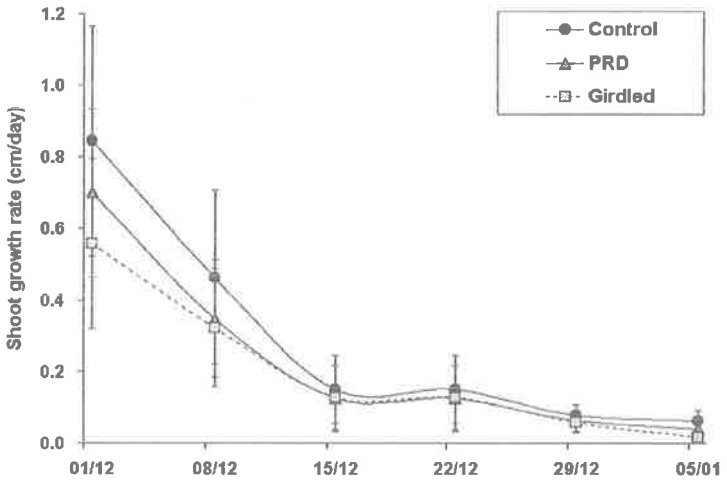


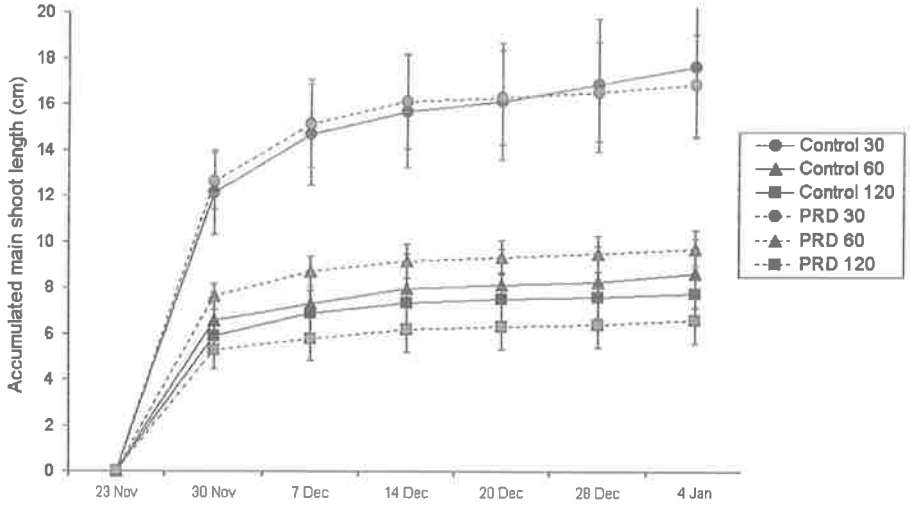
Figure 4.7 PRD and girdling effects on active shoot growth of Alverstoke Cabernet Sauvignon in 2001/2. **(A)** Accumulated shoot length (cm); **(B)** Shoot growth rate (cm/day). PRD started on the 24/11/01 and received the same amount of water as control. (means n = 6; average of 5 measurements per plot; Vertical bars indicate standard errors of the average)

Nuriootpa Shiraz

The effect of PRD and pruning level on Nuriootpa Shiraz grapevines is shown in Figure 4.8. PRD Shiraz vines received half the amount of irrigation water as control vines. There was no main effect of irrigation treatment on accumulated shoot length of Shiraz at Nuriootpa (P=0.8355). PRD therefore did not affect the accumulated shoot length at any pruning level, compared to control. Although a significant main effect of pruning level on accumulated shoot length (P=0.0001) existed with 30 nodes/vine having significantly higher accumulated shoot length than 60 and 120 nodes, there was no interaction between irrigation and pruning level on accumulated shoot length (P=0.8047).

There was also no main effect of irrigation treatment on shoot growth rate of Shiraz at Nuriootpa (P=0.7346). PRD therefore did not affect the shoot growth rates at any pruning level, compared to control. Although a significant main effect of pruning level on initial shoot growth rate (P=0.0001) was found with 30 nodes/vine having significantly higher growth rates, no interaction between irrigation and pruning level on shoot growth rates (P=0.7646) existed.

A



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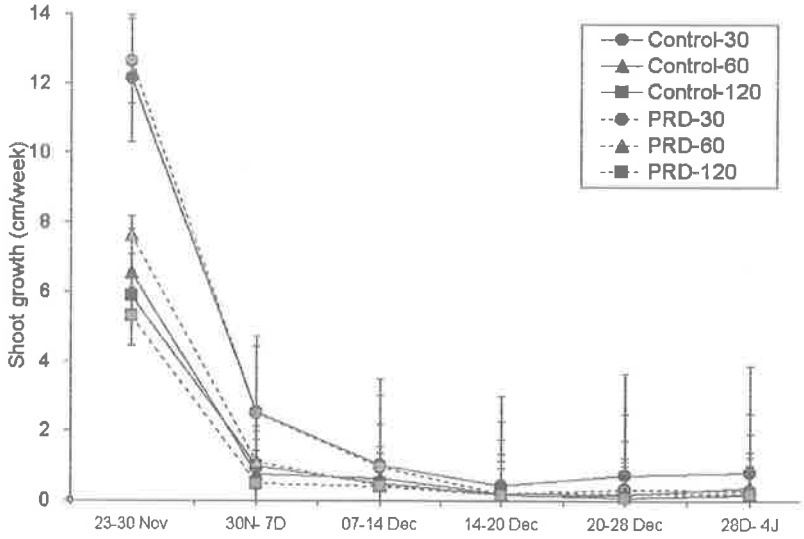


Figure 4.8 PRD and pruning level effects on shoot growth of Nuriootpa Shiraz in 2000/1. **(A)** Accumulated shoot length (cm) and **(B)** average shoot growth rate (cm/week). PRD started on the 23/11/00 and received half the amount of irrigation water as control. Pruning levels consisted of retaining 30, 60 or 120 nodes per vine. (means n = 5; average of 5 measurements per plot; Vertical bars indicate standard errors of the average)

4.3.2 Effects of PRD on leaf and stem water potentials

To investigate the effect of PRD on grapevine plant water relations the leaf water potentials during the three seasons were investigated. The leaf water potentials of the Coombe Cabernet Sauvignon and Shiraz in 2001 are shown in Table 4.1 (Taken at 11:00). No significant differences were found in PRD leaf water potentials of both cultivars in the Coombe vineyard, irrespective of the amount of irrigation water applied.

Table 4.1 Leaf water potentials (MPa) of Coombe Cabernet Sauvignon and Shiraz vines (20/01/01).) Control: vines received water on both sides; PRD: water withheld on one side at any time. (means n = 7 ± s.e.; n.s. = not significant (P<0.05)).

	Control	PRD	% Diff	Significance
Cabernet Sauvignon	- 0.89 ± 0.104	- 0.96 ± 0.091	- 7.9	n.s.
Shiraz	- 0.94 ± 0.072	- 0.92 ± 0.066	+ 1.4	n.s.

The leaf water potentials (midday) of Cabernet Sauvignon in the Alverstoke vineyard are shown in Table 4.2. The leaf water potentials were taken at two times during the growing season; during active shoot growth, three weeks before veraison, and during the ripening period, two weeks after veraison. PRD had no significant effect on leaf water potentials during active shoot growth before veraison. However, girdling of the grapevines significantly increased leaf water potentials by 26% compared to control and PRD. After veraison midday leaf water potentials decreased to much lower values in all vines, however no significant differences could be found between treatments.

Table 4.2 Leaf water potentials (MPa) of Alverstoke Cabernet Sauvignon vines (midday). Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation (means n = 6 ± s.e.; means with different letters are significantly different (P<0.05); n.s. = not significant).

	Control	PRD	Girdled	Significance
18 Dec 2001	$-1.33^{b} \pm 0.026$	$-1.33^{b} \pm 0.065$	$-0.98^{a} \pm 0.064$	<0.01
22 Jan 2002	- 1.72 ± 0.077	- 1.68 ± 0.053	- 1.73 ± 0.062	n.s.

The leaf water potentials and stem water potentials were measured again at harvest in the Coombe Cabernet Sauvignon grapevines in 2003 (Table 4.3). PRD Cabernet Sauvignon grapevines that received half the amount of control irrigation water had significantly lower leaf water potentials (on average by 7% at this late stage of ripening) compared to control. A similar reduction of 8% in stem water potential was also found in PRD compared to control, but was statistically insignificant.

Table 4.3 Leaf and stem water potentials (MPa; midday) of Coombe Cabernet Sauvignon vines (18/03/03). Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation (means n =7 ± s.e.; n.s. = not significant (P<0.05))

	Control	PRD	% Diff	Significance
Leaf water potential	- 1.32 ± 0.044	- 1.41 ± 0.056	- 7.2	<0.05
Stem water potential	- 1.07 ± 0.059	- 1.16 ± 0.039	- 8.3	n.s.

4.3.3 Effects of PRD on stomatal conductance

Coombe Cabernet Sauvignon and Shiraz

The PRD effect on the stomatal conductance of Coombe Cabernet Sauvignon and Shiraz during the 2000/1 season is shown in Figure 4.9 and 4.10 respectively. PRD grapevines had significantly (P<0.05) lower stomatal conductance on most measurement days whether irrigated with half the amount of water as control in Cabernet Sauvignon or with the same amount of water as control in Shiraz. PRD significantly reduced average stomatal conductance by 31% and 16% in Cabernet Sauvignon and Shiraz respectively compared to control during the 2000/1 ripening period from veraison until harvest.

The PRD effect on the stomatal conductance of Coombe Cabernet Sauvignon and Shiraz during the 2001/2 season is shown in Figure 4.11 and 4.12 respectively. Although the 2001/2 season was much milder than 2000/1 (Chapter 3), PRD Cabernet Sauvignon grapevines showed significantly (P<0.05) lower stomatal conductance on most days when irrigated with half the amount of water. However, PRD Shiraz that received the same amount of water did not respond to the same magnitude as in the 2000/1 season but only reduced stomatal conductance significantly once soon after veraison. PRD reduced stomatal conductance on average by 22% and 7% in Cabernet Sauvignon and Shiraz respectively compared to control between veraison and harvest in the 2001/2 season.

The PRD effect on the stomatal conductance of Coombe Cabernet Sauvignon and Shiraz during the 2002/3 season is shown in Figure 4.13 and 4.14 respectively. PRD grapevines

showed significantly ($P<0.05$) lower stomatal conductance on most days when irrigated with half the amount of water and with the same amount of water as control. PRD reduced the stomatal conductance on average by 40% and 15% in Cabernet Sauvignon and Shiraz respectively compared to control between veraison and harvest in 2002/3.

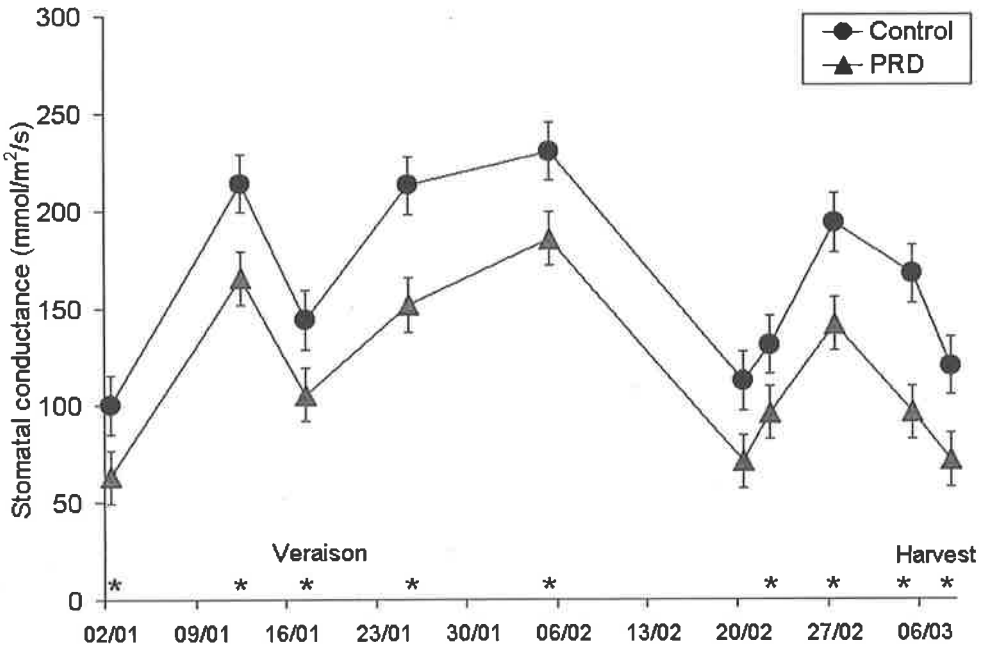


Figure 4.9 Stomatal conductance of Coombe Cabernet Sauvignon in 2001. PRD received half the amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$)).

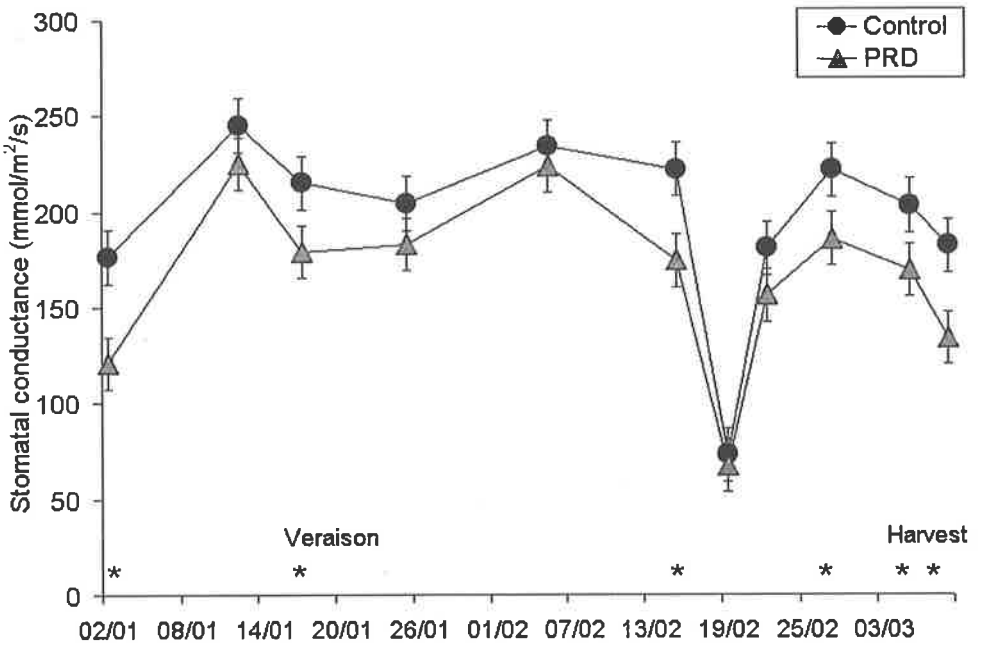


Figure 4.10 Stomatal conductance of Coombe Shiraz in 2001. PRD received the same amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$)).

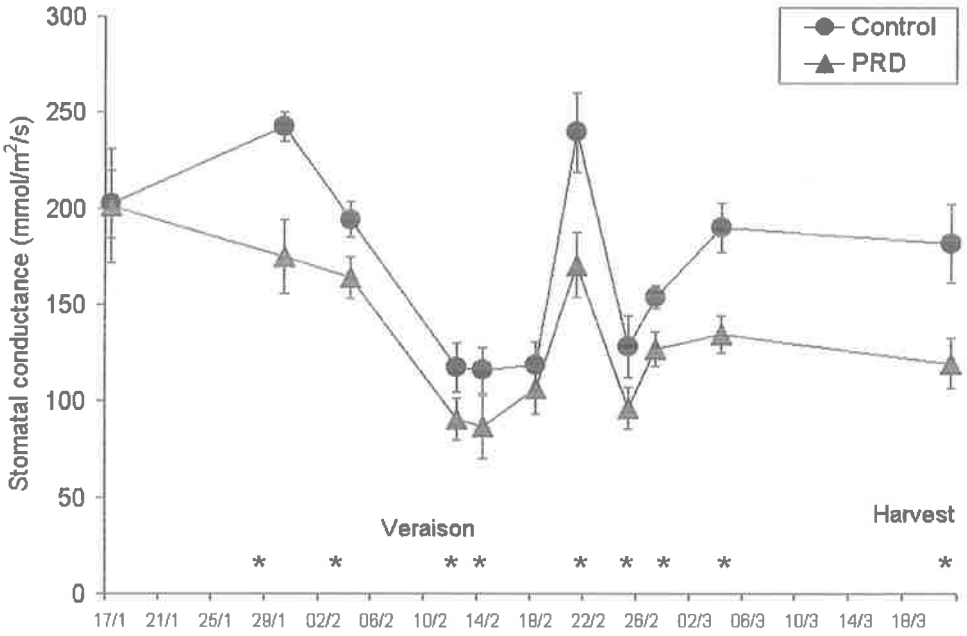


Figure 4.11 Stomatal conductance of Coombe Cabernet Sauvignon in 2002. PRD received half the amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

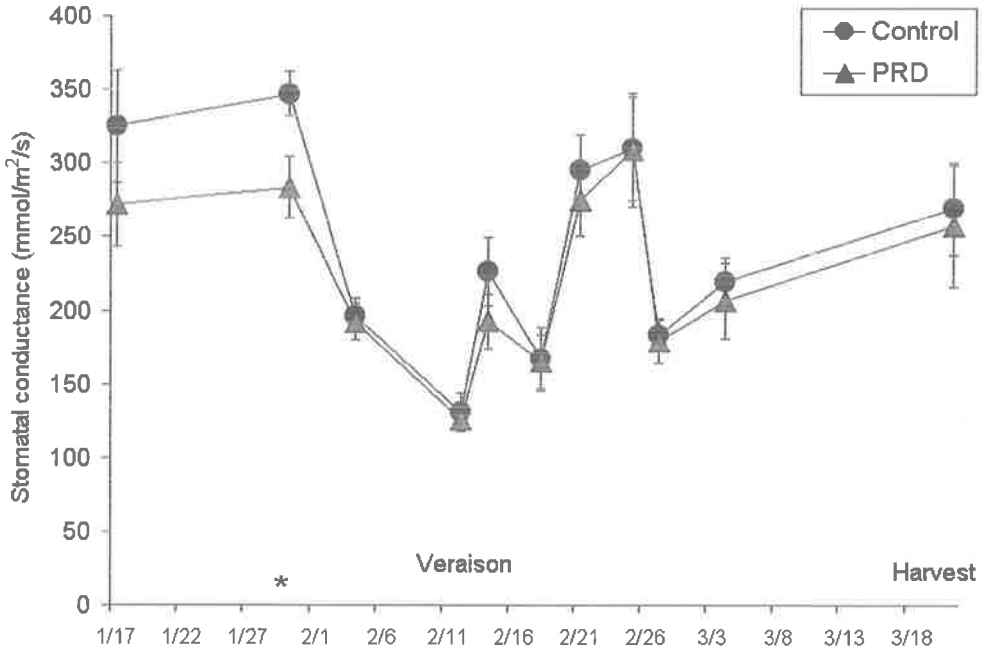


Figure 4.12 Stomatal conductance of Coombe Shiraz in 2002. PRD received the same amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

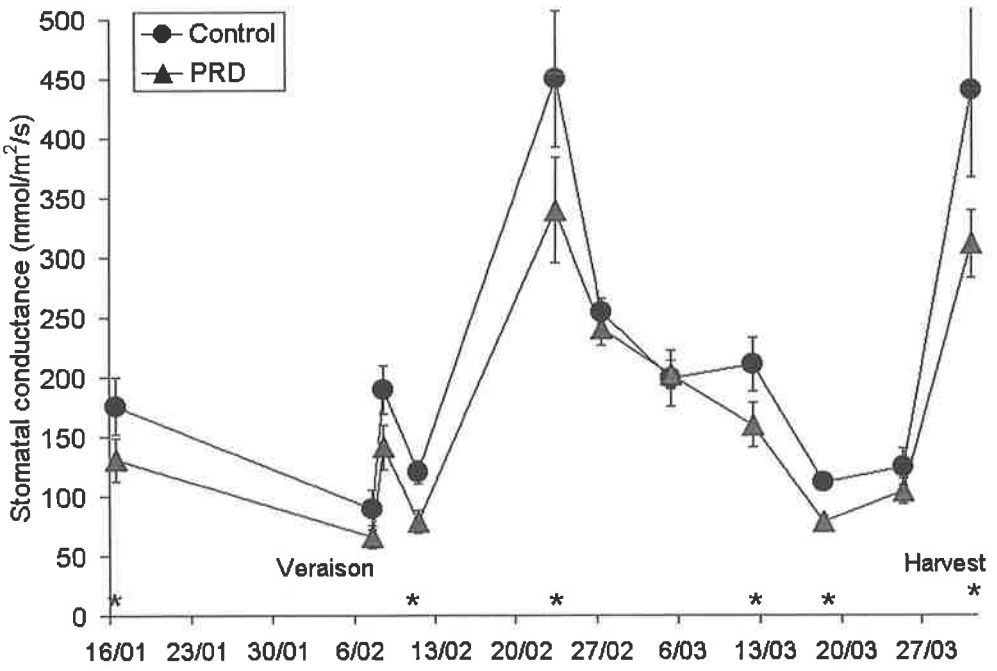


Figure 4.13 Stomatal conductance of Coombe Cabernet Sauvignon in 2003. PRD received half the amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

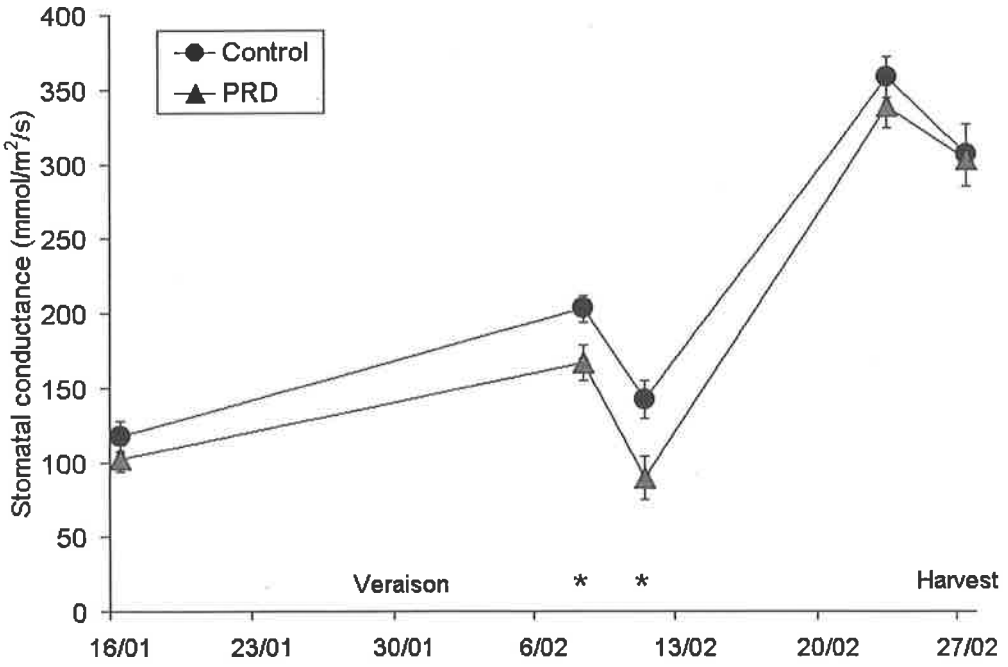


Figure 4.14 Stomatal conductance of Coombe Shiraz in 2003. PRD received the same amount of water as control. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

Alverstoke Cabernet Sauvignon

The effect of PRD and girdling on Alverstoke Cabernet Sauvignon leaf stomatal conductance is shown in Figure 4.15. PRD and girdled vines had significantly higher stomatal conductances early in the growing season, between 55% and 72% respectively compared to control. However, as the vines passed through veraison the stomatal conductance of girdled vines decreased by 59% and became significantly lower than the conductances in control vines ($P<0.05$). PRD stomatal conductances also declined but not to the low levels found in girdled vines. Although not significantly different on most sample days, PRD lowered the stomatal conductance on average by 7% between veraison and harvest.

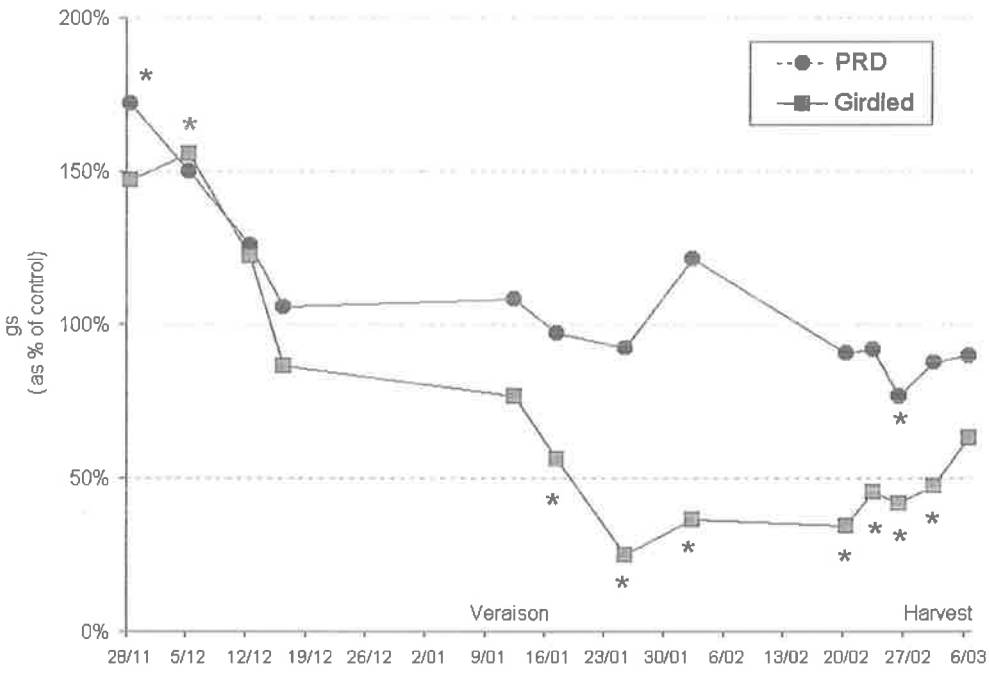


Figure 4.15 A) Stomatal conductance of Alverstoke Cabernet Sauvignon 2000/1 and **B)** PRD and girdle treatments as % of control. PRD and girdled vines received the same amount of irrigation water as control. PRD started on the 24/11/00. (means n = 6; vertical bars represent standard error of the average. * = significantly different ($P<0.05$)).

4.3.4 PRD and Photosynthesis

To investigate the PRD effect on the relationship between stomatal conductance and photosynthetic assimilation, potted Cabernet Sauvignon vines were grown under glasshouse conditions at the Waite campus and subjected to PRD treatment and drying conditions over a 12 day period. Photosynthesis (Pn), stomatal conductance, leaf water potentials (Ψ_L) and soil moisture was measured. The effect of PRD on the relationship between Pn and stomatal conductance is illustrated in Figure 4.16. It was found that although PRD reduced average stomatal conductance significantly by 52% compared to control, the average Pn was only reduced by 10% in comparison. The hyperbolic regression lines fitted for control and PRD treatments were not significantly different (P=0.080).

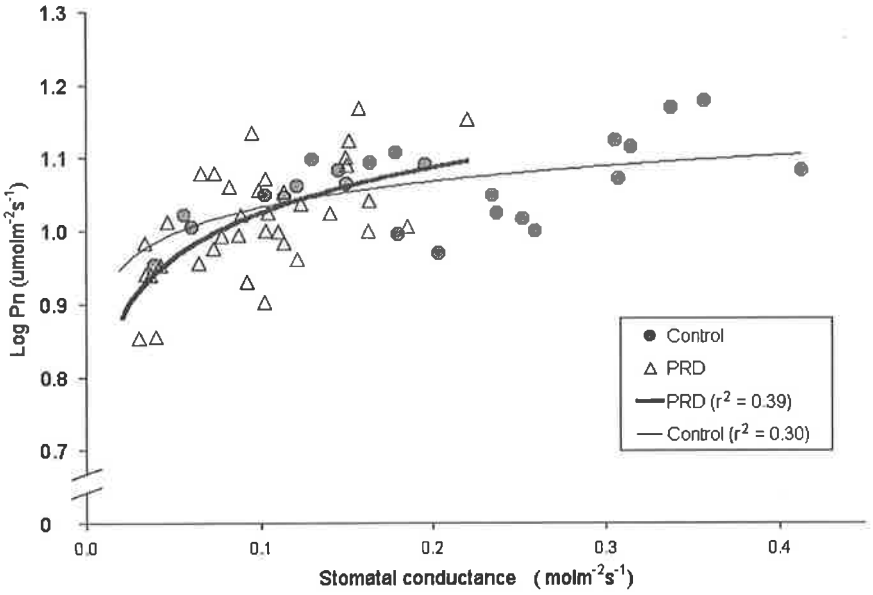


Figure 4.16 Effect of PRD on the relationship between stomatal conductance (mol.m^{-2}s^{-1}) and assimilation rate (log Pn, μmolm^{-2}s^{-1}) at midday in Cabernet Sauvignon grown under glasshouse conditions (2003). (3 observations on each of 6 replicates).

After 4 days PRD significantly reduced stomatal conductance by 50% compared to control (Figure 4.17). This reduction was associated with a non-significant reduction in Pn of 13% compared to control. In comparison, withholding water caused a significant reduction in stomatal conductance of 87% compared to control after only two days. This large reduction in stomatal conductance however was associated with only a 21% reduction in Pn compared to control. Further soil drying reduced stomatal conductance to zero – forcing the vines into a respiratory state.

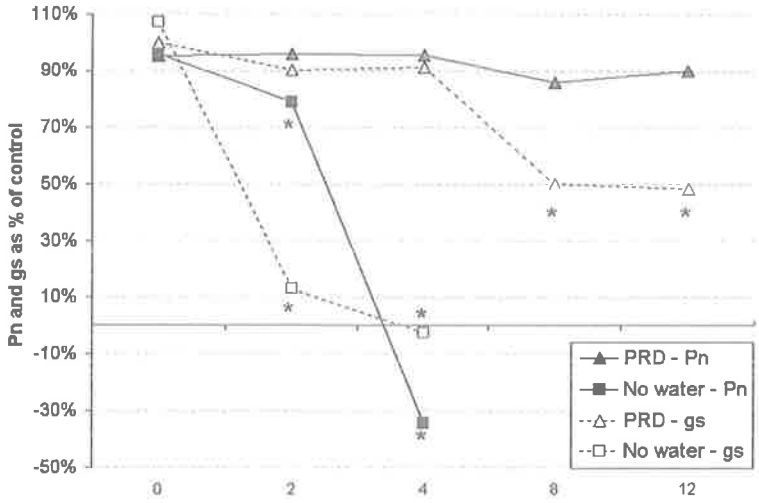


Figure 4.17 The effect of PRD and no irrigation (as % of control) on photosynthesis (Pn) and stomatal conductance (gs) in split-rooted Cabernet Sauvignon grown under glasshouse conditions (2003). (means n=6; PRD received water in only pot at any time; 'No water' received no water in either pot; * = significant ($P<0.05$)).

A

B

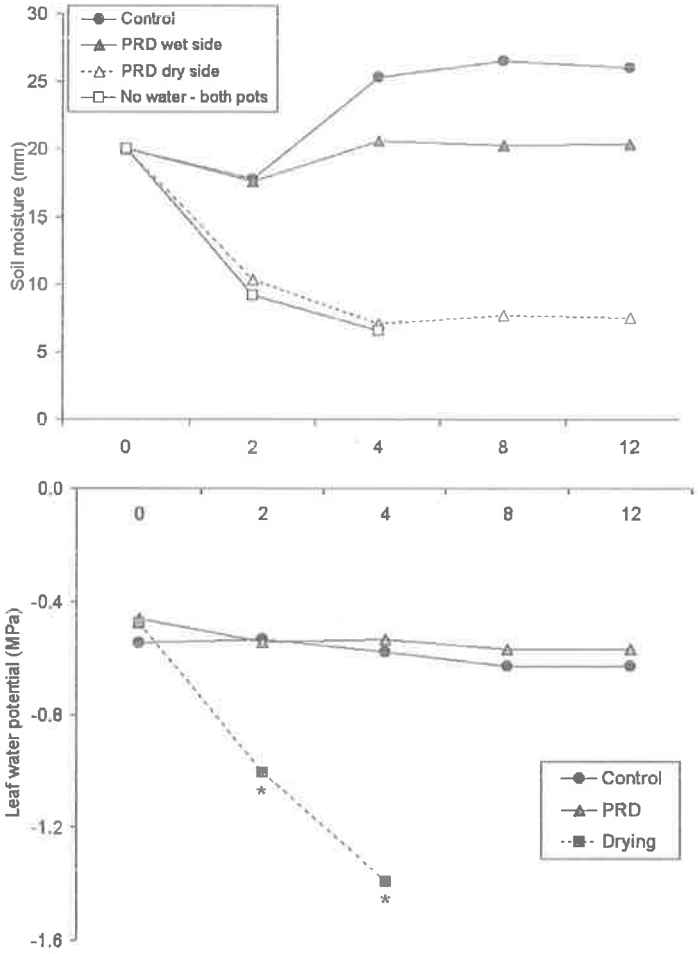


Figure 4.18 (A) Soil moisture and **(B)** leaf water potential measurements (13:00-15:00) in split-rooted Cabernet Sauvignon grown under glasshouse conditions (2003). (Control received water on both sides; PRD received water on only one side at any time (means n = 6 ± s.e.); 'No water' received no water on either side (means n = 2).

The soil water contents and leaf water potentials of the split-rooted Cabernet Sauvignon vines during the experiment are shown in Figure 4.18. PRD vines received irrigation water to saturation point in only one pot while water was withheld from the other. Soil moisture measurements were done 3 hours after the first irrigation of the day and may explain the difference in soil moisture content between average control pots and PRD wet pots. Because PRD had a smaller wetted volume of soil, PRD extracted more water from the irrigated pot than control vines. The reduction in soil water content of the 'no-water' treatment paralleled the reduction in soil moisture of the PRD 'dry' side. 'No-water' treatment of split-rooted vines significantly reduced the soil moisture content and leaf water potentials within 2 days. After 4 days the 'no-water' vines had a leaf water potential of -1.4 MPa, i.e. 240% lower than control potential and considered to be typical of vines under stress. PRD however showed no significant reductions in leaf water potentials compared to control vines during the experimental period.

4.3.5 Effect of PRD on leaf area and canopy density

Coombe Cabernet Sauvignon and Shiraz

Because the Cabernet Sauvignon and Shiraz vines in the Coombe vineyard were hedged several times during the summer it was not useful to calculate their canopy size by leaf area measurements. However, the average light penetration within the bunch zone could be measured with a hand-held ceptometer (Figure 4.19). On average, PRD increased the light penetration in Cabernet Sauvignon by 11% ($P=0.657$) and in Shiraz by 21% ($P=0.367$) compared to control. Increases in light intensity inside the canopy have been a consistent feature in PRD experiments where PRD significantly decreased the total canopy leaf area.

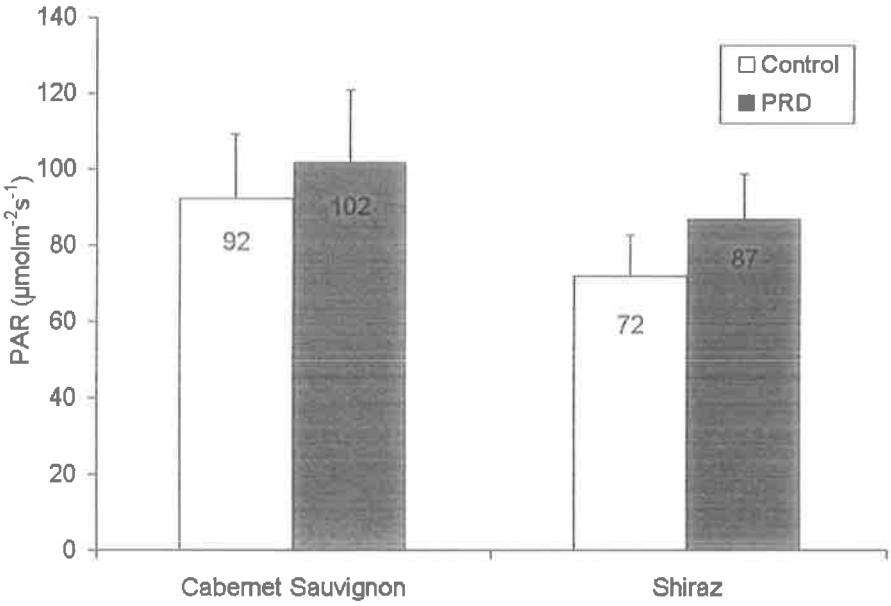


Figure 4.19 Light penetration into the bunch zone at harvest in Cabernet Sauvignon (2002) and Shiraz vines. (means n = 7; Vertical bars indicate standard errors of the average).

Nuriootpa Shiraz at veraison

The effect of PRD and pruning level on shoot growth and canopy leaf area of Nuriootpa Shiraz at veraison is shown in Table 4.4. Although PRD received half the amount of irrigation water than control vines, irrigation treatment had no main effect on shoot numbers at veraison (P=0.3507). As would be expected, pruning level had a significant main effect on the number of shoots at veraison (P=0.0001) with more nodes producing more shoots. No interaction existed between irrigation and pruning level on shoot numbers at veraison (P=0.9852). PRD treatment had no main effect on Shiraz main shoot length at veraison (P=0.3209). However, the main effect on shoot length was found to be pruning level (P=0.0038) with the shoots from more nodes per vine having shorter shoots. There was no interaction between irrigation and pruning level on shoot length at veraison (P=0.8243).

Although PRD received half the amount of irrigation water of control vines PRD had no main effect on leaf area of Shiraz at veraison (P=0.3004). On average though, PRD reduced leaf area for all pruning levels by 5% compared to control. The major factor that determined leaf area was pruning level (P=0.0001) with more nodes per vine having larger canopy sizes at veraison. At this early stage of growth there was no interaction between irrigation and pruning level on Nuriootpa Shiraz leaf area (P=0.9202).

Table 4.4 PRD and pruning level effects on shoot growth components and leaf area (LA) at veraison in Nuriootpa Shiraz (2001). (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation. Pruning levels consisted of retaining 30, 60 or 120 nodes per vine; means n = 5; ± s.e.; n.s. = not significant (P<0.05)).

Shoot number	Control	PRD	% Diff	Sig.
30	50.0 ± 1.4	52.2 ± 1.0	+ 4.4	n.s.
60	66.4 ± 1.4	68.2 ± 1.6	+ 2.7	n.s.
120	82.8 ± 3.4	84.2 ± 3.6	+ 1.7	n.s.
Shoot length (cm)	Control	PRD	% Diff	Sig.
30	129.2 ± 4.9	123.6 ± 3.2	- 4.3	n.s.
60	120.2 ± 3.8	116.6 ± 3.1	- 3.0	n.s.
120	111.9 ± 5.0	111.3 ± 3.1	- 0.6	n.s.
Veraison leaf area (m^2)	Control	PRD	% Diff	Sig.
30	13.24 ± 0.70	12.78 ± 0.53	- 3.5	n.s.
60	16.22 ± 0.70	15.40 ± 0.89	- 5.1	n.s.
120	19.04 ± 1.51	17.78 ± 1.20	- 6.6	n.s.

The correlation matrix of shoot growth components for Nuriootpa Shiraz at veraison in 2001 is shown in Table 4.5. The number of shoots that developed and canopy leaf area at veraison correlated positively with the number of nodes per vine indicating that it was the major factor in determining canopy size. Shoot length was not correlated with veraison leaf area indicating its limited contribution. However, shoot length was negatively correlated with the amount of nodes per vine and the resultant shoot number per vine. Regression analyses with leaf area as the dependant variable also indicated no main effect of shoot length at veraison on leaf area (P=0.2883). However, factors that had a significant main effect included shoot number, irrigation and pruning level (P<0.001)

Table 4.5 Correlation matrix of canopy components at veraison of field-grown Shiraz at the Nuriootpa research station (2001). (PRD received half the amount of control irrigation; light grey cell: P<0.05; dark grey: P<0.01).

	Pruning level	Shoot number	Shoot length	Leaf area
Pruning level	1.00			
Shoot number	0.9248	1.00		
Shoot length	- 0.5819	-0.4620	1.00	
Leaf area	0.7216	0.8683	0.0089	1.00

Df = 28; if r > 0.361, P < 0.05; if r > 0.463, P < 0.01

Nuriootpa Shiraz at harvest

The effect of PRD and pruning level on shoot growth and canopy leaf area of Nuriootpa Shiraz at harvest is shown in Table 4.6. PRD had a significant effect on main shoot length at harvest (P=0.0254) by reducing growth at all pruning levels on average by 13% compared to control. The number of nodes per vine also had an effect on final shoot length (P=0.0018) with the number of nodes per vine being correlated negatively with final shoot length (Table 4.7). There was no interaction between irrigation and pruning level on shoot length at harvest (P=0.7578).

PRD Shiraz receiving half the amount of irrigation water of control vines in 2001 had a significant main effect on leaf area of Shiraz at harvest (P=0.0003). On average PRD reduced leaf area at all pruning levels by 26% compared to control. It seems from the correlation matrix in Table 4.7 that the major factors that determined leaf area were a combination of pruning level, shoot number per vine and shoot length. At the harvest stage there was a stronger but still not significant interaction between irrigation and pruning level on Nuriootpa Shiraz leaf area (P=0.2813). Regression analyses with leaf area as the dependant variable also indicated a significant main effect of shoot length, shoot number and irrigation on leaf area at harvest (P<0.001). However, pruning level did not have the main effect (P=0.1486) on leaf area at harvest as earlier during veraison.

Table 4.6 PRD and pruning level effects on shoot growth components and leaf area at harvest in Nuriootpa Shiraz (2001) (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation. Pruning levels consisted of retaining 30, 60 or 120 nodes per vine; means n = 5; ± s.e.; n.s. = not significant (P<0.05)).

Shoot number	Control	PRD	% Diff	Sig.
30	50.0 ± 1.4	52.2 ± 1.0	+ 4.4	n.s.
60	66.4 ± 1.4	68.2 ± 1.6	+ 2.7	n.s.
120	82.8 ± 3.4	84.2 ± 3.6	+ 1.7	n.s.
Shoot length	Control	PRD	% Diff	Sig.
30	148.45 ± 11.2	131.50 ± 0.6	- 11.4	n.s.
60	143.40 ± 7.4	130.95 ± 6.1	- 8.7	n.s.
120	137.85 ± 17.9	112.70 ± 3.7	- 18.2	n.s.
Harvest LA	Control	PRD	% Diff	Sig.
30	13.08 ± 2.20	11.23 ± 0.25	- 14.1	n.s.
60	18.95 ± 1.21	13.52 ± 0.60	- 28.7	<0.05
120	21.47 ± 3.06	14.05 ± 0.83	- 34.6	<0.01

Table 4.7 Correlation matrix of canopy components at harvest of field-grown Shiraz at the Nuriootpa research station (2001). (PRD received half the amount of control irrigation; light grey cell: P<0.05; dark grey: P<0.01).

	Pruning level	Shoot number	Shoot length	Veraison LA
Pruning level	1.00			
Shoot number	0.9248	1.00		
Shoot length	- 0.5799	- 0.5355	1.00	
Harvest LA	0.3998	0.4607	0.3960	1.00

Df = 28; if r > 0.361, P < 0.05; if r > 0.463, P < 0.01

Alverstoke Cabernet Sauvignon

The PRD effect on the Alverstoke Cabernet Sauvignon shoot growth components and leaf area is shown in Table 4.8. Although PRD received the same amount of irrigation water as control vines, it significantly (P<0.01) reduced the average leaf area per vine by 30% and reduced the final shoot length at harvest on average by 14% compared to control.

Table 4.8 The effect of PRD on and shoot growth components and leaf area at harvest in Cabernet Sauvignon at the Alverstoke site (2001) (Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation; means n = 6; ± s.e.; n.s. = not significant (P<0.05)).

	Control	PRD	% Diff	Sig.
Shoot nr./vine	38.8 ± 2.26	35.3 ± 1.76	- 9.0	n.s.
Shoot length (cm)	151.8 ± 15.8	130.0 ± 5.8	- 14.4	n.s.
Leaf area (m^2)	11.27 ± 0.72	7.95 ± 0.41	- 29.5	<0.01

4.3.6 Effect of PRD on grapevine performance, dry weight accumulation and water use efficiency

Coombe Cabernet Sauvignon

The effect of PRD on Coombe Cabernet Sauvignon yield and berry characteristics at harvest is shown in Table 4.9. Although PRD Cabernet Sauvignon vines received half the amount of irrigation water of control vines, there were no significant differences in the yield in the three years of the experiment. This increased the water use efficiency of Cabernet Sauvignon for the 2001, 2002 and 2003 harvests by 77%, 84% and 106% respectively (Table 4.10). PRD had generally no significant effect on fruit total soluble solids (°Brix), pH or berry numbers per bunch at harvest compared to control. The only exception was in 2003 where PRD had significantly higher pH than control berries. Shoot and bunch numbers were controlled by hand thinning at flowering and no significant differences were expected. However, this seems to have been ineffective because PRD had higher shoot numbers in

2001 and 2002 compared to control. Although there were no differences in bunch numbers per vine between PRD and control over the three years, there was a significant effect on berry weights. PRD reduced the average berry weight of Cabernet Sauvignon in 2001 and 2002 by 11% and 9% respectively.

Vegetative growth can also be expressed by measuring pruning weights. As a consequence of a reduction in vegetative growth in Cabernet Sauvignon grapevines, the mean shoot weights of PRD-treated vines over the three years were reduced on average by 4% compared to control. The use of winter pruning weights was however compromised in the Coombe vineyard due to mechanical canopy hedging that could not be precisely quantified. Table 4.10 shows the winter pruning weights for the Coombe Cabernet Sauvignon vines that received half the amount irrigation water as control. PRD Cabernet Sauvignon grapevines had higher average winter pruning weights in 2001 and 2002 by 8% and 14% respectively compared to control. The only year that PRD reduced winter pruning weights of Cabernet Sauvignon was in 2003 by 6% compared to control.

The dry weights collected from Cabernet Sauvignon during summer hedging in the Coombe vineyard in 2001 are shown in Table 4.11. Cabernet Sauvignon control vines lost 15% more dry weight than PRD vines during summer hedging. When the total pruning weights were calculated, PRD Cabernet Sauvignon grapevines had similar total pruning weights as control vines.

Table 4.9 Effect of PRD on yield and berry characteristics of Coombe Cabernet Sauvignon at harvest. (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation; means n = 7; ± s.e.; n.s. = not significant (P<0.05))

	Control	PRD	%Diff.	Sig.
Yield (kg/vine)				
2001	4.35 ± 0.80	3.88 ± 0.58	- 10.8	n.s.
2002	5.52 ± 0.55	5.08 ± 0.31	- 8.0	n.s.
2003	5.15 ± 0.86	5.28 ± 0.77	+ 2.6	n.s
Juice °Brix				
2001	24.40 ± 0.43	25.40 ± 0.70	+ 4.0	n.s.
2002	24.94 ± 0.31	25.07 ± 0.29	+ 0.5	n.s.
2003	24.71 ± 0.31	24.97 ± 0.30	- 1.0	n.s.
Juice pH				
2001	3.53 ± 0.02	3.45 ± 0.03	- 2.0	n.s.
2002	3.21± 0.02	3.26 ± 0.03	+ 1.4	n.s.
2003	3.55 ± 0.04	3.69 ± 0.05	+ 1.5	<0.05
Bunch no/vine				
2001	73 ± 5.4	65 ± 6.0	- 11	n.s.
2002	62 ± 4.4	64 ± 3.7	+ 3.2	n.s.
2003	76 ± 3.5	78 ± 3.8	+ 2.2	n.s.
Berry weight (g)				
2001	0.98 ± 0.03	0.87 ± 0.04	-11	<0.05
2002	1.08 ± 0.04	0.98 ± 0.03	- 9.3	<0.01
2003	0.92 ± 0.03	0.88 ± 0.03	- 4.3	n.s.
Berry no/bunch				
2001	59.0 ± 5.5	67.0 ± 5.0	+ 14	n.s.
2002	81.9 ± 3.7	81.1± 3.2	- 0.9	n.s.
2003	71.4 ± 7.9	72.3 ± 5.8	+ 1.4	n.s.
Bunch weight (g)				
2001	58.2 ± 7.6	58.8 ± 6.2	+ 0.9	n.s.
2002	88.7 ± 5.7	80.1± 5.1	- 9.7	n.s.
2003	65.9 ± 8.6	65.9 ± 6.7	+ 0.1	n.s.

Table 4.10 Effect of PRD on grapevine shoot growth components and water use efficiency of Coombe Cabernet Sauvignon (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation; means n = 7; ± s.e.; n.s. = not significant (P<0.05)).

	Control	PRD	%Diff.	Sig.
Shoot no/vine				
2001	55.4 ± 4.0	61.7 ± 2.0	+ 13	n.s.
2002	45.4 ± 2.9	54.3 ± 3.5	+ 19	<0.01
2003	61.9 ± 1.9	61.3 ± 2.5	- 0.9	n.s.
Shoot weight (g)				
2001	37.79 ± 2.72	36.60 ± 3.39	- 3.1	n.s.
2002	56.84 ± 1.88	54.55 ± 1.51	- 4.0	n.s.
2003	32.49 ± 3.95.	30.81± 2.38	- 5.2	n.s.
Winter pruning weight (kg fresh wt)				
2001	2.12± 0.27	2.28 ± 0.26	+ 7.6	n.s.
2002	2.59± 0.21	2.97 ± 0.22	+ 14.4	<0.05
2003	2.03 ± 0.29	1.92 ± 0.22	- 5.8	n.s.
Irrigation (mm set to harvest)				
2000/1	107	53	- 50	
2001/2	114	57	- 50	
2002/3	90	45	- 50	
WUE (t/100mm)				
2001	8.13 ± 1.50	14.36 ± 2.12	+ 77	<0.01
2002	9.69 ± 0.96	17.82 ± 1.09	+ 84	<0.01
2003	11.4 ± 1.92	23.47 ± 3.41	+ 106	<0.01

Table 4.11 Summer hedging and winter pruning weights of Cabernet Sauvignon vines under PRD irrigation (Control: vines received water on both sides; PRD: water withheld on one side at any time; means n = 7; ± s.e.; n.s. = not significant (P<0.05)).

Dry weight (kg/vine)	Control	PRD	%Diff	Sig.
Summer hedging	0.52 ± 0.07	0.44 ± 0.05	- 14.5	n.s
Winter pruning	0.95 ± 0.12	1.03 ± 0.12	+ 7.6	n.s.
Total	1.47 ± 0.16	1.47 ± 0.18	- 0.1	n.s.

Coombe Shiraz

The effect of PRD on Coombe Shiraz yield and berry characteristics at harvest is shown in Table 4.12. PRD Shiraz that received the same amount of irrigation water significantly ($P<0.05$) increased the fruit yield in 2001 by 25% compared to control. However, the following two seasons there were no significant differences in yield. Consequently, PRD increased the water use efficiency of Shiraz in the 2001 season by 25% (Table 4.13) compared to control but not during 2002 and 2003. PRD had no significant effect on fruit total soluble solids (°Brix), pH or berry numbers per bunch at harvest compared to control. Similar to the Cabernet Sauvignon the shoot and bunch numbers were controlled by hand thinning at flowering and no significant differences were expected. However, it seems it was ineffective because PRD had increased shoot and bunch numbers in 2001 compared to control and it seems that increased bunch numbers were mainly responsible for increased yield in 2001. PRD had no significant effect on berry weights in Shiraz during the three years.

As a consequence of a reduction in vegetative growth by PRD or increased shoot numbers, the mean shoot weights of PRD Shiraz vines were reduced by 11% in 2001 compared to control. The following years showed no significant differences in shoot numbers and the average shoot weight in PRD vines for 2002 and 2003 was 3% higher than control vines. As in the case of Cabernet Sauvignon vines, the use of winter pruning weights was compromised in the Coombe Shiraz due to mechanical canopy hedging that could not be precisely quantified. Table 4.13 shows the winter pruning weights for the Coombe Shiraz vines that received the same amount of irrigation water as control. PRD Shiraz grapevines had higher average winter pruning weights in 2001, 2002 and 2003 by 12%, 11% and 5% compared to control. The differences were however non significant.

The dry weights collected from Shiraz during summer hedging in the Coombe vineyard in 2001 are shown in Table 4.14. Shiraz control vines lost 26% more dry weight than PRD vines during the 2001 summer hedging. When the total pruning weights were calculated, PRD Shiraz grapevines had similar total pruning weights as control vines.

Table 4.12 Effect of PRD on grapevine yield and berry characteristics in Coombe Shiraz at harvest (Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation; means n = 7; ± s.e.; n.s. = not significant (P<0.05)).

	Control	PRD	%Diff	Sig.
Yield (kg/vine)				
2001	5.53 ± 0.32	6.89 ± 0.33	+ 24.6	<0.05
2002	13.67 ± 0.98	12.37 ± 0.71	- 9.51	n.s.
2003	8.79 ± 0.87	8.54 ± 0.73	- 2.80	n.s.
Juice °Brix				
2001	26.8 ± 0.66	27.3 ± 0.44	+ 2.0	n.s.
2002	24.3 ± 0.71	24.9 ± 0.28	+ 2.6	n.s.
2003	24.8 ± 0.43	24.5 ± 0.36	- 1.2	n.s.
Juice pH				
2001	3.54 ± 0.03	3.53 ± 0.01	0	n.s.
2002	3.27 ± 0.03	3.28 ± 0.01	+ 0.2	n.s.
2003	3.84 ± 0.02	3.72 ± 0.05	- 2.9	n.s.
Bunch no/vine				
2001	74.6 ± 2.8	88.1± 4.0	+ 18.2	n.s.
2002	99.6 ± 4.0	89.7 ± 2.6	- 9.9	n.s.
2003	66.2 ± 3.3	61.9 ± 1.9	- 6.5	n.s.
Berry weight (g)				
2001	1.17 ± 0.02	1.16 ± 0.03	- 0.9	n.s.
2002	1.34 ± 0.03	1.33 ± 0.04	- 0.7	n.s.
2003	1.27 ± 0.03	1.29 ± 0.05	+ 1.2	n.s.
Berry no/bunch				
2001	63.3 ± 1.9	68.1 ± 3.6	+ 7.6	n.s.
2002	102 ± 4.8	103.6 ± 4.6	+ 1.5	n.s.
2003	101.4 ± 5.6	106.4 ± 5.1	+ 5.0	n.s.
Bunch weight (g)				
2001	74.0 ± 2.3	78.9 ± 4.3	+ 6.7	n.s.
2002	136.8 ± 7.1	138.1± 7.4	+ 7.1	n.s.
2003	131.6 ± 8.2	137.2 ± 8.7	+ 4.2	n.s.

Table 4.13 Effect of PRD on grapevine shoot growth components and water use efficiency of Coombe Shiraz (Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation; means n = 7; ± s.e.; n.s. = not significant (P<0.05)).

	Control	PRD	%Diff	Sig.
Shoot no/vine				
2001	58.4 ± 2.1	74.9 ± 2.7	+ 28.2	<0.01
2002	57.1 ± 2.2	61.3 ± 2.2	+ 7.3	n.s.
2003	58.7 ± 1.8	59.9 ± 2.4	+ 1.9	n.s.
Shoot weight (g)				
2001	40.40 ± 2.72	35.95 ± 2.35	- 11	n.s.
2002	51.09 ± 1.70	52.58 ± 2.47	+ 2.9	n.s.
2003	38.37 ± 3.09	39.39 ± 2.60	+ 2.7	n.s.
Winter pruning weight (kg fresh wt)				
2001	2.38 ± 0.23	2.67 ± 0.16	+ 12.3	n.s.
2002	2.91± 0.14	3.25 ± 0.24	+ 11.4	n.s.
2003	2.25 ± 0.19	2.37 ± 0.21	+ 5.2	n.s.
Irrigation (mm set to harvest)				
2000/1	107	107	0	
2001/2	127	127	0	
2002/3	147	147	0	
WUE (t/100mm)				
2001	10.3 ± 0.61	12.9 ± 0.61	+ 25	<0.05
2002	21.5 ± 1.54	19.5 ± 1.12	- 9.3	n.s.
2003	12.0 ± 1.18	11.6 ± 0.99	- 2.8	n.s.

Table 4.14 Summer hedging and winter pruning weights (kg dry weight) of Coombe Shiraz in 2001 (Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation; means n = 7; ± s.e.; n.s. = not significant (P<0.05))

Dry weight (kg/vine)	Control	PRD	%Diff	Sig.
Summer hedging	0.38 ± 0.07	0.28 ± 0.02	- 26.4	n.s.
Winter pruning	1.07 ± 0.10	1.20 ± 0.07	+ 12	n.s.
Total	1.45 ± 0.10	1.48 ± 0.08	+ 2	n.s.

Alverstoke Cabernet Sauvignon

Table 4.15 shows the PRD and girdling effects on yield and shoot growth components of Cabernet Sauvignon grown in the Alverstoke vineyard in 2001. Both PRD and girdled grapevines received the same amount of irrigation water as control vines. Neither PRD nor girdling significant reduced crop yield or berry weight compared to control. PRD and girdling had lower winter pruning weights by 15% and 31% respectively with lower average shoot weights that may indicate lower vegetative growth.

Table 4.15 Yield and shoot components at harvest for Cabernet Sauvignon at the Alverstoke site (2001) (Control: vines received water on both sides; PRD: water withheld on one side at any time and received the same amount as control irrigation; means n = 6; ± s.e.; n.s. = not significant (P<0.05))

	Control	PRD	Girdled	Pr> F
Yield (kg/vine)	3.49 ± 0.41	2.65 ± 0.18	3.29 ± 0.53	n.s.
Berry weight (g)	0.98 ± 0.03	0.98 ± 0.03	0.94 ± 0.06	n.s.
TSS (°Brix)	21.8 ± 0.44	22.1 ± 0.47	23.5 ± 0.62	n.s.
Pruning weight (kg/vine)	2.25 ± 0.56	1.92 ± 0.31	1.56 ± 0.34	n.s.
Shoot weight (g)	56.9 ± 12.6	53.1 ± 5.7	43.7 ± 4.8	n.s.

Nuriootpa Shiraz

The effect of PRD and pruning level on Nuriootpa Shiraz performance components at harvest in 2001 is shown in Table 4.16. PRD Shiraz received half the amount of irrigation water of control. PRD treatment had no effect on yield (P=0.2109). However, yield was significantly affected by the amount of nodes per vine (P=0.0017). There was no interaction (P=0.5138) between irrigation and pruning level on yield at harvest.

PRD vines received 50 mm/ha of irrigation water compared to control vines that received 100 mm/ha. This significantly increased the water use efficiency (WUE) of PRD vines compared to control at all pruning levels (P=0.0001). Pruning level had also a significant effect (P=0.0012) on the WUE of Shiraz with 60 and 120 nodes/vine having significantly higher WUE than 30 nodes/vine. There was however no interaction between irrigation and pruning level on WUE (P=0.8501).

Table 4.16 PRD and pruning level effects on yield components at harvest in Nuriootpa Shiraz (2001). (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation. Pruning levels consisted of retaining 30, 60 or 120 nodes per vine; means n = 5; ± s.e; n.s. = not significant)

Yield (kg/vine)				
Nodes/vine	Control	PRD	% Diff	Sig.
30	6.26 ± 0.64	6.19 ± 0.52	- 1	n.s.
60	8.84 ± 1.08	8.17± 0.45	- 8	n.s.
120	11.06± 1.52	8.89± 0.88	-20	n.s.
Berry weight (g)				
Nodes/vine	Control	PRD	% Diff	Sig.
30	0.83 ± 0.046	0.79 ± 0.050	- 4.9	n.s.
60	0.79 ± 0.048	0.78 ± 0.051	-1.9	n.s.
120	0.73 ± 0.058	0.62 ± 0.021	- 15.2	n.s.
Juice TSS (°Brix)				
Nodes/vine	Control	PRD	% Diff	Sig.
30	25.88 ± 0.43	25.72 ± 0.47	- 0.6	n.s.
60	25.68 ± 0.56	25.08 ± 0.50	- 2.3	n.s.
120	23.72 ± 0.78	22.84 ± 0.48	- 3.7	n.s.
Juice pH				
Nodes/vine	Control	PRD	% Diff	Sig.
30	4.05 ± 0.05	4.04 ± 0.04	- 0.1	n.s.
60	3.99 ± 0.05	3.96 ± 0.02	- 0.8	n.s.
120	3.79 ± 0.06	3.73 ± 0.05	- 1.4	n.s.
Irrigation (mm set to harvest)				
	100	50	- 50	
WUE (t/100mm)				
Nodes/vine	Control	PRD	% Diff	Sig.
30	12.52 ± 1.28	24.76 ± 0.61	+ 98	<0.01
60	17.69 ± 2.16	32.70 ± 1.82	+ 85	<0.01
120	22.13 ± 3.03	35.58 ± 3.51	+ 61	<0.05

There was no significant effect of irrigation treatment on berry weight at harvest of Shiraz at Nuriootpa (P=0.1652) but PRD reduced the berry weight at all pruning levels on average by 7% compared to control. At a pruning level of 120 nodes/vine it seems that the reduction in PRD berry weight by 15% compared to control also reduced PRD yield by 20%. A significant effect of pruning level existed on berry weight at harvest (P=0.0183) with higher number of nodes per vines having smaller berries. There was no interaction between irrigation and pruning level on Shiraz berry weight at harvest (P=0.5861).

No effect of PRD treatment could be found on berry total soluble solids (TSS; °Brix; P=0.2335) or juice pH (P=0.4420) at harvest for Nuriootpa Shiraz. However, there were a significant effect of pruning level on TSS (P=0.0002) and pH (P=0.0001) at harvest with vines that was pruned to higher number of nodes per vine having lower TSS and higher pH than vines pruned to lower numbers. No interaction existed between irrigation and pruning level on berry TSS or pH at harvest (P=0.8045).

The correlation matrix of harvest components at harvest of Nuriootpa Shiraz is shown in Table 4.17. It was found that crop yield was positively correlated with pruning level i.e. the number of nodes per vine, and not with berry weight. Berry weight was furthermore negatively correlated with pruning level.

Table 4.17 Correlation matrix of harvest components at harvest of field-grown Shiraz at the Nuriootpa research station (2001). (PRD received half the amount of control irrigation; dark grey cell: P<0.01).

	Pruning level	Berry weight	Yield
Pruning level	1		
Berry weight	- 0.5350	1	
Yield	0.5885	0.1870	1

Df = 28; if r > 0.361, P < 0.05; if r > 0.463, P < 0.01

4.4 Discussion

Results of field experiments in this chapter have provided evidence that PRD reduces vegetative biomass production and also changes canopy architecture. This study has shown that PRD can lead to long term reductions in shoot growth rates and leaf area per vine irrespective of the amount of irrigation water applied. PRD reduced main and lateral shoot growth when irrigated with the same and up to half the amount of water used in control vines. PRD reduced main and lateral shoot growth but the largest effect was found on lateral shoot growth. Transient reductions in shoot growth rate in response to soil drying were found in earlier work (Dry *et al.*, 1996) and as a consequence it was proposed that a constant effect on shoot growth could be maintained by alternating the wetting zones. Since then, significant work by Dry and Loveys (1999) and Stoll (2000) have provided evidence that manipulating the soil conditions in this way reduces shoot growth rate by 18% to 30% in field-grown grapevines. The results in this study support their findings.

There was a clear difference in shoot growth between the two seasons that may be related to the difference in climatic conditions mentioned in Chapter 3. The 2000/1 growing season was characterized by higher spring and summer temperatures and higher average solar radiation than 2001/2. The mild growing season of 2001/2 may have contributed to lower

evaporative demand and soils drying slower than usual in the spring, thereby postponing the time when PRD could be started and reducing the effect of PRD on shoot growth. This effect was manifested in higher overall shoot growth rates in both Cabernet Sauvignon and Shiraz vines in 2001/2 compared to 2000/1 and the fact that PRD did not significantly affect shoot growth rate and accumulated shoot lengths until late in the measuring period of 2001/2. Therefore, the PRD effect on grapevine shoot growth was less pronounced in 2001/2 than in 2000/1. It is concluded that PRD decreased grapevine shoot growth irrespective of the amount of water applied and predominantly affected lateral shoot growth. Many authors have found similar PRD effects on grapevine shoot growth (Loveys *et al.*, 2000; Stoll, 2000; Dry *et al.*, 2001; dos Santos *et al.*, 2003). Lateral shoots are undesirable in vigorous vineyards, because they lead to dense canopies and an imbalance favouring vegetative growth versus fruit production (Smart, 1985).

PRD had a long-term effect on grapevine leaf physiology by significantly reducing stomatal conductance in field experiments irrespective of the amount of water applied. PRD Cabernet Sauvignon grapevines that received half the amount of irrigation water reduced stomatal conductances between 22% and 41% between veraison and harvest over the three-year experimental period. PRD-treated Coombe Shiraz and Alverstoke Cabernet Sauvignon (both receiving equal amounts of irrigation water as control) also showed periods of significant reduction in stomatal conductance in response to PRD, but only reducing stomatal conductance between 7% and 16% compared to control. It is concluded that the reductions in stomatal conductance appears to be mainly due to a PRD effect and not simply a reduction in amount of water applied. These findings supported the results found by many authors in PRD experiments with half the amount of irrigation water as control (Loveys *et al.*, 2000; Stoll *et al.*, 2000; de Souza *et al.*, 2003; dos Santos *et al.*, 2003). In contrast, experiments with equal amounts of irrigation water by de la Hera Orts et al. (2002) found that although PRD vines increased water use efficiency there was no decrease in stomatal conductance.

It was proposed by Jones (1998) that stomatal control could play a major role in limiting the plant water loss, but a relatively small role in determining the rate of photosynthesis. It was found that large changes in stomatal conductance had only minor effects on assimilation rate under PRD (Stoll, 2000) and situations of mild water stress (Flexas *et al.*, 1999). This is also supported by Barradas and Jones (1996) who reported the assimilation rate in beans can increase faster than the rate of increase of stomatal conductance when exposed to different light conditions. After the leaf CO_2 assimilation rate reaches saturation a further increase in

stomatal conductance has no effect on photosynthesis. The higher water loss from fully open stomata without an increase in CO_2 assimilation could be considered a luxurious consumption and an inefficient use of water if no other processes were affected. A reduction in stomatal aperture without a concurrent reduction in assimilation rate would greatly increase the grapevine's water use efficiency. PRD experiments in this study with grapevines grown under glasshouse conditions also found this non-linear relationship to be true – relatively large changes in stomatal conductance had minor affects on photosynthesis rates. To illustrate the flexibility of the relationship by withholding water from both pots in split rooted grapevines it was found that a large reduction in stomatal conductance by as much as 87% compared to control had only a 21% reduction in assimilation rate. With further soil drying and water stress the assimilation rate quickly declined with small further decreases in stomatal conductance. It is therefore concluded that under field-PRD conditions, with long-term reductions in stomatal conductances up to 41% compared to control, that grapevine assimilation rates would not be appreciably affected and would lead to greatly increasing the grapevine's water use efficiency. These results support the findings of many authors experimenting with PRD in various crops (Stoll, 2000; Kang *et al.*, 2001).

Girdling of the Cabernet Sauvignon grapevine trunks was done in order to examine the effect of interrupting the carbohydrate flow to the roots and the effect on leaf physiology without reducing the amount of irrigated water. Girdling was done shortly after berry set. Girdling shortly after anthesis is used in the cultivation of table grapes to alter the plant's source/sink relationship and increase berry size (Roper and Williams, 1989). Various researchers have shown that girdling decreases the net CO_2 assimilation rate and stomatal conductance of grapevine leaves for a short period (Düring, 1978; Hofaecker, 1978; Harrell and Williams, 1987). After the initial effect of girdling has dissipated, girdling is reported to increase the carbohydrate concentration above the girdle (Roper and Williams, 1989) and the ABA concentration in the leaves (Düring, 1978).

Girdled Cabernet Sauvignon grapevines successfully reproduced the PRD effects in grapevines by significantly lowered stomatal conductances compared to control vines even though they received the same amount of irrigation water. Similarly to PRD, girdling had no effect on plant water relations as measured by leaf water potential. Girdling also reduced the accumulation of vegetative dry weight while maintaining yield and hastening berry maturity (evolution in maturity data not shown).

Earlier PRD experiments with tomato (Davies *et al.*, 2000) and grapevines in pot and field experiments (Loveys *et al.*, 2000; Stoll, 2000) have shown that although PRD treatment significantly reduced stomatal conductance, there was no significant effect on leaf water potential. By contrast, deficit irrigation of grapevines may significantly reduce leaf water potential relative to well-watered controls (Matthews and Anderson, 1988). Grapevines exposed to severe water stress may exhibit mid morning leaf water potentials of the order of -1.5 MPa to –2.3 MPa (Dundon and Smart, 1984). Investigations into plant water status during this experiment have shown that PRD had no significant effect on mid-morning leaf water potentials of field-grown Cabernet Sauvignon or Shiraz, irrespective of the amount of irrigation water applied. Midday leaf water potentials were much lower than mid-morning values and would be the most stressful time for plants during the day. However, neither PRD nor girdling reduced the leaf water potentials of grapevine leaves during the early stages of active growth or during berry ripening after veraison. The reducing effect of PRD on stomatal conductance and shoot growth rates could therefore not be associated with a reduction in leaf water potentials nor a loss in turgor pressure. Therefore, PRD grapevines were not under any hydraulic stress. However, very late in the ripening season when deeper layers of soil had dried significantly, PRD did exhibit some lower midday leaf and stem water potentials. Marginal reductions in leaf water potentials were also found by de Souza *et al.* (2003), dos Santos *et al.* (2003) and de la Hera Orts *et al.* (2002) at the end of growing season in PRD-treated grapevines.

Stoll (2000) observed that canopies with lower leaf area (PRD) became more open, resulting in higher light intensities inside the canopy compared to canopies with a larger leaf area (control). This was also found in the current study with PRD grapevines receiving half and the same amount of water as control irrigation. PRD increased the light penetration into the bunch zone irrespective of canopy hedging or amount of water applied in the Coombe vineyard. However, PRD treatment on Nuriootpa Shiraz had a limited effect on leaf area at veraison and it was rather the shoot number per vine that was positively correlated with canopy density. The leaf area at harvest however was significantly influenced by PRD treatment. PRD reduced harvest canopy density by its effect on final shoot length. This was also found in Alverstoke Cabernet Sauvignon that received the same amount of irrigation water as control. PRD-treatment significantly reduced the leaf area of grapevines at harvest by reducing final shoot length. These findings are in agreement with the conclusions of Dry *et al.* (2001) and support the findings of dos Santos *et al.* (2003) where PRD reduced vegetative growth in grapevines receiving 50% of crop evapotranspiration. The effect of

PRD on vegetative growth was characterized by lower total leaf area, lower leaf layer number, decreased canopy wideness and decreased number of water shoots.

Partial rootzone drying (PRD) is an irrigation management technique developed in grapevines with a consistent feature that there is little or no significant reduction in yield even though the amount of irrigation water is substantially reduced in comparison to normal irrigation practices (Dry *et al.*, 2001) thereby increasing water use efficiency (WUE). This is found in most experimental situations where soil water status is carefully maintained and plant physiology monitored on regular intervals. In the commercial situation however, the application of PRD and a maintained yield compared to control vines seems to be more challenging (Dry *et al.*, 2001). Dry et al. (2001) found in their Australian commercial experience under various different climatic environments that with a reduction in irrigation water between 37 and 50% compared to control, an increase in WUE of between 29 and 90% could be achieved in field-grown PRD-treated grapevines. Significant increases in WUE due to PRD of 80% and 43% have also been found in other areas of the world using half the amount of irrigation water as control (Kang *et al.*, 2002; dos Santos *et al.*, 2003) and an equal amount of irrigation water as control (de la Hera Orts *et al.*, 2002) respectively. Over the three years in this study, PRD vines that received half the amount of irrigation water as control had no significant effect on yield irrespective of amount of water applied, thereby consistently increasing the WUE in Coombe Cabernet Sauvignon significantly ($P<0.01$) between 77% and 106% and Nuriootpa Shiraz on average by 81% compared to control. In Coombe Shiraz the WUE of PRD vines was significantly increased in 2001 by 25%, but had no significant effect in 2002 and 2003. An increased WUE may be one of the most attractive characteristics of the PRD irrigation system with rising economical and environmental pressures such as salinity and increasing planting areas. A restriction in water availability in the future will require more efficient irrigation practices to sustain production in horticultural crops.

PRD significantly reduced the berry size of Cabernet Sauvignon at harvest in 2 years of the 3-year experiment. PRD however did not influence Shiraz berry weight. Berry degree brix and pH at harvest however was never affected irrespective of amount of water applied. This was also found in berries harvested from Nuriootpa Shiraz and Alverstoke Cabernet Sauvignon receiving half and the same amounts of irrigation water compared to control respectively. However, in Nuriootpa Shiraz pruning level had major effects on yield, berry weight, TSS and pH that did not interact with the irrigation treatment.

The pruning weights over the three years of PRD treated grapevines in the Coombe vineyard were characterized by lower mean shoot weights in Cabernet Sauvignon vines that received half the amount of irrigation water as control. In Coombe Shiraz that received the same amount of irrigation water as control there was reduction in mean shoot weight only in 2001, thereafter the mean shoot weight and pruning weights were higher compared to control. It seems contradictory that PRD reduced shoot growth in Cabernet Sauvignon and Shiraz but increased pruning weights. However, this may be due to the summer hedging done just after veraison so that bird netting could be installed. Assuming that hedging pruned vines to a uniform sized canopy, Cabernet Sauvignon and Shiraz control vines lost more accumulated dry weight than PRD vines. When the total pruning weights were calculated PRD had similar total pruning weights as control vines irrespective of the amount of irrigation water applied.

4.5 Conclusions

The experiments described in this chapter were conducted to test the hypothesis that PRD increases water use efficiency and reduces stomatal conductance, shoot growth and canopy density irrespective of the amount of irrigation water applied. Enough evidence has been collected to accept this hypothesis. The major conclusions were:

1) PRD reduced main and lateral shoot growth when irrigated with either the same or up to half the amount of irrigation water used in control vines. The largest effect was found on lateral shoot growth.

2) PRD had a long-term effect on grapevine leaf physiology by significantly reducing stomatal conductance in field experiments irrespective of the amount of water applied.

3) PRD caused long-term reductions in stomatal conductance compared to control without appreciably affecting grapevine assimilation rates, thereby greatly increases grapevine water use efficiency.

4) Girdling significantly lowered stomatal conductances without reducing leaf water potentials compared to control even though they received the same amount of irrigation water. Girdling also reduced the accumulation of vegetative dry weight while maintaining yield.

5) The reducing effects of PRD on stomatal conductance and shoot growth rates were not associated with a reduction in leaf water potentials or a loss in turgor pressure. Therefore, PRD grapevines were not under any hydraulic stress.

6) PRD treatment significantly reduced the leaf area and increased light penetration into the bunch zone of grapevines at harvest by reducing final shoot length.

7) PRD had no significant effect on yield irrespective of the amount of water applied, thereby consistently increasing the WUE.

8) PRD lowered mean shoot weights but increased winter pruning weights. However, when the total pruning weights (summer and winter) were calculated PRD had similar total pruning weights as control vines irrespective of the amount of irrigation water applied.

Chapter 5: PRD and exogenous ABA affect accumulation and partitioning of nitrogen, minerals and assimilated carbon in grapevine.

5.1 Introduction

Plant nutrition, in the biochemical sense, deals with a complex of biosynthetic events by which organic plant tissue is produced from inorganic materials in the environment. Physiologically, the definition should be broadened to include the selective acquisition of these materials from the environment and their internal distribution to organs where they are needed. Grapevines can adapt to a wide range of soil characteristics and they are less exacting than many other horticultural crops (Winkler *et al.*, 1974). The root system will explore the surface soil and the subsoil to depths determined primarily by the soil characteristics but also by the location of soil moisture. Under sufficient soil conditions grapevine roots are active from early spring to late autumn, leaving a long period to absorb the required soil nutrients. Only fifteen elements are known to be absolutely necessary for normal growth and fruiting. Ten of these elements are needed in relatively large amounts, called macronutrients: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, sulphur, iron, calcium and magnesium. The other five elements, although essential, are used in relatively small amounts, called trace elements: boron, manganese, copper, zinc and molybdenum.

Experiments with intact or excised roots have elegantly demonstrated the rate of nutrient absorption by roots over a wide range of ionic concentration (Nissen, 1974) or by depletion of the nutrient solution over time (Claassen and Barber, 1974). However, it has undoubtedly created the false impression that there is a fixed relationship between ionic concentration and transport rates. Clarkson and Hanson (1980) argues that the quantity of nutrient absorbed by a plant over a given period of time is an integral of the growth that has occurred and that the rate of absorption from a given concentration is determined by the "demand" for nutrients created by growth.

Growing tissue depends on actively dividing and expanding cells and the expansion is dependent upon a sufficient water supply from the roots. It has long been known that, since flows of water and of nutrients are coupled both in the xylem and in the phloem, scarcely transpiring organs like developing fruits in certain species tend to be under-supplied with nutrients such as calcium, which are phloem immobile (Marschner, 1995). However, similar limitations of nutrient supply may also arise for phloem mobile elements like nitrogen, since the import via xylem is dependent on the intake of water and phloem flow of solutes on the

hydrostatic pressure gradient (Jeschke and Hartung, 2000). PRD significantly reduces both the vegetative growth and stomatal conductance (transpiration) in grapevines (Dry *et al.*, 2001) and may reduce xylem flow towards transpiring organs. However, no information exists on the effect of PRD on the actual assimilation and partitioning of nutrients and minerals in grapevines.

The grapevine's demand for seasonal nitrogen is fairly well known, but by using conventional methods it is not possible to distinguish between nitrogen absorbed during different periods within one growing season. By using the ^{15}N isotope the amount of recently absorbed nitrogen can be determined. Apart from the permanent structure, leaves and shoots are important transitional reservoirs for absorbed nitrogen and up to harvest nitrogen is turned over at a relatively fast rate in these organs with large contributions to fruit, even though the total nitrogen content does not change (Conradie, 1991). The fate of nitrogen absorbed during a specific period cannot be quantified in field trials because some of the applied nitrogen will be retained in the soil and absorbed later (Conradie, 1986). Furthermore, it is important that the entire plant should be analyzed to measure nutrient utilization and distribution (Titus and Kang, 1982). It is therefore most practical to use potted grapevines in sand culture where excess ^{15}N can be washed out at the end of the labeling period and plants can be harvested cleanly and efficiently.

Studies investigating the hormonal aspects of plant responses to drought have focused on abscisic acid (ABA) with the first evidence that loss of turgor leads to massive increases in foliar ABA by Wright and Hiron (1969). Since then much evidence has been accumulated that ABA plays a central role in the early defense mechanism against excessive water loss by regulating stomatal aperture. The PRD system relies on hormonal signals originating from the roots in response to low soil water potentials within the 'dry' zone. Much evidence has been accumulated that drying roots are the origin of abscisic acid (ABA), which is involved in regulating stomatal aperture (Düring *et al.*, 1996; Dry *et al.*, 2000). The observed effects of ABA in aboveground organs due to PRD are a reduction in shoot growth and partial stomatal closure (Dry and Loveys, 1999). It was suggested that a long-term effect on stomatal conductance and shoot growth in grapevines is only possible if the signal originating from the 'dry' side can be sustained. By alternating the 'wet' and 'dry' sides, it was possible to maintain a long-term response (Dry, 1997) and it became clear that a continuous chemical signal or a certain concentration of the signal is necessary to maintain a physiological response.

There are numerous, and frequently contradictory, reports that growth regulators influences the rates of absorption and transport of ions either directly, by some interaction with membranes (Wood *et al.*, 1974), or indirectly through effects on metabolism (for review see Steveninck (1976)). Hormones transported between roots to shoots may provide the messages to co-ordinate root activity with shoot demand. To test the specificity of any hormonal action in plants, the effects that are proposed to be influenced by a single plant hormone should be able to be induced by either external application or by manipulation of the endogenous hormone level. The criteria of "correlation and duplication" and "deletion and reinstatement" for the significance of a hormone effect was first proposed by Jacobs (1959) and later modified by Jackson (1987).

The PRD irrigation strategy relies on exposing the root system to different soil water gradients at the same time to invoke a hormonal and/or nutritional effect on grapevine vegetative growth. Exogenous ABA successfully reproduced the PRD effect in grapevines in previous experiments by Stoll (2000) i.e. reducing stomatal conductance and shoot growth. Exogenous ABA may therefore be used again to investigate the hormonal effect of PRD with the aspect of soil drying removed. The diminishing effect on vegetative growth may have significant effects on the nutrient absorption and partitioning of grapevines on a physiological and biochemical level.

Experiments described in this chapter were conducted to test the hypothesis that *Partial rootzone drying causes partitioning of more nitrogen, minerals and assimilated carbon to the permanent structure at the expense of vegetative growth and this is mainly due to the hormonal influence of root-sourced ABA on plant growth and function.*

5.2 Materials and methods

Experiments in the field are explained in more detail in Section 2.1 and in Chapter 4. In short, field grown PRD Cabernet Sauvignon (*Vitis vinifera* L.) received half the amount of irrigation water of control vines until harvest and PRD Shiraz received the same amount of irrigation water as control vines until harvest. All vines were own-rooted and the experimental design for both cultivars consisted of a randomized block with two treatments, control and PRD irrigation, and seven replicates. Each plot consisted of three vines and data were only collected from the centre vine.

Field-grown Shiraz vines at the PIRSA research station in the Barossa Valley (Nuriootpa, South Australia) were also used. Experimental design consisted of a 2 x 3 factorial design with two irrigation treatments (Control and PRD) and three pruning levels (30, 60 and 120

nodes/vine) with five repetitions of each. PRD received half the amount of water as control vines.

The potted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) were grown outdoors from cuttings taken from the Coombe vineyard in 1999, split-rooted and established in two 8 L pots filled with standard potting mix (Table 2.1). A year before the experiment (2002) the vines were un-potted, the roots washed and cleaned and re-potted in pure sand. The re-potted vines were then fertilized during the 2002 season with a 0.2% Aquasol® solution. In preparation for the 2003 experiment the vines were pruned to five 2-node spurs and the shoot number was controlled to 5 per vine. The grapevines were left to bear 2 or 3 bunches. The vines received no fertilization, PRD or exogenous ABA from the end of the previous summer until the experiment started on the 13th of January 2003. The grapevines were between the stages where berries are still hard and green and when they start to soften (33 and 34 on the E-L system). The pots were placed on potting trays and bagged in plastic to eliminate any loss of water and labeled nitrogen due to leaching (Figure 5.1). The pots were then covered by a sheet of reflective material (Sisalation™) to minimize extreme temperatures and evaporation from the potting medium.

Figure 5.1 Split-rooted Cabernet Sauvignon used in labeled nitrogen experiment at the Waite campus (summer 2003).

The split-rooted four-year-old grapevines were irrigated daily with 0.8-1.2 L water that was split in two sessions, one in the morning (9:00 am) and one in the late afternoon (5:00 pm). The irrigation was calculated by the amount needed to refill the soil moisture content to field capacity measured by Time Domain Reflectometry (TDR, Trase system) using a 15 cm waveguide (for details see section 2.3). Daily stomatal conductances were measured using the AP4 diffusion porometer (DELTA-T Devices Ltd, Cambridge, UK) described in Section 2.4 on 4 leaves per vine between 11:00 am and 12:00 pm. The experiment consisted of three treatments; control received the same amount of water in both pots every day, PRD received water in one pot only while the other was left to dry and ABA received the same irrigation as control except that one of the pots received water with a 10 μM ABA (synthetic 98%, Sigma-Aldrich, Sydney, Australia) concentration every second day. The PRD irrigation was alternated every 3-4 days when the soil moisture readings in the drying pot showed no further soil drying. The ABA treatment alternated with the PRD alternations.

Labeling was done as described by Conradie (1991) and Glad et al. (1994). Grapevines were fertilized with a Tournament formulation (T-Link, South Australia) containing Nitrogen:Phosphorus:Potassium:Sulphur (22:4.3:19:0.2) with trace elements and KNO_3. Replacing the normal KNO_3 with labeled $K^{15}NO_3$ (5 atom % excess) increased the natural abundance of the isotope. In this season, 15 vines were labeled with ^{15}N during a 56-day period, starting near the end of rapid shoot growth (close to veraison). The fertilizer mixture contained 1 gm/L $^{15}NO_3K$ and 2 gm/L Tournament. One unlabelled vine received a fertilizing mixture where the $^{15}NO_3K$ was replaced with normal KNO_3 to measure the natural abundance of ^{15}N. The daily irrigation was replaced by the fertilizer solution once a week for 8 weeks until 2 weeks before harvest. Every grapevine received 7400 mL fertilizing mixture during the experiment that equaled to 7.4 gm $K^{15}NO_3$ and 14.8 gm Tournament.

Small berry samples (5 berries) were taken at harvest to determine berry soluble solids (°Brix) and pH. Vines were harvested on the 25th of March 2003 and divided into different organs, i.e. thick roots (> 2 mm), fine roots (< 2 mm), permanent structure (wood older than 1 year), new structure (wood younger than 1 year), mature leaves, young/small leaves, berries and rachises. After the fresh weights were recorded and all the organs were dried in a forced-air oven at 60°C, weighed and milled to pass through a sieve with 0.2 mm aperture (Endecotts Ltd. Mesh no 80, London, UK). Sub-samples (10 µg) were carefully weighed into tin capsules, rolled into balls and placed into trays. The ^{15}N isotopic composition and total N of the samples were then determined using a mass spectrometer linked to a combustion nitrogen analyzer. ^{15}N analyses (atom % excess) were done by Stuart McClure (CSIRO Land and Water, Adelaide, South Australia). Further analyses included starch content (Section 2.12), total carbon and nitrogen (Section 2.14), K, Ca, Mg, P, S and Na (Section 2.11).

Partitioning of ^{15}N: The partitioning of ^{15}N was calculated within the whole plant. The amount of ^{15}N (in excess of natural abundance) originating from $K^{15}NO_3$ labeling was calculated by:

Mass of ^{15}N (in excess) arising from labeling =

Dry weight(g) **X** %as total N **X** (% atom ^{15}N excess $_{sample}$ - % atom ^{15}N $_{zero}$)

The sum of the amounts of ^{15}N found in excess in the different organs gave the total amount in the whole plant (S). Subsequently, the relative distribution of ^{15}N in the different organs was expressed as the parameter P(%):

$$P(\%)\ (organ) = Mass\ ^{15}N_{organ}/Mass\ ^{15}N_S\ X\ 100$$

The experiment consisted of three treatments with five repetitions of each. ANOVA's, correlations and the student T-tests to determine which groups were significantly different were done using the SAS System (SAS Institute Inc., Cary, North Carolina, USA). Significance values are indicated by the P-values.

5.3 Results

To mimic the PRD irrigation effect, synthetic ABA was applied to the roots every second day to split-rooted cabernet Sauvignon vines. The effect of PRD and exogenous ABA on stomatal conductance is shown in Figure 5.2. Stomatal conductance measurements started two days after labeling started on the 13th of January 2003.

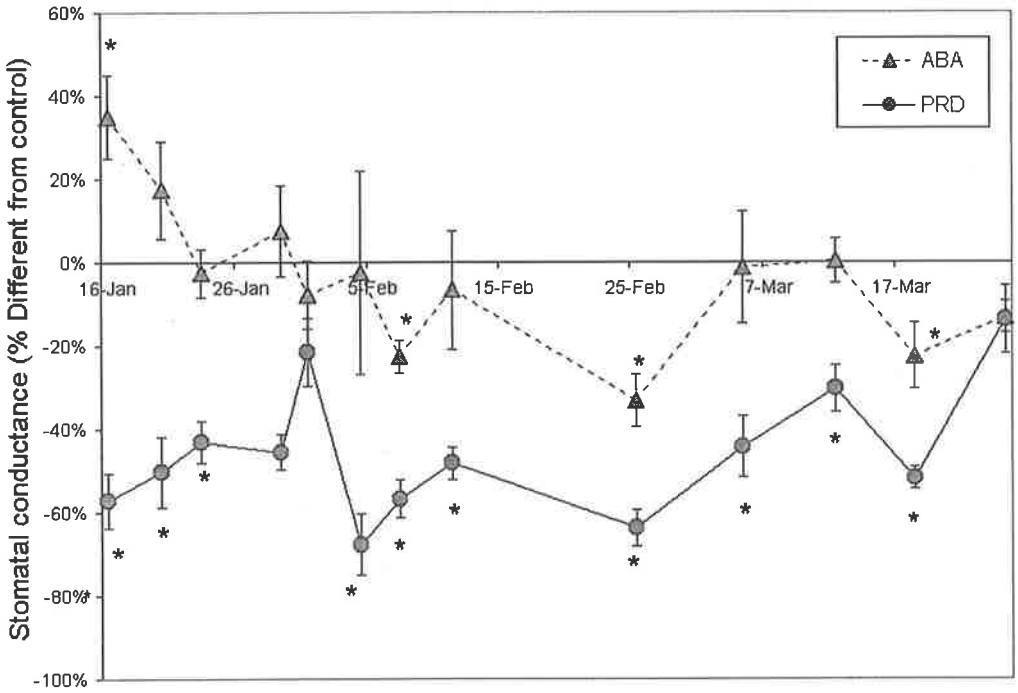


Figure 5.2 Effects of PRD and exogenous ABA on grapevine stomatal conductance ((mmolm^{-2}s^{-1}) as a percentage of control; Cabernet Sauvignon, split-root potted vines). Data points represent the mean of 4 measurements on each of the 5 replicates (± s.e) * = significantly different compared to control ($P<0.05$).

The exogenous ABA treatment had a less pronounced effect on stomatal conductance than experiments of the previous year compared to control (Chapter 6; Figure 6.9). Stomatal conductance was only reduced on three separate occasions during the period until harvest

amounting to an average reduction of 20%. PRD treatment, however, showed significantly larger reductions in stomatal conductance compared to control and ABA treated grapevines, averaging a 60% and 40% reduction respectively. PRD had significant differences in stomatal conductance at the start of the experiment that was sustained throughout the ripening period. Exogenous ABA however started with significantly higher stomatal conductance and slowly reduced over time until it was significantly less about 2 weeks later.

5.3.1 PRD and exogenous ABA effects on fruit and accumulated dry weight

The harvest data are presented in Table 5.1. PRD and exogenous ABA had no significant effect on harvest yield or total soluble solids (°Brix). However, PRD significantly reduced berry weight and increased juice pH. The production of a similar yield in PRD with a reduced amount of irrigation water significantly increased the water use efficiency of PRD vines by 85%.

Table 5.1 Harvest data for pot-grown Cabernet Sauvignon (2003). Means indicated with different letters are significantly different ($P<0.05$) and means without letters are not significantly different ($P<0.05$).

	Yield (g/vine)	Berry weight (g)	Juice TSS (°Brix)	Juice pH	Applied Water (L)	WUE ($t.ML^{-1}ha^{-1}$)
Control	121.3	0.80^{a}	26.7	3.24^{a}	76.2	3.18^{b}
ABA	121.0	0.75^{ab}	24.6	3.39^{ab}	76.2	3.12^{b}
PRD	112.1	0.70^{b}	26.1	3.44^{b}	38.1	5.88^{a}

The dry weights of the harvested plant organs are shown in Table 5.2. Both PRD and exogenous ABA significantly reduced the total canopy dry weights due to the significant reduction in dry weights of leaves and one-year old shoots that constituted the vegetative part of the grapevine. However, the dry weights of the reproductive part, i.e. the berries and rachises, did not differ significantly between treatments. The permanent structure, i.e. wood older than 1 year and roots, also showed no significant differences although PRD had less thick roots (≥ 2mm). Total root weight did not change significantly during the ripening period. Both PRD and exogenous ABA had higher average dry weights of permanent wood (mostly the trunk and spurs) by 35% and 37% respectively, but the increased amounts were not significantly different ($P<0.05$).

Table 5.2 Organ dry-weights for pot-grown Cabernet Sauvignon (2003). Means indicated with different letters are significantly different (P<0.05) and means without letters are not significant different (P<0.05).

	Rachis dry wt (g)	Total berries dry wt (g/vine)	Leaves dry wt (g)	1Year old Shoots dry wt (g)
Control	2.4	41.5	58.7[a]	84.0[a]
ABA	2.2	29.6	42.0[b]	54.7[b]
PRD	2.3	38.9	42.0[b]	69.3[ab]
	Perm Wood dry wt (g)	≥2mm Roots dry wt (g)	≤2mm Roots dry wt (g)	Total Roots dry wt (g)
Control	72.3	106.2[a]	67.1	173.3
ABA	99.3	92.2[ab]	60.1	152.4
PRD	97.3	81.3 b	66.9	148.3

5.3.2 PRD and exogenous ABA effects on grapevine starch accumulation and partitioning.

The results of the analyses of starch concentration per organ are shown in Figure 5.3. Exogenous ABA had no effect on organ starch concentration compared to control, however PRD significantly reduced the starch concentration in both mature and young leaves ($P < 0.05$) amounting to a 73% drop in starch concentration in the total canopy. PRD however, had no further effect on the tissue starch concentration of reproductive or permanent organs compared to control and exogenous ABA.

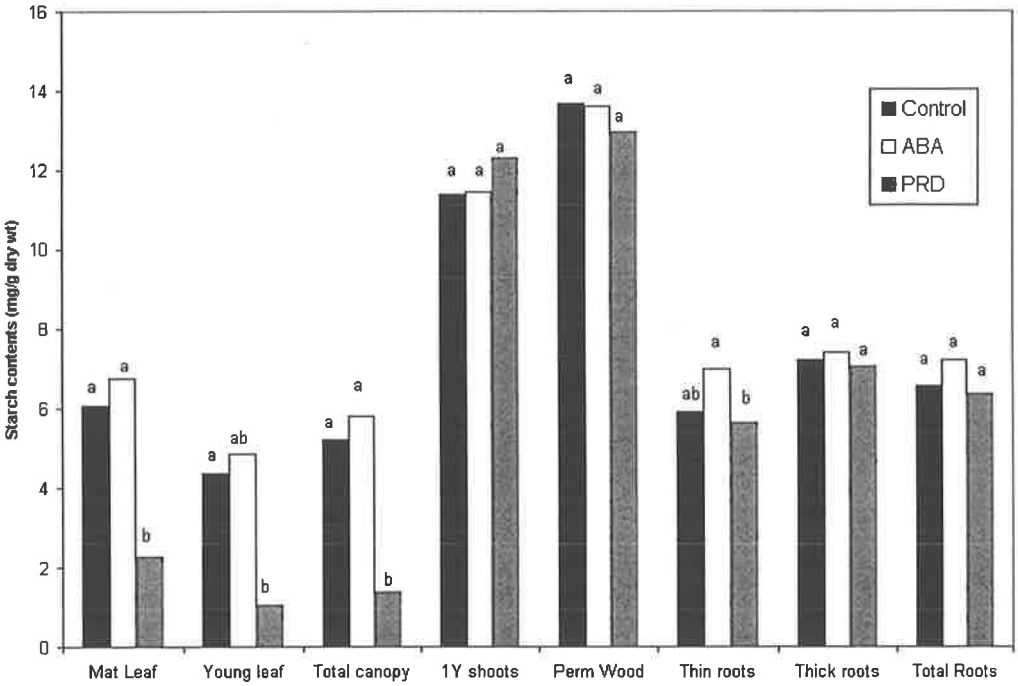


Figure 5.3 Effect of PRD and exogenous ABA on organ starch concentration (mg/g dry weight) of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 µM ABA on one side). Bars represent means of 5 replicates and bars with different letters are significantly different (P<0.05).

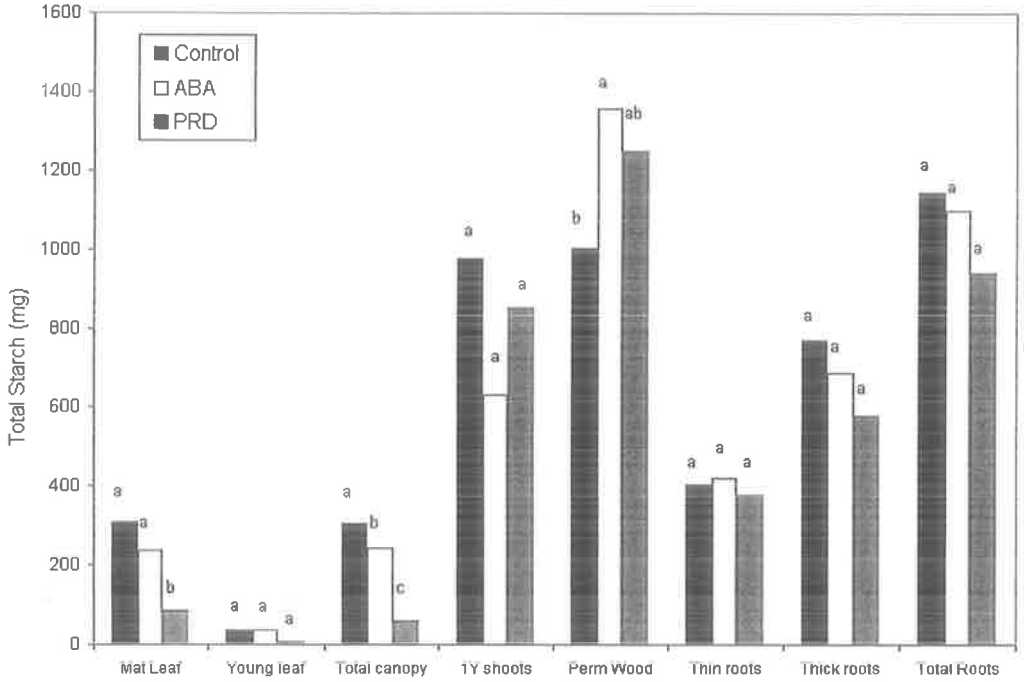


Figure 5.4 Effect of PRD and exogenous ABA on total starch (mg/organ) of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Bars represent means of 5 replicates and bars with different letters are significantly different ($P<0.05$).

The calculated starch per organ is shown in Figure 5.4. PRD had significantly less total starch (mg) in mature leaves and total canopy compared to control and exogenous ABA due to its significantly lower starch concentration and accumulated dry weights. Exogenous ABA-treated vines accumulated less starch in their canopy than control vines mainly due to a lower canopy dry weight, however it was significantly more than PRD-treated vines. One-year old shoots did not have significantly different total starch in either PRD or exogenous ABA compared to control, although the accumulated dry weights were less. This was due to the effect of small increases in tissue starch concentration. PRD and exogenous ABA also averaged higher total starch in permanent wood than control by 25% and 35% respectively. Both PRD and exogenous ABA caused decreases in root starch but this was not significant ($P<0.05$). Ultimately, PRD and exogenous ABA treatments had no significant effect on the total accumulated starch per vine. The average total amounts of starch per vine for PRD was 3.13 g, ABA 3.36 g and control 3.47 g.

5.3.3 PRD and exogenous ABA effects on grapevine mineral accumulation and partitioning.

The PRD and exogenous ABA effects on total inorganic ion content (mg/organ) are shown in Table 5.3 and on tissue concentrations (mg/g dry weight) in Table 5.4. The correlation matrix between mineral content per berry and berry weight (dry weight) is shown in Table 5.5.

Potassium (K)

Neither PRD nor exogenous ABA influenced the total amount of K assimilated or partitioned in the grapevine (Table 5.3). The only notable difference was between K accumulated in the permanent wood and vegetative tissues. In PRD and ABA-treated vines the combined percentages of permanent wood and root K in relation to the whole plant K rose by 5% and 3% respectively ($P=0.034$) and fell by the same margin in one-year old shoots ($P=0.094$) compared to control while K distribution stayed the same in all the other organs.

PRD and exogenous ABA however, increased the percentage K on a dry weight basis in berries and leaves compared to control. PRD furthermore increased the concentration of root and mature leaf K compared to exogenous ABA. ABA berries had the highest concentration of K of all treatments but on amount (mg) per berry basis control berries had the highest K content, followed by exogenous ABA and then PRD. Accumulated K per berry was highly and positively correlated ($P<0.01$) with berry size and the range of other minerals that included N, C, Ca, Mg, P and S.

Calcium (Ca)

Although not influencing total Ca content on a whole plant basis, PRD and exogenous ABA significantly affected the distribution of Ca during the ripening period. The Ca accumulated in leaves was significantly less ($P=0.002$) and amounted to 12% and 10% less Ca partitioned to these organs in PRD and exogenous ABA respectively. The Ca partitioned to one-year old shoots was also significantly less ($P=0.020$) and amounted to 3% and 4% less for PRD and exogenous ABA respectively. However, on a whole plant basis there were no significant differences in the amount of Ca absorbed between the treatments in spite of the significant differences in partitioning/accumulation of Ca in permanent structures. Permanent wood showed significant increases in accumulated Ca for both PRD and exogenous ABA amounting to 10% and 12% respectively ($P=0.0002$). Neither PRD nor exogenous ABA had any effect on berry final Ca concentration or total accumulation compared to control, however control berries had the highest Ca content on the amount (mg) per berry basis, followed by exogenous ABA and then PRD. Accumulated Ca per berry correlated positively ($P<0.05$) with berry size and the other minerals except Na and S.

Magnesium (Mg)

On a whole plant level, both PRD and exogenous ABA accumulated on average 17% less Mg than control ($P=0.081$) with the biggest decrease in partitioning in leaves (7%). Both

PRD and exogenous ABA partitioned significantly more (P=0.001) Mg in permanent wood by 6% and 8% respectively. Control berries had the highest Mg content on amount (mg) per berry basis, followed by exogenous ABA and then PRD. Accumulated Mg per berry was highly and positively correlated (P<0.01) with berry size and the other minerals except Na.

Sodium (Na)

PRD accumulated significantly more sodium than exogenous ABA but no more than control vines. PRD partitioned higher amounts to permanent wood (P=0.0002), amounting to a 3% increase compared to control, while exogenous ABA had an increased Na content in permanent wood but lower partitioning to leaves and roots compared to control. The correlation between berry size and sodium per berry was not strong but slightly negative, which means that with bigger berry sizes the sodium concentration was actually diluted to lower concentrations. This was clear in the Na per berry values where control berries with the larger berries had the lowest Na content, followed by exogenous ABA and finally PRD with the smallest berries having the highest Na content. Na per berry also correlated negatively with all the other minerals with only N, K and S being significantly (P<0.1) correlated.

Phosphorus (P)

PRD and exogenous ABA had no effect on total P content. However, PRD and exogenous ABA accumulated significantly less P in leaves (P=0.041) that amounted to a 5% and 6% decrease in partitioning respectively compared to control. Exogenous ABA also had lower P in one-year old shoots that amounted to a 4% decrease (P=0.048). This was equalized to a degree by greater partitioning to permanent wood. PRD had a significant increase in P concentration in berries and leaves compared to control. However, the amount of P per berry was not significantly affected by treatments but positively correlated (P<0.01) with berry size.

Sulphur (S)

PRD and exogenous ABA had little effect on grapevine total S content. However, due to differences in dry weights PRD and exogenous ABA had significantly less S partitioned to the leaves amounting to a 3% and 5% decrease (P=0.020) in partitioning respectively compared to control. More S seemed to be partitioned to the permanent structure with a 5% and 7% rise for PRD and exogenous ABA respectively (P=0.070). Similar to the accumulation in P, PRD and exogenous ABA increased concentrations of S in berry, leaf and root tissues compared to control. However, the total amount of S per berry indicated no

significant differences between treatments but a positive correlation ($P<0.05$) between berry size and S.

Table 5.3 Total inorganic ion content (mg/organ) at harvest of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Means indicated with different letters are significantly different ($P<0.05$). Percentages represent the relative distribution of total mineral content within the vine.

Total (mg)		Rachis	Berries	Total Leaves	1Year old shoots	Permanent Wood	Total Roots	Total/plant
Potassium	Control	82.7 (2.2%)	499.1 (13.3%)	968.5 (26%)	707.9 (19%)	281.1 (7%)	1184.7 (32%)	3742 (100%)
(K)	ABA	58.8 (1.8%)	412.2 (12.4%)	942.1 (28%)	476.0 (14%)	424.6 (13%)	1010.6 (30%)	3324 (100%)
	PRD	73.3 (1.9%)	511.2 (13.4%)	968.5 (26%)	586.3 (15%)	428.2 (11%)	1235.7 (33%)	3803 (100%)
Calcium	Control	9.1 (0.4%)	42.9 (2.0%)	818.7^{a} (38%)	334.4^{a} (15%)	279.8^{b} (13%)	695.3 (32%)	2180 (100%)
(Ca)	ABA	5.6 (0.3%)	34.8 (1.9%)	517.2^{b} (28%)	200.6^{b} (11%)	470.1^{a} (25%)	627.2 (34%)	1855 (100%)
	PRD	6.6 (0.4%)	41.5 (2.3%)	475.5b (26%)	228.2^{b} (12%)	426.4^{a} (23%)	663.2 (36%)	1841 (100%)
Magnesium	Control	3.9 (0.5%)	29.1 (3.6%)	258.5^{a} (32%)	136.8 (17%)	66.6^{b} (8%)	307.4 (38%)	802^{a} (100%)
(Mg)	ABA	3.0 (0.5%)	22.1 (3.4%)	162.0^{b} (25%)	83.3 (13%)	101.6^{a} (16%)	273.9 (42%)	646^{b} (100%)
	PRD	3.4 (0.5%)	28.2 (4.3%)	170.8^{b} (26%)	101.5 (15%)	93.0^{ab} (14%)	266.6 (40%)	662^{ab} (100%)
Sodium	Control	13.0^{ab} (2.6%)	17.2 (3.4%)	74.8^{a} (15%)	49.1 (10%)	19.8^{b} (4%)	328.3^{ab} (65%)	502^{ab} (100%)
(Na)	ABA	7.8^{b} (2.4%)	15.9 (4.8%)	44.5^{b} (14%)	28.6 (9%)	39.7^{a} (12%)	192.6^{b} (59%)	329^{b} (100%)
	PRD	16.7^{a} (3.0%)	21.9 (3.9%)	65.4^{ab} (12%)	43.2 (8%)	41.8^{a} (7%)	373.4^{a} (66%)	562^{a} (100%)
Phosphorus	Control	6.5 (1.1%)	53.2 (8.8%)	100.7^{a} (17%)	101.8^{a} (17%)	65.4 (11%)	276.2 (46%)	604 (100%)
(P)	ABA	5.4 (1.0%)	40.2 (7.2%)	69.1^{b} (12%)	69.8^{b} (13%)	89.7 (16%)	280.0 (51%)	554 (100%)
	PRD	6.1 (1.1%)	58.2 (10.5%)	63.5^{b} (11%)	88.4^{ab} (16%)	84.8 (15%)	255.1 (46%)	556 (100%)
Sulphur	Control	1.8 (0.4%)	24.3 (5.2%)	98.6^{a} (21%)	61.3 (13%)	49.4^{b} (11%)	228.4 (49%)	464 (100%)
(S)	ABA	1.4 (0.4%)	21.0 (5.3%)	65.4^{b} (16%)	42.3 (11%)	75.1^{a} (18%)	193.6 (49%)	399 (100%)
	PRD	1.4 (0.3%)	28.3 (6.6%)	75.5^{b} (18%)	53.4 (12%)	67.2^{ab} (16%)	205.4 (48%)	431 (100%)

Table 5.4 Inorganic ion concentration (mg/g dry wt) at harvest of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Means indicated with different letters are significantly different ($P<0.05$).

		Rachis	Berry (mg/g)	Berry (mg/berry)	Mature Leaves	Young Leaves	Total Leaves	1Year old shoots	Permanent wood	> 2mm roots	<2mm roots	Total roots
Potassium	Control	31.8	12.2^{c}	3.264	14.3^{c}	19.3^{b}	16.8^{b}	8.49	3.82	4.43 b	9.37^{b}	6.90^{b}
(K)	ABA	29.9	13.9^{a}	3.156	19.4^{b}	25.3^{a}	22.4^{a}	8.67	4.27	4.72 b	8.60^{b}	6.66^{b}
	PRD	32.2	13.1^{b}	3.073	22.3^{a}	23.6^{ab}	22.9^{a}	8.2	4.4	5.45 a	11.13^{a}	8.29^{a}
Calcium	Control	3.5	1.09	0.292	15.1	13.2	14.1	3.95^{a}	3.84^{b}	3.27	5.04	4.16
(Ca)	ABA	2.82	1.19	0.273	13.8	11.1	12.5	3.67^{ab}	4.71^{a}	3.51	4.70	4.10
	PRD	2.86	1.07	0.250	13.2	9.4	11.3	3.25^{b}	4.41^{a}	3.64	5.31	4.48
Magnesium	Control	1.60	0.73	0.195	4.38	4.54	4.46	1.60	0.91	1.32	2.28	1.80
(Mg)	ABA	1.54	0.76	0.173	3.91	3.88	3.89	1.51	1.02	1.45	2.14	1.79
	PRD	1.54	0.73	0.170	4.24	3.87	4.05	1.44	0.96	1.32	2.28	1.80
Sodium	Control	5.07^{ab}	0.41	0.109	1.31^{ab}	1.28	1.3	0.58	0.26^{b}	0.95 b	2.76^{b}	6.90^{b}
(Na)	ABA	4.12^{b}	0.52	0.113	1.06^{b}	1.06	1.06	0.52	0.40^{ab}	0.80 b	1.72^{b}	6.66^{b}
	PRD	7.60^{a}	0.58	0.133	1.78^{a}	1.29	1.54	0.6	0.44^{a}	1.48 a	3.51^{a}	8.29^{a}
Phosphorus	Control	2.68	1.30^{b}	0.347	1.66	1.82^{a}	1.74^{a}	1.23	0.96	1.75	1.56^{b}	1.65
(P)	ABA	2.55	1.31^{b}	0.314	1.73	1.68^{ab}	1.71^{ab}	1.25	0.95	1.91	1.7^{ab}	1.81
	PRD	2.66	1.47^{a}	0.350	1.56	1.49^{b}	1.53^{b}	1.23	0.91	1.85	1.75^{a}	1.8
Sulphur	Control	0.66	0.60^{c}	0.158	1.27^{b}	2.09	1.68	0.72	0.67	1.04	1.51^{b}	1.28^{b}
(S)	ABA	0.63	0.69^{b}	0.161	1.23^{b}	2.10	1.66	0.77	0.71	1.07	1.53^{b}	1.30^{ab}
	PRD	0.63	0.74^{a}	0.169	1.90^{a}	1.89	1.89	0.75	0.67	1.11	1.77^{a}	1.44^{a}

Table 5.5 Correlation matrix for inorganic ion content (mg/berry) and berry dry weight at harvest of split-rooted Cabernet Sauvignon (Grey cell: P<0.05; dark grey: P<0.01).

	N	C	K	Ca	Mg	Na	P	S
N	1.00							
C	0.7579	1.00						
K	0.8210	0.8972	1.00					
Ca	0.5408	0.5650	0.5844	1.00				
Mg	0.4504	0.5947	0.5385	0.8970	1.00			
Na	-0.5674	-0.3762	-0.4896	-0.4115	-0.2069	1.00		
P	0.8699	0.8415	0.8594	0.5929	0.6553	-0.3767	1.00	
S	0.9640	0.7094	0.8433	0.4226	0.3460	-0.5404	0.8354	1.00
Berry size	0.6616	0.9789	0.8525	0.4850	0.5354	-0.3856	0.7834	0.6387

Df = 13; if r > 0.514, P < 0.05; if r > 0.641, P < 0.01;

The correlation matrix between berry mineral concentrations and berry fresh weight for the split-rooted experiment is shown in Table 5.6. The results indicate that berry size (fresh weight) in the split-rooted experiment correlated poorly with most of the mineral concentrations tested, except for Na. Na was negatively correlated (P<0.05) with berry size indicating that larger berries had lower Na concentrations. N was positively correlated with C, K, P and S (P<0.01) concentrations and there were positive correlations between the berry concentrations of K, P and S (P<0.01). A strong positive correlation existed between Mg and Ca concentrations (P<0.01).

Table 5.6 Correlation matrix for inorganic ion concentration (mg/g dry wt) and berry fresh weight at harvest of split-rooted Cabernet Sauvignon (Grey cell: P<0.05; dark grey: P<0.01).

	N	C	K	Ca	Mg	Na	P	S
N	1.00							
C	0.6226	1.00						
K	0.6930	0.3273	1.00					
Ca	0.3301	0.3401	0.4354	1.00				
Mg	0.1482	0.2413	0.2826	0.8597	1.00			
Na	-0.2933	-0.1194	0.0130	-0.0160	0.2186	1.00		
P	0.7417	0.4794	0.5738	0.3920	0.4577	0.1186	1.00	
S	0.9015	0.3247	0.7338	0.2568	0.1163	-0.0871	0.7060	1.00
Berry size	-0.1991	0.2010	-0.2899	-0.3409	-0.3775	-0.5793	-0.4337	-0.4250

df=13; if r>0.514, P<0.05; if r>0.641,P<0.01;

5.3.4 PRD and exogenous ABA effects on the partitioning of newly absorbed nitrogen.

The fate of newly absorbed nitrogen (N) as measured in tissue ^{15}N concentration (atom % excess) is represented in Table 5.7. Analyses of ^{15}N abundance revealed that PRD and exogenous ABA did not have any significant effect on the abundance of labeled N in most organs compared to control. However, exogenous ABA caused significantly higher abundances of labeled N in berries, one-year old shoots and thin roots than PRD. PRD vines had increased abundance only in total canopy leaves compared to both control and exogenous ABA vines. It could therefore be expected that PRD and exogenous ABA partitioning of labeled N would be greatly influenced by organ dry weight.

Table 5.7 Abundance of labeled nitrogen (atom% excess ^{15}N) at harvest of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Data represent means after natural abundance of ^{15}N was subtracted. (Means indicated with different letters are significantly different ($P<0.05$); means n = 5).

	Berries	Rachis	Mature Leaves	Young leaves	Total Canopy
Control	0.97 ^{ab}	1.52	1.94	1.6	1.75 ^{b}
ABA	1.10 ^{a}	1.69	2.08	1.6	1.85 ^{b}
PRD	0.84 ^{b}	1.37	2.02	2.0	2.12 ^{a}
	1Year-old shoots	Permanent wood	Thick roots (≥2mm)	Thin roots (≤2mm)	Total roots
Control	2.01 ^{ab}	1.38	1.60	1.41 ^{ab}	1.50
ABA	2.09 ^{a}	1.37	1.50	1.43 ^{a}	1.46
PRD	1.72 ^{b}	1.27	1.31	1.21 ^{b}	1.26

PRD and exogenous ABA effects on the distribution of total ^{15}N per organ relative to whole plant ^{15}N is shown in Table 5.8. The relative distribution is categorized in vegetative, reproductive and permanent structures. PRD-treated grapevines absorbed significantly less ^{15}N than control during the ripening period, while exogenous ABA-treated and control vines absorbed similar amounts of ^{15}N. Although PRD-treated vines had less ^{15}N in total, it had no effect on N assimilated in reproductive organs. The relative distribution of newly absorbed N increased to fruit by 0.5% compared to control and 0.6% compared to ABA. PRD however partitioned less N to vegetative structures by 2% compared to control but significantly more than exogenous ABA by 8%. Further breakdown of vegetative structures revealed that PRD-treated vines had significantly less ^{15}N partitioned to young leaves by 3% and, although not significant, to one-year old shoots by 3% compared to control. The distribution of ^{15}N to vegetative structures in exogenous ABA was significantly less by 10%

compared to control. The biggest reduction was in mature leaves by 6% and in one-year old shoots by 4% compared to control.

Both PRD and exogenous ABA partitioned more ^{15}N in the permanent structure by 2% and 3% respectively. The biggest significant increase in distribution was in older than one-year old wood with PRD by 4% and exogenous ABA by 6%. Although PRD had significantly less ^{15}N partitioned in thick roots by 8% compared to control, both PRD and exogenous ABA had increased partitioning of ^{15}N to fine roots by 2% an 3% respectively compared to control vines.

5.3.5 PRD and exogenous ABA effects on the assimilation and partitioning of total nitrogen and carbon.

Analysis of newly absorbed N does not include the mobilization of N from storage sources. It is therefore important to include the effects of PRD and exogenous ABA on total N and C contents and the N/C ratio. PRD and exogenous ABA effects on the N% and C% per organ are illustrated in Figure 5.5. Neither PRD nor exogenous ABA had any significant effect on grapevine tissue C concentration on a percentage dry weight basis. However, PRD and exogenous ABA significantly increased grapevine tissue N concentration in leaves by 10% and 11% and roots by 19% and 15% respectively. Furthermore, PRD significantly increased berry and permanent wood N concentration by 22% and 9% respectively. This similarly affected the organ tissue N%/C% ratios of PRD and exogenous ABA treated grapevines (Figure 5.6). Both PRD and exogenous ABA significantly increased the N%/C% in canopy leaves and roots while PRD also increased the N%/C% ratio in berries and permanent wood.

Table 5.8 Total ^{15}N (mg/organ) of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side).

	PRD ^{15}N mg (P%)	Control ^{15}N mg (P%)	ABA ^{15}N mg (P%)
Young leaves	1.7 mg (2.1%) b	4.1 mg (4.7%) a	3.5 mg (3.1%) a
Mature leaves	25.8 mg (32.0%) a	25.4 mg (29.1%) a	20.4 mg (23.6%) b
1Y shoots	12.3 mg (15.0%)	15.3 mg (17.6%)	11.8 mg (13.7%)
Total Vegetative	**39.8 mg (49.3%) a**	**44.8 mg (51.4%) a**	**35.8 mg (41.3%) b**
Rachises	0.7 mg (0.9%)	0.8 mg (0.9%)	0.9 mg (1%)
Berries	4.4 mg (5.4%)	4.3 mg (4.9%)	4.1 mg (4.7%)
Total Reproductive	**5.1 mg (6.3%)**	**5.0 mg (5.8%)**	**4.9 mg (5.7%)**
>1Y wood	10.3 mg (12.7%) a	7.4 mg (8.4%) b	12.1 mg (14%) a
Roots			
>2 mm	11.0 mg (13.6%) b	18.6 mg (21.3%) a	20 mg (23.1%) a
<2 mm	14.5 mg (18.0%)	11.4 mg (13.1%)	13.8 mg (15.9%
Total Permanent	**35.8 mg (44.4%)**	**37.3 mg (42.8%)**	**46.0 mg (53%)**
Total vine	**80.7 mg b**	**87.2 mg a**	**86.7 mg ab**

*Means indicated with different letters are significantly different ($P<0.05$). Percentages represent the relative distribution of total ^{15}N between organs.

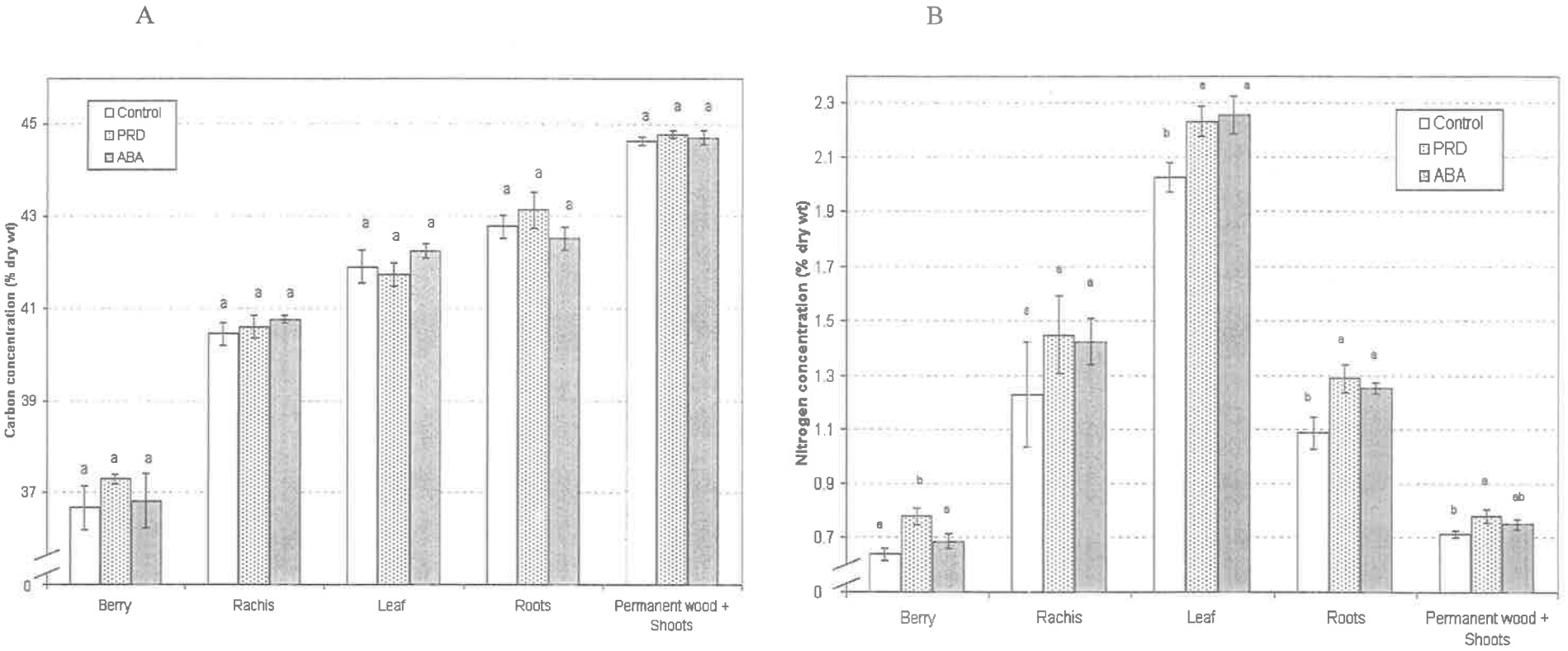


Figure 5.5 Effect of PRD and exogenous ABA on grapevine organ A) carbon (% dry weight) and B) nitrogen (% dry weight) of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 µM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different ($P<0.05$).

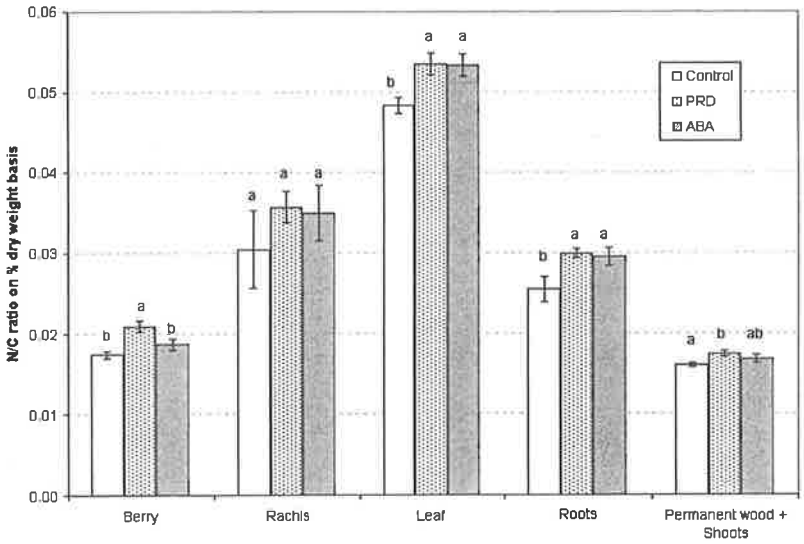
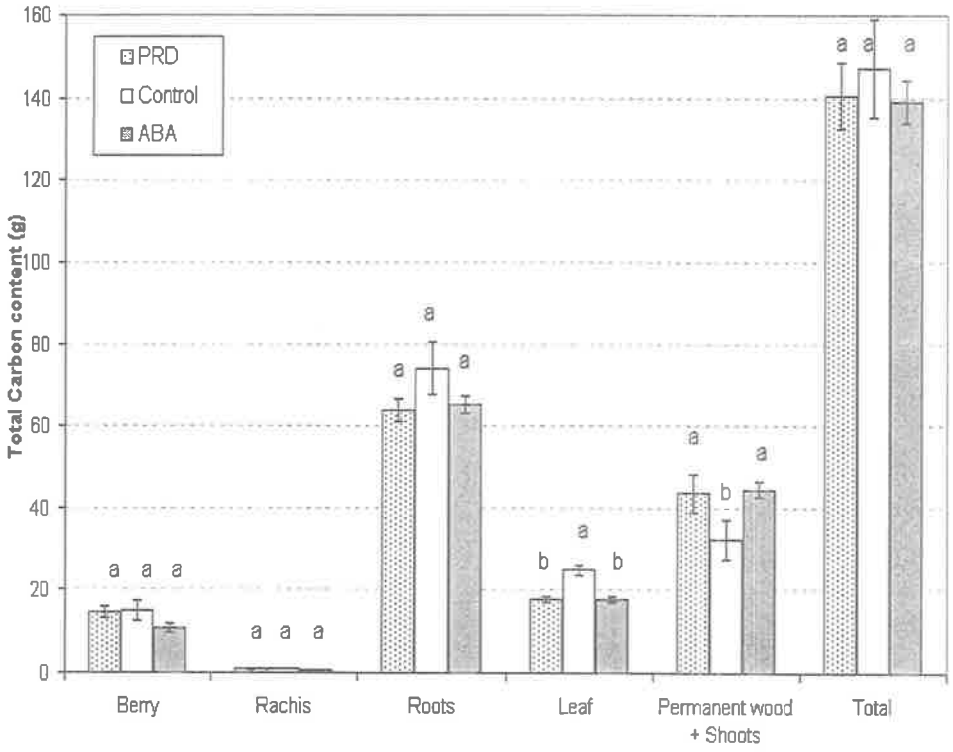


Figure 5.6 Effect of PRD and exogenous ABA on organ %N/%C ratio of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different ($P<0.05$).

When the dry weights of experimental vines were incorporated, the effect of PRD and exogenous ABA on the total accumulation and partitioning of N and C could be compared. PRD and exogenous ABA effects on total C content on a whole plant basis is shown in Figure 5.7 and the effects on N content in Figure 5.8. Neither PRD nor exogenous ABA had any significant effect on total plant C content. However, PRD and exogenous ABA significantly affected the partitioning of total C between organs. PRD and exogenous ABA had no effect on the total C content of berries, rachises or roots, but significantly increased the partitioning to permanent wood and shoots at the expense of canopy leaves. PRD and exogenous ABA increased total shoot and trunk C content by 9% and 11% respectively, however this was balanced by decreased amounts of C partitioned to leaves by 4% and 4% and roots by 5% and 3% respectively. In ABA-treated grapevines the extra 3% needed to balance the equation came from a decrease of carbon partitioning to berries.

Similarly to C accumulation, neither PRD nor exogenous ABA had any significant effect on total plant N content. However, PRD and exogenous ABA significant affected the partitioning of total N between organs. PRD and exogenous ABA had no effect on total N of berries, rachises or roots, but significantly increased the partitioning to permanent wood and shoots at the expense of leaves. PRD and exogenous ABA increased total shoot and trunk N content by 7% and 6% respectively, however this was balanced by decreased amounts of N partitioned to leaves by 6% and 7% respectively.

A



B

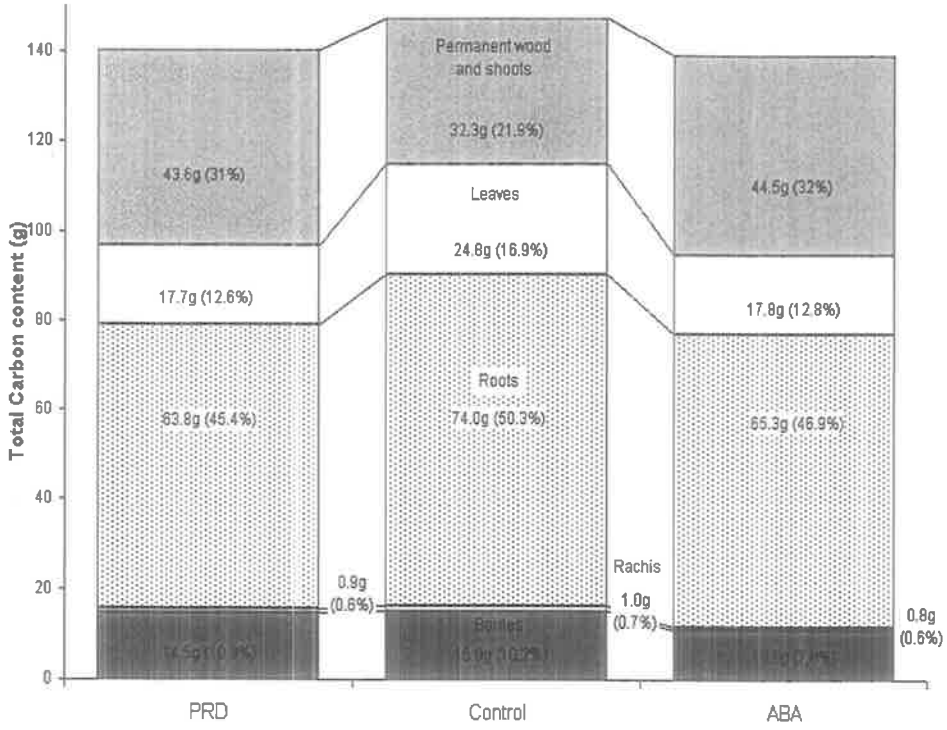
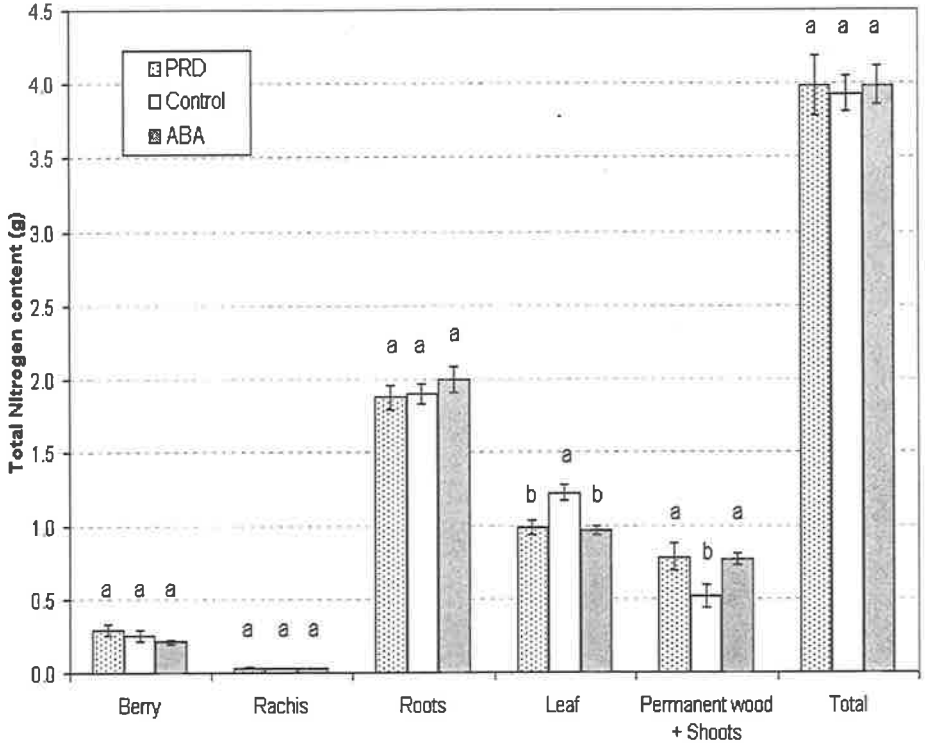


Figure 5.7 A+B) Effect of PRD and exogenous ABA on accumulated organ carbon content (g) of split-rooted Cabernet Sauvignon vines (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 µM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different ($P<0.05$). Percentages represent the relative distribution of total carbon between organs

A



B

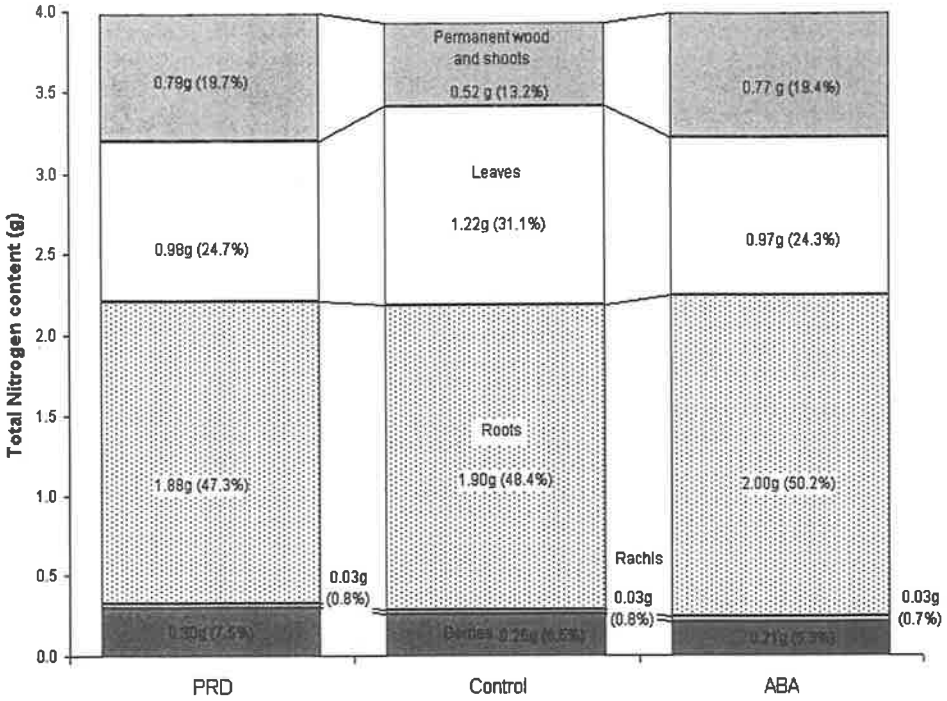


Figure 5.8 A+B) Effect of PRD and exogenous ABA on accumulated organ nitrogen content (g) of split-rooted Cabernet Sauvignon vines (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different ($P<0.05$). Percentages represent the relative distribution of total nitrogen between organs.

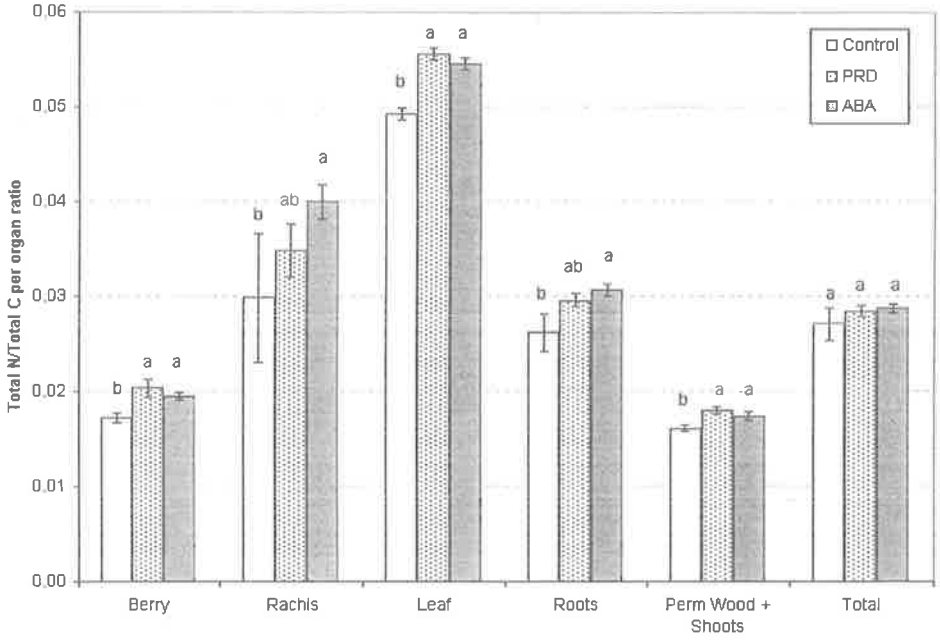


Figure 5.9 Effect of PRD and exogenous ABA on organ total N/C ratio of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different ($P<0.05$).

The PRD and exogenous ABA effects on the ratio between total N and total C in split-rooted Cabernet Sauvignon are illustrated in Figure 5.9. Neither PRD nor exogenous ABA significantly affected the N/C ratio on a whole plant basis. However, both treatments increased the total N/C ratio at the separate organ level. PRD and exogenous ABA both significantly increased the N/C ratio compared to control in berries by 18% and 14% respectively, leaves by 13% and 11% respectively and permanent wood by 11% and 8% respectively. In addition, exogenous ABA significantly increased the total N/C ratio of rachises by 34% and roots by 17% while PRD had smaller increases of 17% in rachises and 13% in roots compared to control which were non significant.

5.3.6 PRD field-experiment: effects on assimilation and partitioning of total nitrogen and carbon.

Berries

The PRD effect on the N and C content of field-grown Cabernet Sauvignon berries at harvest for 3 years is shown in Table 5.9. PRD had no effect on berry N on a percentage dry weight basis in two of the three years compared to control. In 2003 however, PRD significantly increased berry N concentration by 51%. Calculated per berry, PRD decreased N/berry in 2001 and 2002 by 12% and 11% respectively although it was only significant in 2002. This was due to the fact that PRD had smaller berry sizes at harvest in 2000/1 and

2001/2 seasons. However, in 2003 PRD significantly increased the N content per berry by 48% compared to control.

Table 5.9 Berry nitrogen content at harvest of field-grown Cabernet Sauvignon at the Waite campus. Control: vines received water on both sides of the vine; PRD: water withheld to one side – half of control irrigation; (means n = 7 ± s.e.).

		Control	PRD	% Diff. (PRD compared to control)	P
N (% dry wt.)	2001	0.507 ± 0.009	0.512 ± 0.008	0.8	0.7649
	2002	0.423 ± 0.009	0.415 ± 0.013	-2.0	0.5718
	2003	0.466 ± 0.017	0.704 ± 0.051	50.9	0.0059
N (g/berry)	2001	0.1636 ± 0.010	0.1468 ± 0.008	-12.1	0.0652
	2002	0.1515 ± 0.008	0.1351 ± 0.008	-10.8	0.0105
	2003	0.1411 ± 0.007	0.2087 ± 0.014	47.9	0.0088
C (% dry wt.)	2001	38.64 ± 0.17	38.62 ± 0.14	-0.1	0.7071
	2002	38.28 ± 0.08	38.04 ± 0.25	-0.6	0.4473
	2003	38.96 ± 0.12	38.97 ± 0.14	0.0	0.9449
C (g/berry)	2001	12.47 ± 0.74	11.04 ± 0.46	-11.5	0.0251
	2002	13.67 ± 0.52	12.34 ± 0.37	-9.8	0.0025
	2003	11.78 ± 0.35	11.62 ± 0.33	-1.4	0.6858

There was no significant difference between PRD and control berry C concentration (% dry weight) in any year. However, when C content was expressed on a per berry dry weight basis, there was significantly less C per berry during the 2001 and 2002 harvests. The decreases in both berry N and C had the effect that the N/C ratio between PRD and control berries at harvest was not significantly different for 2001 and 2002 (Table 5.11). The significant increase in N in 2003 without a concurrent increase in C however, significantly increased the N/C ratio of Cabernet Sauvignon berries for the 2003 harvest.

The PRD effect on the N and C content of field-grown Shiraz berries at harvest for 3 years is shown in Table 5.10. PRD had no effect on either berry N or C concentration at harvest over the experimental period (2001-2003). No significant effect could be found in percentage dry weight, gram per berry or in the N/C ratio (Table 5.11).

Table 5.10 Berry nitrogen content (g/berry) at harvest of field-grown Shiraz at the Waite campus. Control: vines received water on both sides of the vine; PRD: water withheld to one side – full amount of control irrigation; (means n = 7 ± s.e.)

		Control	PRD	% Diff. (PRD compared to control)	P
N (% dry wt.)	2001	0.554 ± 0.0095	0.567 ± 0.0239	2.4	0.6296
	2002	0.379 ± 0.0254	0.362 ± 0.0098	-4.6	0.4159
	2003	0.579 ± 0.0222	0.526 ± 0.0207	-9.3	0.2181
N (g/berry)	2001	0.207 ± 0.011	0.206 ± 0.008	-0.4	0.9564
	2002	0.169 ± 0.014	0.160 ± 0.008	-5.6	0.3882
	2003	0.242 ± 0.009	0.223 ± 0.009	-8.2	0.2110
C (% dry wt.)	2001	38.05 ± 0.16	38.25 ± 0.43	0.5	0.6512
	2002	38.04 ± 0.12	37.91 ± 0.12	-0.3	0.5629
	2003	39.18 ± 0.17	38.84 ± 0.39	-0.9	0.3653
C (g/berry)	2001	14.20 ± 0.77	13.39 ± 0.36	-1.9	0.6538
	2002	16.84 ± 0.43	16.67 ± 0.45	-1.1	0.6513
	2003	16.45 ± 0.46	16.48 ± 0.51	0.2	0.9729

Table 5.11 Berry N/C ratio at harvest of field-grown Cabernet Sauvignon and Shiraz at the Waite campus. Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; Shiraz PRD received full amount of control irrigation; (means n = 7 ± s.e.)

		Control	PRD	% Diff. (PRD compared to control)	P
Cabernet Sauvignon	2001	0.0131 ± 0.0002	0.0133 ± 0.0002	0.9	0.7317
	2002	0.0111 ± 0.0002	0.0109 ± 0.0006	-1.4	0.6484
	2003	0.0120 ± 0.0004	0.0181 ± 0.0001	50.8	0.0063
Shiraz	2001	0.0146 ± 0.0002	0.0148 ± 0.0005	1.7	0.6782
	2002	0.0100 ± 0.0006	0.0095 ± 0.0003	-4.2	0.4251
	2003	0.0148 ± 0.0006	0.0135 ± 0.0004	-8.7	0.2289

Nuriootpa: PRD Shiraz at Nuriootpa received half the amount of control irrigation and three different levels of nodes per vine were retained. The effects of PRD and pruning level on berry N and C concentration at harvest are shown in Table 5.12. No main effects of irrigation treatment (P=0.8247) or pruning level on berry N concentration at harvest (P=0.5138) could be found. There were also no interactions between irrigation and pruning level on berry N at harvest (P=0.8677). This was also found in berry C concentration with no main effects of irrigation treatment (P=0.6430), pruning level (P=0.4414) or their interaction (P=0.9924).

Table 5.12 Berry nitrogen at harvest of field-grown Shiraz at the Nuriootpa research station (2001). Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of the amount of control irrigation (means; n = 5 ± s.e.).

N (% dry wt.)	Control	PRD	% Diff. (PRD compared to control)
30 nodes/vine	0.70 ± 0.069	0.69 ± 0.030	-0.7
60 nodes/vine	0.77 ± 0.104	0.72 ± 0.048	-6.1
120 nodes/vine	0.76 ± 0.010	0.78 ± 0.064	2.4
C (% dry wt.)	**Control**	**PRD**	**% Diff. (PRD compared to control)**
30 nodes/vine	40.10 ± 0.88	39.71 ± 0.14	-1.0
60 nodes/vine	40.95 ± 1.23	40.63 ± 0.61	-0.8
120 nodes/vine	40.88 ± 0.48	40.69 ± 0.88	-0.5

Leaves

The PRD effect on the N and C concentration (% dry weight) of field-grown Waite Cabernet Sauvignon and Shiraz leaves at harvest for 3 years is shown in Table 5.13. Cabernet Sauvignon PRD that received half the amount of control irrigation had no effect on leaf N on a percentage dry weight basis in the first year of the study. However, in the following year PRD significantly increased leaf N concentration by 12% and decreased it again in 2003 by 11%. Furthermore, PRD had little effect on Cabernet Sauvignon leaf C concentration compared to control; a significant increase in 2002 by 1% was the only exception. Shiraz PRD, receiving the same amount of control irrigation, had no effect on leaf N or C concentration at harvest in any year of the study.

Table 5.13 Leaf nitrogen and carbon concentration (% dry wt) at harvest of field-grown Cabernet Sauvignon and Shiraz at the Waite campus. (Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; Shiraz PRD received full amount of control irrigation; means n = 7 ± s.e.)

Cabernet Sauvignon		Control	PRD	% Diff. (PRD compared to control)	P
N (% dry wt.)	2001	1.96 ± 0.037	1.95 ± 0.057	-0.6	0.9817
	2002	2.05 ± 0.053	2.31 ± 0.059	12.3	0.0179
	2003	1.85 ± 0.069	1.64 ± 0.041	-11.2	0.0069
C (%dry wt.)	2001	45.49 ± 0.270	45.85 ± 0.126	0.8	0.4694
	2002	43.86 ± 0.118	44.35 ± 0.169	1.1	0.0112
	2003	44.75 ± 0.212	44.49 ± 0.204	-0.6	0.3223
Shiraz		Control	PRD	% Diff. (PRD compared to control)	P
N (%dry wt.)	2001	2.08 ± 0.073	2.04 ± 0.055	-2.0	0.5862
	2002	2.35 ± 0.057	2.28 ± 0.075	-2.7	0.4196
	2003	1.92 ± 0.066	1.99 ± 0.050	3.6	0.1003
C (%dry wt.)	2001	43.64 ± 0.24	43.52 ± 0.11	-0.3	0.6780
	2002	42.38 ± 0.15	42.62 ± 0.53	0.6	0.6407
	2003	44.09 ± 0.34	44.01 ± 0.14	-0.2	0.7288

The PRD effect on the N/C ratio of leaves seems to be dominated by the changes in N concentration as represented in Table 5.14. As PRD affected N concentration, the N/C ratio did not change in 2001 but significantly increased in 2002 and decreased again in 2003 by 11 %. The N/C ratio in Shiraz leaves however did not change in any year during the experimental period.

Table 5.14 Leaf N/C ratio (% dry weight) at harvest of field-grown Cabernet Sauvignon and Shiraz at the Waite campus. (Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; Shiraz PRD received full amount of control irrigation; means n = 7 ± s.e.)

Cabernet Sauvignon	Control	PRD	% Diff. (PRD compared to control)	P
2001	0.0431 ± 0.0008	0.0425 ± 0.0012	-1.4	0.8326
2002	0.0468 ± 0.0013	0.0520 ± 0.0014	11.1	0.0320
2003	0.0413 ± 0.0017	0.0369 ± 0.0010	-10.8	0.0161
Shiraz	Control	PRD	% Diff. (PRD compared to control)	P
2001	0.0477 ± 0.0016	0.0468 ± 0.0012	-1.7	0.6246
2002	0.0554 ± 0.0014	0.0537 ± 0.0020	-3.1	0.3415
2003	0.0435 ± 0.0014	0.0451 ± 0.0011	3.8	0.0693

Shoots

The PRD effects on the N and C concentration of field-grown Cabernet Sauvignon and Shiraz shoots during the experimental period are shown in Table 5.15. Cabernet Sauvignon PRD, receiving half the amount of control irrigation, significantly reduced shoot N concentration at harvest in 2001 by 12%. However, measurements later that year on dormant shoots indicated no significant differences compared to control vines. Measurements of shoot N concentration in 2003 indicated no significant differences between PRD and control vines.

Furthermore, PRD had no significant effect on shoot C concentration on field-grown Cabernet Sauvignon. Shiraz PRD, receiving the same amount of control irrigation, showed no significant increases or decreases in shoot N concentration at harvest over the experimental 3 years and similarly for C concentration in 2 of the 3 years. In 2003 however, there was a significant decrease in shoot C concentration compared to control.

Table 5.15 Shoot nitrogen and carbon concentration (% dry wt) at harvest of field-grown Cabernet Sauvignon and Shiraz at the Waite campus. (Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; Shiraz PRD received full amount of control irrigation; means n = 7 ± s.e.)

Cabernet Sauvignon		Control	PRD	% Diff. (PRD compared to control)	P
N (% dry wt.)	2001	0.463 ± 0.007	0.408 ± 0.018	-12.0	0.0136
	Winter 2001	0.568 ± 0.009	0.547 ± 0.012	-3.7	0.1744
	2003	0.422 ± 0.023	0.431 ± 0.012	2.2	0.6467
C (%dry wt.)	2001	44.26 ± 0.054	44.12 ± 0.039	-0.3	0.1372
	Winter 2001	44.30 ± 0.076	44.30 ± 0.034	0.0	1.000
	2003	44.12 ± 0.041	44.17 ± 0.030	0.12	0.3824
Shiraz		Control	PRD	% Diff. (PRD compared to control)	P
N (% dry wt.)	2001	0.429 ± 0.019	0.439 ± 0.014	-2.2.	0.6870
	2002	0.365 ± 0.014	0.353 ± 0.005	-3.2	0.4509
	2003	0.365 ± 0.015	0.409 ± 0.026	12.0	0.1715
C (%dry wt.)	2001	43.71 ± 0.056	43.73 ± 0.049	0.1	0.6952
	2002	44.27 ± 0.064	44.22 ± 0.064	-0.1	0.3401
	2003	44.10 ± 0.076	43.78 ± 0.055	-0.7	0.0240

The N/C ratios in field-grown Cabernet Sauvignon and Shiraz shoots are shown in Table 5.16. As noted in leaves, the PRD effect on the N/C ratio in shoots seems to be dominated by the changes in N concentration. In 2001 Cabernet Sauvignon PRD had a significantly lower N/C ratio at harvest in shoots compared to control but the difference disappeared going into dormancy with winter shoot N concentrations similar to control vines. Shiraz PRD had no differences in the N/C ratios of shoots at harvest during the 3 years of the experiment.

Table 5.16 Shoot N/C ratio (% dry weight) at harvest of field-grown Cabernet Sauvignon and Shiraz at the Waite campus. (Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; Shiraz PRD received full amount of control irrigation; means n = 7 ± s.e.).

Cabernet Sauvignon	Control	PRD	% Diff. (PRD compared to control)	P
2001	0.0105 ± 0.0001	0.0092 ± 0.0004	-11.7	0.0164
Winter 2001	0.0128 ± 0.0002	0.0123 ± 0.0003	-3.7	0.1987
2003	0.0096 ± 0.0005	0.0098 ± 0.0003	2.8	0.9357
Shiraz	Control	PRD	% Diff. (PRD compared to control)	P
2001	0.0098 ± 0.0005	0.0100 ± 0.0003	2.1	0.6972
2002	0.0082 ± 0.0003	0.0082 ± 0.0001	-3.2	0.4659
2003	0.0083 ± 0.0003	0.0093 ± 0.0006	12.8	0.1570

Nuriootpa: The effects of PRD and pruning level on shoot N and C concentration at harvest are shown in Table 5.17. No main effects of irrigation treatment on shoot N (P=0.251) or C (P=0.9045) concentration at harvest could be found. However, a significant main effect in nodes per vine retained (P=0.0088) on shoot N was found with 30 nodes having significantly higher N (% dry wt.) than 60 and 120 nodes at harvest. No significant main effect was found in nodes retained (P=0.2165) on shoot C concentration. There were also no interactions between irrigation treatment and pruning level on shoot N (P=0.0990) or C (P=0.9364) at harvest.

Table 5.17 Shoot nitrogen and carbon content (% dry weight) at harvest of field-grown Shiraz at the Nuriootpa research station (2001) (Control: vines received water on both sides of the vine; PRD: water withheld to one side; Cabernet Sauvignon PRD received half of control amount of irrigation; (means; n = 5 ± s.e.))

N	Control	PRD	% Diff. (PRD compared to control)
30 nodes/vine	0.444 ± 0.011	0.466 ± 0.009	5.0
60 nodes/vine	0.382 ± 0.025	0.437 ± 0.011	14.4
120 nodes/vine	0.409 ± 0.021	0.384 ± 0.023	-6.1
C	**Control**	**PRD**	**% Diff. (PRD compared to control)**
30 nodes/vine	43.59. ± 0.058	43.57 ± 0.053	0
60 nodes/vine	43.56. ± 0.095	43.59 ± 0.157	0.1
120 nodes/vine	43.76. ± 0.097	43.17 ± 0.109	-1.3

5.4 Discussion

Results of experiments in this chapter have provided evidence that PRD affects grapevine physiology and the accumulation of dry weight and minerals. PRD and exogenous ABA affected grapevine physiology by significantly reducing stomatal aperture - PRD more so than exogenous ABA. PRD in potted vines continuously reduced stomatal conductance on average by 60% compared to control and it is considered to increase the vines water use efficiency. Exogenous ABA only managed to reduce stomatal conductance by 20% compared to control. However, this could be expected, because physical drying of the root system will involve additional hormonal, hydraulic and even nutritional stimuli that would alter root to shoot communication (Comstock, 2002; Chaves *et al.*, 2003). Although ABA is arguably the strongest signal (Davies and Zhang, 1991), ABA may not be the only signal involved in the PRD root to shoot response. Stoll (2000b) found that not only did an increase in root and xylem sap ABA influence stomatal aperture with PRD but there was also a decrease in cytokinins (CK), substantially changing the ABA to CK ratio. It was speculated that the reduction in growth associated with PRD was also related to the reduced delivery of CK. Plants respond to drying soil by reducing their CK supply (Itai and Vaadia, 1965) and Pillay & Beyl (1990) reported in tomato that a decrease in root CK within the first 24 h could be an early response to water stress. It seems reasonable that control over stomatal aperture may be augmented by a reduced supply of CK from the roots.

Stoll (2000) found that the stomata of water stressed grapevines had greater sensitivity to ABA than well-watered grapevines at a given CK concentration. The implication is that the stomata of well-watered vines will react less to exogenous ABA than grapevines experiencing a drying soil medium, even if the CK level of well-watered grapevines were lowered to the level found under PRD conditions. Mechanically the effect of physical root

drying influences the rate of delivery of hormones, nutrients and water needed for growth from roots to shoots via the transpiration stream. Many enzymes involved in the C and N biosynthetic pathways are substrate-inducible and their activity would be dependant on the supply stream to incorporate basic organic minerals to produce carbohydrates and amino acids for metabolism and growth (Salisbury and Ross, 1992).

ABA may not only affect stomatal aperture, but also a range of biochemical processes either directly or indirectly. ABA generally decreases shoot elongation, but increases root elongation at low soil water potentials (Saab *et al.*, 1990) due to the important role of ABA to restrict ethylene production (Spollen *et al.*, 2000). Direct ABA influences include increased leaf respiration (Hedrich *et al.*, 2001), induced protein synthesis to counteract water loss and repair cellular damage (Campalans *et al.*, 1999), promoted nitrate reductase and sucrolytic enzyme activity in roots and inhibited activity in leaf tissue (Lu *et al.*, 1992; Chraibi *et al.*, 1995).

Considering the big differences in stomatal conductances between PRD and exogenous ABA treatments it is somewhat surprising that ABA had very similar effects on growth as PRD. Both PRD and exogenous ABA in this split-rooted experiment reduced the accumulation of dry weight in both leaves and one-year old shoots, the vegetative section of the grapevine, while slightly increasing dry weight of permanent wood. Neither treatment however had any effect on the dry weight of roots. This may be as expected because the behaviour of roots is different in the potted environment than in the field situation. Potted roots are exposed to different regimes of temperature flux, soil moisture availability and physical barriers. To eliminate such impacts on experimental vine roots would require very large soil volumes that would be too difficult to manage. Neither PRD nor exogenous ABA had a significant effect on fruit dry weight accumulation. This significantly increased the water use efficiency in PRD vines calculated as yield in tonnes produced per ML of irrigation water.

A reduced berry weight and slightly increased juice pH were only observed in vines treated with PRD. According to Coombe (1992) the characteristics of berry growth indicate a large increase in berry volume after veraison due to the accumulation of both water and dry matter. The amount of water accumulated each day depends on the movement of water into the berry from xylem and phloem minus the losses in transpiration and movement back through the xylem. However, after veraison the contribution of water and minerals by xylem are considerably reduced by embolism and the majority are derived from the phloem

(Findlay *et al.*, 1987). This hydraulic isolation of the fruit may explain why although shoot growth was significantly reduced, exogenous ABA had little effect on berry growth. Davies *et al.* (2000) found that although a marked accumulation of ABA occurred in expanding and mature tomato leaves after only a short period of soil drying, no ABA accumulated in the fruit epidermis of tomato.

The final berry size is greatly dependant on its sink activity to import solutes and increase its osmotic potential. Higher osmotic potential will increase water influx via the phloem to increase berry volume. PRD and exogenous ABA however had no effect of total soluble solids (°Brix). In some way the carbohydrate supply to the fruit was maintained, either by actively increasing the sink strength or decreasing the crop's only major competition during ripening, namely developing lateral shoots. Analysis by Conradie (1980) suggested that the majority of mass increase in the crop is from photosynthesis, predominantly from the leaves closest to the bunch. However, in mature vines a significant portion may be derived from mobilized reserves from the permanent structures such as wood of trunk and roots (Kliewer and Antcliff, 1970).

One such pool of carbohydrate reserve is the accumulation of starch in permanent structures, but also in leaves. Starch accumulation in leaves is believed to be just enough to support sucrose export during the night. Surplus sucrose during the day is diverted to starch that builds up when photosynthesis exceeds the combined rates of respiration and export while the export rate may be finely tuned to average rates of photosynthesis (Sims *et al.*, 1998) and leaf sucrose concentration. PRD treatment in this pot experiment, unlike exogenous ABA treatment, significantly reduced the total leaf starch concentration by reducing mature and young leaf starch concentrations by 73%. However, no significant differences in starch concentration could be found in other organs for both PRD and exogenous ABA. When total starch content was expressed on an organ dry weight basis the accumulation per organ at harvest showed that both PRD and exogenous ABA significantly reduced the accumulated starch in the leaf canopy, mostly mature leaves, compared to control with PRD less compared to ABA. It is therefore clear that PRD caused significantly smaller investment in carbohydrate storage in leaves that could not be attributed to the hormonal influence of root-borne ABA since exogenous ABA leaf starch concentration was not different to control. ABA treatment however caused lower accumulated starch in the leaf canopy purely due to a smaller canopy size.

The accumulation of starch in permanent wood may be the most important carbohydrate reserve in the grapevine. Firstly, it is the carbohydrate reserve in the trunk and arms that is rapidly mobilized at the beginning of berry ripening. The rate of movement of carbohydrates into fruit is found to be too rapid to only be a result from a change in competition for photosynthates (Winkler *et al.*, 1974). Secondly, the stored carbohydrates in permanent wood are utilized for new growth in spring and the amount of available carbohydrates seems to be a significant factor in flower development and fruit set. In this pot experiment, PRD and exogenous ABA had no significant effect on the total starch reserves in one-year old shoots, despite having increased starch concentrations compared to control. Starch concentrations in the older permanent wood were not significantly different but the total amount of starch increased by 25% and 35% respectively. Although PRD and exogenous ABA decreased total starch in roots compared to control, the differences were not significant and not represented in differences of starch concentrations. Ultimately, PRD and exogenous ABA had lower starch on a whole plant basis but the differences were too small to be significant. It is concluded that PRD and exogenous ABA-treated vines invested significant amounts in permanent wood and this is explained by the 'functional equilibrium' model of Poorter and Nagel (2000). Under PRD conditions, the grapevine may "rank" the permanent wood and roots as being the organs most limited and carbohydrates are partitioned to aid in osmotic function and increase vine capacity to overcome limited soil moisture conditions.

The absorption of inorganic ions is high during the periods of rapid root growth that coincides with periods of new growth before veraison and periods of accumulation between harvest and leaf fall. During the crop ripening period, incidentally also the PRD period, inorganic ion accumulation is much less and mainly redistributed from the trunk, shoots and leaves, depending on its phloem mobility. The phloem is the major source of solutes and water to the berry during ripening due to xylem embolism at veraison, restricting the xylem movement into and out of the berry. The major inorganic ion in phloem sap and berries is potassium (K). Excess K in grape berries may have a negative influence on wine quality, mainly because it decreases free tartaric acid resulting in increased pH of grape juice (Mpelasoka *et al.*, 2003). In Australia, high K status is common in most vineyards which necessitate pH adjustments during the vinification process. PRD may have positive effects on canopy microclimate by regulating vine vigor without water stress. Both a reduction in shoot growth and accumulated ABA in the roots may reduce the K uptake of the vine. The experiment with split-rooted vines however showed that neither PRD nor exogenous ABA had any effect on the total amount of K assimilated by the vine during the ripening period.

The only notable difference was that both PRD and exogenous ABA partitioned 3-4% more K in the permanent structures at the expense of the shoots. This may be due to reduced sink demand in shoots compared to the demand in permanent structures. Considering the differences in plant organ dry weights of PRD and ABA treatments, the plant organs did not accumulate K in relation to their organ size, but significantly increased their concentration (mg/g dry weight) of K in organs such as berries, leaves and roots to maintain normal partitioning. This may be due to osmotic potential changes in the regulation of plant water relations. Although the K concentration of berries was higher in response to PRD and exogenous ABA, the amount of K per berry was calculated to be lower but the differences were not large enough to be significantly different.

The majority of Calcium (Ca) absorption occurs about three weeks after budburst and again a small amount before leaf fall. Conradie (1981) also found a small accumulation of Ca in both leaves and shoots during the ripening period that could not be fully accounted by the decrease in root Ca. The low absorption by grapevines during the ripening period may explain why the split-rooted experiment of PRD and exogenous ABA had no effect on the total amount of absorbed Ca. However, PRD and exogenous ABA significantly affected the Ca partitioning within the vine structure by increasing the amount partitioned in permanent wood and roots on average by 14% at the expense of Ca stored in leaves and one-year old shoots. This partitioning may be due to reduced sink demand by shoots or a reduced xylem flow from roots to shoots. Because Ca is phloem immobile its accumulation in berries occurs when xylem influx is high and almost completely stops after veraison. It was therefore expected that PRD and exogenous ABA would have no effect on berry Ca accumulation due to the fact that the experiment started close to veraison. This was actually a good measure of the homogeneity in the experimental population. If there were differences in berry Ca at harvest, it would have meant that there were differences in berry minerals before the experiment started.

Magnesium (Mg) is the only mineral analyzed where PRD and exogenous ABA caused accumulation of significantly less per vine than control. PRD and exogenous ABA accumulated on average 17% less Mg than control with the biggest decrease in partitioning to leaves. However, both PRD and ABA partitioned significantly more Mg in permanent wood. The differences in accumulated Mg may be related to the differences in organ size because there were no differences in tissue concentrations compared to control. Like most of the other minerals, Mg per berry was correlated positively with berry size.

PRD and exogenous ABA had no significant effect on sodium (Na) accumulation per vine compared to control. Both PRD and ABA partitioned significantly more to permanent wood compared to control but only exogenous ABA had lower Na partitioning to leaves and roots. PRD and exogenous ABA may have sequestered Na to certain organs due to significant increases in tissue concentration of Na in organs such as rachises, mature leaves, permanent wood and roots. Na may be involved in osmotic and ionic balances (Clarkson and Hanson, 1980). Na per berry did not correlate with berry size, although the relationship seemed to be negative. Bigger berry sizes may have diluted the Na to lower concentrations. This was clear in the Na per berry values where control berries (with the largest berries) had the lowest Na content, followed by exogenous ABA and finally PRD with the smallest berries having the highest Na content.

Phosphorus (P) content between veraison and harvest generally stays constant in grapevines, but the P content of the fruit increases due to remobilisation from leaves. In the split-rooted experiment PRD and exogenous ABA also had no effect on total P accumulation during ripening, but mobilized significantly more P from leaves compared to control. ABA also had lower P in one-year old shoots that amounted to a 4% decrease. This was equalized to a degree by greater partitioning to permanent wood compared to control. Considering the differences between plant organ dry weights of PRD and control, the plant organs did not only accumulate P in relation to their organ size, but also significantly increased their concentration of P in organs such as berries and thick roots compared to control. However, on a per berry basis there were no significant differences in P content.

PRD and ABA had little effect on grapevine total sulfur (S) content. Calculated on total organ dry weights, PRD and ABA had significantly less S partitioned to the leaves compared to control and more S partitioned to the permanent structure. Similar to the accumulation in P, PRD and ABA had increased concentrations of S in berries, leaves and roots. However, on a per berry basis there were no significant differences in S content.

The correlations between berry mineral concentrations and berry size for the split-rooted experiment indicated that most mineral concentrations were not correlated with berry sizes, but correlations existed between minerals. Berries with a high N concentration were most likely to have high concentrations of C, K, P and S. irrespective of their berry size. This meant that berries kept a certain concentration as the berries enlarged, keeping to the hypothesis that berry size is the major sink strength for nutrients. However, Na concentration correlated negatively with the final berry size, which means that berries may

have accumulated a finite amount of Na before treatment or that Na is used as an osmoticum to increase water movement to smaller berries. Larger berries will therefore have lower Na concentrations due to the dilution effect. There existed a strong positive correlation between Ca and Mg concentrations in berries and may be because very low amounts of both minerals are absorbed by the berry during the ripening period (Conradie, 1981) and amounts in the berry remained very much the same as before the experiment started.

Labeled nitrogen (^{15}N) measurements elucidated the absorption and partitioning of newly absorbed N during the ripening period. Grapevines are "N-hungry" plants and capable of new growth under favorable conditions of water and nutrient supply. Analyses of ^{15}N abundance revealed that the biggest differences were found between PRD and exogenous ABA. Exogenous ABA caused a higher abundance of ^{15}N in berries, one-year old shoots and thin roots than PRD, which was only marginally higher than control. PRD, on the other hand, had only significantly higher ^{15}N in the total canopy that consisted of mature and young leaves, than control and ABA. This is interesting, because unlike the effects on dry mass and inorganic minerals where ABA and PRD had lower accumulations compared to control, ABA and control had higher abundances of ^{15}N compared to PRD. The phenomenon may be explained by the fact that ABA may directly increase nitrate reductase (NR) activity and at the same time decrease growth by its effect on stomatal conductance. With no decrease in N availability the NR activity was not inhibited by exogenous ABA or its concurrent reduction on stomatal conductance (Chapter 6). However, under PRD conditions the N availability may also be reduced and the nitrate reductase activity inhibited by substrate availability. This was also evident in the total amounts of ^{15}N absorbed per vine. PRD absorbed significantly less ^{15}N during the ripening period compared to exogenous ABA and control, but maintained the accumulation of ^{15}N in the reproductive organs by increasing the relative partitioning of ^{15}N to fruit compared to control and exogenous ABA. This indicates that the fruit had a higher sink strength for newly absorbed N than other organs during ripening. As for the rest of the plant, the accumulation of dry weight in vegetative organs had a big influence on the partitioning of newly absorbed N in both PRD and exogenous ABA vines. PRD and exogenous ABA caused significantly less ^{15}N to be partitioned to vegetative organs with the biggest reduction in young leaves in PRD and mature leaves in exogenous ABA compared to control. Similar to other solutes, PRD and exogenous ABA increased the partitioning of ^{15}N to the permanent structure, specifically to the older wood. However, PRD accumulated and partitioned significantly less ^{15}N in the thick roots (> 2mm) compared to control and exogenous ABA. Conversely, PRD increased partitioning to small roots which may indicate new root growth.

The analyses of ^{15}N may indicate the fate of recently absorbed N, however nutrients may also be mobilized from reserve storage for fruit ripening and growth. It was therefore necessary to elucidate the PRD and exogenous ABA effects on total N and C contents of the split-rooted grapevines. Both PRD and exogenous ABA had no significant effect on any grapevine tissue C concentrations (% dry weight), but significantly increased the N concentration in leaf and root tissues while PRD also increased the berry and permanent wood tissue N concentration compared to control. This significantly increased the N/C concentration ratio of PRD vines in every plant tissue and also in the leaf and root tissue of exogenous ABA compared to control.

By calculating the total amount of accumulated N and C in different organs the partitioning of total N and C in the split-rooted experiment could be elucidated that would include both recently absorbed and stored reserves. Neither PRD nor exogenous ABA had any effect on total vine C or N status at harvest compared to control. However, both treatments affected the partitioning of N and C by significantly increasing the accumulation in permanent wood and shoots at the expense of the leaf canopy. The relative partitioning of total N and C in berries, rachises and roots was not significantly different to control vines. However, the ratio of total N to total C per organ indicated that both PRD and exogenous ABA affected grapevine physiology by significantly increasing the total N/C ratio in berries, leaves, permanent wood and roots. Ultimately, total plant accumulated N/C ratio indicated no significant differences.

A simple model of the relative partitioning of total C and N in the split-rooted grapevine experiment is shown in Figure 5.10. However, certain assumptions were made to construct the model. Firstly, the permanent wood and shoots play a central role in the partitioning of N and C because they connect all the sources and sinks via the phloem and xylem network and they are also storage organs. They may therefore be regarded as a 'reservoir' for nutrients that can be easily mobilized when needed for berry, shoot or root growth – the vine capacity. Secondly, movement of nitrogenous compounds and photosynthates into berries can only originate from the leaves via a concentration gradient in the phloem, since there is little xylem sap flux due to xylem embolism at veraison. Thirdly, nutrients can be freely distributed between the roots and permanent wood according to sink demand. Both PRD and ABA significantly altered N partitioning by reducing the amount of N accumulated in leaves by 6% and 7% respectively and balancing it by a significant increase in shoots and

trunks by 7% and 6%. Neither treatment greatly affected root N accumulation. PRD increased and exogenous ABA decreased berry N by 1% respectively compared to control. PRD and ABA similarly altered C partitioning by significantly increasing shoot and trunk total C content by 9% and 11% respectively compared to control, however this was balanced by significant decreases in total leaf C in both treatments by 4% and root C by 5% and 3% in PRD and ABA respectively. In ABA treated grapevines the extra 3% needed to balance the C equation came from a significant decrease in berry total C contents.

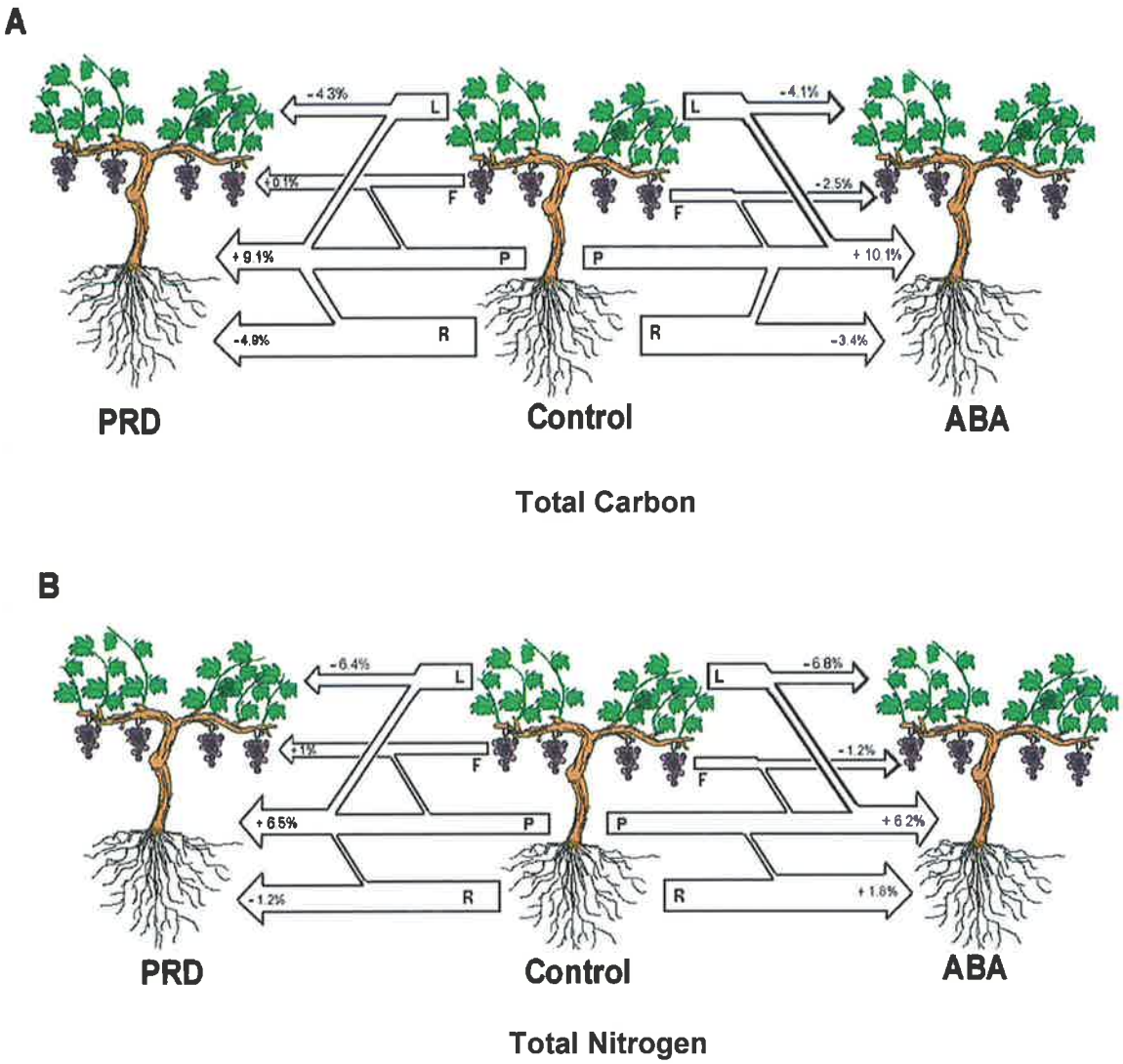


Figure 5.10 Relative partitioning of **A)** total carbon and **B)** total nitrogen between organs at harvest in split-rooted grapevines (2003). (L = leaves; F = fruit; P = permanent wood; R = roots)

It is clear that both PRD and exogenous ABA treatment caused investment of significantly more resources to permanent structures at the expense of vegetative growth. Both PRD and exogenous ABA reduced stomatal conductance and probably photosynthesis, but shoot growth is much earlier affected by mild water stress than photosynthesis via the hormonal

influence of ABA. A significant reduction in shoot growth would reduce the nutrient demand of the vegetative organs and may be the reason behind the increased N/C ratio in PRD and exogenous ABA tissues since there is no feedback inhibition for the uptake of N. According to the 'functional equilibrium of Poorter and Nagel (2000), the physiological reaction of plants to the inhibitory effect of soil-borne ABA on shoot growth is to use the excess nutrients to increase root volume in order to overcome the 'most limiting' factor i.e. low soil moisture, by exploring deeper layers of the soil. This is aptly demonstrated by Mingo *et al.* (2004) in root activities of tomatoes subjected to PRD, where root biomass was increased by 55%. In the current study however, the volume of soil used in the experiment may have restricted the growth and utilization of the excess nutrients in PRD and exogenous ABA grapevines that would have led to greater root activity in the field environment. Stoll *et al.* (2000a) have shown that PRD-treated grapevine roots grew to greater depths in the field and pot environment.

Grapevines respond differently in pots than in the field environment and it is therefore necessary to evaluate the PRD effect on field-grown grapevines. The N and C concentrations of field-grown Cabernet Sauvignon and Shiraz grapevines receiving half and the full amount of control irrigation respectively were investigated over a period of 3 years (2000-2003). Contrary to the split-rooted experiment, PRD increased the berry N concentration of Cabernet Sauvignon at the Waite campus only once during the three years compared to control vines (in 2003). The C content was not affected and therefore the N/C ratio increased significantly. However, in 2001 and 2002 the PRD Cabernet Sauvignon berries had significantly lower C and N on a gram per berry basis that was related to PRD having smaller berries. The N/C ratios therefore indicated that there was no PRD effect on berry nutrition in 2001 and 2002. PRD receiving the same amount of irrigation as control had no significant effect on Shiraz berry N and C concentration (% dry weight), content (g/berry) or N/C ratio compared to control. PRD Shiraz grown at the Nuriootpa Research Station similarly had no effect on berry N and C concentration compared to control irrespective of the pruning level.

Results obtained from leaf N and C analyses were also inconclusive. PRD Cabernet, receiving half the amount of control irrigation, significantly increased leaf N in 2002 but reduced it in 2003 by the same margin compared to control. The same situation was found in the N/C ratio of leaves. One pattern that did emerge was that in the year when N and C per berry was significantly reduced, the N and C concentration in leaves was increased compared to control and in the year when berry N was significantly increased, leaf N

concentration was decreased compared to control. PRD Shiraz, receiving the same amount as control irrigation, showed no significant differences in either N or C contents compared to control and it was apparent in the N/C ratios.

The results of PRD treatment on shoot N and C concentration at harvest were also inconclusive. After a significant decrease in PRD Cabernet Sauvignon shoot N and N/C ratio compared to control during the 2001 harvest, the shoots were analyzed again later that year during dormancy to investigate the stored reserves for the next growing season. PRD however accumulated enough nutrients during the post-harvest period to have similar N and C concentrations during the winter compared to control. This may indicate similar vine capacity. PRD Shiraz grown at the Nuriootpa Research Station in 2001 showed no effect of PRD on shoot N or C concentration. However, a negative correlation existed in shoot N concentration and nodes retained, fewer nodes retained having the higher shoot N concentration at harvest.

5.5 Conclusions

Experiments in this chapter investigated the effects of partial rootzone drying and exogenous ABA on accumulation and partitioning of N, minerals and assimilated C in the pot and field-grown environments. Enough evidence has been collected to accept the hypothesis that Partial rootzone drying causes partitioning of more nitrogen, minerals and assimilated carbon to the permanent structure at the expense of vegetative growth and this is mainly due to the hormonal influence of root-sourced ABA on plant growth and function. The major conclusions were:

1) PRD and exogenous ABA treatments affected grapevine physiology by significantly reducing stomatal aperture - PRD more so than exogenous ABA.
2) Both PRD and exogenous ABA in this split-rooted experiment reduced the accumulation of dry weight in leaf canopy and one-year old shoots, the vegetative section of the grapevine, while slightly increasing dry weight of permanent wood. Both treatments however had no effect on the dry weight of roots.
3) A reduced berry weight and slightly increased juice pH were only observed in vines treated with PRD. PRD and exogenous ABA, had no effect of total soluble solids (°Brix).
4) PRD had significantly smaller investment in carbohydrate storage as starch in leaves that could not be attributed to the hormonal influence of root-borne ABA.

5) PRD and exogenous ABA had no effect on the total amount of accumulated inorganic ions such as K, Ca, Na, P and S at harvest. However, both treatments accumulated significantly less Mg compared to control.
6) Both PRD and exogenous ABA showed increased partitioning of ions to the permanent structure at the expense of the shoots without affecting the total vine ion content.
7) Both PRD and exogenous ABA treatments increased the berry concentrations of K, P and S. However, calculated on a gram per berry basis both PRD and exogenous ABA did not affect berry mineral contents.
8) Exogenous ABA-treated and control vines absorbed significantly more ^{15}N during the ripening period than PRD vines. This was manifested in higher abundances in berries, leaf canopy, one-year old shoots and thin roots. Although there were differences in abundance, the total amount of ^{15}N partitioned to the fruit was the same. As for the rest of the plant, similarly to situations with starch and minerals, PRD and exogenous ABA increased the partitioning of ^{15}N to the permanent structure, specifically to the older wood at the expense of the vegetative structure. PRD also partitioned significantly less ^{15}N to the thick roots in favor of small roots: this may indicate new root growth.
9) Both treatments had no significant effect on the total vine C or N at harvest compared to control. However, both treatments affected the partitioning of N and C by significantly increasing the accumulation in permanent wood and shoots at the expense of the leaf canopy.
10) The ratio of total N to total C per organ indicated that both PRD and exogenous ABA affected grapevine physiology by significantly increasing the total N/C ratio in berries, leaves, permanent wood and roots. It is clear that PRD and exogenous ABA treatment caused investment of significantly more resources to permanent structures and roots at the expense of vegetative growth.
11) Investigations into the PRD effect on field-grown grapevine berry, leaf and shoot tissue N and C content resulted in inconclusive results. No conclusions could be drawn from N/C ratios due to the same reason. It is concluded that unlike the pot environment where roots have constraints in terms of volume and moisture, the excess nutrients partitioned to permanent structures in field-grown grapevines would be used to enlarge its root structure to exploit the deeper layers of soil.

Chapter 6: Partial rootzone drying reduces grapevine root and leaf nitrate reductase: the role of ABA and soil drying

6.1 Introduction

PRD has the effect of reducing vegetative growth in grapevines, leading to a reduced canopy density and better vine balance (Dry *et al.*, 1996). PRD reduces canopy density due to a reduction in total leaf area, the result of an effect on both main and lateral shoot growth. Shoot growth and development are limited by nitrogen (N) availability more than any other nutritional factor (Crawford and Glass, 1998). Knowledge about the movement, compartmentation and turnover of N is therefore critical to understanding the impact of the PRD system.

The absorption of nitrate (NO_3^-) and ammonium (NH_4^+) by plants allows them to form numerous nitrogenous compounds, mainly proteins, essential to growth and metabolism. The first step in N assimilation is the reduction of NO_3^- to NO_2^-. This step is energy dependent and catalyzed by the enzyme nitrate reductase (NR) (Lewis *et al.*, 2000). NR activity is assumed to be the rate-limiting step in NO_3^- assimilation pathway and the main regulatory site of the reduction of NO_3^- to NH_4^+ (Crawford, 1995; Tischner, 2000). Hunter and Ruffner (1997) found NR activity in leaves, roots and berries of grapevines. NR activity in grapevine leaves was found one to two orders of magnitude higher than NR activity in roots and berries - a difference that increased steadily with increased N supply. By implication, grapevine leaves have a much higher capacity for NO_3^- reduction than do grapevine roots, and any contribution by roots to whole-vine NO_3^- assimilation declines even further as NO_3^- availability increases (Zerihun and Treeby, 2002). Berry NR activity generally increases towards ripeness and is affected only slightly by canopy management that improves light microclimate and photosynthetic activity (Hunter and Ruffner, 1997).

NR activity is substrate-inducible and it has been proposed that nitrate flux is the most important factor in the regulation of NR activity (Gojon *et al.*, 1991). However, its activity can be altered by environmental, hormonal or metabolic factors that may impact on the metabolic state of the plant cell. The nitrate assimilation pathway requires carbon skeletons for amino acid biosynthesis and consumes as much as 25% of the energy of photosynthesis (Solomonson and Barber, 1990). Consequently, NR is regulated by sugar metabolism. Therefore most fast growing plants reduce nitrate in their leaves where the main reducing power arises directly from light and photosynthesis (Huber *et al.*, 1992; de Cires *et al.*, 1993). It is therefore assumed

that a reduced NR activity at adequate nitrogen levels may be associated with a reduced availability of both energy and reducing power. Conversely, at adequate levels of energy and reducing power a reduction in NR activity may be attributed to low levels of nitrate. Some data are available showing that only small amounts of nitrate are sufficient for induction and that either the N-flux or the plant N-status controls NR expression (Samuelson *et al.*, 1995).

Using split-rooted plants, Gowing *et al.* (1990) demonstrated that many effects of water stress could be explained by the transport of chemical signals from the roots to shoots and that the excising of the drying rootzone would eliminate the source, thereby leading to the recovery of water stressed plants. Drying soils have been correlated with increases in root abscisic acid (ABA) and much evidence has been accumulated that drying roots under PRD conditions are the source of ABA, which is involved in reducing stomatal aperture and shoot growth (Loveys, 1984; Düring *et al.*, 1996; Dry *et al.*, 2000). Although elevated ABA levels usually reduce stomatal aperture and limit growth it is often found with exogenous ABA treatment that there is a concurrent increase in NR activity, a higher consumption of sucrose and a significant decrease in the level of reducing sugars (Chraibi *et al.*, 1995; Goupil *et al.*, 1998). Chraibi et al. (1995) explained that the increase in NR activity might be due to increased availability of reductants that are less diverted towards growth. Increased levels of phosphate-esters such as Glc-6-P and Triose-phosphate by ABA may also contribute to an increase in NR activity due to the modification in the phosphorylation state of NR (Chraibi *et al.*, 1995).

Various researchers have shown that girdling decreases the net CO_2 assimilation rate and stomatal conductance of grapevine leaves for a short period (Düring, 1978; Hofaecker, 1978; Harrell and Williams, 1987). After the initial effect of girdling has dissipated, girdling is reported to increase both the carbohydrate concentration above the girdle (Roper and Williams, 1989) and the ABA concentration in the leaves (Düring, 1978). This girdling effect may be used to study the effect of stomatal closure, due to an increase in endogenous ABA levels, on leaf NR activity in grapevines without exposing the roots to low soil moisture conditions.

Foyer *et al.* (1998) found a clear relationship between NR activity and the rate of photosynthetic CO_2 assimilation in water-stressed maize leaves. The inhibition of photosynthesis caused by water stress correlated with a marked decrease in total NR activity. Furthermore, the inhibition of NR activity during mild water stress could be reversed by an

increase in the external CO_2 concentration (Kaiser and Förster, 1989). A hyperbolic correlation was found between extractable NR activity and CO_2 fixation in barley by de Cires *et al.* (1993). This was an indication that NR activation follows saturation kinetics with respect to CO_2 fixation.

The next step in nitrogen assimilation is where ammonium (NH_4^+) is converted to glutamine via the glutamine synthase/glutamate synthase (GS/GOGAT) cycle (Givan, 1979; Roubelakis-Angelakis and Kliewer, 1992). The GS/GOGAT cycle is thought to be the most important process in the production of amino acids in grapevine leaves and roots (Roubelakis-Angelakis and Kliewer, 1992).

Since NR activity indicates the turnover of newly absorbed nitrogen, elucidating the effects of PRD on NR activity will also contribute to our understanding of nitrogen partitioning and source:sink relationships. The experiments described in this chapter were conducted to test the hypothesis that *PRD reduces shoot and root growth by affecting nitrate reductase in response to soil drying due to changes in hormone production and substrate availability.*

6.2 Materials and methods

6.2.1 Experimental material and design

Field experiments

Experimental sites consisted of field-grown Cabernet Sauvignon grapevines in the Coombe vineyard where PRD received half the amount of irrigation water of control vines until harvest and Shiraz where PRD received the same amount of irrigation water as control vines until harvest as described in Section 2.1. All vines were own rooted and experimental design for both cultivars consisted of a randomized block with two treatments, control and PRD irrigation, and seven replicates. Each plot consisted of three vines and data were only collected from the centre vine.

Cabernet Sauvignon vines in the Alverstoke vineyard were planted in 1997 in a trench with roots divided by a plastic membrane. The vines were trained to VSP trellis system and allowed to sprawl. Experimental design consisted of randomized treatment plots within one row. There were 6 replicates of three treatments (Control, PRD and girdling). Girdling was done shortly

after set, before irrigation started, by removing ± 3 mm strip of bark with a double-bladed knife. Vines were irrigated with drip emitters, either on both sides of the membrane at 1 L/h (control) or only on one side at any time at 2 L/h (PRD). The consequence is that all vines received the same amount of irrigation water throughout the growing season.

Pot experiments

To continue research during the winter, two-year-old Cabernet Sauvignon vines were grown in pots in a greenhouse located at the Waite Campus, Glen Osmond, South Australia. The vines were split rooted into two separate 12 L pots filled with standard UC potting mix (Section 2.2). PRD treatment consisted of daily irrigation of 20 min of 2 L/h drippers in only one side while water was withheld from the other side at any time. Watering sides were switched every 5 days. Control treatment received water in both pots for the same amount of time. When irrigated, soil moisture was increased to soil capacity.

To investigate the effect of exogenous ABA on leaf NR activity four-year old split-rooted Cabernet Sauvignon vines were grown under 50% shadecloth in two 8 L pots filled with pure washed sand (Figure 6.1). The pots were covered with a reflective sheet (Sisalation™) to reduce heat and evaporation and all vines were irrigated twice daily with a 0.02% w/v fertilizer mixture (Aquasol™) solution to field capacity. The vines were allowed to have 5 shoots and to bear three bunches. Both treatments received the same amount of irrigation water during the experiment and the soil matric potential was monitored using a hand-held tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA) as described in Section 2.3. Every second day the irrigation water in ABA-treated grapevines was spiked with synthetic ABA to achieve a final concentration of 10 μM. The final concentration was determined over a 2 week period by starting at an ABA concentration typically found in the xylem sap of PRD treated grapevines of 3 μM by Stoll (2000) and progressively increasing the concentration until a significant reduction in stomatal conductance compared to control was detected. Experimental design consisted of 5 single vines per treatment randomly selected for either exogenous ABA or control.

Figure 6.1 Split-rooted Cabernet Sauvignon used for exogenous ABA treatment at the Waite campus (summer 2002).

Two-year old split-rooted Cabernet Sauvignon grapevines (Figure 6.2) were used that grew in two 3 L pots filled with a standard potting mix (Section 2.2). The bottom of the pots were cut out and replaced with plastic mesh to allow roots to grow through into two secondary pots filled with vermiculite. This allowed easy harvest of clean and actively growing roots. The vines received a top dressing of 1 g of slow release fertilizer (Osmocote®) once at fruit set and the vines were allowed to have 3 shoots and to bear 2 bunches. In this 'double-pot' experiment irrigation water was applied either to one side of the root system at any time (PRD) or to both sides (Control) as described in Section 2.1. Soil matric potential was monitored in the top pots using a hand-held tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil moisture equipment corp., Santa Barbara, USA) as described in Section 2.3. To protect the roots from excessive heat, intermittent rain and sunlight the pots were enclosed in polystyrene boxes with holes cut through the lid for the extruding vine trunk. Experimental design consisted of 10 single vines per treatment randomly selected for either PRD or control.

Figure 6.2 Pot configuration for Split rooted Cabernet Sauvignon used for root analyses. Top two pots filled with standard potting mix and the bottom two pots with vermiculite.

6.2.2 Stomatal conductance

Stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day.

6.2.3 Soil moisture measurements

Soil moisture measurements were done in field and pot-grown experiments as described in Section 2.3.

6.2.4 Glutamine synthase (GS) activity

The activity of GS was determined by the method described by Lin and Kao (1996). Plant tissue was homogenized with 10 mM Tris-HCl buffer (pH 7.6), containing 1 mM $MgCl_2$, 1 mM EDTA and 1 mM 2-mercaptoethanol in a chilled pestle and mortar. The homogenate was centrifuged at 15000 g for 30 min and the supernatant was used for the enzyme assay. The whole extraction procedure was carried out at 4°C. GS assay was done on the supernatant by the method described by Oaks *et al.* (1980). The reaction mixture contained of a final volume

of 1 mL, 80 μM Tris-HCL buffer, 40 μM L-glutamic acid, 8 μM ATP, 24 μM $MgSO_4$ and 16 μM NH_2OH (final pH was 8.0). Reactions were started by the addition of the enzyme extract and after incubation of 30min at 30°C the reaction was stopped by the addition of 2 mL 2.5% (w/v) $FeCl_3$ and 5% (w/v) trichloroacetic acid in 1.5 M HCl. The mixture was centrifuged at 3000 g and the absorbance of the supernatant was read at 540 nm. One unit of GS activity was defined as 1μmol L-glutamate γ-monohydroxamate formed per min.

6.2.5 Nitrogen reductase (NR) activity assay by infiltration method

NR activity was determined in leaves, berries and shoots by the method described by Hunter and Ruffner (1997). After the removal of leaf veins, leaves were cut into 2 mm^2 disks. Berries and roots were cut into 2 mm wide slices. Representative samples of leaves (0.2 g), berries (1 or 2 g) and roots (1 g) were immediately infiltrated under vacuum in pre-cooled 50 mL Erlenmeyer flasks containing 5 mL 0.1 M KNO_3 and 5 mL 0.1 M phosphate ($Na_2HPO_4 \cdot 12H_2O$- KH_2PO_4) buffer at pH 7.5. In controls, KNO_3 was substituted with water. The infiltration of the tissue comprised of repetitive (5 x 30 s) removal of oxygen by vacuum and replacing it with nitrogen gas. After infiltration, nitrogen gas was bubbled into the incubation medium for 60 s. Flasks were then sealed with rubber stoppers, wrapped in aluminum foil and incubated with gentle shaking in a water bath for 1 hour at 40°C. After incubation the flasks were vortexed for 10 s and 1 mL aliquots removed for nitrite determination. Nitrite was estimated by the addition of 1 cm^3 1% (w/v) Sulphanilamide in 1.75 M HCl, 1 mL 0.01% (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride and 5 mL H_2O. Absorbance was read at 540 nm with a spectrophotometer after 30 min. The NR activity was expressed as nmol nitrite produced per gram fresh tissue per hour.

6.2.6 Nitrogen Reductase (NR) assay by extraction method

NR was extracted (Foyer *et al.*, 1998) from leaf tissue powdered in liquid nitrogen. The extraction buffer (50 mM Mops-KOH, pH 7.8, 5 mM NaF, 1 μM Na_2MoO_4, 10 μM FAD, 1 μM Leupeptin, 1 μM microcystin, 0.2 g^{-1} fresh weight PVP, 2 mM β-mercaptoethanol and 5 mM EDTA) was then added to the leaf tissue powder (1 mL/50mg fresh weight). The homogenate was centrifuged at 4°C for 5 min at 12000 g. NR activity was measured immediately on the supernatant. The reaction mixture consisted of 50 mM Mops-KOH buffer, pH 7.5, supplemented with 1 mM NaF, 10 mM KNO_3, 0.17 mM NADH, and either 10 mM $MgCl_2$ or 5 mM EDTA. The reaction was terminated after 8 or 16 min by the addition of an equal volume

of sulfanil-amide (1% w/v in 3 N HCl) and then naphthylethylene-diamine dihydrochloride (0.02% w/v) to the reaction. The A_{540} was then measured in a spectrophotometer. The activation state of NR was defined as the activity measured in the presence of 10 mM MgCl divided by the activity measured in the presence of 5 mM EDTA (expressed as a percentage).

6.2.7 Determination of ammonium

The determination of ammonium in grapevine tissue was carried out using the Berthelot reaction, modified according to Lin and Kao (1996). Two hundred µL of xylem sap were diluted by 0.3 mM sulphuric acid to a final volume of 4 mL. The color reaction was achieved by addition of 0.5 mL of solution A (5 g phenol, 25 mg nitroprusside dissolved in 100 mL water) and 0.5 mL of solution B (40 mL 5% sodium hypochlorite and 2.5 g NaOH mixed and made up to a final volume of 100 mL with distilled water). Gentle shaking in a water bath at 37°C for 20min carried out incubation. Absorbance was measured at 625 nm against the control without extract. Ammonium levels were calculated using standard NH_4Cl concentration curves and expressed as µM $NH_4^+ mL^{-1}$.

6.2.8 Determination of nitrate

Nitrate and nitrite concentration in xylem sap were measured using a nitrite/nitrate assay kit from Roche Diagnostics (Mannheim, Germany) to the method specification. The principle of the method is the colorimetric reaction of nitrite with a diazo dye and its absorbance is visible at 540 nm. Nitrate was reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase. The nitrite formed reacted with sulphanilamide and N-(1-naphthyl)-ethylene-diamine dihydrochloride to give a red-violet diazo dye. The A_{540} of the diazo dye was then measured in a spectrophotometer against a blank. Sample measurements were compared to standard absorption curves for nitrite and nitrate to calculate sample concentrations.

6.3 Results

6.3.1 PRD effect on ammonium levels and glutamine synthesis

To determine the effect of PRD and girdling on the accumulation of ammonium (NH_4^+) in grapevines, the NH_4^+ concentration in exudates from leaves were measured after leaf water potential measurements in Alverstoke Cabernet Sauvignon (Table 6.1). PRD treatment had no

effect on leaf exudate NH_4^+ concentration compared to control. Girdling however increased the NH_4^+ level in exudates by 44% on average compared to control.

Table 6.1 Xylem sap NH_4^+ of PRD-treated and girdled Cabernet Sauvignon grapevines in the Alverstoke vineyard (19/12/2000). (PRD and girdled vines received the same amount of irrigation water as control; means n = 6 ± s.e.)

	Control	PRD	Girdled	Sig.
Xylem sap NH_4^+	2.25 ± 0.72	2.11 ± 0.35	3.24 ± 0.29	n.s.

To determine the effect of PRD on the metabolic rate of glutamine synthesis in the GS/GOGAT cycle the glutamine synthase (GS) activity was measured in leaves of Coombe Cabernet Sauvignon and Shiraz that received half and equal amounts of irrigation water compared to control respectively. It was found that GS activity in grapevine leaves were not significantly influenced by PRD in Cabernet Sauvignon or Shiraz irrespective of the amount of irrigation water applied. On average, the leaves of PRD treated Cabernet Sauvignon and Shiraz had lower GS activity by 13 and 10% respectively (Table 6.2) compared to control.

Table 6.2 Glutamine synthase activity measured in leaves of field-grown grapevines in the Coombe vineyard (8/02/2001) (PRD received half the amount of control irrigation) and Shiraz (PRD receiving the same amount as control). (means n = 7 ± s.e.; n.s. = Not Significant ($P<0.05$); GS activity is defined as μmol L-glutamate γ-monohydroxamate/min)

	Control	PRD	% Diff (PRD as % of Control)	Sig.
Cabernet Sauvignon	0.268 ± 0.04	0.233 ± 0.01	- 13	0.416
Shiraz	0.137 ± 0.02	0.123 ± 0.01	- 10	0.559

6.3.2 PRD effect on berry NR activity

The effect of PRD on the NR activity in Coombe Cabernet Sauvignon berries is shown in Table 6.3. Although the Cabernet Sauvignon grapevines received half the amount of irrigation water as control there was no significant difference in berry NR activity. On average, the berry NR activity in PRD-treated grapevines decreased by 11%.

Table 6.3 NR activity measured in berries of field-grown Cabernet Sauvignon grapevines in the Coombe vineyard (7/02/2001) (PRD received half the amount of control irrigation; means n = 7 ± s.e.; n.s. = Not Significant ($P<0.05$); NR activity measured as nmol $NO_2.gFw^{-1}.h^{-1}$)

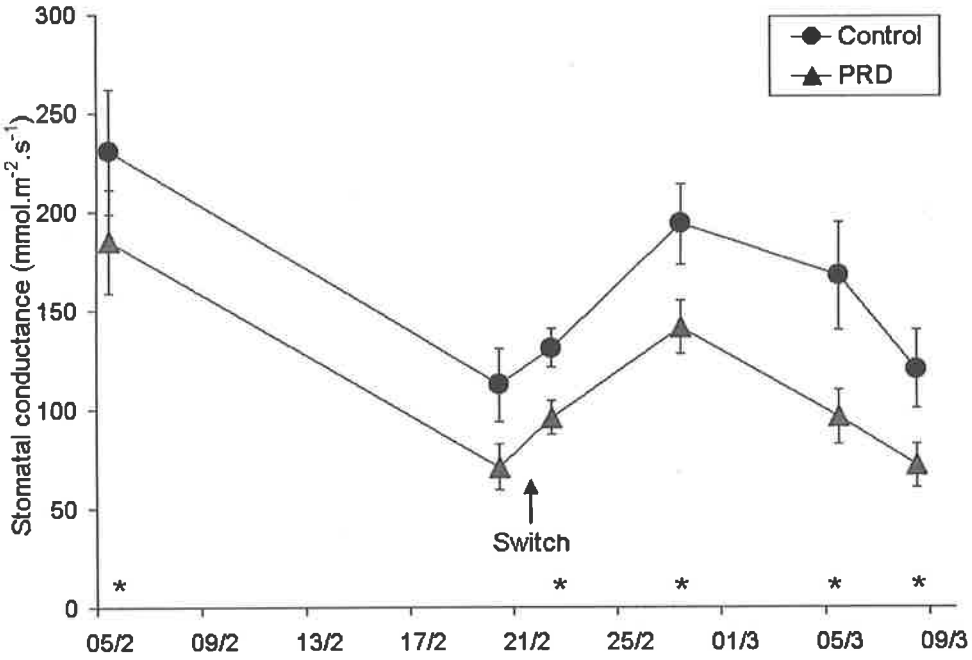
	Control	PRD	% Diff (PRD as % of Control)	Sig.
Berry NR activity	14.98 ± 1.0	13.54 ± 1.2	- 11	n.s.

6.3.3 PRD effect on leaf NR activity

The effect of PRD on the stomatal conductance and NR activity of Coombe Cabernet Sauvignon and Shiraz leaves receiving half and the same amount of irrigation water as control are shown in Figures 6.3 and 6.4 respectively. PRD significantly reduced the stomatal conductance and NR activity in both Cabernet Sauvignon and Shiraz on most of the sampling days compared to control. On average, PRD reduced stomatal conductance by 35 and 17% compared to control in Cabernet Sauvignon and Shiraz respectively and reduced NR activity by 29 and 9% respectively compared to control over the PRD cycle.

Figure 6.5 shows the relationship found between stomatal conductance and NR activity in Coombe Cabernet Sauvignon during some of the days when a significant inhibition of NR activity was found relative to the control. There existed a hyperbolic correlation between stomatal conductance and NR activity in both PRD treated and control grapevine leaves. No significant difference existed between the regression lines fitted for PRD and control ($P=0.2456$).

A



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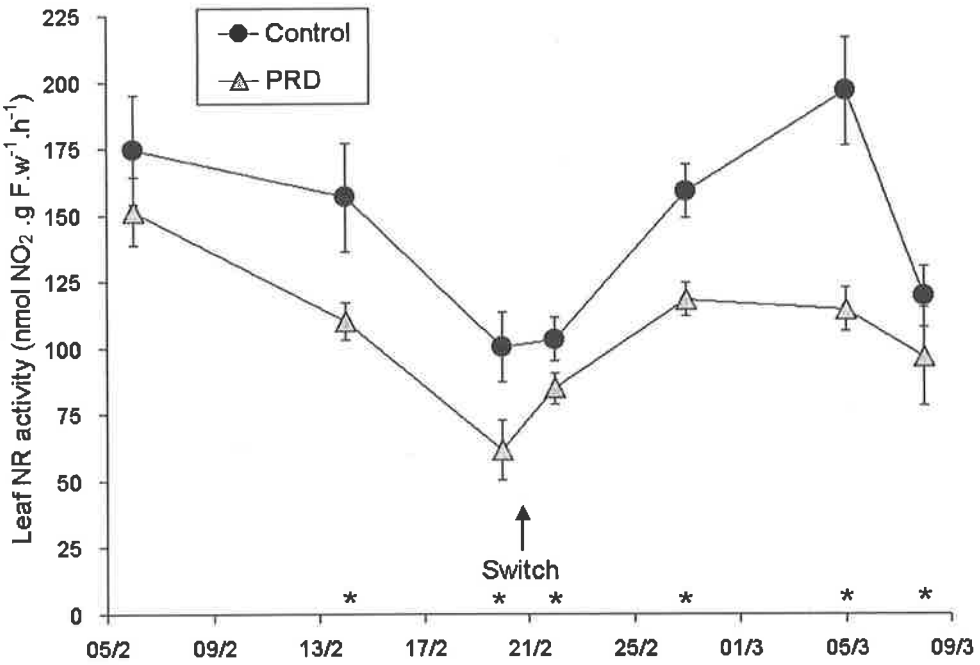


Figure 6.3 Effect of PRD treatment on **(A)** stomatal conductance and **(B)** NR activity in leaves of field-grown Cabernet Sauvignon over one PRD cycle in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$)).

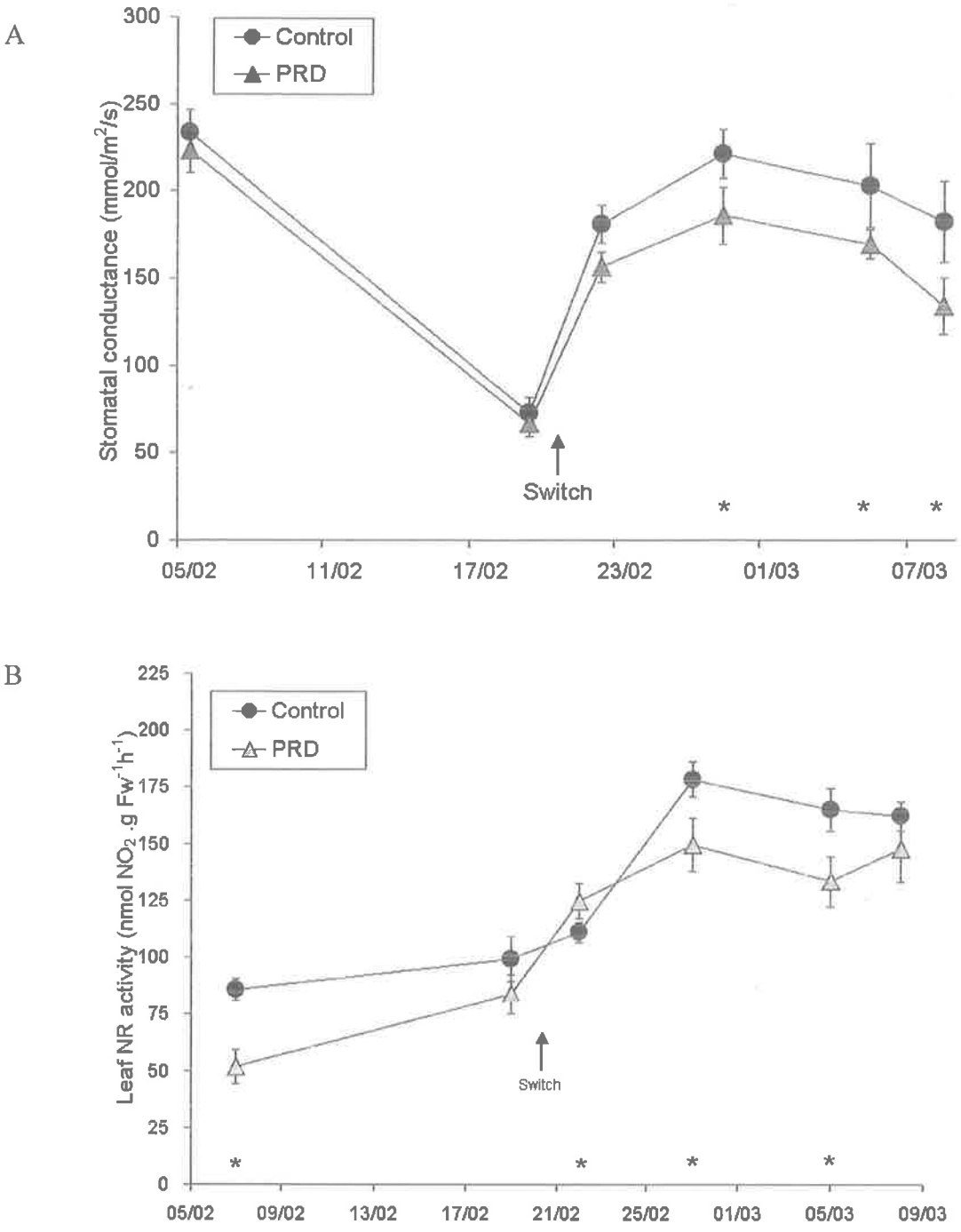


Figure 6.4 Effect of PRD treatment on **(A)** stomatal conductance and **(B)** NR activity in leaves of field-grown Shiraz over one PRD cycle in 2001. (PRD received the same amount of irrigation as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$).

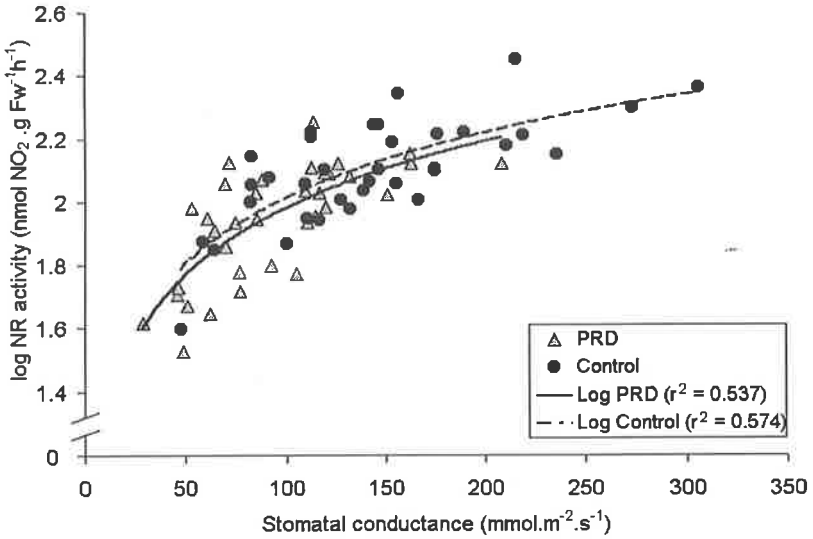


Figure 6.5 Effect of PRD on the relationship between stomatal conductance ($mmol.m^{-2}.s^{-1}$) and nitrate reductase activity (log NR activity, nmol $NO_2.g\ Fw^{-1}.h^{-1}$) in field grown Cabernet Sauvignon in 2001. (Data from 3 sampling days (20/02/2001, 27/02/2001, 5/03/2001); 7 replicates per treatment)

The effect of PRD on both stomatal conductance and NR activity (expressed as a percentage of control) is shown in Figure 6.6. It was found that PRD caused a long-term reduction in stomatal conductance compared to the control for both Cabernet Sauvignon and Shiraz by 35 and 17% respectively and for most of the sampling days corresponded with similar percentage deceases in NR activity. However, transient increases or decreases in inhibition occurred in NR activity that corresponded with the periods of extended soil drying and the time when the switch of the wetting zones in PRD took place.

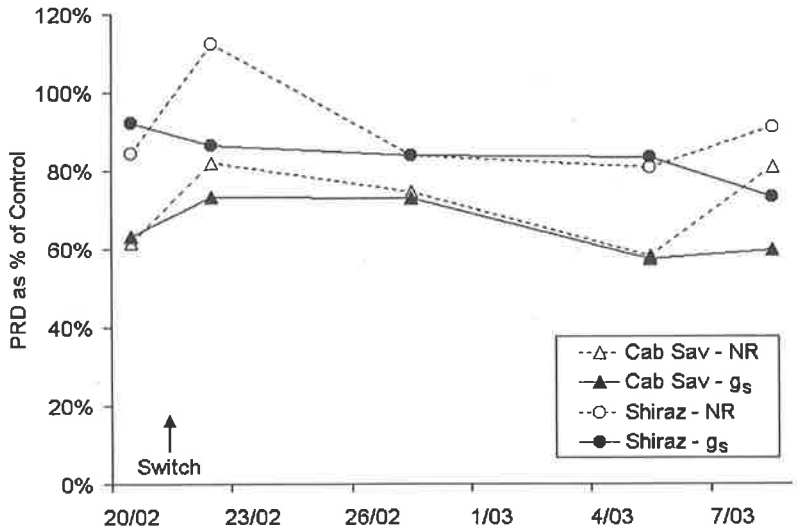
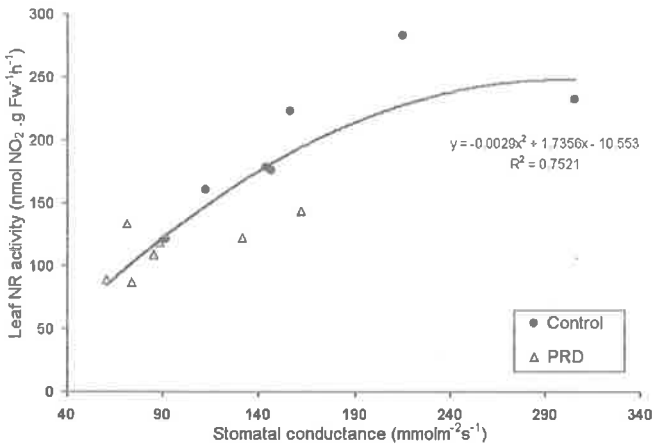


Figure 6.6 The effect of PRD on stomatal conductance (g_s) and NR activity as a percentage of control in Coombe Cabernet Sauvignon and Shiraz grapevines.

Figure 6.7 shows the relationship between stomatal conductance and NR activity in Cabernet Sauvignon leaves on two different days within the PRD cycle. The 05/03/2001 is a typical day during the PRD cycle where the PRD vine leaves had on average an equal percentage decrease in stomatal conductance and NR activity compared to control (similar on most PRD days between switches in irrigation pattern). Regression analysis showed that a strong hyperbolic relationship existed (Figure 6.7 A) between these processes during most sample days of the PRD cycle. However, after an extended period of soil drying the inhibition of NR activity decreased without any change in the inhibition of stomatal conductance compared to control. This was typically found at the stage when the wetting was switched (22/02/2001 and 08/03/2001). Regression analysis shows that a weaker relationship existed (Figure 6.7 B) between these processes at those times, but the nature of the relationship stayed the same. This implies that stomatal conductance might not have been the causal factor that influenced NR activity under PRD conditions.

A



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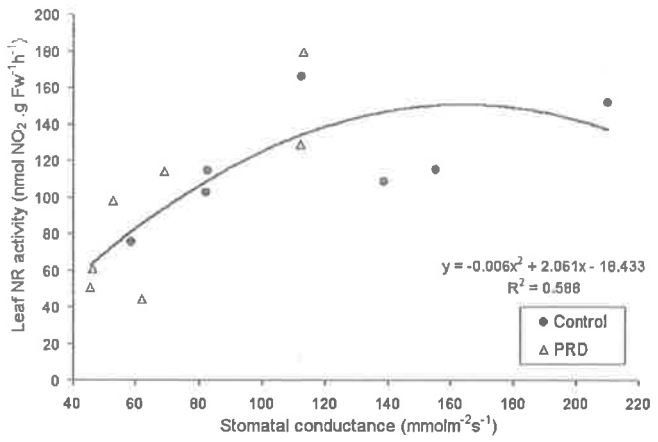


Figure 6.7 Correlation between leaf stomatal conductance (mmol.m^{-2}.s^{-1}) and nitrate reductase (nmol NO_2.g Fw^{-1}.h^{-1}) in field grown Coombe Cabernet Sauvignon on **(A)** 05/03/2001 and **(B)** 08/03/2001.

Because the energy state of the cell can exert some control over NR activity, a second method to analyze NR activity was then applied on two-year-old Cabernet Sauvignon vines grown in pots in a glasshouse to test the validity of the previously used infiltration method. The vines were split-rooted into two separate pots for PRD purposes. Because this method required the addition of a low concentration of NADH, the possible negating effect of limited NADH that may arise during analyses was avoided. The results obtained with the crude extract method on pot-grown Cabernet Sauvignon are shown in Table 6.4 and were very similar to the above-mentioned findings in field-grown vines. PRD-treated vines showed significantly lower NR activity and stomatal conductance by 16 and 24% respectively compared to control vines.

Table 6.4 NR activity (%) and stomatal conductance ($mmol.m^{-2}.s^{-1}$) measured in leaves of split-rooted Cabernet Sauvignon under glasshouse conditions in 2001. (means n = 6 ± s.e.)

	Control	PRD	% Diff (PRD as % of Control)	Sig.
NR activity (%)	107.5 ± 1.2	90.2 ± 4.5	- 16	<0.05
Stomatal conductance	78.8 ± 1.4	59.8 ± 5.4	- 24	<0.01

6.3.4 Factors affecting stomatal conductance and NR activity

As a result of the previously described results, experiments were conducted to investigate methods that would test the relationship between stomatal conductance and the NR activity in leaves and the effect of exogenous ABA. The first experiment involved field-grown Alverstoke Cabernet Sauvignon that was treated with PRD receiving the same amount of irrigation as control and girdling of the vine trunk shortly after berry set. It is generally found that girdling increases ABA in leaves and reduces shoot growth and stomatal conductance for a short period. My experiments with girdled Cabernet Sauvignon showed significant reductions in stomatal conductance and shoot growth compared to control without influencing plant water relations (see Chapter 4). Along with a reduction in stomatal conductance, girdling significantly reduced leaf NR activity (Figure 6.8). PRD treatment did not have a significant effect on stomatal conductance on most of the sample days and the effect on NR activity was inconsistent.

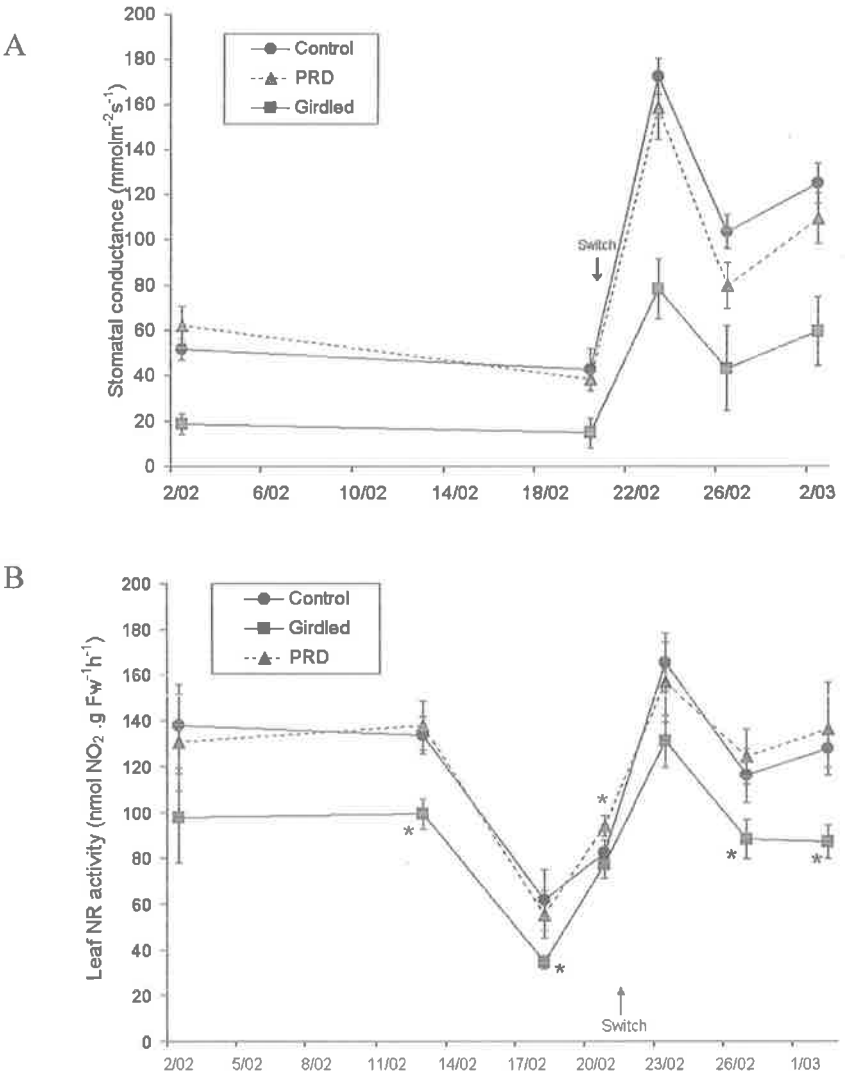


Figure 6.8 A) Stomatal conductance and **B)** Nitrate reductase activity of Alverstoke Cabernet Sauvignon in 2000/1. PRD and girdled vines received the same amount of irrigation water as control (means n = 6; vertical bars represent standard error of the average. * = significantly different ($P<0.05$)

The second experiment involved treating split-rooted Cabernet Sauvignon (grown in sand in pots) with exogenous ABA to mimic the root-derived ABA signal found in PRD treatments. All vines received the same amount of irrigation water and fertilizer. Exogenous ABA (10 μM) was applied directly to the roots on one side of the treated vines every second day. The effect of exogenous ABA on the stomatal conductance of the split-rooted Cabernet Sauvignon is shown in Figure 6.9. Exogenous ABA reduced the stomatal conductance on most of the sample days compared to control without any soil drying. On average, exogenous ABA reduced the stomatal conductance by 16% compared to control over the experimental period.

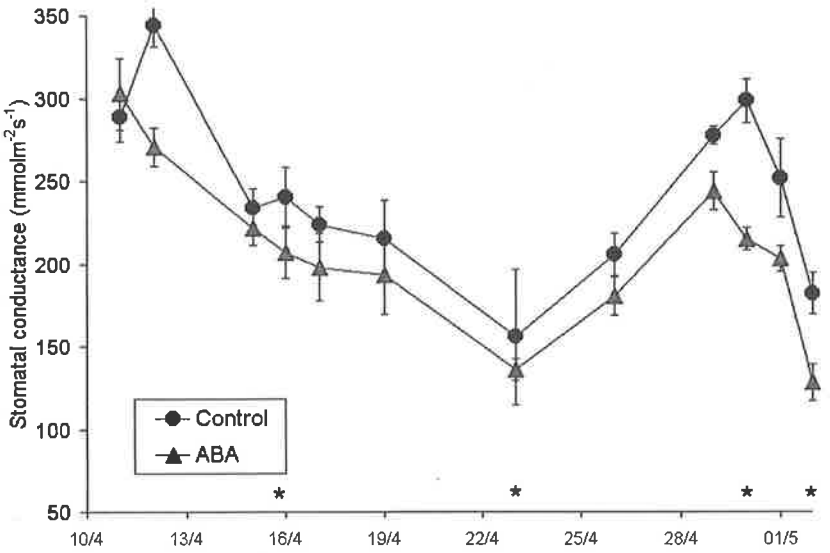


Figure 6.9 Effect of exogenous ABA on the stomatal conductance (g_s) of split-rooted Cabernet Sauvignon in 2002 (Control: water on both sides; ABA: water on both sides with additional 10 μM ABA on one side). (means n = 5; Bars represent the standard error of the mean; * = significantly different ($P<0.05$).

The effect of exogenous ABA on leaf NR activity in the split-rooted Cabernet Sauvignon grapevines grown in pots is shown in Figure 6.10. The leaf NR activity was measured on sampling days when exogenous ABA treated vines had significantly lower stomatal conductance compared to control. Although exogenous ABA reduced stomatal conductance on average by 20% there was no significant effect on leaf NR activity compared to control. On the contrary, on two of the sampling days exogenous ABA increased the NR activity by 25% compared to control.

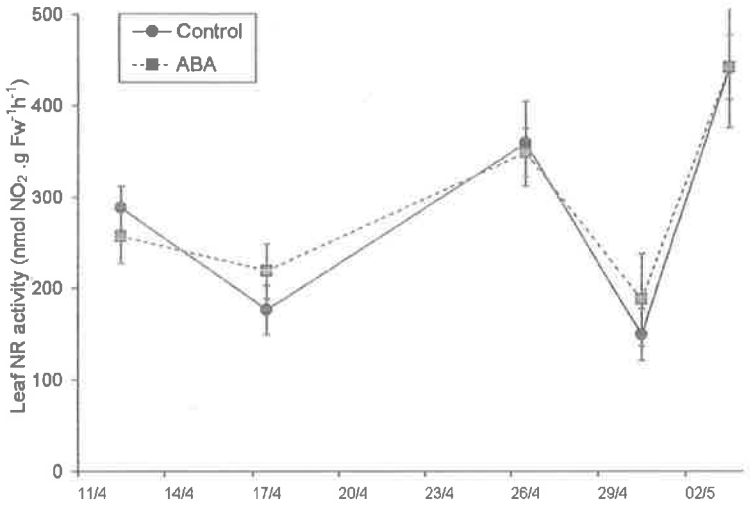


Figure 6.10 Effect of exogenous ABA on the leaf nitrate reductase activity of split-rooted Cabernet Sauvignon in 2002 (Control: water on both sides; ABA: water on both sides with additional 10 μM ABA on one side). (means n = 5; Bars represent the standard error of the mean)

6.3.5 PRD effect on xylem sap nitrate concentration

The effect of PRD on xylem sap nitrate (NO_3^-) concentration in field-grown Cabernet Sauvignon and Shiraz in the Coombe vineyard during the 2002 growing season is shown in Figure 6.11. PRD is known to reduce sap flow and growth in grapevines irrespective of the amount of irrigation water applied (Stoll, 2000; de Souza *et al.*, 2003) and therefore an increase in xylem sap NO_3^- concentration may be expected in PRD vines due to lower N utilization, especially those that received the same amount of irrigation water as control. This was found to be the case in PRD Shiraz with a significant increase in xylem sap NO_3^- by 7% compared to control. However, PRD Cabernet Sauvignon that received half the amount of irrigation water as control had a significant decrease in xylem sap NO_3^- by 24% compared to control.

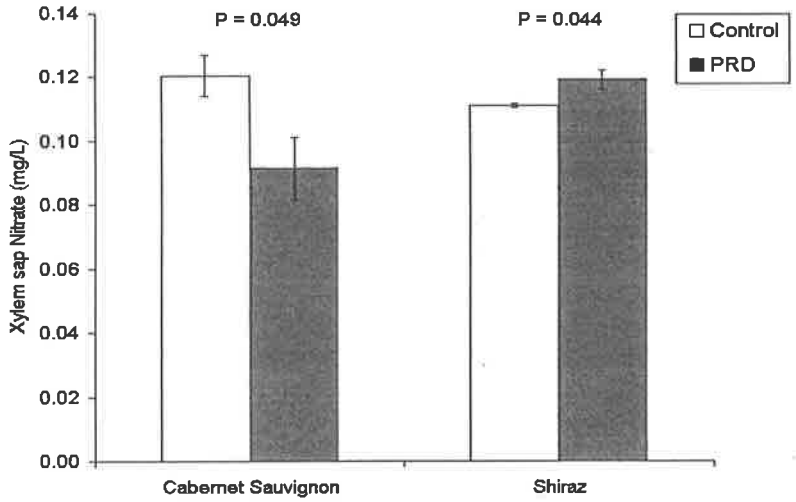


Figure 6.11 The PRD effect on xylem sap nitrate concentration in Coombe Cabernet Sauvignon and Shiraz grapevines during the 2001/2 growing season (15/03/2002). PRD Cabernet Sauvignon received half the amount of control irrigation and PRD Shiraz received the same amount of irrigation as control. (means n = 7 ± s.e.)

6.3.6 PRD effect on root NR activity

To investigate the effect of PRD on grapevine roots, split-rooted Cabernet Sauvignon vines were grown in 'double-pot' systems and treated either with PRD that received water on only one side at any time or control that received water on both sides during the whole season of 2002. The effect of PRD on soil matric potential and stomatal conductance during one PRD cycle is shown in Figure 6.12. There were no significant differences in the soil matric potentials of control and PRD 'wet' pots during the PRD cycle. Due to a rain event 5 days after the drying cycle started, the wetting pattern was switched over sooner than expected. However, as expected withholding water from the PRD 'dry' side rapidly decreased soil matric potential on a daily basis indicating a steady drying rate. PRD significantly reduced stomatal conductance on average by 29% compared to control.

The NR activity in roots that had grown through into the vermiculite was analyzed on two dates after the switch of wetting patterns. The first date was 4 days after the switch and at a time when there was a 24% reduction in PRD stomatal conductance compared to control (Figure 6.13 A). PRD treated grapevine roots had significantly lower NR activity on the 'dry' side compared to both control and its own 'wet' side by 22% and 14% respectively. The difference between root NR activity in the PRD 'wet' side compared to well-watered control roots was not significant. After a further 4 days of soil drying (Figure 6.13 B), the stomatal conductance was significantly reduced by 34% compared to control and the roots on the 'dry' side of PRD

treated grapevines had reduced NR activity by 54% and 64% compared to control and its own 'wet' side respectively. Root NR activity on the PRD 'wet' side compared to well-watered control roots after 8 days of PRD was 25% higher.

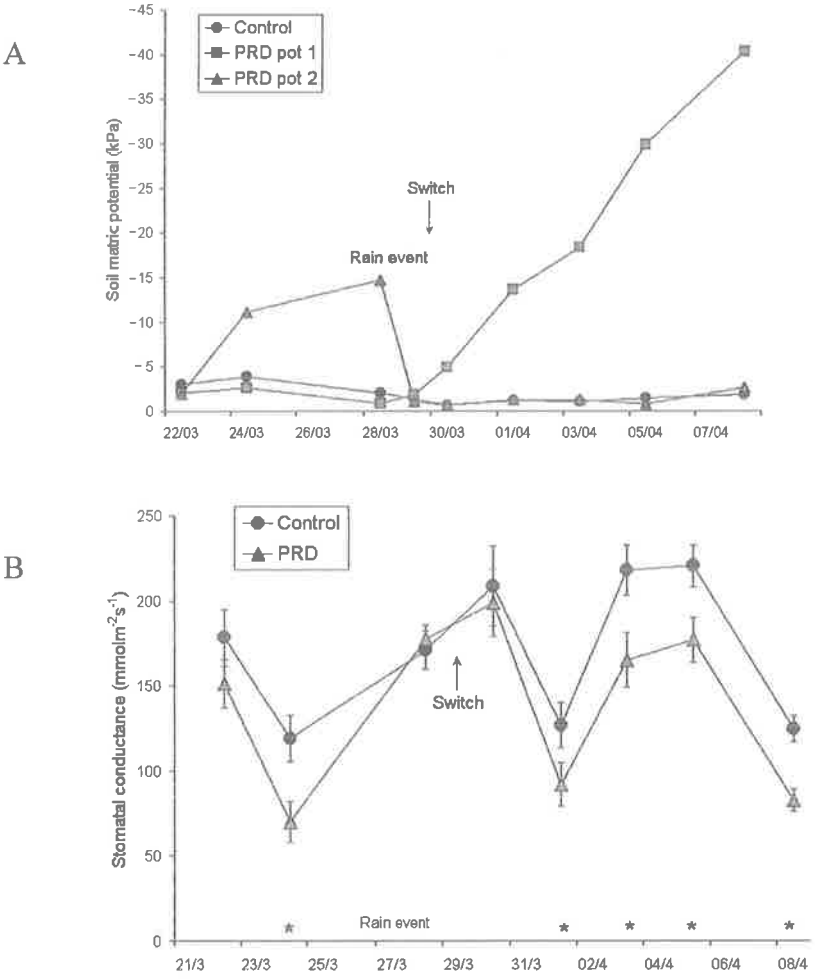
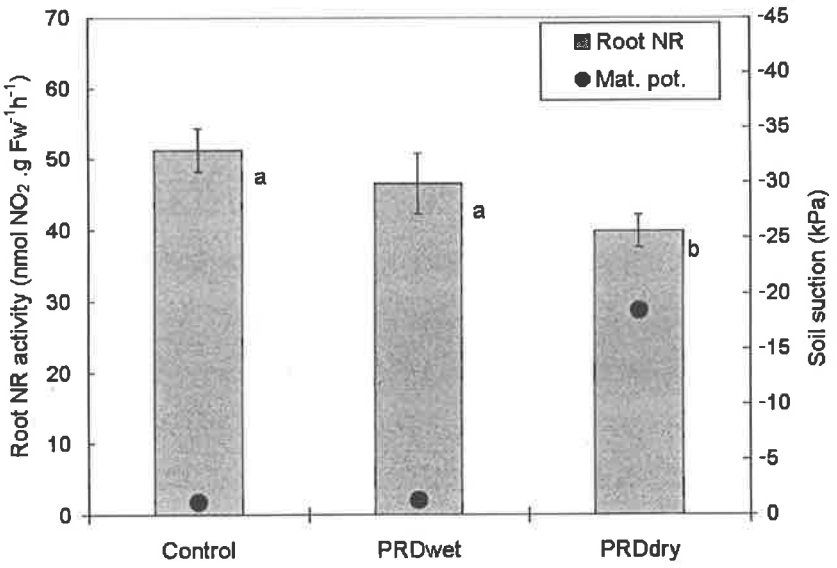


Figure 6.12 A) Soil matric potential (kPa) and B) stomatal conductance ($mmolm^{-2}s^{-1}$) of split-rooted 'double-pot' Cabernet Sauvignon grapevines in 2002. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10; vertical bars represent the standard error of the mean; * = significantly different ($P<0.05$)).

A



B

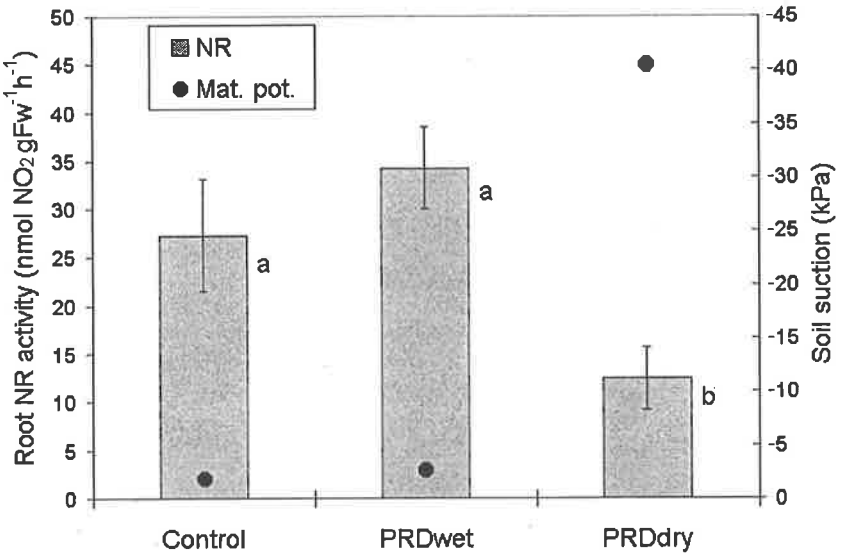


Figure 6.13 Soil matric potential and root NR activity after **(A)** 4 days and **(B)** 8 days of PRD treatment of split-rooted 'double-pot' Cabernet Sauvignon grapevines. PRDwet = PRD pot currently under irrigation; PRDdry = PRD pot with water withheld. (Control: vines received water on both sides; PRD: water withheld from one side at any time. (means n = 5; vertical bars represent the standard error of the mean; Bars with different letters are significantly different ($P<0.05$).

6.4 Discussion

To elucidate the effect of PRD on the turnover of newly absorbed nitrogen it was important to investigate the activity of enzymes involved in the biochemical assimilation of nitrate and their intermediate substrates. PRD did not significantly influence the endogenous ammonium levels or glutamine synthase activity of grapevine leaves that would indicate an inhibited rate of amino acid synthesis via the GS/GOGAT cycle. The rate-limiting step in nitrogen assimilation is the conversion of nitrate to NH_4^+ by nitrate reductase (NR) and activity of this enzyme was detected in berries, leaves and roots of grapevines in this study. A PRD effect on grapevine leaf NR activity would have major implications for whole vine turnover of newly absorbed nitrogen and elucidate organ sink:source relationships.

Hunter and Rufner (1997) found that although berry NR activity corresponded to the influx of sugars and increased substantially during ripening, it is not an indicator of berry amino acid content or total nitrogen status. Results from the current study in field-grown Cabernet Sauvignon indicated that PRD marginally decreased berry NR activity when PRD vines received half the amount of irrigation water as control.

Aruajo and Williams (1988) found a linear relationship between vine nitrogen content and leaf dry mass and suggested that leaves are a major factor in determining the bulk of the vine nitrogen content. Most fast growing plants reduce nitrate in their leaves where the main reducing power arises directly from photosynthesis. Accordingly, the NR activity in grapevine leaves is one to two orders of magnitude higher than NR activity in roots and berries and therefore grapevine leaves have a much higher capacity for NO_3^- assimilation. This order of magnitude was also found in this study with grapevine leaves exhibiting roughly a ten and five-fold higher NR activity in leaves compared to berries and roots respectively. PRD-treated and control grapevines showed similar hyperbolic relationships between leaf NR activity and stomatal conductance. The reduction of stomatal conductance was also correlated with similar reductions in leaf NR activity in PRD-treated vines compared to control.

Leaf stomatal conductance and NR activity were significantly reduced in response to PRD treatment in both field-grown Cabernet Sauvignon and Shiraz grapevines irrespective of the amount of irrigation water applied. Changes in leaf NR activity in response to PRD followed

the development of the PRD cycle. Although the effect of PRD was less pronounced in Shiraz in this respect, differences were still significant and the trend was still obvious. This difference in response between Cabernet Sauvignon and Shiraz vines may either be due to differences in treatments or it may be due to a difference in anisohydric nature of the two cultivars. Differences in anisohydric behaviour are fundamentally linked to stomatal behaviour and marked differences in leaf water potential exist between cultivars during water stress (Jones and Sutherland, 1991; Schultz, 1996; Schultz, 2003). For the most of the PRD cycle the PRD-treated grapevines showed equal percentage reductions in stomatal conductance and leaf NR activity compared to control and there existed a strong hyperbolic relationship between the processes, irrespective of treatment. However, during the periods shortly before and after the switch in wetting patterns, where one rooting zone was wet and the 'dry'-side had dried extensively or just started to dry, NR activity in PRD leaves increased without an increase in stomatal conductance. This was also characterized by a weaker relationship between stomatal conductance and leaf NR activity. This indicated that the effect of PRD on stomatal conductance and leaf NR activity was independent of each other. Earlier PRD results (Section 4.3.4) have shown that a strong hyperbolic relationship existed between stomatal conductance and CO_2 assimilation, with large decreases in stomatal conductance correlated with only minor decreases in CO_2 assimilation (Stoll, 2000; Kang *et al.*, 2001). It may therefore be possible that the leaf NR activity was, in fact, affected linearly by the reduction in photosynthesis. Foyer (1998) found a linear relationship between NR activity of water stressed maize leaves and the rate of photosynthetic CO_2 assimilation. The inhibition of photosynthesis caused by water stress correlated with a marked decrease in total NR activity. However, under PRD conditions leaves are not hydraulically stressed and the minor decreases in photosynthesis caused by PRD cannot be solely responsible for the large decrease in leaf NR activity compared to control. The changes in leaf NR activity may be caused by another factor in response to PRD, which is reduced or absent during times of extended soil drying and switching of the wetting pattern.

Different experiments were conducted to mimic the PRD effect in field and pot-grown grapevines by cultural and chemical means to investigate the stomatal control on leaf NR activity. Firstly, well-watered field-grown Cabernet Sauvignon grapevines were girdled to induce a reduction in shoot growth and stomatal conductance without reducing the amount of water supplied or changing the wetting pattern compared to control. Reportedly, the girdling of grapevines reduces stomatal conductance (Hofaecker, 1978; Harrell and Williams, 1987) and

growth by increasing the endogenous ABA levels in leaves (Düring, 1978). Unfortunately, PRD-treated Cabernet Sauvignon that received the same amount of water as control did not have consistent effects on stomatal conductance nor NR activity during the particular cycle for a comparison to be made. However, girdling successfully reduced stomatal conductance and NR activity compared to control vines for extended periods without a reduction of the amount of water applied or a change of wetting pattern. This provided evidence that cultural practices that reduce shoot growth and stomatal conductance may be associated with a reduction in leaf NR activity. The effect of trunk girdling on NR activity however may or may not be attributed to hormonal influences. Conversely, phloem disruption increases the build-up of sugars above the girdle (Salisbury and Ross, 1992) and may have a feedback inhibition on growth. Secondly, to investigate the hormonal effect of ABA, well-watered pot-grown Cabernet Sauvignon grapevines were treated with exogenous ABA to mimic the PRD effect of a reduced stomatal conductance and to investigate the direct effect on leaf NR activity without soil drying. Exogenous ABA treatment successfully reduced stomatal conductance on most of the sampling days, but did not reduce the leaf NR activity. On the contrary, exogenous ABA application increased leaf NR activity measured on more than one occasion. This may be due to increased availability of reductants that are less diverted towards growth, or ABA may contribute to an increase in NR activity due to the modification in the phosphorylation state of NR (Chraibi *et al.*, 1995). Results from this experiment indicate that soil drying may be a necessity to reduce leaf NR in PRD grapevines since ABA action on its own would reduce stomatal conductance but generally increases leaf NR activity. Furthermore, stomatal closure without soil drying did not affect leaf NR activity.

PRD may have a direct hormonal effect on leaf NR activity by reducing the rate of cytokinins delivered to vegetative organs. Stoll *et al.* (2000) have found that the cytokinin concentrations in roots, shoot tips and buds of PRD-treated vines significantly decreased compared to well-watered vines. This decrease in cytokinins may have contributed to the reduction in shoot growth associated with PRD treatments. Loveys *et al.* (2000) have shown that this reduction in shoot growth and inhibition of cytokinins (according to Stoll *et al.* (2000)) could only be maintained by alternating the wetting sides as soon as a recovery in stomatal conductances were observed. It may be possible that in my experiments there were a slight recovery in cytokinin production before the switch in PRD irrigation that might have increased leaf NR activity.

On the other hand, soil drying under PRD conditions may have influenced the amount of nitrate absorbed and delivered to shoots. Consequently, nitrate (NO_3^-) analyses were done on xylem sap collected in 2002 in field-grown Cabernet Sauvignon and Shiraz grapevines that received half and equal amounts of irrigation water as control. PRD-treated Cabernet Sauvignon showed a marked decrease in xylem sap NO_3^- concentration compared to control that was sufficient to inhibit leaf NR activity by limiting its substrate availability. It is well known that NR is substrate-inducible and it has been proposed that nitrate flux is the most important factor in the regulation of NR activity. However, PRD Shiraz vines that received equal amounts of irrigation water had significantly higher xylem sap NO_3^- concentration compared to controls. A range of possibilities may explain the increase in xylem sap NO_3^- compared to control. By wetting the rootzone with equal amounts of irrigation water PRD and control vines may have had the same root absorption of N, but a decrease in NO_3^- demand in shoots or a decrease in delivery rate by lower sap flow in PRD may increase the xylem sap NO_3^- concentration compared to control.

It is therefore hypothesized that, at least in PRD grapevines receiving less irrigation water than control, the significant reduction in leaf NR activity may be caused by changes in the hormone(s) and NO_3^- supply from the roots. It may be possible that during periods of extended soil drying under PRD conditions, when NR activity is increased without an increase in stomatal conductance, there is a change in root physiology to increase delivery of NO_3^- from the vine's 'wet' side. Evidence of this was found in a PRD root experiment with split-rooted pot-grown Cabernet Sauvignon. Drying roots of PRD-treated vines showed a significant reduction in NR activity compared to both control and its own 'wet' side roots a few days after the PRD cycle started. The root NR activity on the 'wet' side of the PRD vines was also lower than that of control roots, but only marginally. During this period the N-assimilation of PRD-treated roots would have been significantly impaired and the NO_3^- supply to shoots significantly reduced. After an extended period of soil drying the drying roots of PRD-treated vines still had a marked reduction in NR activity compared to control and its own 'wet' side, however the 'wet' side of PRD-treated vines exhibited a marked increase in root NR activity compared to control. This would increase NO_3^- supply to the shoots and alleviate the inhibition on leaf NR activity without any change in stomatal conductance.

6.5 Conclusions

Experiments in this chapter investigated grapevine nitrogen assimilation under PRD conditions by measuring berry, leaf and root NR activity and the effects of soil drying and ABA on stomatal conductance and substrate availability. Enough evidence has been collected to accept the hypothesis that PRD reduces shoot and root growth by affecting nitrate reductase in response to soil drying due to changes in hormone production and substrate availability. The major conclusions were:

1) PRD did not significantly influence the endogenous ammonium levels nor amino acid synthesis as measured in terms of glutamine synthase activity.

2) PRD marginally decreased berry NR activity when PRD vines received half the amount of irrigation water as control.

3) Leaf stomatal conductance and NR activity were significantly reduced in response to PRD treatment in both field-grown Cabernet Sauvignon and Shiraz grapevines regardless of the amount of irrigation water applied and a strong hyperbolic relationship exists between the processes, irrespective of treatment.

4) However, although related, PRD may have independent effects on stomatal conductance and leaf NR activity, because transient increases in NR activity in field vines were not directly associated with increases in stomatal conductance.

5) It is proposed that the transient increases in NR activity may be associated with the recovery of cytokinin production after extended periods of soil drying.

6) Exogenous ABA treatment provided further evidence that stomatal conductance and leaf NR activity are independently affected by PRD treatment since ABA action on its own reduces stomatal conductance without influencing leaf NR activity.

7) Soil drying in PRD-treated experiments caused a marked decrease in grapevine xylem sap NO_3^- concentration compared to control. This could inhibit leaf NR activity by limiting its substrate availability.

8) The significant decrease in PRD xylem sap NO_3^- concentration may be due to a significant decrease in the 'dry' side root nitrogen assimilation as measured by its NR activity. During periods of extended soil drying however, there may be a change in root physiology to increase production of NO_3^- in roots that are well-supplied with water, thereby increasing leaf NR activity.

Chapter 7: Osmotic regulation and sucrolytic enzyme activity in roots of partial rootzone drying: accumulation of sugars, amino acids and polyamines.

7.1 Introduction

The PRD system is designed as a strategy to control shoot vigor and improve water use efficiency in grapevines by drying half of the rootzone at any given time. However, PRD does not only affect vine shoot growth and physiology but also the development in roots. Root responses to PRD have received generally less attention than shoot physiology and yield aspects, however the increased ability to access soil resources may be an important mechanism of plant response to PRD. Dry *et al.* (2000) and Kang *et al.* (2002) found that, in half-dried pot vines, there was a relative increase in root development in the moist soil layers in the 'wet' side as a whole and the deeper layers of the 'dry' side compared to control. They proposed that the part of the root system in the dry soil could survive because of water movement from the 'wet' roots to the 'dry' roots. These results are supported by Dry *et al.* (2001) who reported that PRD roots of field-grown grapevines grew to deeper soil layers and had significantly higher abundance of roots of the 1 mm to 3 mm diameter at the 0.4 m to 0.7 m depth compared to control vines. The implication is that a more exploratory root system of PRD-treated vines may contribute to greater water stress tolerance and a better soil environment for root growth. The authors concluded however that the total dry weight of PRD-treated and control grapevine roots did not differ.

PRD treatment of pot-grown tomato plants showed a marked increase in root biomass by 55% as resources were partitioned away from shoots (Mingo *et al.*, 2004). Similar findings were reported by Kang *et al.* (2001) and Kang *et al.* (1998) with PRD treatment on hot peppers and maize plants where an alternating wetting pattern significantly increased the root/shoot ratio in both crops and in the total root biomass in maize compared to control and treatment with a fixed irrigation pattern (withholding water from one side). Tomatoes and grapevines however may differ in the way they store resources since grapevines are perennial plants and may partition significant resources into permanent wood (Chapter 5) compared to tomato plants that are annual and herbaceous.

Episodes of soil drying and rewatering that form the basis of PRD treatment can enhance the extension and initiation of secondary roots (Liang *et al.*, 1996), and increase root hydraulic

conductivity (North and Nobel, 1991). Root growth however is regulated by the maintenance of turgor by water influx (Nonami *et al.*, 1997) facilitated by low membrane resistance (Pritchard, 1994) and a water potential difference between the cell and wall. Plants may control root expansion by the supply of solutes (osmolytes) that are required to lower the osmotic potential within cells (Patrick, 1997). The uptake of osmolytes may be apoplastic and/or symplastic (Pritchard *et al.*, 2000). In the first instance there are probably two sources of apoplastic osmolytes; inorganic ions can be absorbed from the external soil solution or solutes (inorganic or organic) can be unloaded from cells in the surrounding tissue. Secondly, the alternate route for osmolyte supply is via the symplastic route and mainly via a direct connection between cells through plasmodesmata. The sieve elements are the symplastic source of osmolytes to growing root cells and the driving force behind phloem supply is the accepted Munch hypothesis (Minchin *et al.*, 1993) as described in Section 1.3.2. Cells can exert some control over supply by decreasing plasmodesmatal resistance, as seen during periods of drought (Schultz, 1994) and increasing their sink strength or demand of solutes as required for metabolism e.g. respiration and construction of new walls and components (Pritchard *et al.*, 2000).

Root growth can continue at very low soil water potentials because, even though the growing zone may not have hydraulic contact with the soil, it is not unreasonable to expect that osmolyte and water requirements for growth can be sustained by symplastic import alone (Pritchard *et al.*, 2000). Other authors have suggested that a significant amount of water enters growing cells through the plasmodesmata; in pea and maize it was calculated that 50% and up to 81% respectively of the water required for growth could be supplied symplastically (Schmalstig and Cosgrove, 1990; Bret-Harte and Silk, 1994). This may also involve the observed movement of water in PRD-treated grapevines from 'wet' roots to 'dry' roots (Dry *et al.*, 2000; Stoll, 2000; Dry *et al.*, 2001).

Sink unloading has been positively linked to sucrolytic enzyme activities that would hydrolyse the sucrose to hexose sugars (Section 1.3). The degradation of sucrose can be catalyzed by at least two different classes of enzymes (Winter and Huber, 2000) namely sucrose synthase (SucSy) and the invertases. Invertases catalyze the irreversible hydrolysis of sucrose to glucose and fructose while SucSy catalyses a reversible cleavage of sucrose. According to Roitsch (1999) the regulation of extracellular invertase is not only important for supplying carbohydrates to sink tissues but also plays a crucial role to mediate source-sink regulation in

response to a variety of stimuli. SucSy activity has been correlated with starch synthesis, cell wall synthesis and overall sink strength (Winter and Huber, 2000). The assay of these sucrolytic enzymes in the non-photosynthetic tissue of PRD-treated roots may broaden our knowledge of relative supply of carbohydrates and sink strength compared to control vines.

While water use efficiency (WUE) is a component of drought tolerance, the accumulation of osmoprotectants increases WUE (Naidu, 1995), protects plant macromolecules (Paleg *et al.*, 1985) and increases plant survival in stressful situations (Naidu *et al.*, 2000). These solutes include sugars, sugar alcohols, amino acids (e.g. proline) and their N-methyl derivatives (which include betaines). The osmoprotective properties of glycine betaine towards proteins and enzyme activities (Rhodes and Hanson, 1993) are well known and large quantities accumulate under water stress and in response to ABA treatment (Xing and Rajashekar, 2001).

In his review of a wide range of perennial plants, Rabe (1990) concluded that under conditions of water stress, proline and the polyamine putrescine usually accumulate. The role of proline accumulation is widely ascribed to that of a cytoplasmic osmoticum that lowers the cell water potential during drought (Rabe, 1990). According to Aspinall and Paleg (1981) other possible functions of proline accumulation may include (a) the hydration of biopolymers, (b) serving as a readily utilizable energy and (c) nitrogen source. Work done on drought stressed maize roots (Sharp, 1990) has shown that increased rates of proline deposition are a key factor in osmotic adjustment of the slightly vacuolated cells close to the root apex. This is where root elongation is particularly insensitive to low water potentials. In maize and several other species (Sharp and Davies, 1989) primary root growth continues at water potentials far below that which causes complete cessation of shoot development. Sharp and Davies (1989) stated that increased rates of solute deposition play a major role in the osmotic adjustment of growing regions in higher plants. Sharp and Davies (1989) found that, for at least maize roots, the ability to increase the deposition of proline is a key factor in maintaining root elongation at low water potentials.

Spermidine and spermine are synthesized from putrescine by the addition of aminopropyl groups from decarboxylated S-adenosylmethionine (SAM), which is an intermediate of ethylene biosynthesis. In many studies enhanced rooting was associated with increased levels of spermine and spermidine (Shyr and Kao, 1985; Kakkar and Rai, 1987; Faust and Wang, 1992) and conversely in *Phaseolus* (Jarvis *et al.*, 1985) the application of a SAM decarboxylase

inhibitor, methylglyoxal bis(guanylhydrazone) (MGBG), reduced endogenous levels of spermine and spermidine and inhibited root induction. Polyamines, especially spermine and spermidine, are therefore associated with enhanced rooting, especially adventitious root formation. If elevated polyamine concentrations in PRD vines exist they may contribute to our understanding of a more exploratory root system observed by Dry and Loveys (1999).

The experiments in this chapter were conducted to test the hypotheses that *the osmotic potential of grapevine roots is increased by the accumulation of solutes such as sugars, amino acids and inorganic ions in response to partial rootzone drying*

and that

PRD increases rooting activity and sink strength by increasing both polyamine production and sucrolytic enzyme activity.

7.2 Materials and methods

7.2.1 Experimental material and design

The same 'double-pot' two-year old split-rooted Cabernet Sauvignon grapevines that were used for nitrate reductase experiments in Chapter 6 were used for the investigation into the accumulation of osmolytes under PRD treatment. Cabernet Sauvignon grapevines were used that were grown in two 3 L pots filled with a standard potting mix (Section 2.2). The bottom of the pots were cut out and replaced with plastic mesh to allow roots to grow through into two secondary pots filled with vermiculite. This allowed easy harvest of clean and actively growing roots. All measurements of soil moisture and stomatal conductance done during the NR activity experiment applied to this experiment since root sampling and measurements were done during the same period. The experimental design and layout is exactly the same as described in Chapter 6. Irrigation water was applied either to one side of the root system at any time (PRD) or to both sides (Control) as described in Section 2.1. PRD therefore received half the amount of irrigation water at any given time compared to control. Experimental design consisted of ten repetitions of single vines randomly selected for each of the two treatments (PRD or control).

In 2003 the 'double pot' split-rooted Cabernet Sauvignon grapevines were moved into a temperature-controlled greenhouse at the CSIRO Horticultural unit (Waite Campus, Adelaide) and used again for PRD experiments to investigate sucrolytic enzyme activity in roots. The

vines received a top-dressing of 1 g Osmocote™ and were allowed to have 5 shoots and to bear 3 bunches. In all PRD experiments irrigation water was applied by an automatic drip system (2 L/h) either to one side of the root system at any time (PRD), to both sides (Control) or received no water (Non-irrigated) as described in Section 2.1. Soil suction was measured in potted experiments using portable tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA) as described in Section 2.3. Experimental design consisted of five repetitions of single vines randomly selected for each of the two treatments (PRD or control) and two single vines that were not irrigated. This 'no-irrigation' treatment served only as a reference to the progression of water stress compared to control, and these data were not included in the statistical analysis of results.

7.2.2 Measurements of leaf physiology

In 2002 the stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day. In 2003, the stomatal conductance were measured using a LICOR open photosynthesis system (Li 6400, Lincoln, Nebraska, USA) with an infrared gas analysis instrument (IRGA) as described in Section 2.5. Leaf water potential was measured on fully matured leaves as described in Section 4.2.9.

7.2.3 Root tissue sampling and analyses

Roots were sampled from pots as described in Section 2.7.1. To determine the level of hydration of the root samples a sub-sample of the powdered roots was weighed and placed into an oven for 36 h at 60°C. After the samples were totally dry and cooled down they were re-weighed and the percentage water content calculated. This is however not a measure of hydration of PRD roots but a measure of the water content in root samples for osmolyte analyses, since the roots were washed and re-hydrated at harvest.

7.2.4 Root osmolality

Root sap osmolality was measured by a freezing point depression method using an osmometer (Fiske® ONE TEN™ osmometer, Fiske Associates, Norwood, MA). Root sap was extracted from root material that was powdered in liquid nitrogen. Frozen root powder was allowed to

thaw and transferred to 1 mL modified eppendorf tubes. The eppendorf tubes were perforated in the bottom by a needle and a small amount of non-absorbent cotton wool was compacted on the bottom to stop any material escaping. The 1 mL eppendorf was then placed inside a 2 mL eppendorf and centrifuged at 12000 rpm for 10 min and the root sap in the lower tube was used for analysis. The osmometer was calibrated before operation to manufacturer's specifications. 15 µL of root sap was loaded into the osmometer and the freezing point measured by super cooling of the sample. The freezing point of the sample was then compared to the standard curve for solutions of known osmolality and the results reported in mOsm/kg H_2O.

7.2.5 Soluble sugars and osmolyte analysis

Sugar analysis was conducted as described by Naidu (1998). On average, 1 g of root tissue was powdered in liquid nitrogen and analyzed as described in Section 2.8.

7.2.6 Amino acid analysis

Amino acid analyses were done by the method of Hernandez-orte *et al.* (1997) described in Section 2.9.

7.2.7 Free polyamine analysis

Free polyamine analysis was done as described by Flores and Galston (1982). Crude extracts was prepared from root tissue in cold 5% $HClO_4$ at a ratio of about 100 mg fresh weight/mL and analyzed as described in Section 2.10.

7.2.8 Inorganic mineral analysis

Inorganic mineral analyses were done by optical emission spectrometry (CSIRO Div. Soils) as described in Section 2.11.

7.2.9 Sucrolytic enzyme activity

Sucrose synthase (SucSy) and invertase enzyme activity was assayed on 1 g of root tissue using the methods described in Section 2.13.

7.4 Results

7.4.1 Root osmolality

To investigate the effect of PRD on grapevine roots, split-rooted Cabernet Sauvignon vines were grown in 'double-pot' systems and PRD-treated by withholding water from one pot at any given time or control by watering both pots at the same time. The measured changes in soil matric potential and stomatal conductance during this PRD experiment have been shown in Section 6.3.6. In short, there were no significant differences between the soil matric potential of control and PRD 'wet' pots during the PRD cycle. Due to a rain event 5 days after the drying cycle started, the wetting pattern was switched over sooner than expected. However, as expected, withholding water from the PRD 'dry' side rapidly decreased soil matric potential on a daily basis indicating a steady drying rate. The effect of PRD on stomatal conductance during this experiment can be seen in the previous chapter (Figure 6.12). PRD significantly reduced stomatal conductance by 29% on average compared to control.

The root sap osmolality in the split-rooted Cabernet Sauvignon vines after eight days of soil drying is shown in Figure 7.1. Roots in the PRD 'dry' and 'wet'-side showed a significant ($P<0.01$) increase in root sap osmolality by 80% and 15% on average respectively compared to control. The root sap osmolality of the PRD 'dry'-side was also significantly ($P<0.01$) higher by 57% on average compared to the 'wet'-side.

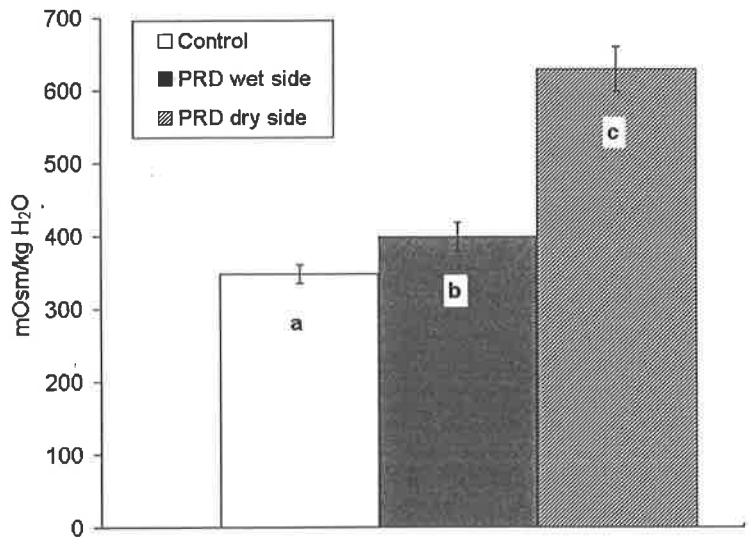


Figure 7.1 Root sap osmolality of split-rooted 'double-pot' Cabernet Sauvignon grapevines (8/04/2002). Eight days after the switch of wetting pots in PRD. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e).

7.4.2 Sugars and osmolytes

The effect of PRD after 8 days of soil drying on the accumulation of sugars and osmolytes in the roots in split-rooted Cabernet Sauvignon is shown in Table 7.1. PRD-treated roots showed significant increases in levels of sucrose and fructose in both the 'dry' and the 'wet'-side compared to control vines. Furthermore, nitrogen-containing compounds such as hydroxy methyl proline, glycine betaine and proline were also significantly increased in the 'dry'-side roots of PRD vines compared to control by 30%, 39% and 139% respectively. Compounds that are formed under highly stressful conditions, e.g. drought and salinity, like mannitol and methyl-proline (Naidu *et al.*, 2000) did not show a significant increase in response to PRD-treatment. In total, PRD-treated grapevine roots showed a significant increase in sugars and osmolytes in the 'dry'-side compared to control roots.

The PRD 'dry' side roots of Cabernet Sauvignon vines showed significantly ($P<0.05$) higher concentrations of sucrose, fructose, hydroxy-N-methyl proline, glycine betaine and proline compared to its own 'wet' side. PRD 'dry' side roots therefore showed a significantly higher total amount of sugars and osmolytes by 32% compared to the 'wet' side roots.

The average concentrations of sucrose, fructose, hydroxy-N-methyl proline, and proline in PRD 'wet' and 'dry' side roots was significantly ($P<0.05$) higher than control roots. The concentrations of sucrose and proline in PRD roots showed a 2-fold increase while fructose and hydroxy-N-methyl proline increased by 3-fold and 30% compared to control roots. Compounds like glucose, mannitol, glycine betaine, methyl proline and stachydrine however showed no significant differences between PRD and control roots. In total, PRD roots had on average a 19% significant ($P<0.05$) increased amount of sugars and osmolytes compared to control roots.

Table 7.1 Root sugars and osmolytes (μMol/g fresh wt) for split-rooted pot-grown Cabernet Sauvignon (8/04/2002). PRD received water in only one pot while water was withheld from the other at any given time. Control received water in both pots. 'PRD average' is the mean between PRD 'dry' side and PRD 'wet' side roots. (means n = 10 ± s.e.; means indicated with different letters are significantly different (P<0.05)).

	Control	PRD wet side	PRD dry side	PRD average	P
Sucrose	$3.41^{c} \pm 0.45$	$6.55^{b} \pm 0.75$	$9.62^{a} \pm 1.05$	$8.08^{ab} \pm 0.69$	0.0001
Glucose	$4.51^{a} \pm 0.74$	$5.69^{a} \pm 0.92$	$6.04^{a} \pm 0.90$	$5.91^{a} \pm 0.55$	0.4575
Fructose	$1.04^{c} \pm 0.34$	$2.34^{b} \pm 0.37$	$3.75^{a} \pm 0.58$	$3.05^{ab} \pm 0.35$	0.0143
Mannitol	$32.67^{a} \pm 14.15$	$26.09^{a} \pm 5.08$	$27.63^{a} \pm 5.34$	$26.86^{a} \pm 2.59$	0.4924
Hydroxy-N-methyl-Proline	$6.86^{c} \pm 0.26$	$8.33^{b} \pm 0.34$	$9.53^{a} \pm 0.50$	$9.02^{ab} \pm 0.18$	0.0001
Glycine betaine	$33.10^{ab} \pm 4.56$	$32.21^{b} \pm 2.95$	$42.67^{a} \pm 2.16$	$37.44^{ab} \pm 2.13$	0.0681
Methyl Proline	$4.49^{ab} \pm 0.81$	$4.16^{b} \pm 0.79$	$7.01^{a} \pm 1.23$	$5.43^{ab} \pm 0.66$	0.1502
Stachydrine	$9.12^{ab} \pm 1.45$	$6.56^{b} \pm 0.91$	$10.53^{a} \pm 1.65$	$8.30^{ab} \pm 0.83$	0.2160
Proline	$0.33^{c} \pm 0.05$	$0.61^{b} \pm 0.03$	$0.78^{a} \pm 0.07$	$0.72^{ab} \pm 0.03$	0.0001
Total	$85.9^{c} \pm 7.9$	$102.2^{bc} \pm 9.33$	$135.3^{a} \pm 10.5$	$102.6^{ab} \pm 4.65$	0.0053

The changes in concentration of sugars and osmolytes in PRD 'wet' and 'dry' side roots and control over the PRD cycle is shown in Figure 7.2. Sugar contents (sucrose and fructose) in PRD-treated roots showed a 2 to 4-fold increase in response to soil drying compared to the well-watered roots of control vines. When the PRD 'dry'-side was rewetted, the sugar levels in roots decreased substantially to similar or slightly higher levels found in control roots. However, after days of extended soil drying even the 'wet'-side roots of PRD vines had significant increased (up to 2-fold) sucrose and fructose contents compared to control vines. Glucose levels seemed to be less responsive to soil drying and rewetting with no significant increases in PRD roots compared to control vines.

The nitrogen-containing osmolytes (e.g. hydroxy-methyl-proline, glycine betaine and proline) also showed periods of significant higher concentrations in PRD 'dry' roots compared to control (Figure 7.3). Hydroxy-methyl-proline (*cis* and *trans* isoforms) showed transient increases of 2 to 3 fold in both the 'dry' and the 'wet' side of PRD-treated roots compared to control. Levels of glycine betaine increased with soil drying in PRD roots compared to control and returned to values similar to control when irrigated again. On average, the glycine betaine content of PRD roots in the 'dry' side was increased by 40% compared to control. The concentration of DL-proline was generally higher in both 'wet' and 'dry' side roots of PRD

compared to control and increased with extended soil drying to significant levels between 150% and 200% compared to control. The total osmolyte concentration (Figure 7.4) in PRD roots increased in both the 'dry' and the 'wet' side compared to control and more so with extended soil drying. The total osmolyte content of PRD roots over the experimental period in the 'dry' and 'wet' side increased on average by 60% and 20% respectively compared to control roots.

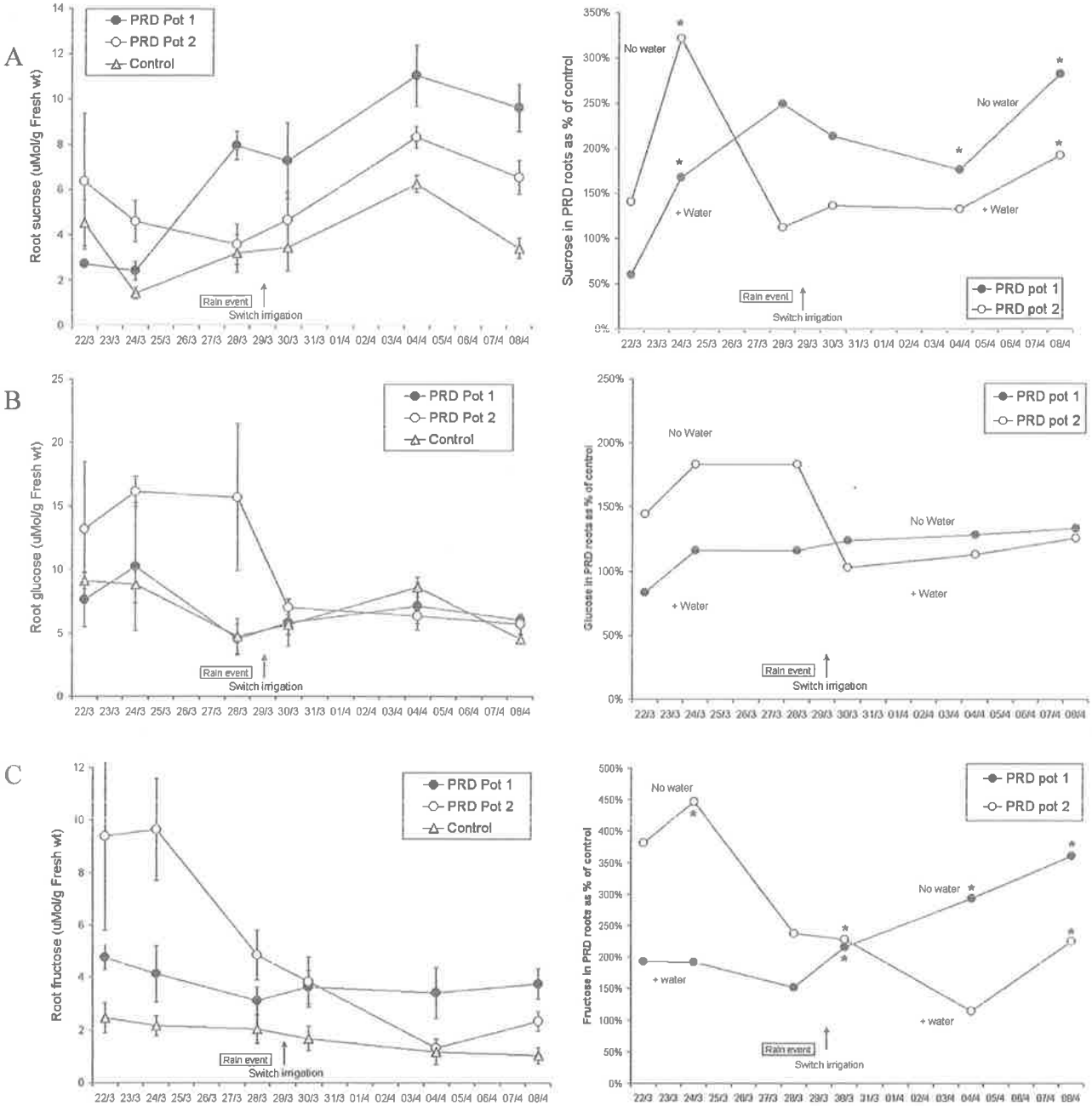


Figure 7.2 Sugar contents of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines in 2002. **(A)** Sucrose, **(B)** glucose and **(C)** fructose. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e.; * = significantly different (P<0.05)).

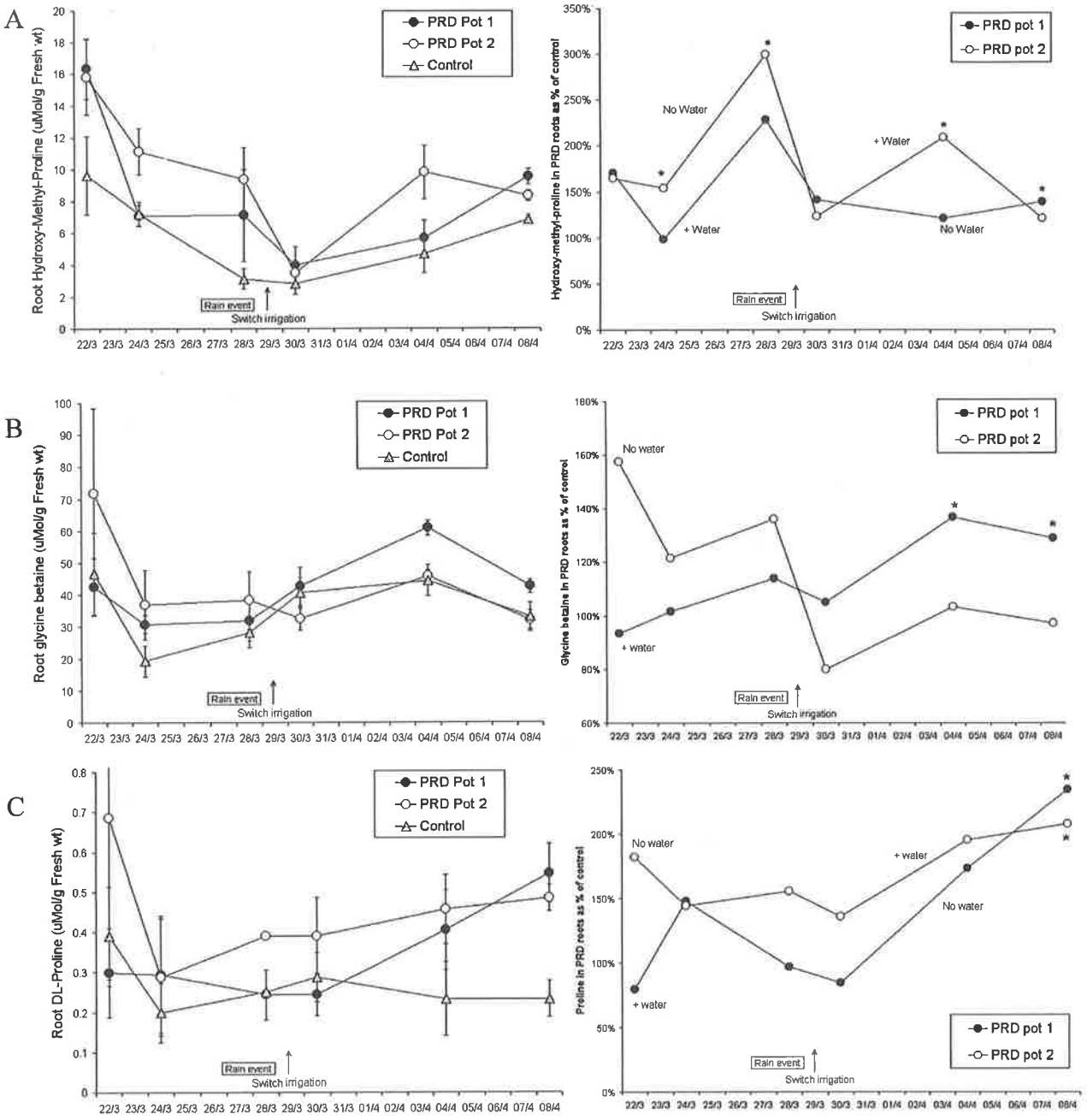


Figure 7.3 Osmolyte contents of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines in 2002. **(A)** Hydroxy-methyl-proline, **(B)** glycine betaine and **(C)** DL-Proline. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e.; * = significantly different ($P<0.05$)).

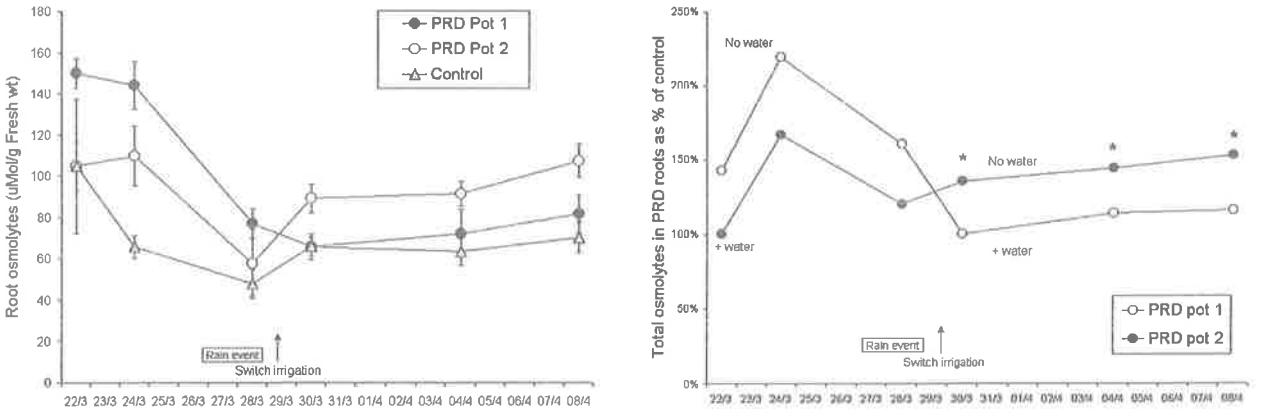


Figure 7.4 Total osmolyte contents of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines in 2002. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e.; * = significantly different (P<0.05)).

7.4.3 Amino acids

The amino acid contents after 8 days of PRD treatment in roots of the 'wet' and 'dry'-side compared to control levels are shown in Table 7.2. PRD treatment significantly increased many of the major and minor amino acids in the roots of both the 'dry' and 'wet' side compared to control vine roots. Major amino acids that were increased in the 'dry' side of PRD plants included aspartic acid, glutamic acid, glutamine, asparagine, threonine and proline with average increases between 60% and 100% compared to control. Increases in the minor amino acids included valine, methionine, isoleucine, norleucine and phenylalanine with average increases between 90% and 190% compared to control. In total, the amino acid content of PRD 'dry' side roots increased significantly by 38% compared to control.

Major amino acids that were increased in the 'wet' side of PRD plants included aspartic acid, glutamic acid and especially proline with average increases of 83%, 46% and 131% respectively compared to control. Increases in the minor amino acids included valine, methionine, isoleucine, norleucine and phenylalanine with average increases between 80% and 160% compared to control. In total, the amino acid content of PRD 'wet' side roots increased by 26% compared to control.

Arginine and proline constituted the largest part of the total free amino acid in grapevine roots (>50%) and changes in either amino acid would have a large impact on the contribution of amino acids to osmotic adjustment. The PRD 'dry' side roots of Cabernet Sauvignon vines

showed a significantly ($P<0.05$) higher concentration of asparagine compared to its own 'wet' side, but in total there was no significant difference between PRD 'wet' and 'dry' side roots.

On average, the concentrations of aspartic acid, glutamic acid, threonine, proline, valine, norleucine, and phenylalanine in PRD 'wet' and 'dry' side roots were significantly ($P<0.05$) higher than control roots. The amino acids that significantly increased in PRD roots showed on average a 1.6 to 2-fold increase compared to control roots. In total, PRD roots had on average a 35% significant ($P<0.05$) increased amount of amino acids compared to control roots.

Table 7.2 Amino acid contents (µmol/g fresh wt) of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines (8/04/2002). 'PRD average' is the mean between PRD 'dry' side and PRD 'wet' side roots. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e.).

	Control	PRD 'dry' side	PRD 'wet' side	PRD average	P
Trigonelline	$58.5^{a} \pm 10.1$	$49.4^{a} \pm 5.8$	$62.5^{a} \pm 9.7$	$55.9^{a} \pm 5.2$	0.874
Aspartic acid	$3.07^{b} \pm 0.9$	$5.13^{ab} \pm 0.5$	$5.63^{a} \pm 0.7$	$5.36^{a} \pm 0.5$	0.043
Glutamic acid	$11.1^{b} \pm 1.5$	$17.8^{a} \pm 1.2$	$16.3^{a} \pm 1.2$	$18.1^{a} \pm 0.7$	0.001
Serine	$8.20^{a} \pm 1.2$	$9.88^{a} \pm 1.3$	$9.65^{a} \pm 1.5$	$9.90^{a} \pm 0.8$	0.757
Asparagine	$28.0^{b} \pm 12.2$	$59.0^{a} \pm 3.1$	$35.7^{b} \pm 8.7$	$49.1^{ab} \pm 5.8$	0.040
Glycine	$44.8^{a} \pm 7.3$	$27.6^{b} \pm 2.2$	$32.1^{ab} \pm 6.6$	$30.6^{ab} \pm 3.5$	0.153
Glutamine	$49.6^{a} \pm 4.4$	$62.1^{a} \pm 5.9$	$51.5^{a} \pm 7.1$	$56.8^{a} \pm 6.0$	0.512
Threonine	$5.9^{b} \pm 0.8$	$12.2^{a} \pm 1.5$	$10.0^{ab} \pm 2.0$	$11.3^{a} \pm 1.5$	0.046
Histidine	$16.5^{a} \pm 2.3$	$21.3^{a} \pm 2.4$	$12.0^{a} \pm 3.2$	$20.6^{a} \pm 2.6$	0.594
Alanine	$11.6^{a} \pm 1.4$	$11.8^{a} \pm 1.8$	$17.0^{a} \pm 2.9$	$14.8^{a} \pm 1.0$	0.199
Arginine	$134.6^{a} \pm 16.8$	$176.1^{a} \pm 32.5$	$134.3^{a} \pm 26.6$	$154.0^{a} \pm 22.9$	0.260
Proline	$145.7^{b} \pm 18.6$	$252.1^{a} \pm 27.7$	$337.2^{a} \pm 41.8$	$288.4^{a} \pm 26.6$	0.001
Tyrosine	$13.9^{a} \pm 2.6$	$16.4^{a} \pm 1.9$	$17.2^{a} \pm 1.7$	$17.4^{a} \pm 1.2$	0.617
Valine	$2.55^{b} \pm 0.3$	$6.44^{a} \pm 1.3$	$4.58^{ab} \pm 0.9$	$5.51^{a} \pm 0.9$	0.036
Methionine	$1.08^{b} \pm 0.1$	$2.06^{a} \pm 0.4$	$1.81^{ab} \pm 0.3$	$1.93^{ab} \pm 0.2$	0.110
Cystine	$10.2^{a} \pm 1.9$	$16.9^{a} \pm 2.7$	$14.1^{a} \pm 3.4$	$16.0^{a} \pm 1.6$	0.255
Isoleucine	$0.88^{b} \pm 0.1$	$2.52^{a} \pm 0.5$	$2.25^{a} \pm 0.5$	$2.39^{a} \pm 0.3$	0.026
Leucine	$3.50^{a} \pm 1.2$	$1.86^{a} \pm 0.4$	$2.58^{a} \pm 0.8$	$2.25^{a} \pm 0.4$	0.504
Norleucine	$6.68^{b} \pm 1.4$	$15.30^{a} \pm 1.2$	$14.62^{a} \pm 2.9$	$14.58^{a} \pm 1.5$	0.015
Phenylalanine	$0.46^{b} \pm 0.08$	$0.83^{ab} \pm 0.14$	$1.07^{a} \pm 0.17$	$0.98^{a} \pm 0.13$	0.018
Tryptophan	$3.21^{a} \pm 0.5$	$3.59^{a} \pm 0.4$	$4.07^{a} \pm 0.8$	$3.81^{a} \pm 0.6$	0.780
Lysine	$2.76^{a} \pm 0.5$	$4.54^{a} \pm 0.6$	$3.57^{a} \pm 0.9$	$4.17^{a} \pm 0.6$	0.266
Total	$554.2^{b} \pm 40.5$	$767.3^{a} \pm 56.1$	$699.5^{ab} \pm 54.3$	$746.8^{a} \pm 49.3$	0.044

7.4.4 Free polyamines

The free polyamine content of split-rooted 'double pot' Cabernet Sauvignon after 8 days of soil drying in the PRD experiment is shown in Figure 7.5. PRD had no significant effect on putrescine levels in the roots of both the 'wet' and 'dry' side compared to roots of control vines. However, PRD showed significantly higher spermidine concentrations in both the 'wet' and 'dry' side roots by 46% and 55% respectively compared to control. Furthermore, the spermine levels in the 'dry' side of PRD roots increased by 55% and 61% compared to control and its own 'wet' side respectively.

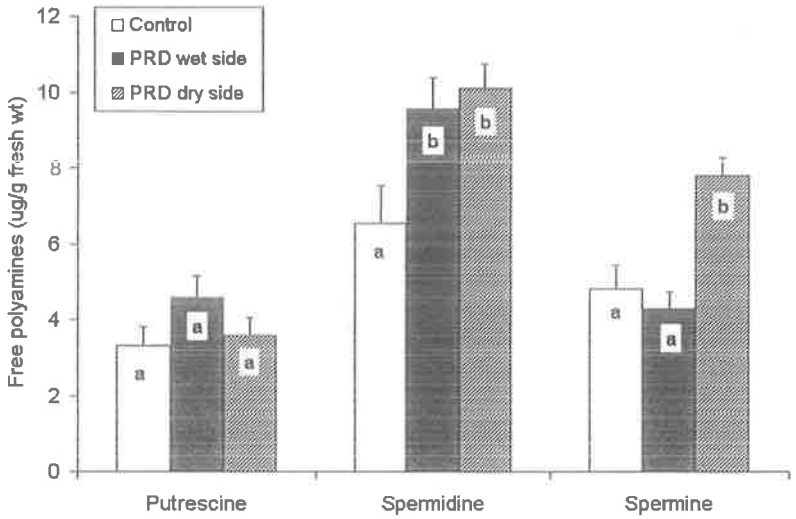


Figure 7.5 Free polyamine contents of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines (8/04/2002). (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 + s.e.; bars with different letters are significantly different ($P<0.05$)).

To investigate the effect of PRD on root sucrolytic enzyme activity and inorganic ion accumulation the split-rooted 'double-pot' Cabernet Sauvignon grapevines were grown in the temperature-controlled greenhouse at the CSIRO Horticultural unit during the winter of 2003. PRD vines received irrigation on only one side at any time, control vines received water on both sides and 'No water' vines received no irrigation on any side. The soil matric potential measurements during this experiment and the effect of PRD and no irrigation on stomatal conductance are shown in Figure 7.6. Soil matric potential in both the PRD 'dry' side and in non-irrigated pots rapidly decreased compared to control over the first 4 days. After 4 days the non-irrigated vines showed severe signs of water stress e.g. leaf wilting, and had to be re-watered. By comparison, the low soil matric potential in PRD 'dry' side could be maintained without affecting the vine water status (measured as leaf water potential) after 12 days (Table

7.3). This was made possible by frequently irrigating the PRD 'wet' side to maintain a similar soil matric potential as the control.

Table 7.3 Leaf water potential (MPa) after 12 days of PRD treatment in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions (2003). (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 6 ± s.e.; n.s.= not significant (P<0.05).

	Control	PRD	% Diff	P
Leaf water pot. (MPa)	-0.966 ± 0.08	-0.986 ± 0.06	- 2	0.834

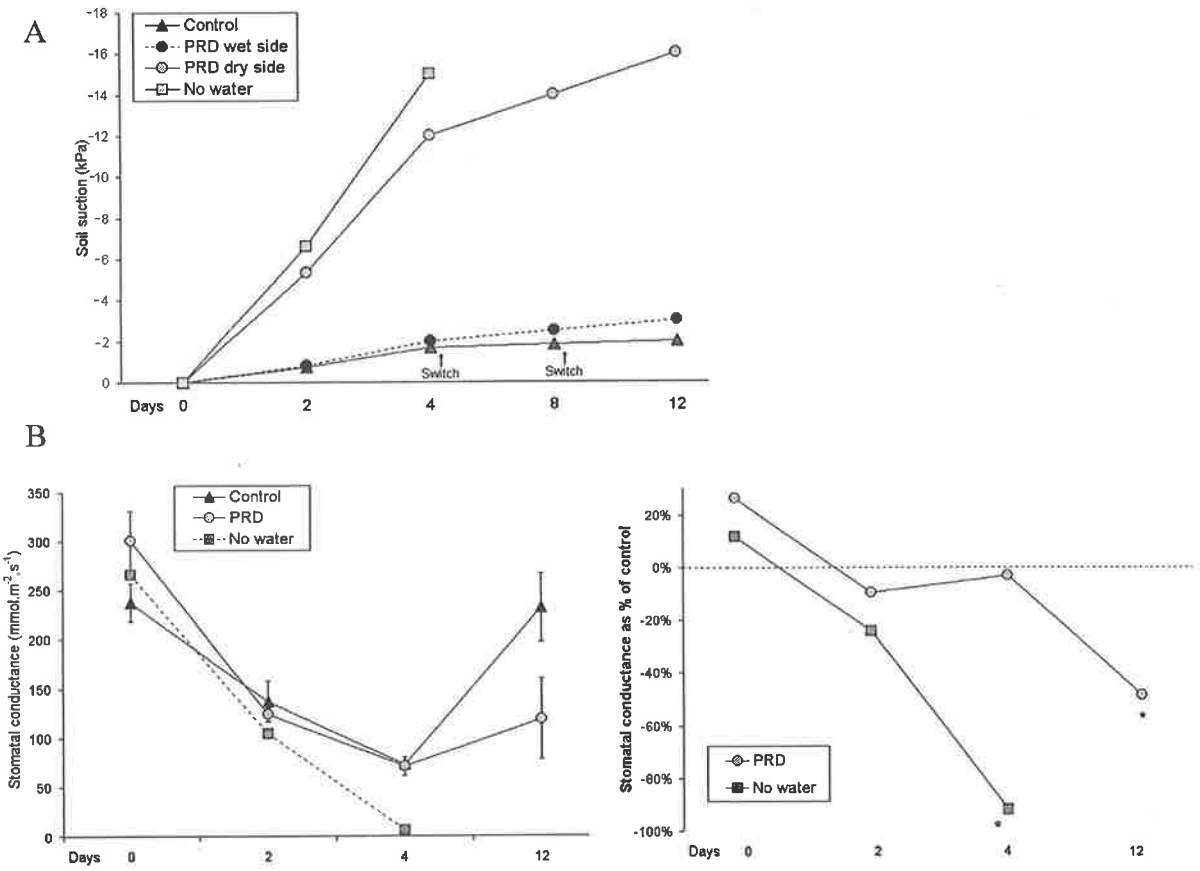


Figure 7.6 (A) Soil matric potential and **(B)** Stomatal conductance of split-rooted 'double-pot' Cabernet Sauvignon grapevines in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.; Non-irrigated vines received no water for the duration of the experiment; means n = 2; * = significantly different (P<0.05)) PRD irrigation was switched on average every 4 days.

The stomatal conductance of non-irrigated vines progressively reduced compared to control vines by 24% after 2 days and 92% after 4 days. For PRD-treated vines the stomatal conductance was not significantly influenced until after 4 days of soil drying. However, after 12 days and two switches in irrigation, PRD vines showed a significant (P<0.05) reduction in stomatal conductance by 49% compared to control vines.

The water content of powdered root samples from the split-rooted Cabernet Sauvignon grapevines are shown in Table 7.4. It is important to note that the water contents (%) of the root sample material used in analyses did not differ significantly for either the PRD 'wet' and 'dry' side compared to control after 2 days. The water contents of PRD 'dry' side root samples did however differ significantly from control after 12 days of soil drying, but this amounted to only a 5% decrease compared to control samples. The water content in PRD 'wet' side roots never differed significantly from control samples.

Table 7.4 Water content (%) of powdered root samples for analyses of split-rooted Cabernet Sauvignon under glasshouse conditions during PRD treatment in 2003. (means n = 5 ± s.e.; means indicated with different letters are significantly different (P<0.05)).

	Control	PRD 'wet' side	PRD 'dry' side	Sig.
Water content (after 2d)	$80.2^{a} \pm 1.3$	$80.9^{a} \pm 1.0$	$79.7^{a} \pm 1.7$	n.s.
Water content (after 12d)	$86.6^{a} \pm 0.7$	$85.3^{a} \pm 1.3$	$82.2^{b} \pm 1.0$	<0.05

The root osmolyte contents after 2 days of PRD is shown in Table 7.5. The root sugars and osmolytes measured in PRD-treated roots showed very similar concentrations as in the previous root experiment. Roots on the PRD 'dry' side showed a 2-fold increase in sucrose, glycine betaine and methyl proline, while proline increased on average by 65% compared to control. The roots in the PRD 'wet' side did not show a significant change in sugar and osmolyte concentration compared to control roots. This was, however, after only 2 days of PRD treatment and significant changes in the 'wet' side compared to control were only seen after longer periods (as seen in previous experiment). PRD 'dry' side roots showed roughly a 2-fold significant increase in the concentration of glycine betaine and proline compared to its own 'wet' side. Although PRD 'dry' side roots had a 26% increase in total sugar and osmolyte concentration compared to control, it was not statistically significant.

Table 7.5 Root sugars and osmolytes (μMol/g fresh wt) in split-rooted Cabernet Sauvignon under glasshouse conditions after 2 days of PRD treatment in 2003. PRD received water in only one pot while water was withheld from the other at any given time. Control received water in both pots. (means n = 5 ± s.e.; means indicated with different letters are significantly different (P<0.05)).

	Control	PRD 'wet' side	PRD 'dry' side	P
Sucrose	$4.31^{a} \pm 1.13$	$5.46^{a} \pm 0.82$	$8.88^{a} \pm 1.91$	0.806
Glucose	$13.88^{a} \pm 2.06$	$7.73^{b} \pm 2.37$	$8.21^{ab} \pm 0.76$	0.069
Fructose	$5.84^{a} \pm 1.41$	$4.28^{a} \pm 1.09$	$6.22^{a} \pm 1.31$	0.266
Mannitol	$4.80^{a} \pm 2.49$	$5.32^{a} \pm 1.12$	$8.73^{a} \pm 2.59$	0.647
Hydroxy-N-Methyl Proline	$3.98^{a} \pm 1.69$	$3.92^{a} \pm 0.83$	$3.39^{a} \pm 1.62$	0.848
Glycine betaine	$5.60^{b} \pm 1.33$	$4.25^{b} \pm 1.46$	$11.29^{a} \pm 1.97$	0.021
Methyl Proline	$1.68^{b} \pm 0.60$	$2.41^{ab} \pm 0.55$	$3.81^{a} \pm 0.74$	0.094
Proline	$1.03^{a} \pm 0.29$	$0.87^{a} \pm 0.47$	$1.70^{a} \pm 0.23$	0.240
Total	$42.8^{a} \pm 4.9$	$38.9^{a} \pm 1.1$	$54.1^{a} \pm 7.9$	0.185

7.4.5 Sucrolytic enzyme activity

The root sucrolytic enzyme activity over the 12-day period in split-rooted 'double-pot' Cabernet Sauvignon vines was investigated to understand changes in sink strength and root growth. The effect of PRD and no irrigation on root SucSy activity is shown in Figure 7.7. The SucSy activity in grapevine roots was generally low compared to the invertase activity. The roots of non-irrigated vines showed no significant differences in SucSy activity compared to control over the 4 days of soil drying. The roots of PRD-treated vines however showed 8-fold and 3-fold increases in the SucSy activity in the 'wet' and 'dry' side respectively after 2 days compared to control.

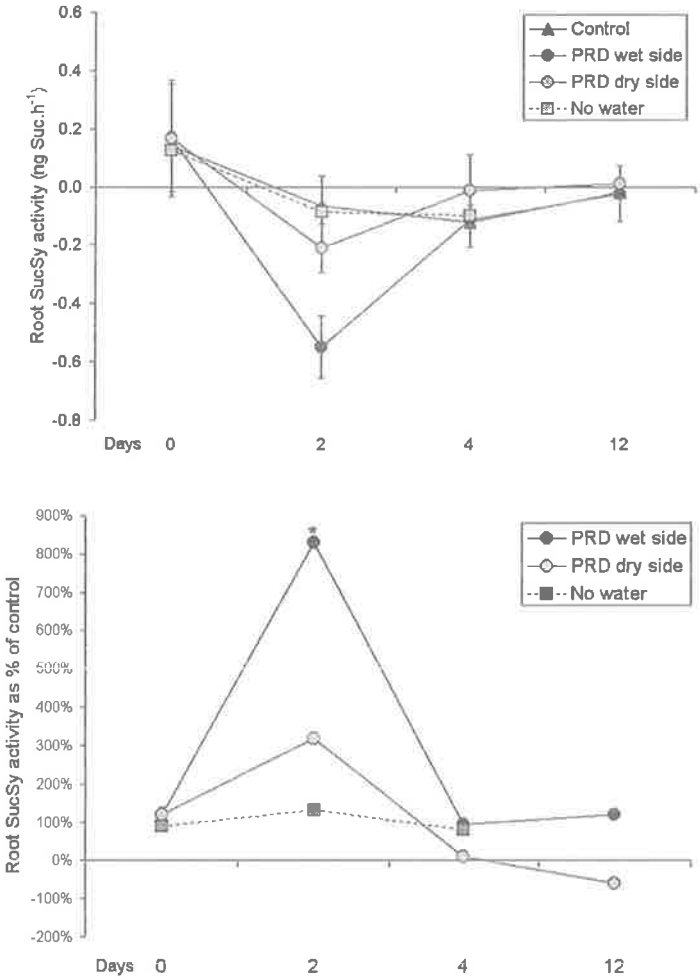


Figure 7.7 Root SucSy activity of non-irrigated, control, PRD 'wet' and 'dry'-side roots in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.; * = significantly different ($P<0.05$)). Non-irrigated vines received no water for the duration of the experiment; means n = 2)

By comparison, the roots of the PRD 'wet' side significantly increased SucSy activity 8-fold after 2 days, but returned to similar levels compared to control after 4 days. After 12 days the SucSy activity in PRD 'wet' side roots showed a 20% increase in SucSy activity compared to control roots which may indicate an increase in sink strength or growth rate.

The effect of PRD and no irrigation on root acid invertases (AI) is shown in Figure 7.8. Although not significant, withholding water from roots slightly decreased the AI activity in non-irrigated roots after 2 days and in PRD 'dry' roots after 4 days by 15% and 10% respectively compared to control. However, after an additional two days of soil drying the AI activity in non-irrigated roots increased by 8% compared to control roots. Similarly, after 12 days of PRD treatment a significant increase was also found in root AI activity in the PRD 'dry' side by 76% compared to control roots. Root AI activity in the PRD 'wet' side showed an

initial increase of 13% compared to control after 2 days, but after 4 days onwards it was decreased by an average of 23% compared to control.

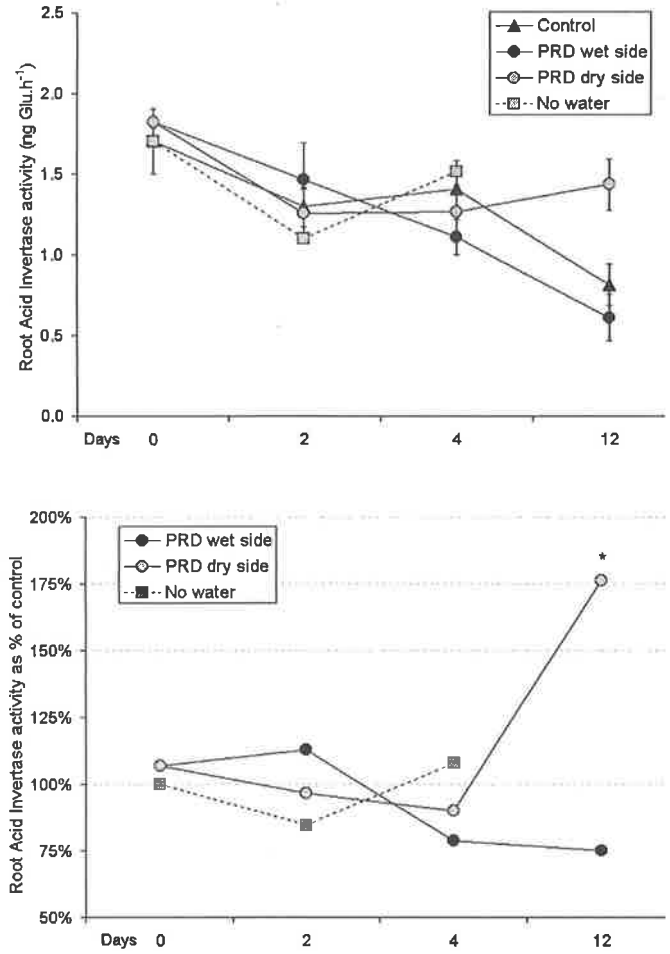


Figure 7.8 Root AI activity of non-irrigated, control, PRD 'wet' and 'dry'-side roots in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.). Non-irrigated vines received no water for the duration of the experiment; means n = 2; * = significantly different ($P<0.05$))

The effect of PRD and no irrigation on root neutral invertase (NI) is shown in Figure 7.9. Withholding all water from roots only slightly decreased the NI activity by 5% in non-irrigated vines compared to control. Conversely, the PRD 'wet' side roots showed a slight increase in NI activity by 4% compared to control for the duration of the experiment. The PRD 'dry' side roots showed the only large and significant change in NI activity compared to control. After 4 days the PRD 'dry' side roots had significantly reduced NI activity by 11% compared to control, however after 12 days of PRD treatment a significant increase by 34% was found.

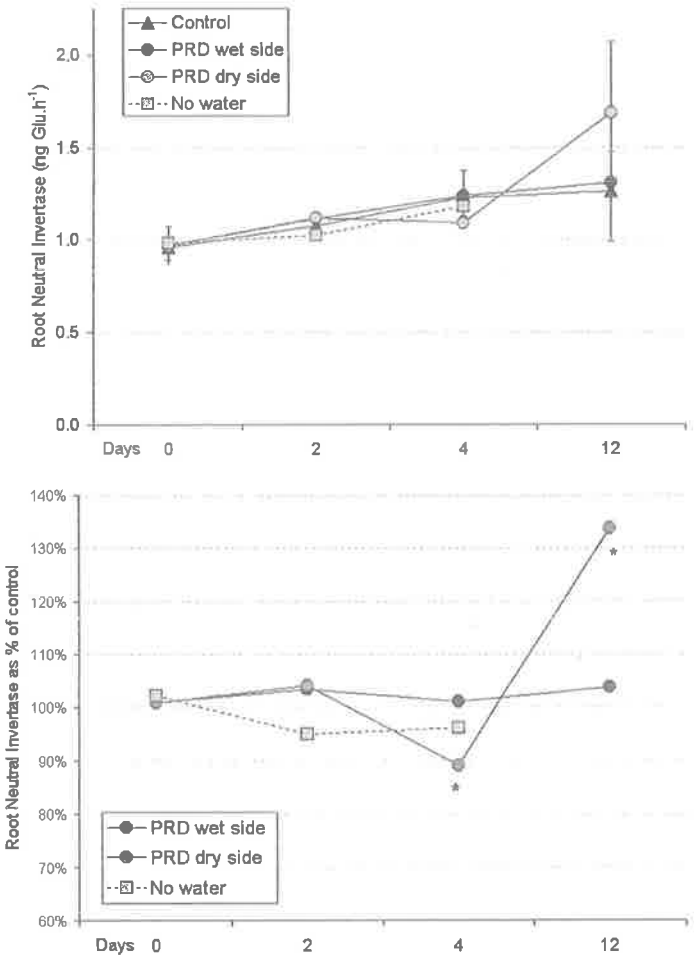
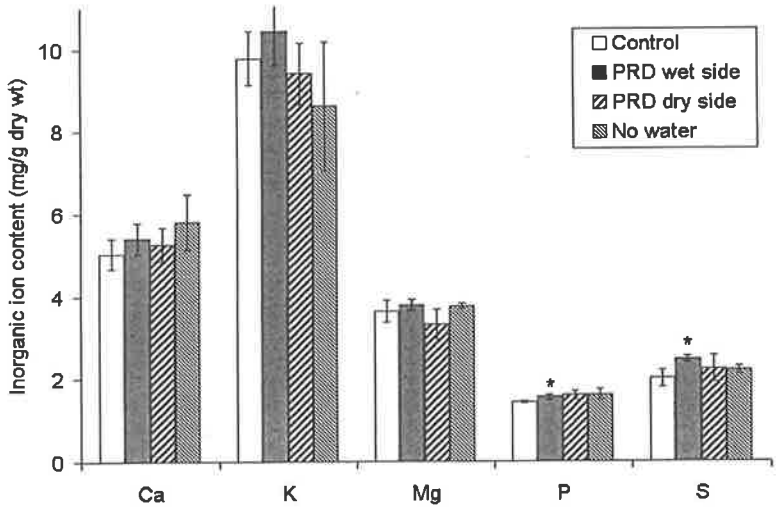


Figure 7.9 Root NI activity of non-irrigated, control, PRD 'wet' and 'dry'-side roots in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.; * = significantly different ($P<0.05$)). Non-irrigated vines received no water for the duration of the experiment; means n = 2)

7.4.6 Inorganic ions

The inorganic ion contents of control, PRD and non-irrigated roots after 2 and 12 days of treatment are shown in Figure 7.10. There were no significant differences in ion contents of Ca, K, Mg, P or S between 'dry' side roots of PRD-treated vines and control vines after 2 days. PRD roots in the 'wet' side however showed a significantly higher concentration of P and S by 8% and 23% respectively compared to control roots. The ion contents of non-irrigated roots did not differ significantly from control roots. After 12 days no significant differences could be found in the ion contents of Ca, K, Mg, P or S between the roots of either PRD 'wet' and 'dry' side compared to control vines.

A



B

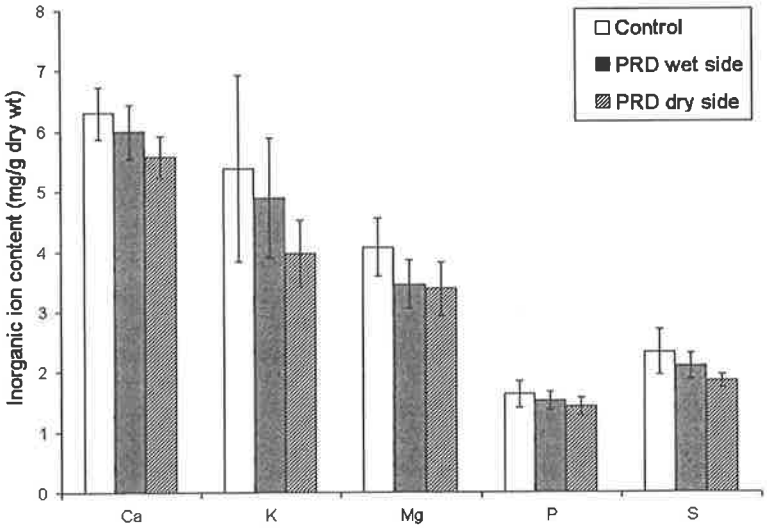


Figure 7.10 Inorganic ion contents (mg/g dry wt) of control, PRD 'wet' and 'dry'-side and non-irrigated roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines after A) 2 days and B) 12 days of PRD treatment and soil drying. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e; * = significantly different from control ($P<0.05$); Non-irrigated vines received no water for the duration of the experiment; means n = 2)

7.5 Discussion

Most of the extractable soil water is held in large pores from which water is readily removed by roots. As the soil dries, this water is used first, leaving behind the small amount of water more tightly held in small pores. Osmotic adjustment enables the plant to extract more of this tightly held water, but the availability of water will diminish as the soil dries until the cost of osmotic adjustment in the dry roots becomes too great or there is no more extractable water left. Results of experiments in this Chapter have provided evidence of osmotic adjustment in the roots of grapevines in response to PRD. Osmolality was increased in both the PRD 'wet' and 'dry' side roots compared to control vines, by increasing concentration of solutes, mainly sugars,

nitrogen-containing compounds and amino acids. The increases in root osmolality cannot be explained by a 'passive' concentration effect as the osmolality and solute concentration were determined after the roots were harvested and re-hydrated. The minor decrease in water content of PRD roots compared to control roots may have increased the solute concentration to a small degree, but considering the large increase in osmolality and solute concentrations, it is certain that dehydration was not a major factor responsible for the increase in osmolality.

As far as these results show, sucrose and fructose were the sugars that contributed the most to the increase in osmolality in PRD 'wet' and 'dry' side roots with 2-fold increases compared to control roots. As for nitrogen-containing compounds, hydroxy methyl proline, glycine betaine, methyl proline and proline also contributed with significant increases compared to control roots. Glycine betaine was found to be consistently increased in the PRD 'dry' side roots compared to control in both root experiments. The significant increase of glycine betaine in drying roots may be important in the tolerance of water stress and may be induced (Ishitani *et al.*, 1995) by the presence of ABA that is produced in response to PRD (Loveys, 1984; Stoll *et al.*, 2000b). PRD 'dry' side roots also significantly accumulated higher amounts of sugars and osmolytes than its own 'wet' side, indicating a concentration gradient for water towards the 'dry' roots. Stoll *et al.* (2000b) have shown that water moves from the 'wet' side roots towards the 'dry' side in PRD-treated grapevines to maintain the hormonal signal under low soil moisture conditions. It was also found that very little water is absorbed by 'dry' side roots at low water potentials and that the dry roots are mostly reliant on the water derived from the wet side. It is therefore proposed that the increase in root osmolality by the accumulation of solutes in the 'dry' side roots of PRD-treated plants creates a water potential gradient that facilitates the water movement from wet to dry roots.

There was also a clear trend in the accumulation of sugars and nitrogen-containing compounds in response to soil drying and rewetting during PRD that could not be attributed to dehydration of the root tissue. With the switch in irrigation the solute concentration in the re-wetted side quickly returned to near-normal levels while the drying side rapidly started accumulating solutes so that the total amount of osmolytes showed significantly higher levels after only 2 days of soil drying compared to control. If osmotic adjustment and turgor maintenance allows root growth to continue in drying soils (Turner and Jones, 1980), this enables a greater volume of soil to be explored or leads to a greater density of roots in a fixed volume of soil. If the plant

can not maintain transpiration by exploring a greater volume of soil or can not exploit the water in a given soil volume more fully, it is thought that osmotic adjustment would be of little use except for survival and adaptation (Turner and Jones, 1980).

In addition to sugars, amino acids have been found to contribute significantly to osmotic adjustment and osmotic potential (Turner and Jones, 1980). In this study, there were significant increases in the free amino acid concentration in PRD roots compared to control. Significant increases in a range of individual amino acids were found in both the PRD 'dry' and 'wet' side compared to control that included major amino acids such as aspartic acid, glutamic acid, glutamine, asparagine and proline. There were also increases in the minor amino acids, but their contribution to total amino acid content was small by comparison. The two amino acids that were present in the largest quantities in roots were proline and arginine: they contributed more than 50% of total root amino acids. Therefore, significant changes in either arginine or proline would influence root amino acid content more than any other amino acid. It was found that arginine did not increase significantly in response to PRD-treatment but proline increased in both the PRD 'dry' and 'wet' side roots compared to control. Proline was therefore the amino acid that potentially would influence osmotic potential the most in PRD roots.

Polyamines, especially spermine and spermidine, are associated with enhanced rooting, especially adventitious root formation (Jarvis *et al.*, 1985; Shyr and Kao, 1985; Kakkar and Rai, 1987; Faust and Wang, 1992). PRD-treated roots in this study showed significant increases in free polyamine levels compared to control roots. It was found that, in both PRD 'dry' and 'wet' side roots, spermidine increased compared to control while putrescine, which usually accumulates under water stressed conditions, did not. In addition to an increased spermidine concentration, PRD 'dry' side roots showed a significant increase in spermine compared to control and PRD 'wet' side roots. The increased spermine and spermidine may be an indication that PRD roots were conditioned to produce more adventitious roots to increase root density within the confined volume of the pot environment compared to the control roots that were well watered and had no problem accessing available water. Spermine and spermidine are synthesized from putrescine by the addition of one or two aminopropyl groups respectively from S-adenosylmethionine, an intermediate of ethylene biosynthesis (Faust and Wang, 1992). It could therefore be speculated by its association with polyamine production, that ethylene biosynthesis in PRD roots may be involved in the conditioning of roots to increase density in

order absorb more water per volume of soil. This hypothesis would support the findings of Dry *et al.* (2001) who reported a significantly higher abundance of roots of the 1 mm to 3 mm diameter at the 0.4 m to 0.7 m depth in PRD roots of field-grown grapevines compared to control vines. The implication is that a more exploratory root system of PRD-treated vines may contribute to water stress tolerance and a better soil environment for root growth. Stoll *et al.* (2000a) found evidence of a more exploratory root system in field-grown PRD-treated vines with roots that grew to greater depths than control.

Roots are non-photosynthetic organs and therefore root osmolality can be increased by increasing solute accumulation either by absorbing more inorganic ions from the soil environment or attracting more photoassimilates from shoots. Roots are capable of amino acid synthesis but still need sugars to drive the process. Consequently, root cells rely on solutes from the phloem to support osmotic adjustment and root growth. The rate of phloem unloading and therefore the sink strength of plant tissues is very much dependent on sucrolytic enzyme activities, mostly sucrose synthase (SucSy) and the invertases. Results from this study have shown that sugars and osmolytes increased in drying roots 2 days after the start of partial rootzone drying, before a significant decrease in stomatal conductance was measured. This is expected because stomatal closure should only occur after significant amounts of ABA have been produced and translocated to the leaves in response to soil drying. Responses in root SucSy activity indicated that, both the PRD 'wet' and 'dry' side roots increased their sink strength and/or growth rate compared to control roots within 2 days of treatment and PRD 'wet' side roots substantially more so than the 'dry' side. In comparison, no-irrigation had no effect on root SucSy activity. This may be an important finding that supports the reports of increased root growth in response to PRD by a number of authors (Dry *et al.*, 2000; Kang *et al.*, 2002; Mingo *et al.*, 2004). After a longer period, that included a few switches in irrigation, the root SucSy activity in the 'wet' side of PRD-treated vines was still higher than control roots, but the SucSy activity in the 'dry' side of PRD-treated plants reduced to lower levels than that in control roots. Normally SucSy activity would be towards the breakdown of sucrose (negative), but because the cleavage of sucrose by SucSy is readily reversible, it is possible to have formation of sucrose and therefore a positive measurement of SucSy activity. Consequently, it was found that SucSy activity in PRD 'dry' side roots changed from initially hydrolyzing sucrose towards the formation of sucrose. This may indicate that the roots in the PRD 'dry' side initially increased growth rate compared to control and thereafter, with extended soil drying

slowed root growth rate compared to control since SucSy activity is also correlated with the rate of cell wall synthesis.

SucSy activity has been correlated with starch synthesis, cell wall synthesis and overall sink strength (Winter and Huber, 2000). The conversion of an osmotically active sugar into an insoluble polymer, such as starch and cell walls creates a strong sink, making cell wall invertase activity relatively irrelevant in the determining of sink strength in growing tissues (Sturm and Tang, 1999). The soluble invertases are therefore proposed to function in osmoregulation, cell enlargement and channeling sucrose into metabolism. In this study the partial drying of grapevines roots did not have a significant effect on the activities of invertase compared to control roots for the first few days of PRD treatment. By comparison, withholding water completely caused an initial decrease and then a slight increase in acid invertase (AI) that was however insignificant compared to control. The response of AI and neutral invertase (NI) to soil drying in PRD 'dry' side roots was similar to some degree but delayed by 2 days and amplified. After 4 days both AI and NI activities were slightly reduced, but after an extended period of PRD treatment the 'dry' side roots showed significant increases in both AI and NI activity compared to control. The NI activity in PRD 'wet' side roots did not change much compared to control, but AI activity was reduced by 23% compared to control after 4 days. The increase in invertase activity in PRD 'dry' roots may be involved in osmoregulation by regulating sugar accumulation (Winter and Huber, 2000) or an increased sugar flow into respiration as roots are progressively exposed to greater soil drying conditions. Invertase activity, especially NI, has been positively correlated with increased metabolism (Rose and Botha, 2000). Sugars would be necessary in root metabolism to produce ABA, amino acids, etc. to tolerate the stressful conditions in the 'dry' side of PRD vines and to increase root growth in the 'wet' side to compensate.

When inorganic ions are utilized for osmotic adjustment it is usually potassium that accumulates (Turner and Jones, 1980). In this study however, no significant differences in inorganic ions could be found in roots treated with PRD or when irrigation water was totally withheld compared to control. There was a transient increase in phosphorus and sulfur in the PRD 'wet' side roots after 2 days compared to control, but this was not enough evidence to draw any conclusions.

7.6 Conclusion

The experiments in this Chapter were conducted to test the hypothesis that the osmotic potential of grapevine roots is increased by the accumulation of solutes such as sugars, amino acids and inorganic ions in response to partial rootzone drying and that PRD increases rooting activity and sink strength by increasing polyamine levels and sucrolytic enzyme activity. Enough evidence has been collected to accept these hypotheses. The major conclusions were:

1) Osmolality was increased in both the PRD 'wet' and 'dry' side roots compared to control roots. This indicates active osmoregulation in roots.

2) Sucrose and fructose contributed the most to the increase in sugars in PRD 'wet' and 'dry' side roots with 2-fold increases compared to control roots. With regard to nitrogen-containing compounds, hydroxy methyl proline, glycine betaine methyl proline and proline also contributed with significant increases compared to control roots

3) Significant increases in a range of individual amino acids were found in both the PRD 'dry' and 'wet' side roots compared to control. These included major amino acids such as aspartic acid, glutamic acid, glutamine, asparagine, and proline. Proline is the amino acid that would potentially influence osmotic potential the most in PRD roots.

4) Polyamines, i.e. spermidine and spermine, increased in PRD roots compared to control while putrescine - which usually accumulates under water stressed conditions - did not. This may be an indication that PRD roots are conditioned to produce more adventitious roots in order to increase root density within the confined volume of the pot environment.

5) Responses in root SucSy activity indicated that both the PRD 'wet' and 'dry' side roots increased their sink strength and/or growth rate compared to control roots within 2 days of treatment; PRD 'wet' side roots substantially more so than the 'dry' side.

6) The increase in invertase activity in PRD 'dry' roots may be involved in osmoregulation by regulating sugar accumulation or an increased sugar flow to respiration in order to

tolerate the stressful conditions on the 'dry' side of PRD vines and to increase growth in the 'wet' side.

7) Inorganic ions appear to make little or no contribution to osmoregulation under PRD conditions.

Chapter 8: Effects of PRD on the enzymes of carbohydrate metabolism: the effect on accumulation of sugars, amino acids and polyamines.

8.1 Introduction

Sucrose plays a central role in higher plants as a transport sugar, nutrient and potential signal molecule. Sucrose is synthesized in the leaves as one of the primary end products of photosynthesis. The rate of photosynthesis is not only affected by environmental conditions such as light and temperature, but also is strongly influenced by the demand of developing sinks. The removal of sinks during their development markedly decreases the source's photosynthetic output (Gifford and Evans, 1981) and indicates a possible feedback mechanism in operation. Translocation of assimilates from photosynthetically-active cells in the leaves to growing tissues, seeds and storage organs is the basis of plant performance and agricultural yield and despite the presence of other phloem solutes such as amino acids, raffinose sugars, inorganic ions and fructans, sucrose is the osmotically dominant solute in sieve tube sap. The loading, and unloading, of sucrose is the major driving force behind mass flow and its availability is determined by several metabolic pathways, especially the enzymes involved in sucrose turnover (Komor, 2000)

Sucrose turnover in leaves depends on three enzymes: sucrose phosphate synthase (SPS), sucrose synthase (SucSy) and invertase. It is thought that SPS is an important control point in the sucrose and starch formation pathway (Stitt *et al.*, 1987b). SPS is located in the mesophyll cell cytoplasm and, similar to nitrate reductase, responds to light/dark signals. The activity of SPS also increases in parallel with a rise in photosynthesis (Stitt *et al.*, 1988) and may be linked to changes in the availability of inorganic phosphate that can be fixed into triose phosphates (ADP and ATP). This activation can however be modified by factors related to the accumulation of sucrose - resulting in a 'coarse' control of SPS activity (Stitt *et al.*, 1988). A close correlation therefore exists between sucrose synthesis and SPS activity in both photosynthetically active plant tissues and in some non-green sucrose forming tissues, such as ripening banana fruit. The regulation of sucrose synthesis provides a way of altering the partitioning of photosynthates to allow, for example, more starch synthesis (Stitt *et al.*, 1987a) or in the case of water stress, an increase in sucrose for osmoregulation (Quick *et al.*, 1989). This 'coarse' control of SPS activity occurs during the photoperiod and in response to sink manipulation (Kerr *et al.*, 1985; Huber *et al.*, 1986). Zhou and Quebedeaux (2003) aptly demonstrated the increase in SPS activity and photosynthesis in response to an increased sink

demand in apple leaves by partial defoliation. Quick et al. (1989) also reported an increase in SPS activity in spinach leaves in response to lowered leaf water potentials in the range of 0 and –1.2 MPa where there was no marked change in the rate of CO_2-saturated photosynthesis. This increase in SPS activity caused a progressive stimulation of sucrose synthesis and inhibition of starch synthesis.

The degradation of sucrose can be catalyzed by at least two different classes of enzymes (Winter and Huber, 2000). Firstly, invertase catalyses the irreversible hydrolysis of sucrose to glucose and fructose. According to Roitsch (1999) the regulation of extracellular invertase is not only important for supplying carbohydrates to sink tissues but also plays a crucial role to mediate source-sink regulation in response to a variety of stimuli. The invertases are a group of β-fructosidases that differ in pH optimum for activity (acidic, neutral and alkaline) and solubility (soluble and insoluble). Soluble acid invertases (AI) are exclusively located in the vacuole, insoluble AI in the cell wall and neutral/alkaline invertases (NI) are cytosolic (Winter and Huber, 2000).

Secondly, SucSy is a glycosyl transferase located in the cytosol that catalyses a reversible cleavage of sucrose:

$$\text{Suc} + \text{UDP} \leftrightarrow \text{UDP-Glc} + \text{Fru}$$

Uridine-5-diphosphate-glucose (UDP-Glc) is the substrate for cellulose and callose synthesis or may enter glycolysis via the reaction:

$$\text{UDP-Glc} + \text{PPi} \leftrightarrow \text{UTP} + \text{Glc-1-P}$$

$$\leftrightarrow \text{Glc-6-P}$$

In non-green tissues from most plants, glycogen-1-phosphate (Glc-1-P) or glycogen-6-phosphate (Glc-6-P) is the preferred hexose-phosphates taken up by amyloplasts for the synthesis of starch. Therefore SucSy activity has been correlated with starch synthesis, cell wall synthesis and overall sink strength (Winter and Huber, 2000). Sink unloading has been positively linked to invertase and SucSy activities that would hydrolyse the sucrose to hexose sugars (Section 1.3)

ABA not only affects stomatal aperture, but also a range of biochemical processes either directly or indirectly. ABA generally decreases shoot elongation, but increases root elongation at low soil water potentials (Saab *et al.*, 1990) due to the important role of ABA to restrict

ethylene production (Spollen *et al.*, 2000). ABA may also promote nitrate reductase and sucrolytic enzyme activity in roots, but cause an inhibition in leaf tissue (Lu *et al.*, 1992; Chraibi *et al.*, 1995). Exogenous application of ABA in soybean leaves inhibited the formation of fruc-1.6-bisphosphatase but not SPS activity, both of which are key points in sucrose synthesis (Cheikh and Brenner, 1992). ABA was also positively and significantly correlated with SPS activity and the remobilisation of pre-stored carbohydrates in water stressed rice leaves (Yang *et al.*, 2002). Furthermore, spraying rice with ABA also significantly increased SPS activity and caused the remobilisation of carbon reserves. The authors concluded that ABA might act as a regulator of SPS activity.

Amino acids, the first products of ammonium assimilation, are the building blocks of proteins in grapevines (Roubelakis-Angelakis and Kliewer, 1992). Arginine, one of the essential amino acids, is regarded as a major storage form of nitrogen for grapevines (Kliewer and Cook, 1974). Arginine stored in the roots, trunks and canes largely supplies the vine with its nitrogen needs during rapid shoot growth in the spring (Gao and Cahoon, 1990). Many plant species accumulate amino acids in response to nutrient and water stresses (Rabe, 1990). The reduction in growth due to stress can lead to a build-up of ammonium and unlike many other molecules and ions, ammonium is difficult to compartmentalize because it is very membrane mobile and toxic to plant tissues. Consequently plants are unable to use compartmentalization as a protective strategy against elevated ammonium (Roubelakis-Angelakis and Kliewer, 1992). The removal of toxic levels of ammonium therefore forces its sequestering into amides and other NCCs (Givan, 1979).

Two particular NCCs warrant further attention due to their strong correlation with the accumulation of ammonium during water stress (Yang and Kao, 2000) and other special functions during plant tolerance of stress. Water stressed leaves are known to synthesize proline and glycine betaine (Hanson and Hitz, 1982; Rhodes, 1987) and export it via the phloem to sinks (Ladyman *et al.*, 1980). Several reports indicate that water stress leads to changes in the activities of enzymes involved in the synthesis and turnover of proline and glycine betaine (Rhodes, 1987). The onset of stress frequently causes an initial decrease in proline oxidation and decreased protein synthesis, both of which contribute to proline accumulation (Stewart, 1978).

Proline synthesis from glutamate was found to be induced by the application of ABA in non-stressed barley (Stewart, 1980) and wheat (Pesci, 1992). The link between ABA and proline accumulation has also been cited by other authors in rice, *Arabidopsis* and maize (Finkelstein and Somerville, 1990; Chou *et al.*, 1991; Ober and Sharp, 1994). According to Hare and Cress (1997), ABA may elicit its effect on proline accumulation at different levels, ranging from metabolic effects arising from stomatal closure to transcriptional activation of the gene encoding the enzyme Δ^1-pyrroline-5-carboxylate synthetase (P5CS). P5CS is the key enzyme that regulate the synthesis of proline from glutamate and is also present in berries throughout their development (Stines, 1999).

The role of proline accumulation is widely ascribed to that of a cytoplasmic osmoticum that lowers the cell water potential during drought (Rabe, 1990). According to Aspinall and Paleg (1981) other possible functions of proline accumulation may include (a) the hydration of biopolymers, (b) serving as a readily utilizable energy and (c) nitrogen source. The synthesis of proline from glutamate in water stressed plants requires a high level of carbohydrates (Stewart, 1978; Pesci, 1993). Carbohydrates supply the needed carbon and hydrogen for proline synthesis. The proline biosynthetic pathway from glutamine also involves a high consumption of reductants. Equally, when proline is oxidized there is a high-energy output (Hare and Cress, 1997). The accumulation of proline appears to be an excellent means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents (Atkinson, 1977). Accordingly, Hare and Cress (1997) suggested that the positive effect of proline accumulation is that it augments growth upon the relief from stress in addition to its direct function during the stress period.

Glycine betaine is found to accumulate under conditions of cold acclimation, drought and ABA treatment (Xing and Rajashekar, 2001). Typically, the increase in glycine betaine levels ranged from two- to five –fold but even a small increase has been shown to induce freezing and drought tolerance (Rajashekar *et al.*, 1999; Xing and Rajashekar, 2001). The osmoprotecting properties of glycine betaine are well known, and under water stress glycine betaine protects proteins and enzyme activities and even stabilizes membranes during freezing (Rhodes and Hanson, 1993). ABA and glycine betaine are known to accumulate in response to water stress and cold acclimation. This is consistent with Ishitani et al. (1995) who found that when they

treated barley plants with ABA, it led to an increased expression of the BADH gene, which codes for the enzyme that catalyses the last step of glycine betaine synthesis.

Researchers have concluded that in addition to arginine, polyamines serve as N storage compounds during stress (Rabe, 1990). The anti-senescence effects of polyamines are well documented (Tiburco *et al.*, 1993) and are not unlike the anti-senescence effects of Ca^{2+}. Both Ca^{2+} and PAs are known to contribute to the rigidification of membranes and keep the cell wall rheology intact. Adams et al. (1992) found that feeding grapevine leaves with agmatine caused the accumulation of putrescine while ornithine and arginine did not. This indicated that healthy, unstressed grapevine leaves have the enzymes necessary to convert agmatine to putrescine but not to convert arginine to agmatine. The synthesis of arginine to agmatine is mediated by arginine decarboxylase (ADC) and this is thought to be the rate-limiting step in putrescine synthesis (Adams *et al.*, 1992). The activity of ADC is sharply increased when osmotic stress is applied to a particular plant tissue not undergoing active cell division, resulting in elevated putrescine levels (Galston *et al.*, 1983).

Experiments in this chapter were conducted to test the hypothesis that *although partial rootzone drying receives less irrigation water, reduces stomatal conductance and shoot growth, normal levels of sugars, amino acids and polyamines are maintained in shoots by increasing the activities of sucrolytic enzymes involved in sucrose turnover and decreasing the enzymes involved in growth and storage.*

8.2 Materials and methods

8.2.1 Pot experiments

Four-year old split-rooted Cabernet Sauvignon vines were grown under a 50% shade-net in two 8 L pots filled with pure washed sand. The pots were covered with a reflective sheet (Sisalation™) to reduce heat and evaporation and all vines were irrigated twice daily with a 0.02% w/v fertilizer mixture (Aquasol™) solution to field capacity. The vines were allowed to have 5 shoots and to bear three bunches. Both treatments received the same amount of irrigation water during the experiment and the soil matric potential was monitored using a hand-held tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA) as described in Section 2.3. Every second day the irrigation water

in ABA-treated grapevines was spiked with synthetic ABA to achieve a final concentration of 10 μM. The final concentration was determined over a 2 week period by starting at an ABA concentration typically found in the xylem sap of PRD treated grapevines of 3 μM by Stoll (2000) and progressively increasing the concentration until a significant reduction in stomatal conductance compared to control was detected. Experimental design consisted of 5 single vines per treatment randomly selected for either exogenous ABA or control. Stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day.

Two-year old split-rooted Cabernet Sauvignon vines were grown under a 50% shade-net in two 8 L pots filled with standard UC mix (Section 2.2). PRD vines received water twice daily on only one side at any time through 2L/h drippers and therefore received half the amount of water as control vines that received water on both sides. When watered, the soil was irrigated to field capacity. The soil matric potential was monitored using a hand-held tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA) as described in Section 2.3. The vines received a top dressing of 2g of Osmocote™ per pot at the start of fruit set and allowed to have 4 shoots and 3 bunches. Experimental design consisted of 7 single vines per treatment randomly selected for either PRD treatment or control. Stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day.

In 2003 'double pot' split-rooted Cabernet Sauvignon grapevines were grown in a temperature-controlled greenhouse at the CSIRO Horticultural unit (Waite campus, Adelaide). The three-year old split-rooted Cabernet Sauvignon grapevines (Section 6.2.1) grew in two 3 L pots filled with a standard potting mix (Section 2.2). The bottom of the pots were cut out and replaced with plastic mesh to allow roots to grow through into two secondary 3 L pots filled with vermiculite. This allowed easy harvest of clean and actively growing roots. The vines received a top dressing of 1 g of slow release fertilizer (Osmocote®) per pot once at fruit set and the vines were allowed have 5 shoots and to bear 3 bunches. In this 'double-pot' experiment

irrigation water was applied either to one side of the root system at any time (PRD) or to both sides (Control) as described in Section 2.1. Soil matric potential was monitored in the top pots using a Model 6050X1 Trase system (Soil moisture equipment corp., California, USA) to measure instantaneously the volumetric water content of the soil (Section 2.3). Experimental design consisted of 6 single vines per treatment randomly selected for each of the two treatments (PRD or control) and two single vines that were not irrigated.

8.2.2 Field experiments

Cabernet Sauvignon vines in the Coombe vineyard (Waite Campus, Adelaide) were trained to a vertical shoot positioning (VSP) trellis system and in all of the three seasons were positioned manually between the foliage wires three weeks after flowering. Roughly five and nine weeks after flowering all vines were mechanically trimmed and netted for bird protection with the onset of veraison. Cabernet Sauvignon vines were irrigated with 2L/h drip emitters 0.3 m from the trunk. PRD received water on only one side at any given time while water was withheld from the other side. Control vines received water on both sides amounting to 4 L/h. No means of soil moisture measurement was available in the Coombe vineyard in 2001, so the amount of water to be used for the controls was predetermined from an average commercial water usage for viticulture in this climatic region (Stoll, 2000). However, in 2001/2 and 2002/3 the soil moisture was measured using a Diviner 2000®, (Sentek, Adelaide, South Australia) as described in Section 2.1. Irrigation management was done manually during the 2000/1 season, but was replaced by a Galcon 7001D computerized irrigation control unit (Plasflo, Adelaide, South Australia) in 2001/2 and 2002/3. Additional applications of water were done when necessary to adjust soil moisture content. On average, irrigation was done 3 times a week for 3 h, pulsed with 1.5 h in the morning (06:00) and 1.5 h in the late afternoon (17:00). Experimental design consisted of a randomized block design with two treatments, control and PRD irrigation, and seven replicates within one row. Each plot consisted of three grapevines and data were only collected from the centre grapevine thereby leaving 2 buffer grapevines between each treatment. Grapevines were pruned to leave 30 nodes/kg winter pruning weight and bunch thinning was done before flowering aiming for 60 bunches per grapevine.

Shiraz vines were used that grew right next to the row of Cabernet Sauvignon in the Coombe vineyard. Both cultivars were planted in 1992 and the climatic and soil conditions between the two cultivars were regarded to be the same during the three years of the experiment. Pruning,

training and vineyard management were also done in the same manner as above. The only difference was in the amount of irrigation water applied in addition to annual rainfall. Shiraz vines were irrigated with 2 L/h and 4 L/h drip emitters 0.3 m from the trunk. PRD received 4 L/h of irrigation water on only one side at any given time while water was withheld from the other side. Control vines received water on both sides amounting to 4 L/h. Irrigation of Shiraz was applied and monitored similarly to Cabernet Sauvignon above. Experimental design consisted of a randomized treatment layout in one row with two treatments, PRD and control, and 7 replicates.

Field-grown mature Shiraz vines were used in 2000/1 that grew at the Nuriootpa Research Station (Barossa Valley, South Australia). The vines were trained to a single wire trellis system and allowed to sprawl. Drip emitters (2 L/h) were installed in the planting line 0.4 m from the trunk of each vine on either side. PRD vines received half the amount of water of control vines. Experimental plots consisted of four vines where the centre two vines were used for analysis and the two border vines as buffers. Experimental design consisted of a total randomized treatment layout spanning over three rows. Treatments consisted of two irrigations, PRD and control, and three pruning levels (30, 60 and 120 nodes/vine) with 5 repetitions of each treatment.

8.2.3 Stomatal conductance

Stomatal conductances of leaves were determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations described in Section 2.4. Measurements were conducted on cloudless periods on exposed leaves at the same time of the day.

8.2.4 Sucrose Phosphate Synthase (SPS) activity

Sucrose phosphate synthase (SPS) activity was assayed by measuring fructose-6-P dependent sucrose-P (+sucrose) formation from UDPG (Kerr *et al.*, 1984). Plant tissues were frozen in liquid nitrogen, powdered with either a mortar and pestle or commercial coffee grinder and stored at –80°C until needed. The powder was extracted in 4 mL of extraction buffer (8 mL/g) for 30 s using a homogenizer. The extraction buffer contained 50mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2% (w/v) PEG-20, 1% (w/v) BSA, 5 mM DTT and 2 mM GSH. The brei was filtered through Miracloth and the filtrate centrifuged at 38000 g at 4°C for 10 min.

The supernatant was immediately used for enzyme activity analyses. The assay mixture (70 µL) containing 7.5 mM UDPG, 7.5 mM fructose-6-P, 15 mM $MgCl_2$, 50 mM Hepes-NaOH (pH 7.5) and an aliquot of enzyme extract was incubated at 25°C for 10 min and terminated by addition of 70 µL 1.0 N NaOH. Heating the mixtures in a boiling water bath for 10 min destroyed unreacted fructose-6-P. After cooling, 0.25 mL of 0.1% (w/v) resorcinol in 95% ethanol and 0.75 mL of 30% HCl were added and the mixtures were incubated at 80°C for 8 min. The tubes were then cooled to room temperature and the A_{520} is measured and compared to standard absorption curves of sucrose content.

8.2.5 Sucrose Synthase (SucSy) and invertase enzyme activity

Basal and apical leaves were harvested in field-grown grapevines and immediately frozen in liquid nitrogen. Basal leaves were selected that were fully sun-exposed and located within the first 5 nodes close to the base of shoots. Apical leaves were selected that were fully sun-exposed and located within the last 7 nodes of shoots. Sucrose synthase (SucSy), Acid invertase and neutral invertase enzyme activity was assayed on 1 g of tissue using the methods described in Section 2.13.

8.2.6 Shoot sap and leaf exudate collection

Shoot sap was collected during the season using a collection apparatus coupled to a vacuum pump and collected in 1.5 mL Eppendorf vials as described in Section 2.6. Leaf exudates were collected when the leaf water potential was measured on a fully matured leaf wrapped in a polyethylene bag and removed with a single cut across the petiole with a razor blade. The leaf was placed into a pressure bomb (Scholander *et al.*, 1965) attached to a nitrogen gas cylinder. Pressure was increased slowly until exudation of petiole sap from the cut end of the petiole was observed under a magnifying glass. Pressure was increased (by no more than 1 bar) until 50 – 100 µL was collected per leaf. The exudates were placed on ice until all plants were measured and thereafter frozen at –40°C until they could be analyzed.

8.2.7 Starch analysis

Starch analyses were done on samples (500 mg) as described in Section 2.12.

8.2.8 Soluble sugars analysis

Sugar analyses were conducted as described in Section 2.8 by the method of Naidu (1998).

8.2.9 Amino acid analysis

Amino acid analyses were done as described in Section 2.9 by the method of Hernandez-orte (1997).

8.2.10 Free polyamine analysis

Free polyamine analyses were done as described in Section 2.10 by the method of Flores and Galston (1982).

8.3 Results

8.3.1 The effect of ABA on enzymes involved in sucrose turnover in leaves

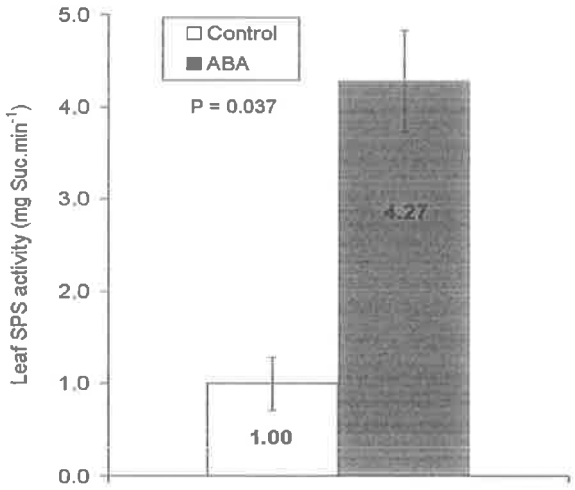


Figure 8.1 Leaf SPS activity in split-rooted Cabernet Sauvignon grapevines of control and with synthetic ABA (10μM) applied to the roots measured on the 02/05/2002. (Both treatments received the same amount of water on both sides; means n = 5 ± s.e.).

The direct effect of root-sourced abscisic acid (ABA) on grapevine leaf sucrose phosphate synthase (SPS) activity is shown in Figure 8.1. The leaf SPS activity was measured on the last day of the exogenous ABA experiment (for more detailed results on stomatal conductance and nitrate reductase activity, see Section 6.3.4). On this particular day, exogenous ABA significantly reduced stomatal conductance by 30% compared to control. However, exogenous ABA significantly increased leaf SPS activity 4-fold compared to control.

8.3.2 The effect of PRD on enzymes involved in sucrose turnover in leaves

As a result of this experience with exogenously applied ABA, I was interested to know what the actual PRD effect would be on leaf sucrolytic enzyme activity since ABA is known to accumulate in response to PRD and soil drying (Düring *et al.*, 1996; Dry *et al.*, 2000). Consequently, the PRD effect on sucrolytic enzymes was investigated in split-rooted Cabernet Sauvignon grown in pots filled with standard potting mix. PRD vines received water on only one side and therefore received half the amount of water as control vines that received water on both sides. The measured soil matric potentials and stomatal conductances during one PRD cycle are shown in Figure 8.2. PRD-treatment was applied for several weeks before the experiment started; consequently the stomatal conductance was significantly reduced on all days on average by 59% compared to control.

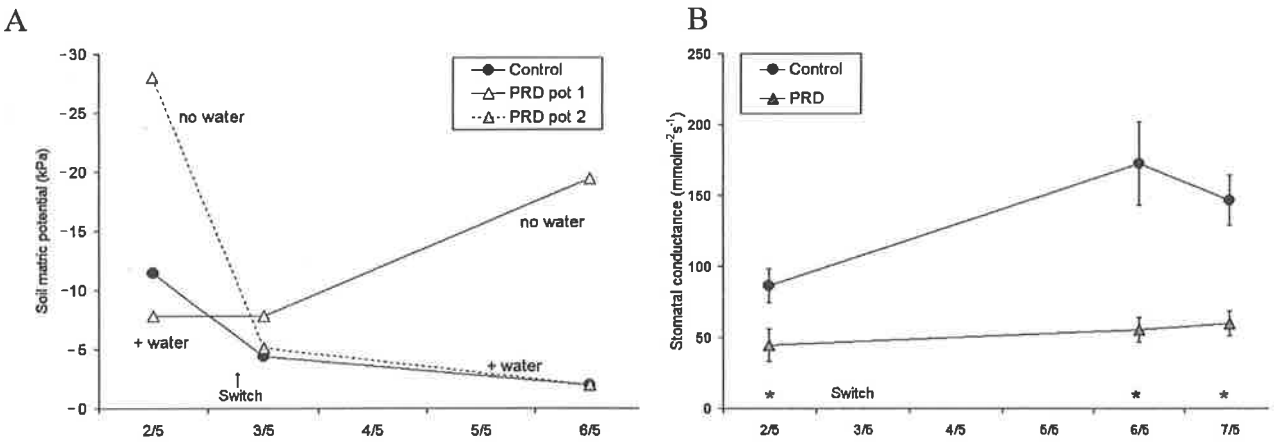


Figure 8.2 (A) Soil matric potential (kPa) and **(B)** stomatal conductance ($mmol.m^{-2}.s^{-1}$) of split-rooted Cabernet Sauvignon in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$))

The effect of PRD treatment on the sucrolytic enzyme activity in split-rooted Cabernet Sauvignon leaves is shown in Table 8.1. In contrast to the ABA-treated vines, the PRD-treated vine leaves showed no significant difference in SPS activity compared to control leaves. Furthermore, PRD-treated leaves also showed no significant difference in acid invertase (AI) activity compared to control. There was however a significant ($P<0.05$) decrease in neutral invertase (NI) activity in PRD-treated vines by 45% on average compared to control.

Table 8.1 Leaf sucrolytic enzyme activity in split rooted Cabernet Sauvignon grapevines on 07/05/2002 (4 days after a switch in PRD; means n = 7 ± s.e).

	Control	PRD	% Diff	P
SPS activity (mg suc/min)	1.93 ± 0.20	1.35 ± 0.29	- 30	0.491
NI activity (mg Glu.h^{-1})	0.820 ± 0.08	0.450 ± 0.09	- 45	0.037
AI activity (mg Glu.h^{-1})	0.127 ± 0.03	0.157 ± 0.06	+ 23	0.738

Further investigations on leaf SPS activity were done on field-grown grapevines in the Coombe vineyard in 2002. The stomatal conductance and leaf SPS activity of Cabernet Sauvignon vines are shown in Figure 8.3. PRD grapevines showed significantly ($P<0.05$) lower stomatal conductance on most days when irrigated with half the amount of water as control. PRD reduced the stomatal conductance on average by 22% compared to control during the experimental period. However, PRD-treatment on average did not have a significant effect on leaf SPS activity over the experimental period, except on the first date where PRD had significantly ($P<0.05$) higher SPS activity compared to control by 48%. After another 10 days the PRD-treated vines showed another increase in SPS activity compared to control by 26% but was not significant ($P=0.1181$).

The stomatal conductance and leaf SPS activity of Shiraz vines are shown in Figure 8.4. In contrast to PRD Cabernet Sauvignon vines, PRD Shiraz received the same amount of irrigation water as control and did not show significantly different stomatal conductances compared to control on most sample days. However, PRD did reduce the stomatal conductance significantly ($P<0.05$) on the 14/02/2002 by 15% compared to control and this corresponded to a significant increase ($P<0.05$) in leaf SPS activity on the same day by 30% compared to control. After another 7 days the PRD-treated vines showed another increase in SPS activity compared to control by 28% but this was not significant ($P=0.1275$). However, PRD-treatment did not have a significant effect on leaf SPS activity on most of the sample days.

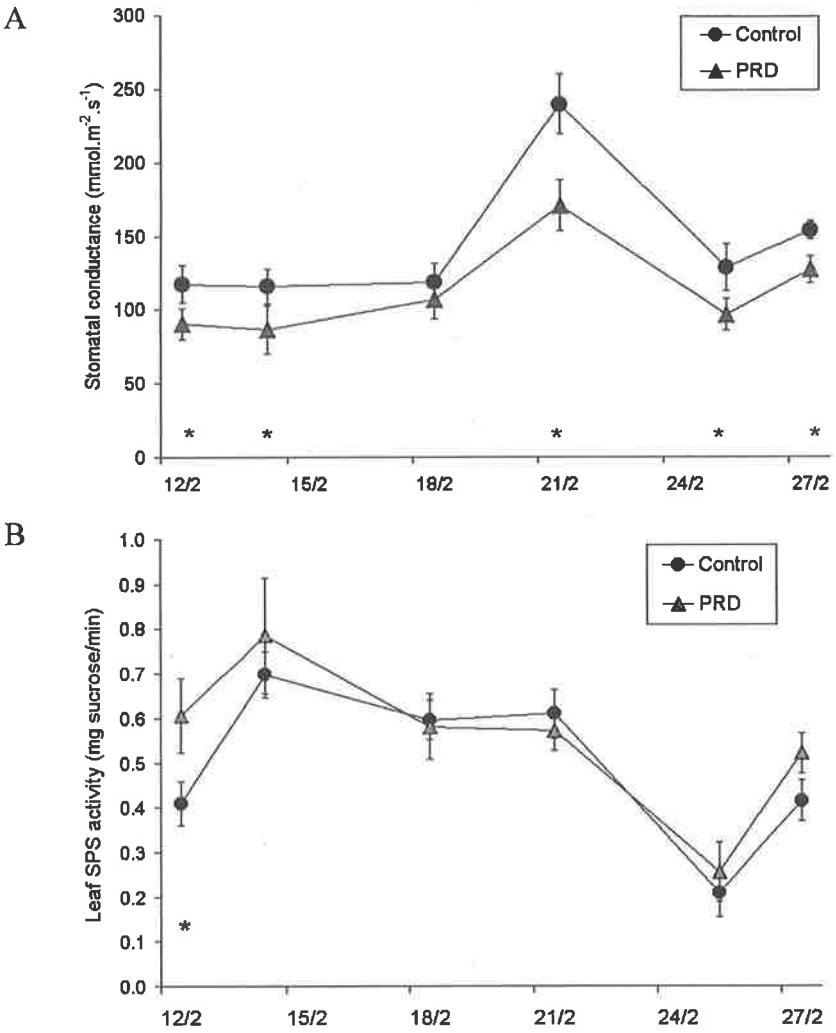
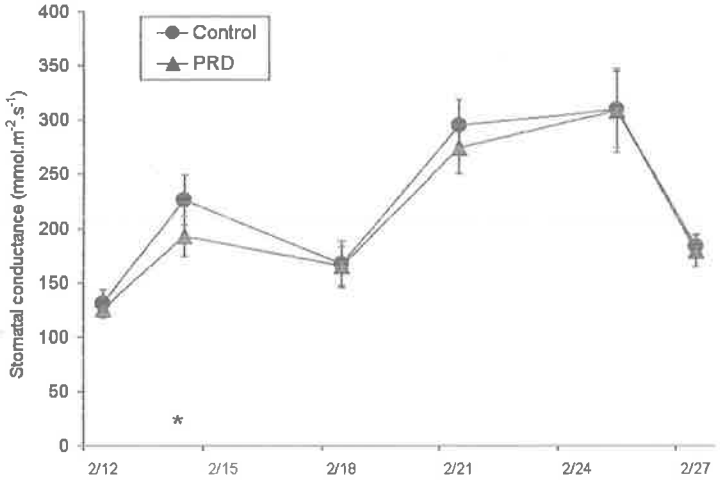


Figure 8.3 (A) Stomatal conductance ($mmol.m^{-2}.s^{-1}$) and **(B)** leaf SPS activity of field-grown Cabernet Sauvignon in the Coombe vineyard (2002). (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$))

A



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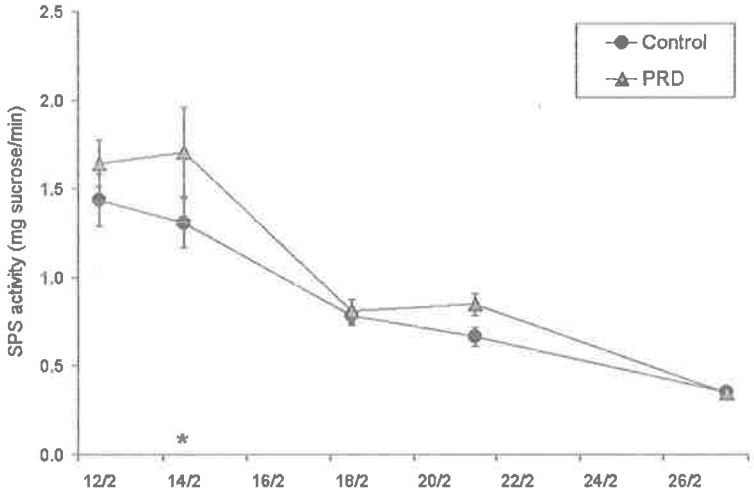


Figure 8.4 (A) Stomatal conductance ($mmol.m^{-2}.s^{-1}$) and **(B)** leaf SPS activity of field-grown Shiraz in the Coombe vineyard (2002). (PRD received the same amount of irrigation as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$).

The effect of PRD on sucrolytic enzyme activity in field-grown Cabernet Sauvignon leaves in 2003 is shown in Table 8.2. Similar to pot experiments, the NI activity in PRD-treated vines was significantly reduced in both the basal and apical leaves by 58% and 57% respectively compared to control. The sucrose synthase (SucSy) and AI activity in PRD-treated Cabernet Sauvignon vines did not show any significant differences compared to control for both the basal and apical leaves.

Table 8.2 Sucrolytic enzyme activity in apical and basal leaves of field-grown Coombe Cabernet Sauvignon grapevines (2003). (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

Basal Leaves	Control	PRD	% Diff	P
NI activity (mg Glu.h^{-1})	2.16 ± 0.04	0.77 ± 0.04	- 58	0.057
AI activity (mg Glu.h^{-1})	1.03 ± 0.21	1.16 ± 0.10	+ 12	0.540
SucSy activity (μM suc.h^{-1})	1.20 ± 0.10	1.38 ± 0.09	+ 15	0.419
Apical leaves	Control	PRD	% Diff	P
NI activity (mg Glu.h^{-1})	0.190 ± 0.03	0.082 ± 0.026	- 57	0.037
AI activity (mg Glu.h^{-1})	1.20 ± 0.16	1.12 ± 0.10	- 7	0.763
SucSy activity (μM suc.h^{-1})	1.60 ± 0.10	1.65 ± 0.04	+ 3	0.414

8.3.3 The effect of PRD on the accumulation of sugars, amino acids and free polyamines in shoots.

a) Starch accumulation in shoots

To investigate the effect of PRD treatment on grapevine shoot physiology the accumulation of solutes and storage compounds were analyzed in both field-grown Cabernet Sauvignon and Shiraz grapevines where PRD treatments received half the amount of irrigation water as control. The effect of PRD on the accumulation of starch in leaves of field-grown Coombe Cabernet Sauvignon vines during the 2000/1 season is shown in Figure 8.5. PRD did not have a significant effect on leaf starch content compared to control on most days during the period between veraison and harvest. Also leaf starch content was especially low during the period leading up to harvest in both control and PRD vines. However, during the four weeks after veraison the PRD-treated leaves showed, on average, lower starch content compared to control that gradually decreased until it was significantly lower by 40% compared to control.

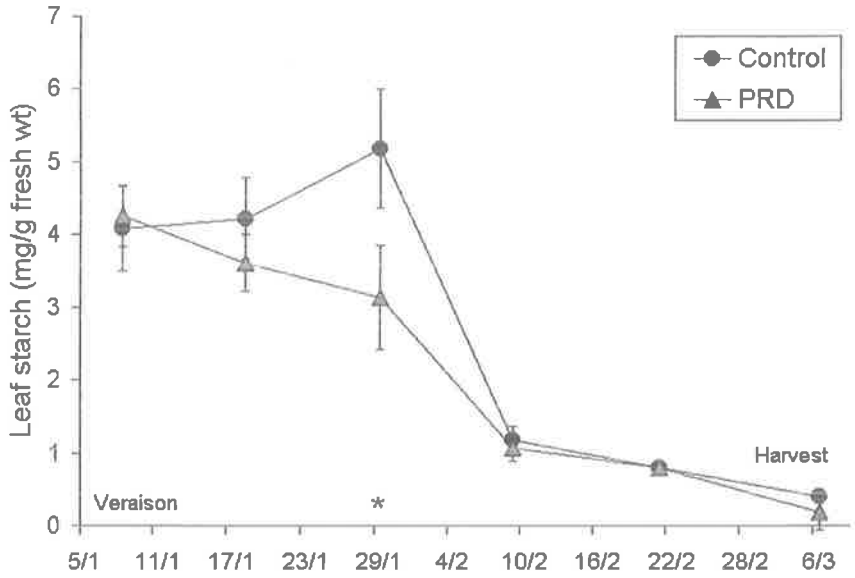


Figure 8.5 Leaf starch contents of field-grown Coombe Cabernet Sauvignon in 2001 (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

The effect of PRD and pruning level on leaf starch concentration in Nuriootpa Shiraz grapevines is shown in Table 8.3. PRD Shiraz vines received half the amount of irrigation water as control vines and vines were treated with three pruning levels (30, 60 and 120 nodes/vine). There was no main effect of irrigation treatment (P=0.7752) or pruning level (P=0.8613) on leaf starch at harvest. However, a significant interaction existed between irrigation and pruning level (P=0.002). The PRD-treated vines pruned to 30 and 60 nodes/vine reduced leaf starch concentration by 54% and 55% respectively compared to control but only the difference in vines pruned to 30 nodes/vine was significant (P<0.05). This result supports the previous findings of reduced leaf starch in response to PRD in field-grown Coombe Cabernet Sauvignon vines and pot experiments in Section 5.3.2. However, PRD-treated vines pruned to 120 nodes/vine showed an unexplainable 4-fold increase in leaf starch concentration compared to control.

Table 8.3 Leaf starch concentration of field-grown Nuriootpa Shiraz at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 5 ± s.e.).

Nodes/vine	Control	PRD	% Diff	P
30	1.53 ± 0.14	0.70 ± 0.26	-54	0.044
60	1.32 ± 0.61	0.60 ± 0.26	-55	0.390
120	0.42 ± 0.08	1.75 ± 0.17	+ 314	0.002

b) Sucrose and nitrogen containing compounds (NCCs) in sap extracted from shoots

The sugar and NCC analysis of sap extracted from shoots in Coombe Cabernet Sauvignon in 2002 is shown in Figure 8.6. Appreciable levels of sucrose and glycine betaine were found in the extracted sap of both PRD-treated and control shoots, but little to no glucose, fructose, mannitol and other nitrogen containing compounds (NCCs) were found. Sucrose, mannitol and glycine betaine levels were found to be higher on average in PRD-treated vines but not significantly so.

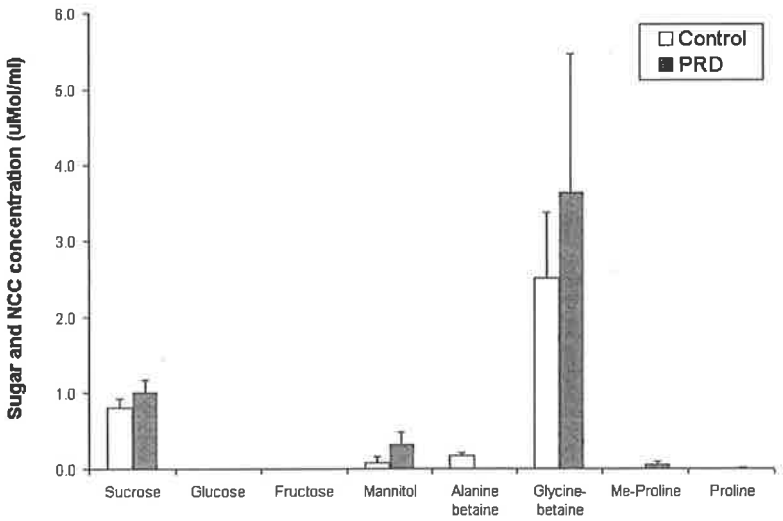


Figure 8.6 Shoot sap sugar and NCC concentrations of field-grown Coombe Cabernet Sauvignon in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 + s.e.)

The evolution in sucrose concentration of leaf exudates during the first 8 days of a 12-day experiment of 'double-pot' split-rooted Cabernet Sauvignon vines grown under glasshouse conditions is shown in Figure 8.7. The effect of PRD and no-irrigation on stomatal conductance, photosynthesis and leaf water potential during this 12-day experiment are

reported in Section 4.3.4. In short, PRD treatment had no significant effect on vine leaf water potential or photosynthesis during the experiment but significantly reduced stomatal conductance after 8 days compared to control. 'No water' vines however showed a significant reduction in leaf water potential, stomatal conductance and photosynthesis compared to control after 2 days. After 4 days there was a 20% increase in sucrose concentration in PRD-treated petiole sap compared to control. However, after 8 days there was a 36% decrease in PRD sucrose concentration compared to control (P=0.173). Vines that received no water for the duration of the experiment showed an initial increase in sucrose concentration after 2 days by 78% compared to control, but this reduced after 4 days to non-detectable levels.

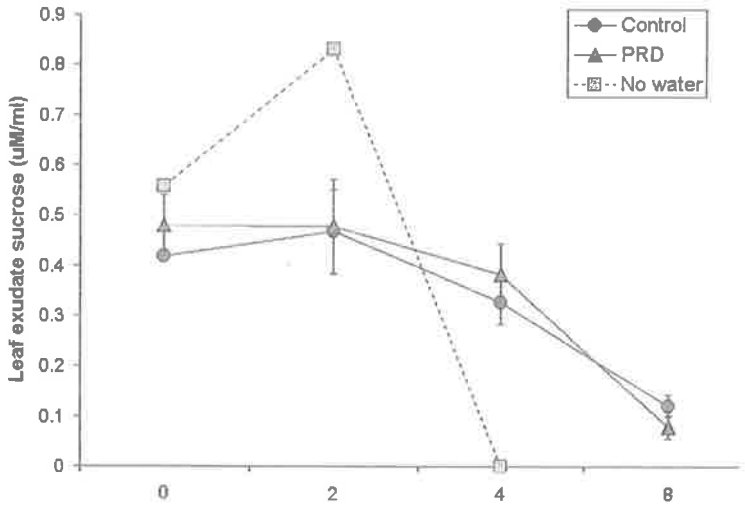
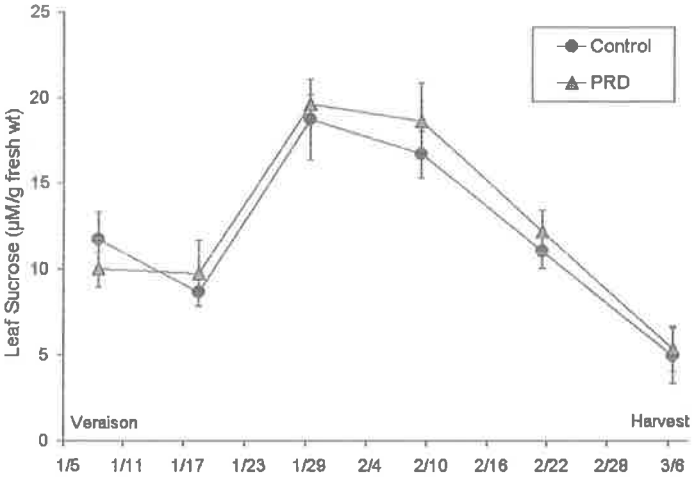


Figure 8.7 Sucrose concentration of petiole sap of split-rooted Cabernet Sauvignon under glasshouse conditions in 2003. (Control received water on both sides; PRD received water on only one side at any time (means n = 6 ± s.e.); 'No water' received no water on either side (means n = 2)

c) Sugars and NCCs in shoots

The evolution in leaf sugars, sugar-alcohols and NCCs in field-grown Coombe Cabernet Sauvignon vines for the period between veraison and harvest in 2001 are shown in Figures 8.8, 8.9 and 8.10. PRD-treated vines received half the amount of irrigation water as control vines and significantly reduced shoot growth (Section 4.3.1) and stomatal conductance (Section 4.3.3) during the 2000/1 season without any associated influence on leaf water potential compared to control (Section 4.3.2). Leaf solute analyses however never showed a significant effect of PRD-treatment on the concentration of sugars (sucrose, glucose and fructose), sugar-alcohols (pinitol and mannitol) or NCCs (glycine betaine, methyl proline and proline) compared to control during the 2000/1 season between veraison and harvest. It is interesting to note that PRD treated leaves had, although not significant, consistently a higher sucrose concentration compared to control during the season. Although there were higher average concentrations in hydroxy-methyl-proline and methyl proline in leaves of PRD-treated vines, the variation in data was too large and therefore it was statistically insignificant.

A



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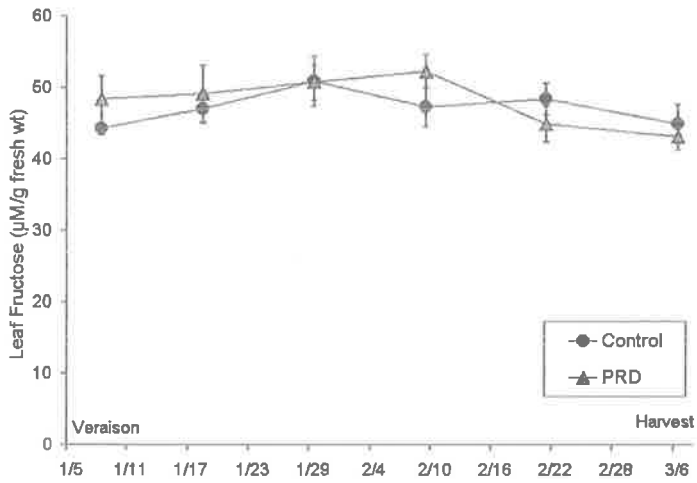

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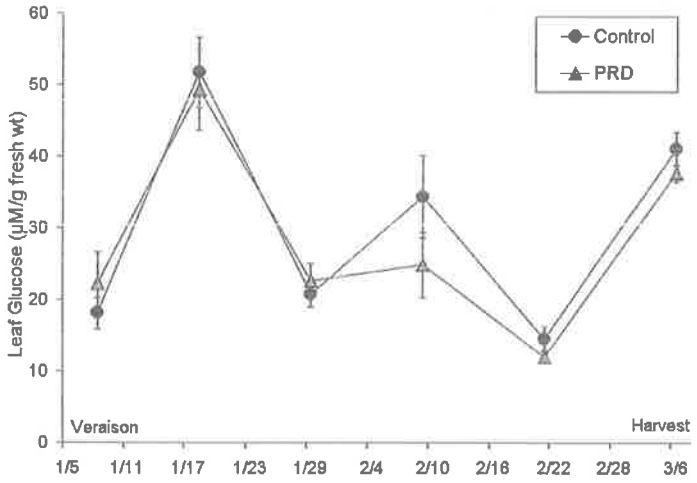


Figure 8.8 The evolution in leaf **(A)** sucrose, **(B)** fructose and **(C)** glucose concentration (µM/g fresh wt) of field-grown Coombe Cabernet Sauvignon vines during the 2000/1 season from veraison to harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different ($P<0.05$))

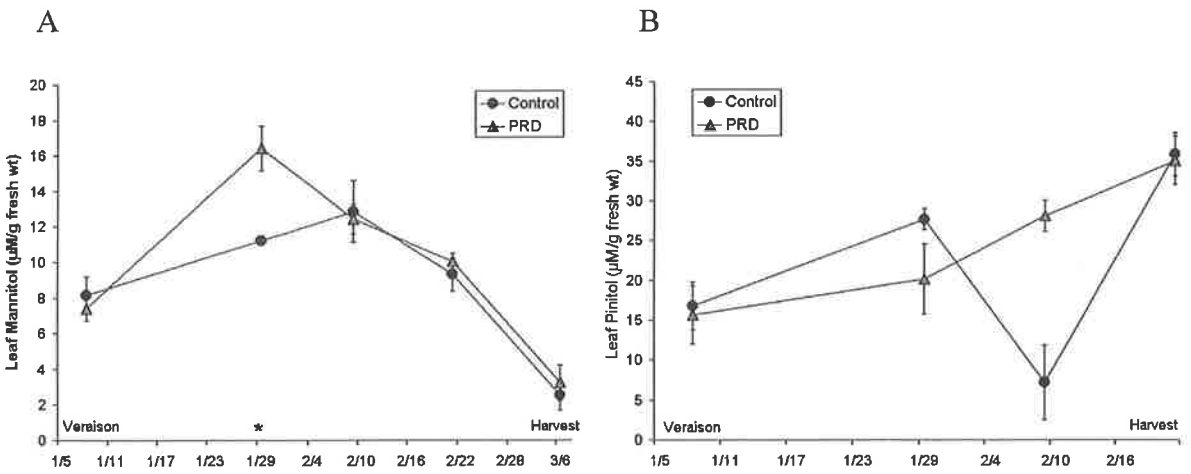


Figure 8.9 The evolution in leaf sugar-alcohols **(A)** mannitol and **(B)** pinitol concentration (µM/g fresh wt) of field-grown Coombe Cabernet Sauvignon during the 2000/1 season from veraison to harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different (P<0.05))

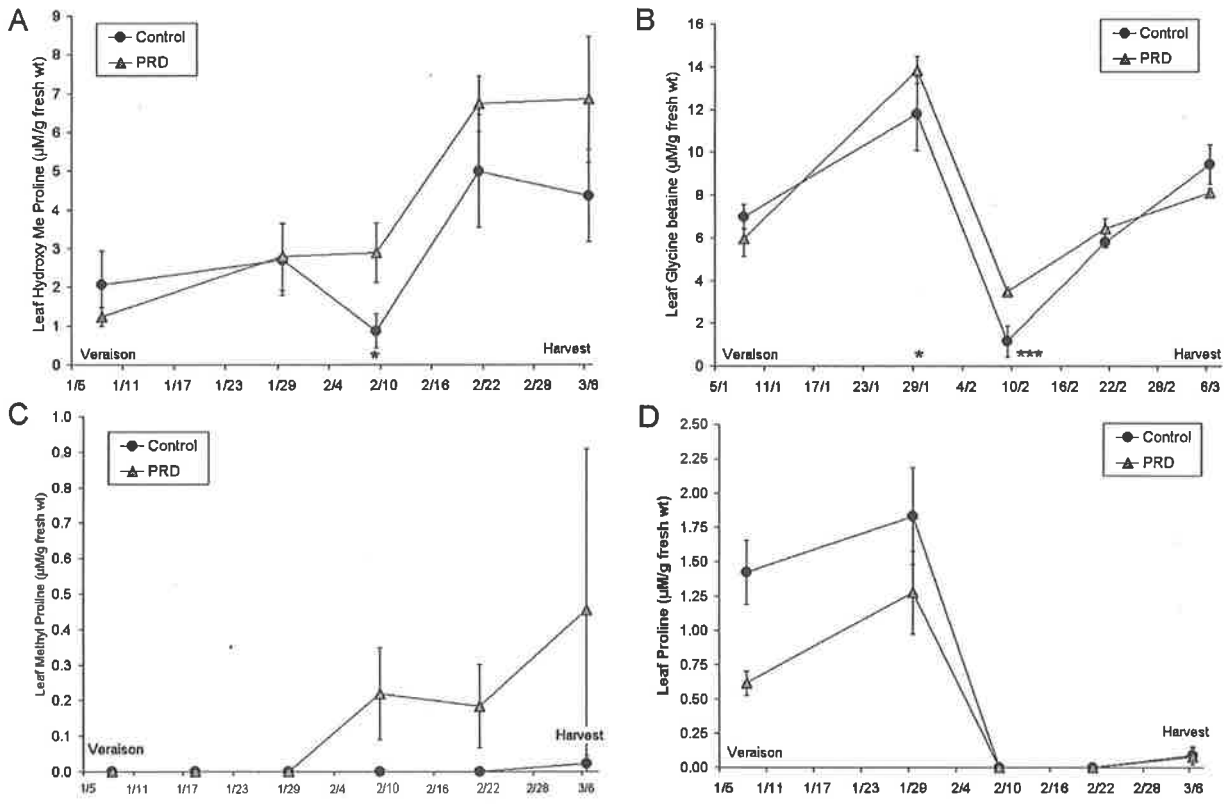


Figure 8.10 The evolution in leaf NCCs **(A)** hydroxy-methyl-proline, **(B)** glycine betaine, **(C)** methyl proline and **(D)** proline concentration (µM/g fresh wt) of field-grown Coombe Cabernet Sauvignon during the 2000/1 season from veraison to harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different (P<0.05))

The analyses of sugar and NCC concentration in the shoots of Coombe Cabernet Sauvignon at harvest in 2001 are shown in Table 8.4. PRD treatment had no significant effect on the sugar (sucrose, glucose and fructose) or sugar-alcohol (mannitol) content of vine shoots at harvest compared to control. The concentration of hydroxy-methyl-proline however significantly increased by 6% in response to PRD treatment. Glycine betaine concentration in PRD shoots was higher on average by 19% compared to control, but not statistically significant.

Table 8.4 Sugars and NCCs (μM/g fresh wt) in shoots of field-grown Coombe Cabernet Sauvignon at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff.	P
Sucrose	5.1 ± 0.4	5.7 ± 0.6	+ 12	0.2810
Glucose	45.8 ± 3.0	42.7 ± 2.4	- 7	0.6199
Fructose	32.3 ± 3.3	27.0 ± 1.8	- 17	0.2163
Mannitol	13.3 ± 0.6	13.4 ± 0.7	+ 1	0.9778
Hydroxy-Me-Pro	47.9 ± 0.5	50.8 ± 0.5	+ 6	0.0003
Glycine betaine	15.9 ± 0.8	19.0 ± 1.6	+ 19	0.3470

The analyses of sugars and NCCs of field-grown Shiraz vine leaves at the 2001 harvest are shown in Table 8.5. PRD-treated Shiraz vines received the same amount of irrigation water as control. Similar to the results found in Cabernet Sauvignon leaves there were no significant differences in leaf sugar or NCC concentrations between PRD and control vines at harvest.

Table 8.5 Sugars and nitrogen containing compounds (μM/g fresh wt) in leaves of field-grown Coombe Shiraz at harvest in 2001. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	0.57 ± 0.22	0.51 ± 0.18	- 11%	0.673
Glucose	41.7 ± 3.2	44.5 ± 2.4	+ 7%	0.427
Fructose	52.5 ± 2.9	49.5 ± 2.2	- 6%	0.287
Mannitol	5.97 ± 1.7	6.51 ± 1.6	+ 9%	0.758
Hydroxy-Me-Pro	17.6 ± 4.6	11.4 ± 3.4	- 35%	0.078
Glycine betaine	7.82 ± 0.35	8.02 ± 0.19	+ 3%	0.579

The analyses of sugars and NCCs of field-grown Shiraz vine shoots at the 2001 harvest are shown in Table 8.6. No significant differences could be found in shoot sugars (sucrose and fructose), mannitol or NCC (Hydroxy-methyl-proline and glycine betaine) concentrations of

PRD and control vines at harvest. However, a significant ($P<0.01$) increase in glucose concentration by 16% was found in PRD-treated vine shoots compared to control.

Table 8.6 Sugars and NCCs (μM/g fresh wt) in shoots of field-grown Coombe Shiraz at harvest in 2001. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	4.7 ± 1.0	5.1 ± 0.6	+ 9	0.567
Glucose	34.5 ± 2.1	39.9 ± 1.3	+ 16	0.009
Fructose	24.8 ± 1.7	27.1 ± 1.1	+ 9	0.156
Mannitol	11.8 ± 0.7	12.8 ± 0.7	+ 9	0.436
Hydroxy-Me-Pro	41.6 ± 1.4	42.8 ± 1.4	+ 3	0.475
Glycine betaine	10.7 ± 0.3	10.7 ± 0.4	0	0.980

d) Amino acids in shoots

The amino acid concentrations of field-grown Cabernet Sauvignon leaves at the 2001 harvest are shown in Table 8.7. Large concentrations of arginine, glutamic acid, aspartic acid, cystine, glutamine, histidine and tyrosine were found in the leaves that represented more than 60% of the total amount of free amino acids. Arginine is regarded as the most important amino acid as a storage form of nitrogen in plants (Kliewer and Cook, 1974; Rabe, 1990) and will therefore receive greater attention. PRD-treated vines showed a 3% increase in leaf arginine concentration compared to control vines. Changes in individual amino acid concentrations were however insignificant and PRD treatment did not affect the total amount of amino acid concentration in leaves compared to control.

Table 8.7 Amino acid concentration (nMol/g fresh wt) in leaves of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Arginine	4498 ± 604	4615 ± 193	+ 3	0.3553
Glutamic acid	1366 ± 284	1183 ± 148	- 13	0.8387
Aspartic Acid	536 ± 72	334 ± 41	- 38	0.1190
Cystine	494 ± 131	651± 171	+ 32	0.6302
Glutamine	401 ± 60	296 ± 32	- 26	0.3879
Histidine	300 ± 38	476 ± 90	+ 52	0.2649
Tyrosine	329 ± 50	404 ± 16	- 5	0.9636
Alanine	241± 101	206 ± 94	- 15	0.2494
Glycine	180 ± 26	205 ± 24	+ 14	0.4279
Norleucine	116 ± 78	72 ± 45	- 38	0.6776
Proline	103 ± 70	94 ± 65	- 9	0.3497
Threonine	101 ± 40	86 ± 35	- 15	0.4320
Asparagine	95 ± 34	55 ± 19	- 42	0.6932
Isoleucine	92 ± 21	102 ± 11	+ 12	0.5662
Valine	85 ± 27	88 ±19	+ 3	0.9721
Leucine	78 ± 24	54 ± 17	- 30	0.6029
Methionine	51 ± 17	35 ± 13	- 30	0.3809
Lysine	46 ± 11	18 ± 9	- 60	0.2728
Serine	39 ± 9	25 ± 6	- 37	0.2825
Phenylalanine	27 ± 13	40 ± 17	+ 50	0.9267
Tryptophan	26 ± 19	52 ± 24	+ 96	0.5504
Hydroxy-L-pro	6.0 ± 0.9	9.3 ± 1.9	+ 54	0.5638
Total	12363 ± 1832	14124 ± 1516	+ 14	0.4818

The amino acid concentrations of Coombe Cabernet Sauvignon shoots at harvest in 2001 are shown in Table 8.8. Proline, arginine, histidine, glutamic acid, cystine and aspartic acid were found in large concentrations in shoots that constituted more than 97% of the total amino acids in shoots of which proline and arginine contributed the most. There were no significant changes in these major amino acid concentrations in response to PRD treatment, however the arginine level decreased on average by 23% compared to control. This decrease was balanced to some degree by an increase in proline concentration and minor amino acids that resulted in PRD having similar total amino acid contents as control shoots. The only significant increase in amino acid was that of alanine by 141% compared to control ($P<0.01$).

Table 8.8 Amino acid concentration (nMol/g fresh wt) in shoots of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; n.d. = not detected)

	Control	PRD	% Diff	P
Proline	4380 ± 247	5044 ± 385	+ 15	0.5449
Arginine	4334 ± 383	3320 ± 90	- 23	0.2541
Histidine	2445 ± 183	2323 ± 118	- 5	0.7360
Glutamine	1260 ± 188	1340 ± 92	+ 6	0.3737
Glutamic acid	1269 ± 287	787 ± 107	- 38	0.5189
Cystine	847 ± 369	756 ± 147	- 11	0.2652
Aspartic Acid	711 ± 76	710 ± 107	0	0.8682
Norleucine	250 ± 77	208 ± 26	- 17	0.7927
Lysine	159 ± 29	147 ± 16	- 8	0.2599
Serine	156 ± 27	193 ± 38	+ 24	0.1079
Glycine	138 ± 3	145 ± 8	+ 5	0.4466
Tryptophan	110 ± 31	104 ± 9	- 5	0.7272
Isoleucine	104 ± 50	24 ± 18	- 77	0.3462
Valine	101 ± 12	225 ± 36	+ 123	0.2687
Tyrosine	87 ± 44	n.d.		
Alanine	46 ± 14	111 ± 21	+ 141	**0.0076**
Asparagine	43 ± 23	86 ± 26	+ 99	0.1287
Methionine	36 ± 20	16 ± 5	- 56	0.6909
Threonine	28 ± 13	46 ± 19	+ 63	0.5538
Hydroxy-L-pro	9 ± 2	5 ± 1	- 44	0.9060
Phenylalanine	n.d.	n.d.		
Leucine	n.d.	29 ± 22		
Total amino acids	15636 ± 809	15977 ± 598	- 2	0.5568

The amino acid concentrations of field-grown Coombe Shiraz leaves at the 2001 harvest are shown in Table 8.9. PRD-treated Coombe Shiraz received the same amount of irrigation water as control. Large concentrations of arginine, glutamic acid, histidine, aspartic acid, proline and glutamine were found in the leaves that represented more than 75% of the total amount of free amino acids. PRD-treated vines showed a 37% increase in leaf arginine concentration compared to control and was the most predominant amino acid found. Changes in individual amino acid concentrations were however insignificant except for glutamic acid and methionine that was significantly reduced by 39% and 66% respectively and leucine that was increased 5-fold compared to control. However, PRD treatment did not affect the total amount of amino acids in leaves compared to control.

Table 8.9 Amino acid concentration (nMol/g fresh wt) in leaves of field-grown Coombe Shiraz at harvest in 2001. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; n.d. = not detected).

	Control	PRD	% Diff	P
Arginine	2551 ± 547	3504 ± 399	+ 37	0.1300
Glutamic acid	1805 ± 202	1105 ± 87	- 39	**0.0213**
Histidine	814 ± 47	760 ± 140	- 7	0.8460
Aspartic Acid	539 ± 46	471 ± 75	- 13	0.5839
Proline	429 ± 179	273 ± 23	- 36	0.5223
Glutamine	385 ± 62	374 ± 32	- 3	0.6810
Tyrosine	296 ± 69	357 ± 32	+ 21	0.7430
Norleucine	265 ± 19	126 ± 59	- 53	0.1403
Cystine	296 ± 56	796 ± 125	+ 169	**0.0718**
Asparagine	247 ± 76	275 ± 31	+ 11	0.1503
Alanine	200 ± 33	132 ± 104	- 34	0.9881
Glycine	162 ± 26	177 ± 27	+ 9	0.1849
Valine	154 ± 19	141 ± 29	- 8	0.8970
Threonine	129 ± 21	147 ± 20	+ 14	0.2683
Isoleucine	98 ± 16	102 ± 22	+ 4	0.6960
Tryptophan	98 ± 30	49 ± 10	- 50	0.3523
Methionine	75 ± 18	25 ± 8	- 66	**0.0270**
Lysine	49 ± 20	59 ± 19	+ 19	0.3205
Phenylalanine	47 ± 8	29 ± 11	- 38	0.6885
Serine	43 ± 6	55 ± 18	+ 27	0.9021
Leucine	16 ± 9	74 ± 4	+ 366	**0.0026**
Hydroxy-L-pro	9 ± 1	7 ± 2	-23	0.2809
Total	8399 ± 786	8710 ± 288	+ 4	0.4712

The amino acid concentrations of Coombe Shiraz shoots at harvest in 2001 are shown in Table 8.10. Similar to levels found in Cabernet Sauvignon shoots, proline, arginine, histidine, glutamic acid, cystine, aspartic acid and glutamine were found in large concentrations in shoots that constituted more than 90% of the total amino acids of which proline and arginine contributed the most. There were no significant changes in these major amino acid concentrations in response to PRD treatment, however the proline, arginine and glutamic acid levels increased on average by 25%, 49% and 47% respectively compared to control. Further changes in individual amino acid concentrations were insignificant, but in total the PRD-treated shoots showed a significant ($P<0.05$) increase in amino acid content by 22% compared to control.

Table 8.10 Amino acid concentration (nM/g fresh wt) in shoots of field-grown Coombe Shiraz at harvest in 2001. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; n.d. = not detected).

	Control	PRD	%	P
Proline	4498 ± 427	5622 ± 309	+ 25	0.1268
Arginine	2947 ± 143	4396 ± 707	+ 49	**0.0745**
Histidine	2135 ± 484	2406 ± 528	+ 13	0.7547
Cystine	1691 ± 100	1742 ± 73	+ 3	0.7656
Glutamic acid	889 ± 161	1305 ± 147	+ 47	**0.0887**
Aspartic Acid	686 ± 72	672 ± 49	- 2	0.8450
Glutamine	552 ± 158	430 ± 222	- 22	0.7503
Alanine	339 ± 46	308 ± 65	- 9	0.7571
Tryptophan	132 ± 21	131 ± 21	- 1	0.9845
Glycine	91 ± 3	103 ± 11	+ 13	0.3028
Norleucine	86 ± 42	113 ± 54	+ 32	0.7293
Valine	76 ± 17	132 ± 27	+ 73	0.2348
Lysine	76 ± 23	41 ± 16	- 45	0.3539
Asparagine	74 ± 19	46 ± 26	- 38	0.5109
Serine	44 ± 13	38 ± 18	- 14	0.8538
Methionine	41 ± 9	49 ± 9	+ 20	0.6016
Threonine	36 ± 12	20 ± 12	- 44	0.3206
Isoleucine	30 ± 11	63 ± 26	+ 111	0.3735
Hydroxy-L-pro	9 ± 4	6 ± 3	- 37	0.5778
Phenylalanine	7 ± 4	3 ± 2	- 53	0.5346
Tyrosine	4 ± 3	4 ± 4	- 1	0.9963
Leucine	3 ± 3	n.d.		
Total	14445 ± 683	17631± 1074	+ 22	**0.0455**

e) Polyamines in shoots

The polyamine concentrations of sap extracted from shoots in field-grown Coombe Cabernet Sauvignon and Shiraz vines near harvest in 2002 are shown in Table 8.11. PRD-treated Cabernet Sauvignon showed a significant ($P<0.05$) increase in shoot sap putrescine concentration by 73% compared to control. No significant differences could be found in spermine and spermidine concentrations between PRD and control vines. In comparison, PRD-

treated Shiraz showed no significant differences in shoot sap polyamine concentration compared to control vines.

Table 8.11 Shoot sap polyamine concentration (ng/ml) of Coombe Cabernet Sauvignon and Shiraz (15/03/2002). (PRD Cabernet Sauvignon received half the amount of irrigation water as control by irrigating on only one side at any time; PRD Shiraz received the same amount of irrigation as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.).

Cabernet Sauvignon	Control	PRD	% Diff	P
Putrescine	136 ± 22	234 ± 30	+ 73	0.038
Spermidine	216 ± 29	219 ± 23	+ 2	0.630
Spermine	157 ± 24	161 ± 31	+ 2	0.797
Shiraz	Control	PRD	% Diff	P
Putrescine	147 ± 11	133 ± 15	- 9	0.206
Spermidine	145 ± 27	136 ± 15	- 6	0.749
Spermine	50 ± 9	78 ± 24	+ 56	0.257

The polyamine content of leaves in field-grown Coombe Cabernet Sauvignon vines (Table 8.12) was measured roughly in the middle of the ripening season of 2001/2 (14/02/2002). No significant differences were found in leaf free polyamine concentrations in response to PRD treatment, although on average spermine increased by 20% and putrescine decreased by 20% compared to control.

Table 8.12 Free polyamine content (ug/g fresh wt) in leaves of field-grown Coombe Cabernet Sauvignon (14/02/2002). (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff.	P
Putrescine	2.77 ± 0.36	2.21 ± 0.39	- 20	0.246
Spermidine	12.61 ± 0.23	13.21 ± 0.53	+ 5	0.401
Spermine	5.27 ± 0.28	6.35 ± 0.46	+ 20	0.138

8.4 Discussion

Results of experiments in this chapter have provided evidence that PRD maintained normal levels of sugars and nitrogenous compounds in shoots but affected growth and sucrose turnover by its effects on sucrolytic enzyme activities. Sucrose phosphate synthase (SPS) is an important control point in the sucrose formation pathway (Stitt *et al.*, 1987b) and its activity is closely linked to changes in photosynthesis and regulated by sink demand. Under water stress

conditions it is generally found that there is a decline in leaf SPS activity (sucrose formation) in photosynthetic active tissues (Vassey and Sharkey, 1989; Du *et al.*, 1998; Foyer *et al.*, 1998) due to the stomatal restriction of the CO_2 supply. Although PRD significantly reduced stomatal conductancc in grapevine leaves, the SPS activity in pot and field-grown experiments in this study were not significantly affected on most days. On occasion the leaf SPS activity was actually significantly increased and this correlated with days of significant reduction in stomatal conductance. The occasional increases measured in SPS activity in response to PRD treatment were found irrespective of the amount of irrigation water applied to PRD vines. It must be said, however, that situations did occur where SPS activity was not affected although stomatal conductance was significantly reduced.

The increase in leaf SPS activity in PRD-treated vines may be related to the increase in ABA in shoots (Stoll *et al.*, 2000), because pot experiments in this study have shown that exogenous application of ABA at levels that reduced stomatal conductance to similar levels as PRD-treated plants, could increase the leaf SPS activity significantly by 4-fold compared to control vines. The reason why PRD-treated leaves did not show the same large increase in SPS activity as the exogenous ABA experiment may be because the rate of sucrose and starch formation, as measured in SPS activity, may be controlled in PRD vines by its sink demand more than its endogenous ABA concentration. A reduction in shoot growth would cause an end-product inhibition in photosynthesis (Flore and Lakso, 1989) when sugars are not utilized as rapidly the demand for carbohydrates is lower and hence SPS activity would be suppressed. On the contrary, over-expression of SPS activity in tomato has clearly demonstrated that a high SPS activity can improve photosynthetic capacity in leaves (Galtier *et al.*, 1993; Galtier *et al.*, 1995). It may therefore be possible that the transient increases found in SPS in the leaves of field-grown Cabernet Sauvignon and Shiraz vines were caused by an ABA-induced effect to improve the photosynthetic capacity of PRD-treated grapevines in response to soil drying conditions. The improvement in photosynthetic capacity has already been seen in the effect of PRD on stomatal conductance and photosynthesis (Section 4.3.4). PRD caused long-term reductions in stomatal conductances without appreciably affecting the CO_2 assimilation rates compared to the control.

Acid invertase (AI) is absent from the cytosol, but exists in both the soluble form in the vacuole and in the cell-wall-bound forms (Sturm and Tang, 1999). Several authors have proposed the

hypothesis that vacuolar AI activity regulates the amount of sucrose in leaves (Hammond *et al.*, 1984; Huber, 1989). It was proposed that high vacuolar AI activity would decrease the amount of sucrose available for both export and storage, while low AI activity would favor sucrose accumulation. However Kingston-Smith *et al.* (1998) found that due to an absence of an inverse relationship between leaf sucrose, hexose or starch contents and endogenous AI activity in grapevine leaves and the leaves of various other species, that soluble AI was not directly involved in carbon partitioning in leaves, but serves an auxiliary function. The AI activity in PRD-treated leaves in this study showed no significant difference compared to control leaves. PRD treatment therefore had no effect on leaf AI activity compared to control and because AI is localized in the vacuole it is concluded that AI activity did not contribute to the turnover of sucrose in grapevine leaves in response to PRD treatment but rather played a role in maintaining adequate glucose and fructose concentrations.

Unlike AI, the neutral invertase (NI) is located in the cytosol and its functions are largely unknown, but are probably involved in the channeling of sucrose into metabolism for growth (Winter and Huber, 2000). Rose and Botha (2000) reported a positive correlation between NI activity and respiration/growth in sugarcane nodes. In this study, the NI activity in both the basal and apical leaves of field-grown PRD-treated vines was significantly reduced by 58% compared to control. This result was further supported by a significant reduction in leaf NI activity of pot-grown Cabernet Sauvignon in response to PRD treatment by 45% compared to control. This may be an indication that the rate of sucrose channeled into the metabolism or growth in shoots is significantly reduced in response to PRD. This result supports the observed reductions in shoot growth rates in PRD-treated grapevines by several authors in earlier work (Dry *et al.*, 1996; Dry and Loveys, 1999; Stoll, 2000). However, whether the reduction of NI is the cause of the reduced growth rate in PRD-treated grapevine shoots or just an effect is not known.

Carbohydrate in leaves are stored in leaves mainly as starch, and its accumulation may be finely tuned to average rates of photosynthesis (Sims *et al.*, 1998) and to an upper limit in accordance to the export rate of sucrose (Komor, 2000). The export of sucrose however would be determined by overall vine sink demand relayed to the source by the concentration gradient in the phloem (Minchin *et al.*, 1993). Surplus sucrose is diverted to starch that builds up in daylight when photosynthesis exceeds the combined rates of respiration and export. To achieve

a steady state of sucrose synthesis, starch synthesis begins and ends gradually during the daily light period (Chatterton and Silvius, 1979). Sucrose synthase (SucSy) and NI are both localized in the cytosol and their activities are proposed to channel sucrose into metabolism (Sturm and Tang, 1999; Winter and Huber, 2000). SucSy activity is also correlated with starch synthesis, cell wall synthesis and overall sink strength (Winter and Huber, 2000). Results obtained in this study showed no significant effect of PRD treatment on SucSy activity compared to control in either basal or apical leaves. However, on occasion PRD-treated vines showed significantly lower starch concentrations in leaves compared to control vines. Whether or not these decreases in starch concentration were correlated with changes in SucSy activity or increases in SPS activity is unknown. An increase in SPS activity in leaves in response to short-term water stress has been shown to cause a stimulation of sucrose synthesis and inhibition of starch synthesis (Quick *et al.*, 1989) that may be important in osmoregulation or an increase in sucrose export. Conversely, it was found in this study that at high node numbers per vine PRD caused a significant increase in leaf starch levels compared to control. The reason for this increase in PRD leaf starch compared to control is unclear and not expected. Further research needs to be done to verify this result.

Smith and Milburn (1980) suggested that phloem loading does not respond to changes in phloem sucrose concentration but rather to the decreasing turgor pressure that can be rapidly transmitted through the phloem from the sink to the source created by phloem unloading and growth of the sink. Leaf sucrose export therefore responds to general sink demand expressed as a decrease in turgor pressure in connecting phloem. The petiole exudate sucrose concentration and the extracted shoot sap sucrose concentration should give a good indication of the individual leaf export rate (Tiaz and Zeiger, 1991) and the overall shoot sucrose supply respectively. Results from the current study indicated that PRD treated shoots and leaves showed a slight increase in sucrose concentration compared to control that may indicate that the production of sucrose in PRD treated shoots was not affected or slightly increased compared to control. A slight increase in supply in every leaf could have a large impact on a whole vine level. There has never been any evidence of an increased photosynthetic rate in PRD-treated leaves and it is therefore proposed that an increase, if any, in leaf sucrose efflux is caused by an increase in leaf SPS activity. This hypothesis is supported by transient increases in SPS activity and associated decreases in leaf starch concentration compared to control leaves, because an

increase in SPS activity would cause a shift in the leaf sucrose/starch fraction (Quick *et al.*, 1989; Zhou and Quebedeaux, 2003).

The ultimate goal of irrigation and canopy management is to benefit fruit quality without disturbing growth and development in other sinks in an unsustainable way. The results obtained from the analysis of sucrolytic enzyme activities have revealed some information on the PRD effect on the source:sink relationship in the leaves of grapevines compared to control. However, to fully evaluate the effect of PRD treatment on shoot physiology and the turnover of nutrients it was important to measure the evolution in sugars and amino acid contents in shoots. Firstly, the sugars and nitrogen containing compounds (NCCs) in leaves and shoots of field-grown Cabernet Sauvignon and Shiraz grapevines, that received half and the same amounts of irrigation water as control, was measured during the 2000/1 season. Although PRD treatment significantly affected leaf physiology in both cultivars, no significant effect of PRD treatment could be found in the evolution of sugars (sucrose, glucose and fructose), sugar-alcohols (pinitol and mannitol) or NCCs (glycine betaine, methyl proline and proline) in leaves during the 2000/1 season compared to control. PRD treatment also did not affect the sugar and NCC concentrations in the shoots of both cultivars compared to control. It is therefore concluded that PRD treatment maintained adequate sugar and NCC levels in grapevine leaves and shoots, irrespective of the amount of irrigation water applied. In general, soluble sugar content tends to be maintained in the leaves of droughted plants, in spite of lower rates of carbon assimilation (Du *et al.*, 1998; Chaves *et al.*, 2003). This is achieved at the expense of starch, which drastically declines (Chaves, 1991). This response favors osmoregulation and enhances desiccation tolerance.

Secondly, the amino acids were measured in the leaves and shoots of Coombe Cabernet Sauvignon and Shiraz vines during the 2000/1 season. Amino acids are the products of nitrogen assimilation and the building blocks for structural growth, enzymes, proteins, nucleotides etc. (Salisbury and Ross, 1992). The dominant organ in amino acid synthesis and distribution is the leaf, which uses the energy and carbon skeletons produced by photosynthesis to assimilate nitrogen into primary amino acid products (glutamine and glutamic acid). The amino groups of these products are then allocated through transamination reactions to form hosts of amino acids necessary for protein synthesis and other functions. It was noted as early as 1974 by Kliewer and Cook (1974) that arginine contents in vine shoots and mature fruit were closely correlated.

Arginine is regarded as the most important amino acid as a storage form of nitrogen in plants (Kliewer and Cook, 1974; Rabe, 1990).

In this study, 22 amino acids were measured in leaves and shoots of PRD-treated and control vines. The most abundant amino acid found in leaves was arginine, and in shoots, proline and arginine dominated. PRD treatment of Cabernet Sauvignon vines had no significant effect on the amino acid profile nor the total amino acid contents in leaves or shoots compared to control. This indicated that there was no detrimental effect of PRD on the production and accumulation of amino acids in grapevine leaves compared to control even though PRD-treated vines received half the amount of irrigation water as control vines. PRD treatment of Shiraz vines also had no effect on the total amount of amino acids in leaves compared to control, but there were some significant changes in the amino acid profile. Arginine concentration increased by 37% while there was a significant decrease in glutamic acid by 39% compared to control. There were also some significant changes in the minor amino acids with significant increases in cystine and leucine and decreases in methionine. The overall effect of the change in amino acid profile is unknown, but in total the PRD treatment showed a similar concentration in leaf amino acids compared to control which meant that there was no detrimental effect of PRD on the production and accumulation of amino acids in grapevine leaves compared to control. It is speculated that the change in amino acid profile may be a result of hormonal influences on protein production for enzymes in response to PRD treatment. The accumulation in shoots however indicated that PRD-treated Shiraz significantly increased its amino acid concentration compared to control vines by 22%. This increase in total amino acids was mainly due to significant increases in major amino acids that included proline, arginine and glutamic acid. The major amino acids were present at high concentrations and are most closely linked to primary carbon metabolism and nitrogen assimilation. An increase in the total amino acid content of shoots may increase the overall vine capacity and fruitfulness.

Polyamines are important in plant developmental processes and may be considered to have hormone-like characteristics (Martin-Tanguy, 1997). It has already been found that PRD increased the polyamine concentrations of spermine and spermidine in roots (Section 7.4.4) compared to control, but the putrescine concentration was the same. In this study, the putrescine concentration in the shoot sap of PRD-treated Cabernet Sauvignon vines was significantly increased by 73% compared to control. It is possible that the putrescine in roots was used to

synthesize spermine and spermidine via the ethylene biosynthesis pathway, however in shoot sap there were no increases in spermine or spermidine concentrations. The increase in putrescine concentration may have a similar hormonal role as ABA in the response of plants to soil drying conditions. Todorov *et al.* (1998) have shown that the exogenous application of putrescine has similar effects as ABA in alleviating plant damage under water stress conditions. However, the polyamine concentrations in the leaves of PRD-treated vines showed no significant differences compared to control. This could however be expected, because the leaves of PRD Cabernet Sauvignon vines did not experience any osmotic stress compared to control as measured in the leaf water potentials (Section 4.3.2). Conversely, the sap extracted from shoots in PRD Shiraz and control that received the same amount of irrigation did not show any significant differences in the polyamine concentration compared to control. The PRD effect on putrescine concentration may therefore be attributed to the amount of water applied. It is hypothesized that putrescine is produced in the roots in response to low soil moisture conditions and partly metabolized into spermine and spermidine and partly translocated up into the shoots via the xylem sap.

8.5 Conclusion

The experiments in this chapter were conducted to test the hypothesis that although partial rootzone drying receives less irrigation water, reduces stomatal conductance and shoot growth relative to control, adequate levels of sugars, amino acids and polyamines are maintained in shoots by increasing the activities of sucrolytic enzymes involved in sucrose turnover and decreasing the enzymes involved in growth and storage. Enough evidence has been collected to accept this hypothesis. The major conclusions were:

1) The SPS activity in pot and field-grown experiments in this study were not significantly affected on most days - on occasion the leaf SPS activity was actually significantly increased in response to PRD and this correlated with days of significant reduction in stomatal conductance. Occasional increases in SPS activity in PRD-treated grapevines were found irrespective of the amount of irrigation water applied.

2) It may be possible that the transient increases found in SPS in the leaves of PRD treated vines were caused by increased ABA and associated with improved photosynthetic capacity of PRD-treated grapevines in response to soil drying conditions.

3) The AI activity in PRD-treated leaves showed no significant difference compared to control leaves. AI activity may not be involved in the turnover of sucrose in grapevine leaves in response to PRD treatment, but may possibly play a role in maintaining glucose and fructose levels.

4) The NI activity in both the basal and apical leaves of field-grown PRD-treated vines was significantly reduced compared to control. However, whether the reduction of NI is the cause of the reduced growth rate in PRD-treated grapevine shoots or just a consequence is not known.

5) PRD-treated vines showed on occasion significantly lower starch concentrations in leaves compared to control vines. Results obtained in this study showed no significant effect of PRD treatment on SucSy activity compared to control and therefore the transient reductions in starch may be related to increases in SPS activity.

6) A PRD-induced increase, if any, in leaf sucrose efflux may be caused by a shift in the sugar/starch fraction by an increase in leaf SPS activity.

7) PRD treatment had no effect on the evolution of sugars (sucrose, glucose and fructose), sugar-alcohols (pinitol and mannitol) or NCCs (glycine betaine, methyl proline and proline) in leaves during the season compared to control. PRD treatment did not affect sugar and NCC levels in grapevine leaves and shoots, irrespective of the amount of irrigation water applied.

8) The most abundant amino acids were arginine in leaves and proline and arginine in shoots. PRD treatment of Cabernet Sauvignon vines had no significant effect on the amino acid profile nor the total amino acid contents in leaves or shoots compared to control. This indicated that there was no detrimental effect of PRD on the production and accumulation of amino acids in grapevine leaves compared to control even though PRD-treated vines received half the amount of irrigation water as control vines.

9) The production and accumulation of amino acids in PRD Shiraz leaves compared to control was also unaffected. The accumulation in shoots however indicated that PRD-treated Shiraz significantly increased its amino acid concentration compared to control vines. An increase in the total amino acid content of shoots may increase the vine capacity and fruitfulness.

10) The increase in putrescine level in extracted shoot sap in response to PRD may be attributed to the reduction in the amount of water applied and/or to some degree of water stress.

Chapter 9: The accumulation of sugars, amino acids and polyamines in PRD berries: effects of sucrolytic enzyme activity and berry size

9.1 Introduction

Before veraison, sink strength is related to the development of seeds, flesh and skin and to metabolic activities such as storage of organic acids in the vacuole. After veraison, sink strength can be described in terms of hexose storage activity. The sink strength of the berry, estimated by carbon import, increases substantially after veraison (Ollat and Gaudillere, 1996). During ripening, the increase in berry volume is accompanied by an increase in berry softness, accumulation of hexoses and in red varieties the skin becomes colored due to the accumulation in anthocyanins (Coombe, 1992). Grape berries are non-climacteric fruit and during the ripening of grape berries, sucrose is transported from the leaves and accumulates in the berry vacuoles as glucose and fructose (Coombe, 1989). The hydrolysis of sucrose is therefore essential in the unloading of sucrose from the phloem and in the sequestering of the formed hexoses into the vacuole. Two classes of invertases are involved in the unloading of sucrose in berries. Most reports deal with either soluble invertase, which has an acidic pI and acid pH optima (AI) and is located in the vacuole, or with insoluble acid invertase, which has basic pI values and is bound to the cell wall. The vacuolar invertases are likely to be important in the regulation of the hexose levels in fruit tissues while the cell-wall forms are associated with rapidly growing tissues and phloem unloading (Davies and Robinson, 1996). The invertase activity in grape berries increases after flowering until it reaches a maximum after 8 weeks, thereafter the invertase activity remains constant on a per berry basis throughout ripening (Hawker, 1969b; Davies and Robinson, 1996). The hexoses however do not start to accumulate until 8 weeks post-flowering (veraison), but thereafter they continue to accumulate up to harvest after 16 weeks. SucSy is another enzyme that also increases at veraison and may be involved in the breakdown of sucrose, but even at its maximal level the activity is low compared to that of the invertases (Hawker, 1969b).

Amino acids, the first products of ammonium assimilation, are the building blocks of proteins in grapevines and play a major role in the fermentation of musts (Roubelakis-Angelakis and Kliewer, 1992). Because sugars are usually in excess to what is required by yeasts, the N content is effectively the metabolic factor that determines the rate of fermentation (Salmon, 1989). The N content of musts can be highly variable and 50%-90% is in the form of free amino acids (Kliewer, 1969; Ough, 1988). Both the concentration and type of nitrogenous

compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine (Stines *et al.*, 2000). Stines *et al.* (2000) concluded that the basic pattern of amino acid composition in mature fruit is determined by genetic factors and that environmental and cultural factors have only a modifying effect. Arginine, proline, histidine and glutamine are the most prominent amino acids in berries, with proline being predominant in Cabernet Sauvignon (Hernandez-orte *et al.*, 1999). Total free amino acids accounted for 40% – 87% of total Kjeldahl nitrogen. Stines *et al.* (2000) found that proline and arginine accumulated to much higher concentrations in Cabernet Sauvignon and Riesling cultivars compared to other amino acids and that their accumulation was confined to the later stages of ripening. The role of proline in fermentation is still uncertain since proline is relatively non-assimilable under anaerobic fermentation conditions. Arginine, on the other hand, is readily assimilable and is the highest source of nitrogen for the yeasts in the fermentation of must into wine since it has four nitrogen atoms per molecule (Jiranek *et al.*, 1990).

The combination of high free α - amino acids (FAA) and low total nitrogen content in berries enhances wine quality (Bena-Tzourou *et al.*, 1999; Hernandez-orte *et al.*, 1999). Musts that were rich in amino acids did not only positively influence the fermentation process but also enriched volatile components of the wines. According to Ingledew and Kunkee (1985), sluggish or stuck fermentations may result from nitrogen deficiencies in grape musts. Although free amino nitrogen (FAN) is proposed as an indicator of total available nitrogen in juice, Monteiro and Bisson (1991) warned that this measure does not account for compounds that can be utilized by *Saccharomyces* and those that cannot. They state that this can lead to overestimation of available nitrogen. Conversely, total nitrogen contribution of certain compounds such as arginine, which contains more than one nitrogen atom, will be underestimated in a FAN assay.

It has been reported that conjugated and wall bound polyamines are important for plant developmental processes such as floral induction, and reproductive processes, and hence may be considered to have hormone-like activity (Martin-Tanguy, 1997). Other findings also suggested that polyamines are essential in the processes of bloom and fruit set (Geny *et al.*, 1997). A polyamine deficiency at these stages has a direct effect on flower drop and berry malformation.

Experiments in this chapter were conducted to test the hypothesis *that PRD increases the sugar and amino acid concentration due its effect on sucrolytic enzyme activity and/or berry size.*

9.2 Materials and methods

9.2.1 Plant material

Field-grown Cabernet Sauvignon and Shiraz vines in the Coombe vineyard were used that was trained, irrigated and managed it the way already described in Section 8.2. PRD Cabernet Sauvignon was irrigating with half the amount of irrigation water as control. Shiraz vines were used that grew right next to the row of Cabernet Sauvignon in the Coombe vineyard. The only difference was in the amount of irrigation water applied in addition to annual rainfall. PRD received the same amount of irrigation water as control, but only on one side at any given time while water was withheld from the other side. Experimental design of both cultivars consisted of a randomized treatment layout in one row with two treatments, PRD and control, and 7 replicates.

Mature Shiraz vines were used that grew at the Nuriootpa Research Station (Barossa Valley, South Australia). The way the vines were treated and managed is already described in Section 8.2. Treatments consisted of two irrigations, PRD and control, and three pruning levels (30, 60 and 120 nodes/vine) with 5 repetitions of each treatment.

9.2.2 Sucrose Synthase (SucSy) and invertase enzyme activity

Sucrose synthase (SucSy), Acid invertase and neutral invertase enzyme activity was assayed on 1 g of tissue using the methods described in Section 2.15.

9.2.3 Soluble sugars analysis

Sugar analyses were conducted as described in Section 2.8 by the method of Naidu (1998).

9.2.4 Amino acid analysis

Amino acid analyses were done as described in Section 2.9 by the method of Hernandez-orte (1997).

9.2.5 Free polyamine analysis

Free polyamine analyses were done as described in Section 2.10 by the method of Flores and Galston (1982).

9.3 Results

9.3.1 The effect of PRD on berry characteristics and growth

The effect of PRD on the increase in berry weight (g) and total soluble solids (TSS; °Brix) of Coombe Cabernet Sauvignon vines for the period between veraison and harvest during the 2000/1 and 2001/2 season is shown in Figure 9.1. PRD-treated Cabernet Sauvignon vines received half the amount of irrigation water as control and significantly influenced berry growth in both seasons. PRD reduced the berry size in 2001 and 2002 by 11% and 9% respectively compared to control. The reduction in berry size at harvest however did not influence the final TSS concentration between PRD-treated and control berries. During the ripening season, PRD significantly ($P<0.05$) increased the accumulation in TSS during the first 3 weeks after veraison in 2001 and in 2002 compared to control by 11% and 10% respectively. However, thereafter and until harvest PRD-treated and control berries had similar TSS values.

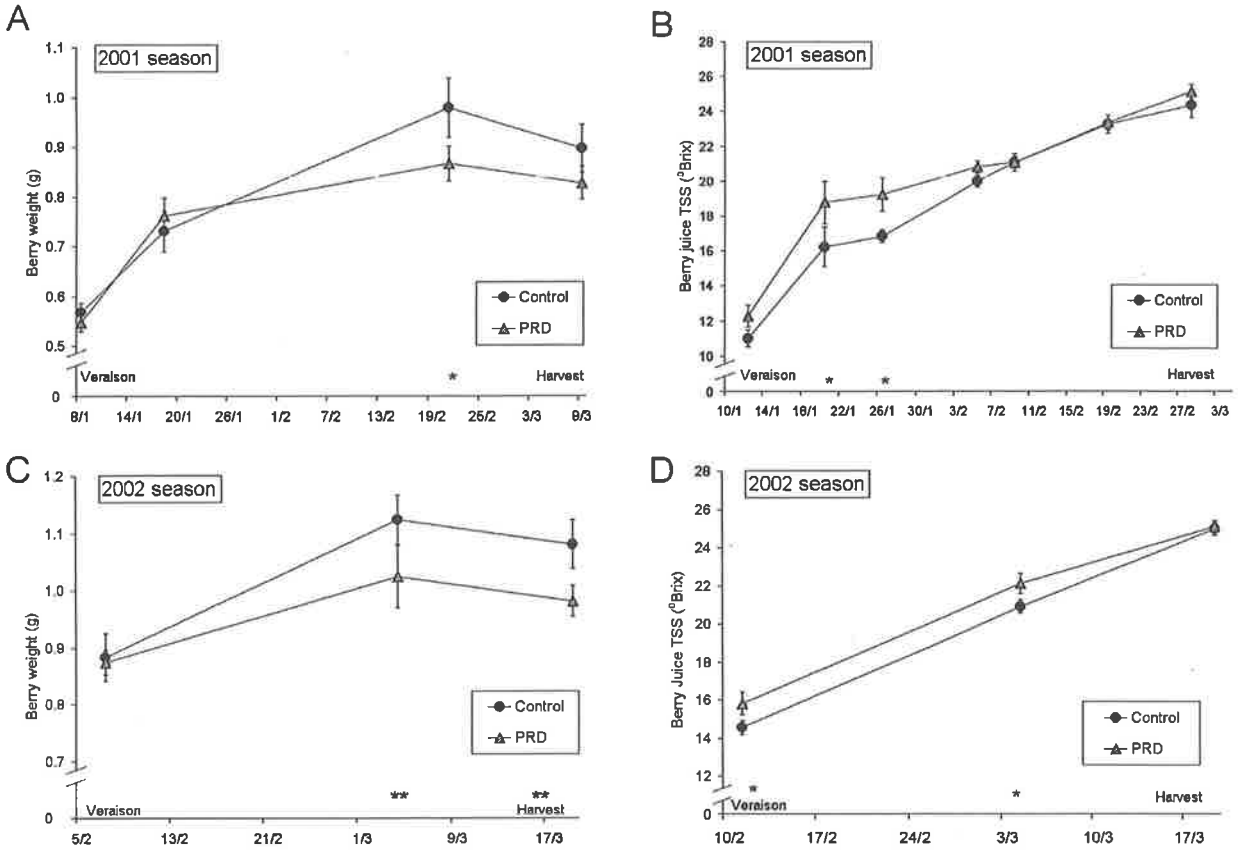


Figure 9.1 Effect of PRD on the increase in **(A+C)** berry weight (g fresh wt) and **(B+D)** evolution in TSS (°Brix) of field-grown Coombe Cabernet Sauvignon vines during the 2001 and 2002 period from veraison to harvest (PRD received half the amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significant (P<0.05); ** = significant (P<0.01)).

The effect of PRD on the increase in berry weight (g) and total soluble solids (TSS; °Brix) of Coombe Shiraz vines for the period between veraison and harvest during the 2000/1 and 2001/2 season is shown in Figure 9.2. PRD-treated Shiraz vines received the same amount of irrigation water as control and did not influence berry growth significantly in either season compared to control. PRD treatment had also no significant effect on the increase of TSS compared to control in either season.

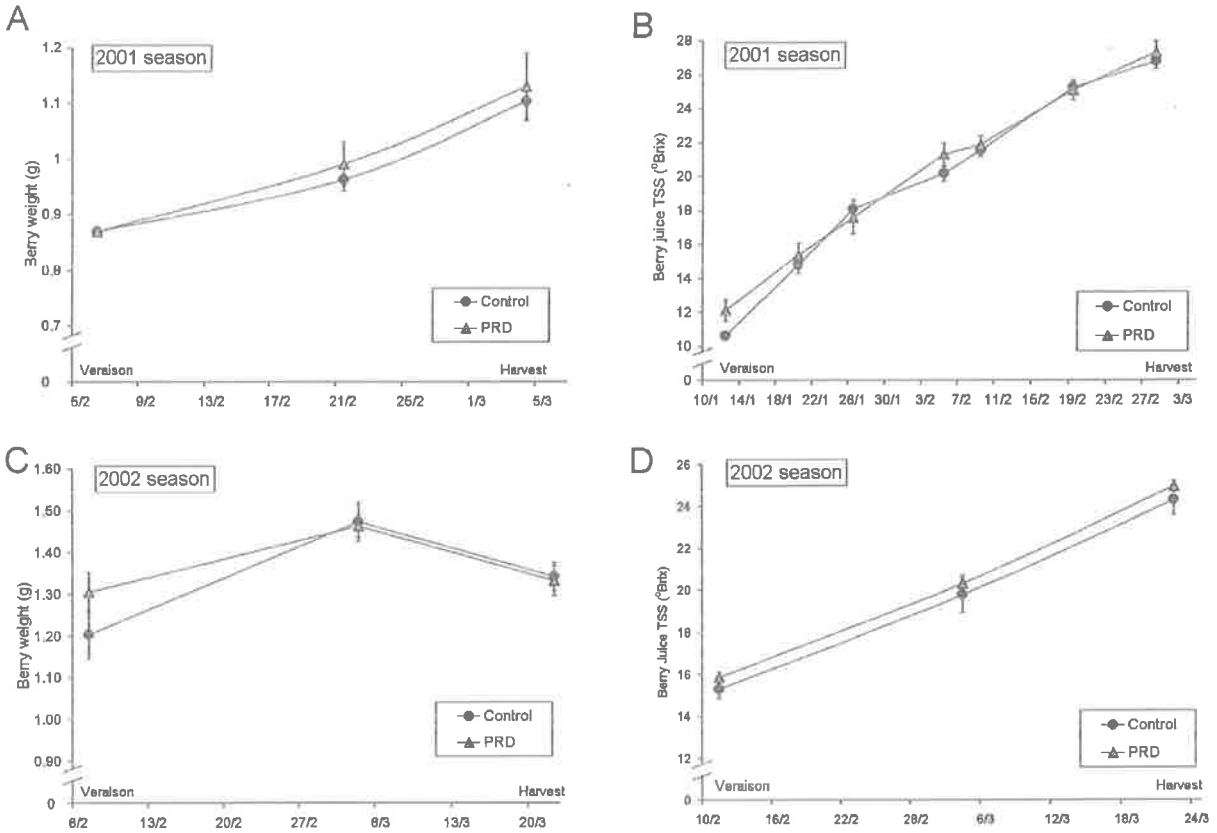


Figure 9.2 Effect of PRD on the increase in **(A+C)** berry weight (g fresh wt) and **(B+D)** evolution of TSS (°Brix) of field-grown Coombe Shiraz vines during the 2001 and 2002 period from veraison to harvest (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.).

The effect of PRD on the berry weight (g fresh wt) of Coombe Cabernet Sauvignon and Shiraz vines at veraison and at harvest in 2003 is shown in Table 9.1. PRD Cabernet Sauvignon that received half the amount of irrigation water as control showed a significant ($P<0.10$) reduction in berry weight at veraison by 10% compared to control. Ultimately, the berries of PRD vines at harvest were not significantly different. In comparison, PRD Shiraz that received the same amount of irrigation water as control vines showed no significant changes in berry weight compared to control at veraison and at harvest. Means of total soluble solids (TSS) have already been reported in Section 4.3.6. In short, the PRD treatment on both Cabernet Sauvignon and Shiraz vine had no significant effect on berry TSS at harvest compared to control berries.

Table 9.1 Berry size (g/berry) of Coombe Cabernet Sauvignon and Shiraz at harvest in 2003. (PRD Cabernet Sauvignon received half the amount of irrigation water as control by irrigating on only one side at any time; PRD Shiraz received the same amount of irrigation as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

Cabernet Sauvignon	Control	PRD	% Diff	P
Veraison	0.68 ± 0.03	0.61 ± 0.01	- 10	0.062
Harvest	0.92 ± 0.03	0.88 ± 0.01	- 4	0.537
Shiraz	Control	PRD	% Diff	P
Veraison	1.16 ± 0.03	1.17 ± 0.04	0	0.898
Harvest	1.27 ± 0.03	1.29 ± 0.05	+ 1	0.847

9.3.2 The effect of PRD on enzymes involved in carbohydrate accumulation in berries that determines sink strength

The sucrolytic enzyme activities in the berries of field-grown Coombe Cabernet Sauvignon measured at two different times during the ripening period in 2003 are shown in Table 9.2. The first time the sucrolytic enzyme activity was measured was 10 days after veraison and thereafter it was measured again just before harvest. The AI activity in berries was substantially greater than the NI activity in the berries of Cabernet Sauvignon vines. During the period shortly after veraison there was no significant effect of PRD treatment on berry sucrolytic enzyme activity compared to control, however AI activity was slightly reduced by 12% ($P<0.10$). Closer to harvest PRD-treatment had a greater effect on berry sucrolytic enzyme activity by significantly reducing SucSy and AI activity by 48% and 32% respectively compared to control. However, PRD treatment significantly ($P<0.05$) increased NI activity by 146% compared to control.

Table 9.2 Sucrolytic enzyme activity in berries of field-grown Coombe Cabernet Sauvignon grapevines (2003) measured at two different times during ripening. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

Shortly after veraison (11/02/03)	Control	PRD	% Diff	P
AI activity (mg Glu.h^{-1})	14.09 ± 0.36	12.42 ± 0.45	-12	0.056
NI activity (mg Glu.h^{-1})	1.55 ± 0.16	1.96 ± 0.24	+ 26	0.285
SucSy activity (μMol Suc.h^{-1})	0.116 ± 0.04	0.089 ± 0.05	- 24	0.689
Close to harvest (12/03/03)	Control	PRD	% Diff	P
AI activity (mg Glu.h^{-1})	6.94 ± 0.74	4.73 ± 0.37	-32	0.077
NI activity (mg Glu.h^{-1})	0.82 ± 0.23	2.01 ± 0.40	+ 146	**0.044**
SucSy activity (μMol Suc.h^{-1})	0.491 ± 0.11	0.255 ± 0.05	- 48	**0.023**

The sucrolytic enzyme activities in the berries of field-grown Coombe Shiraz as measured shortly after veraison during the ripening period in 2003 are shown in Table 9.3. There were no differences in berry growth and accumulation of TSS between PRD-treated vines and control vines during the 2000/1 and 2001/2 seasons and therefore it was expected that there would also be no significant differences in sucrolytic enzyme activities. Accordingly, PRD-treated berries showed no significant differences in AI and SucSy activities compared to control. NI activity in PRD-treated Shiraz berry was, similarly to Cabernet Sauvignon berries, significantly increased ($P<0.01$) by 116% compared to control.

Table 9.3 Sucrolytic enzyme activity in berries of field-grown Coombe Shiraz grapevines measured shortly after veraison in 2003. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

Shortly after veraison (11/02/03)	Control	PRD	% Diff	P
AI activity (mg Glu.h^{-1})	2.77 ± 0.33	3.35 ± 0.33	+ 21	0.470
NI activity (mg Glu.h^{-1})	0.37 ± 0.05	0.79 ± 0.12	+ 116	**0.005**
SucSy activity (μMol Suc.h^{-1})	0.159 ± 0.05	0.239 ± 0.03	+ 50	0.363

9.3.3 The effect of PRD on the accumulation of sugars, amino acids and polyamines in berries.

a) Sugars and NCCs

The evolution of berry sugars (sucrose, glucose and fructose) in field-grown Coombe Cabernet Sauvignon vines for the period between veraison and harvest in 2001 is shown in Figure 9.3. During the 4 weeks after veraison when PRD-treated berries had higher TSS values compared to control, PRD berries also had significantly higher glucose and fructose concentrations (20% and 27% respectively) compared to control berries. However, at harvest there were no significant differences in hexose concentration between PRD and control berries, indicating that PRD berries after veraison may have had a higher sink strength and hexose import rate, but as ripening progressed this was reduced relative to the control.

There were no significant differences in sugar contents between PRD-treated Cabernet Sauvignon berries and control berries at harvest. However, when calculated on a solute per berry basis (Table 9.4) the PRD-treated vines with significantly smaller berries had significantly lower ($P<0.05$) glucose and fructose contents by 7% and 8% respectively compared to control berries.

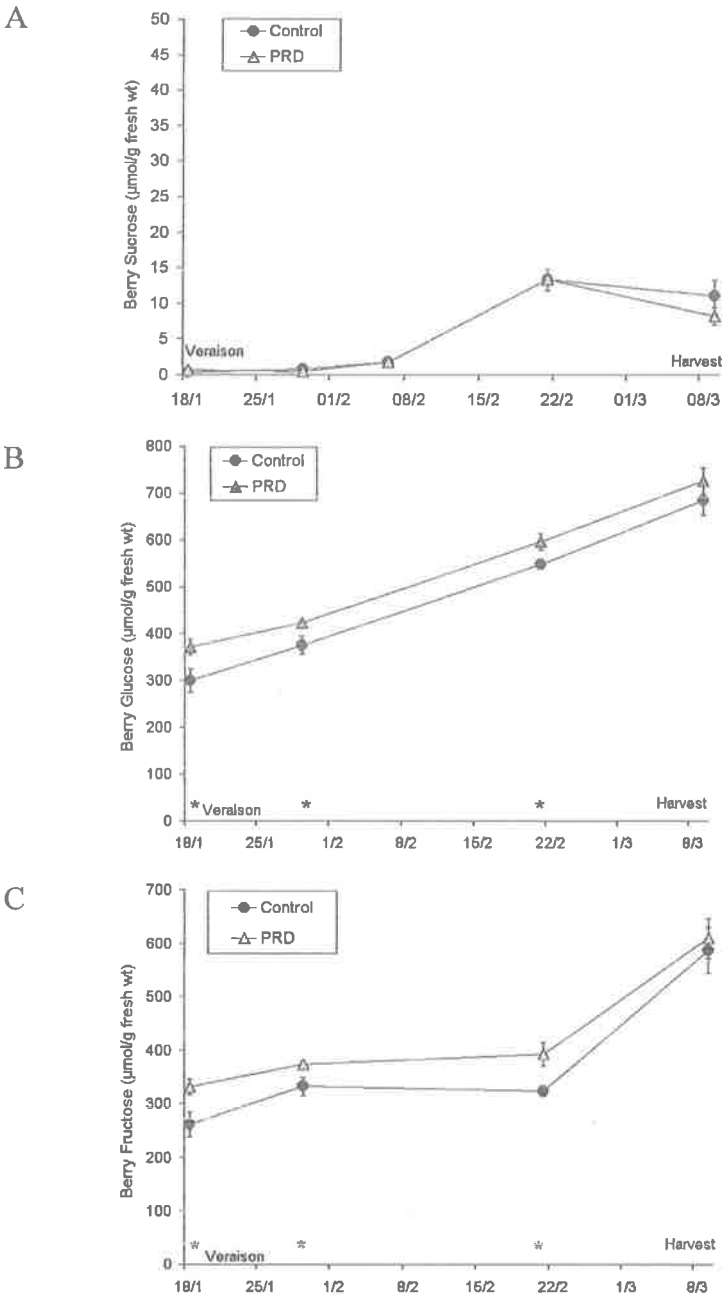


Figure 9.3 The evolution in berry **(A)** sucrose, **(B)** glucose and **(C)** fructose concentration (µMol/g fresh wt) of field-grown Coombe Cabernet Sauvignon vines during the 2000/1 season from veraison to harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different ($P<0.05$))

Table 9.4 Sugars and NCCs (μMol per berry) in berries of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	2.32 ± 0.53	3.00 ± 0.58	+ 30	0.9499
Glucose	565.0 ± 21.1	523.2 ± 9.92	- 7	**0.0381**
Fructose	503.7 ± 18.1	462.2 ± 6.62	- 8	**0.0331**
Glycine betaine	1.16 ± 0.17	1.19 ± 0.17	+ 2	0.7734
Hydroxy-Me-Proline	4.11 ± 0.77	2.82 ± 0.54	- 31	0.3954
Methyl Proline	0.66 ± 0.11	0.61 ± 0.06	- 8	0.7682
Proline	25.21 ± 1.9	24.60 ± 0.86	- 2	0.5832

The evolution in berry NCC concentration (glycine betaine, methyl-proline and proline) of the field-grown Coombe Cabernet Sauvignon vines for the period between veraison and harvest in 2001 is shown in Figure 9.4. No significant differences could be found in the concentrations of glycine betaine or methyl-proline of PRD berries compared to control. Proline is one of the major amino acids in ripening berries and accumulates to large quantities in red varieties. PRD-treated Cabernet Sauvignon berries showed a significantly higher accumulation of proline per gram fresh weight compared to control during the 2000/1 season. PRD berries had significantly higher ($P<0.05$) concentrations of proline both at veraison and at harvest by 64% and 12% respectively compared to control berries. However, when the NCC content was calculated on a per berry basis there were no significant differences between PRD-treated and control berries (Table 9.4).

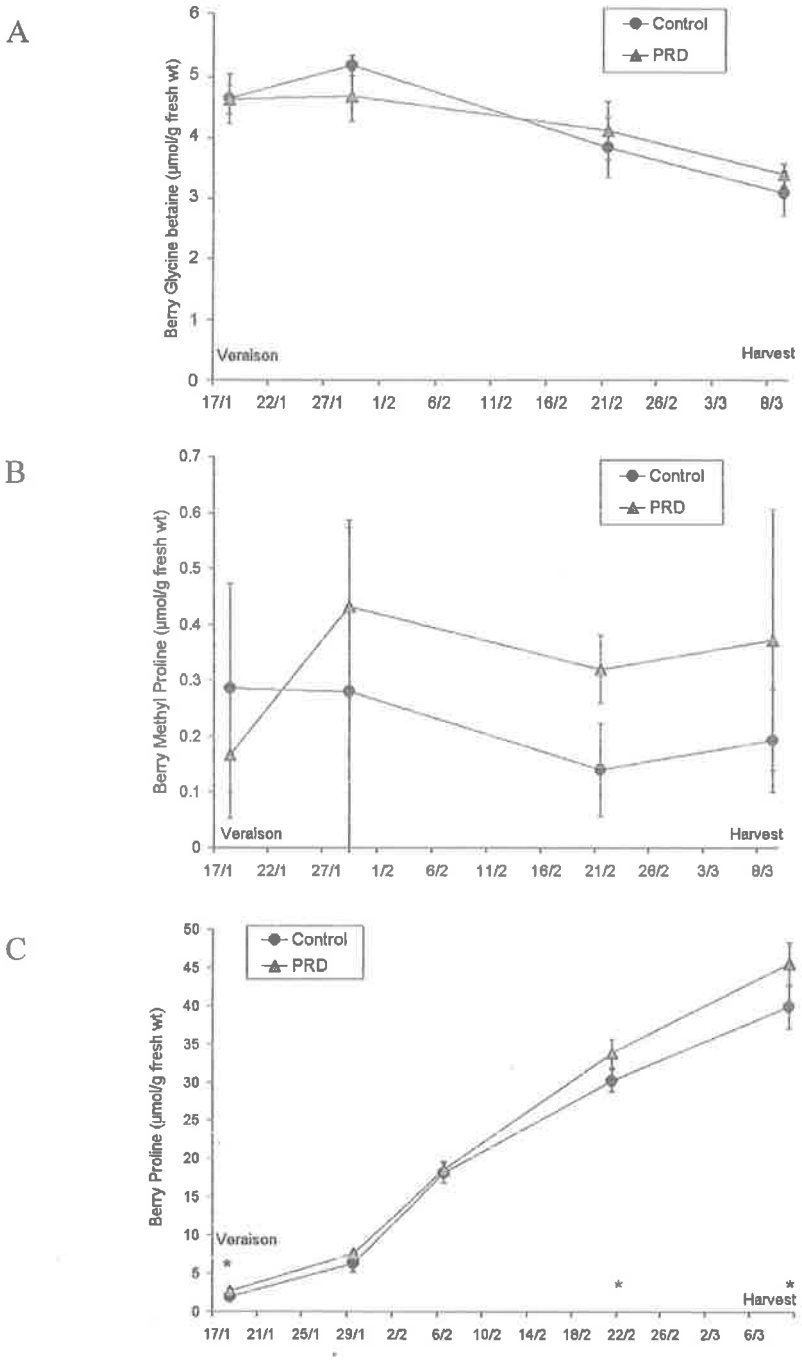
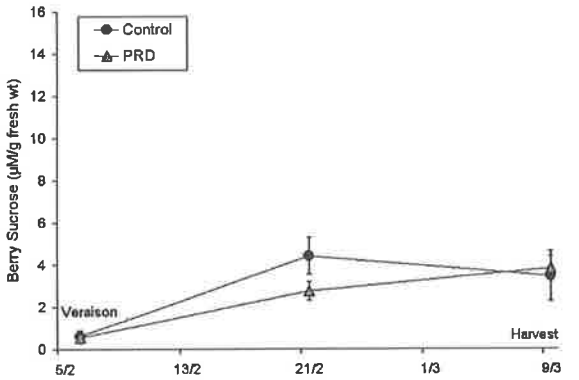


Figure 9.4 The evolution in berry NCC **(A)** glycine betaine, **(B)** methyl proline and **(C)** proline concentration (µMol/g fresh wt) of field-grown Coombe Cabernet Sauvignon vines during the 2000/1 season from veraison to harvest (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different ($P<0.05$)).

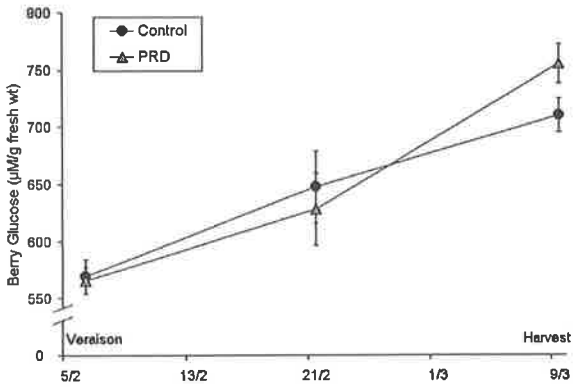
The evolution in berry sugars (sucrose, glucose and fructose) in field-grown Coombe Shiraz vines for the period between veraison and harvest in 2001 is shown in Figure 9.5. PRD Shiraz vines received the same amount of irrigation water as control vines, but only on one side at any time. Both PRD and control berries showed large increases in glucose and fructose concentration from veraison until harvest while the sucrose concentration remained very low in

comparison. PRD-treatment, as the TSS results indicated, did not affect the sugar concentrations in berries of sucrose, glucose or fructose compared to control.

A



B



C

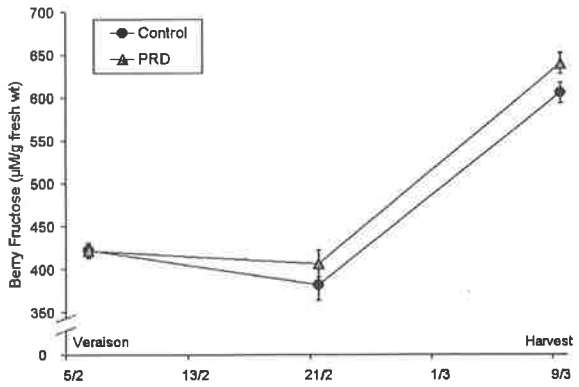


Figure 9.5 The evolution in berry **(A)** sucrose, **(B)** glucose and **(C)** fructose concentration (µMol/g fresh wt) of field-grown Coombe Shiraz vines during the 2000/1 season from veraison to harvest (PRD received the same amount of irrigation water as control but only on one side at any time; control received water on

The evolution in berry NCC concentration (glycine betaine, hydroxy-methyl-proline, methyl-proline, proline) during the same period is shown in Figure 9.6. Similar to berry sugars PRD-treatment in 2001 had no effect on the evolution of NCC concentration in berries compared to control. Furthermore, there were no significant differences in sugar and NCC content on a per berry basis (data not shown) at harvest in 2001, because there was no significant difference in berry weight at harvest between PRD-treated and control in Coombe Shiraz berries.

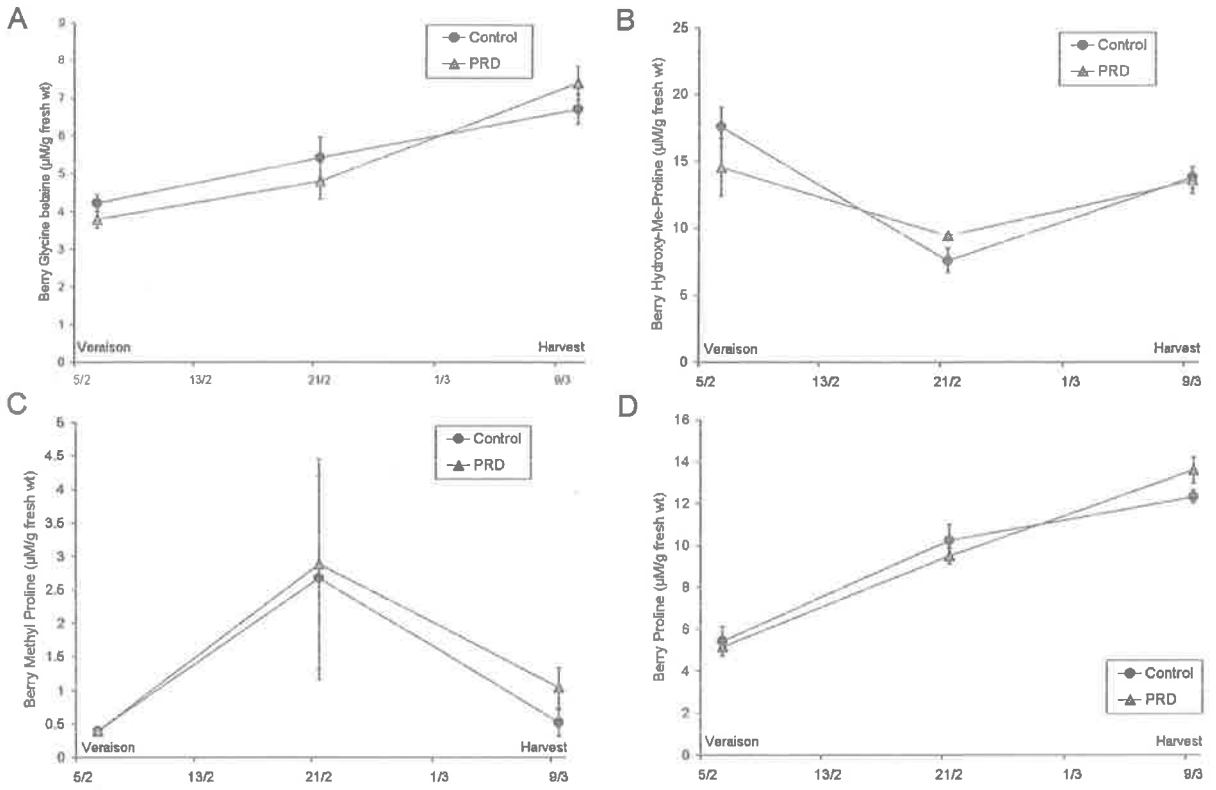


Figure 9.6 The evolution in berry NCC **(A)** glycine betaine, **(B)** hydroxy-methyl-proline, **(C)** methyl proline and **(D)** proline concentration (µMol/g fresh wt) of field-grown Coombe Shiraz vines during the 2000/1 season from veraison to harvest (PRD received the same amount of irrigation water as control, but only on one side at any time; control received water on both sides; means n = 7 ± s.e.).

The concentrations of sugars and NCCs in berries (µMol/g fresh wt) of field-grown Coombe Cabernet Sauvignon vines at harvest in 2002 are shown in Table 9.5. There were no significant differences in sugar or NCC concentrations between PRD-treated berries and control berries at harvest. However, when calculated on a solute per berry basis (Table 9.6) the PRD-treated berries had lower calculated contents of all solutes compared to control due to the fact that PRD had significantly smaller berries at the 2002 harvest. PRD berries had lower contents of glucose and glycine betaine ($P<0.10$) per berry by 17% and 39% respectively compared to control. Furthermore, PRD-treated berries showed a significantly lower ($P<0.05$) content of proline by 19% compared to control.

Table 9.5 Sugars and NCCs (μMol/g fresh wt) in berries of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	17.5 ± 2.3	16.1 ± 0.9	- 8	0.578
Glucose	555.4 ± 30.8	501.4 ± 45.6	- 10	0.339
Fructose	396.1 ± 28.1	357.6 ± 36.6	- 10	0.428
Mannitol	13.0 ± 6.8	11.5 ± 5.7	- 11	0.761
Hydroxy-Me-Proline	1.7 ± 1.2	3.0 ± 2.8	+ 76	0.481
Glycine betaine	18.1 ± 4.6	12.6 ± 2.9	- 30	0.134
Proline	23.6 ± 1.5	20.9 ± 2.0	- 12	0.177

Table 9.6 Sugars and NCCs (μM per berry) in berries of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	19.4 ± 3.1	15.8 ± 0.8	-19	0.238
Glucose	600.0 ± 38.8	496.5 ± 53.0	-17	**0.086**
Fructose	427.3 ± 32.7	354.3 ± 41.5	-17	0.154
Mannitol	35.7 ± 6.9	26.7 ± 2.0	-25	0.543
Hydroxy-Me-Proline	8.0 ± 0.6	6.9 ± 0.1	-14	0.117
Glycine betaine	23.8 ± 4.6	14.6 ± 2.2	-39	**0.074**
Proline	25.4 ± 1.5	20.7 ± 2.4	-19	**0.019**

The concentrations of sugars and NCCs in berries (μMol/g fresh wt) of field-grown Coombe Shiraz vines at harvest in 2002 are shown in Table 9.7. Contrary to the Cabernet Sauvignon berries, the PRD-treated Shiraz berries showed no significant differences compared to control berries in the concentration of sugars and NCCs at the 2002 harvest. This is however not surprising because there were no significant differences in berry growth and evolution in TSS during the 2002 ripening period from veraison until harvest.

Table 9.7 Sugars and NCCs (μMol/g fresh wt) in berries of field-grown Coombe Shiraz grapevines at harvest in 2002. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

	Control	PRD	% Diff	P
Sucrose	13.7 ± 1.3	13.9 ± 3.0	+ 1	0.738
Glucose	573.2 ± 26.0	623.5 ± 21.7	+ 9	0.492
Fructose	413.7 ± 19.2	450.8 ± 11.2	+ 9	0.310
Mannitol	13.5 ± 1.6	13.1 ± 4.7	- 3	0.846
Hydroxy-Me-Proline	22.2 ± 5.7	20.1 ± 6.1	- 9	0.961
Glycine betaine	10.3 ± 2.3	8.3 ± 0.6	- 20	0.399
Proline	19.5 ± 2.3	22.2 ± 3.2	+ 14	0.144

The concentrations of sugars and NCCs in berries (μMol/g fresh wt) of field-grown Nuriootpa Shiraz vines at harvest in 2001 are shown in Table 9.8. PRD-treated vines received half the amount of irrigation water as control and both PRD and control vines were treated with three different levels of pruning (30, 60 or 120 nodes/vine). It has already been shown that PRD treatment had no significant effect on berry weight or TSS at harvest compared to control (Section 4.3.6), however pruning level significantly influenced both berry weight and TSS. It was found that Shiraz vines with a higher number of nodes per vines had significantly smaller berries and lower TSS. It was therefore not surprising that a significant effect of pruning level existed in both the glucose (P=0.002) and fructose (P=0.007) concentrations of Nuriootpa Shiraz berries with the higher number of nodes/vines treatment having the lowest concentrations in glucose and fructose.

No main effect of irrigation treatment existed in the berry glucose and fructose concentrations, however individual pruning level analyses showed that vines pruned to 60 nodes/vine had a significant increase (P<0.05) in glucose and fructose concentration in PRD-treated berries by 6% and 4% respectively compared to control at harvest. This increase could not be explained by a decrease in berry size because there was no significant difference between PRD and control berries at the 60 nodes/vine pruning level. Therefore the hexose accumulation in PRD-treated Shiraz pruned to 60 nodes/vine measured as per berry content was also significantly higher in glucose (P=0.037) and fructose (P=0.087) by 6% and 4% respectively compared to control (data not shown). PRD-treated vines pruned to 120 nodes/vine however showed an average reduction in berry weight of 15% compared to control and therefore the measured glucose (P=0.037) and fructose (P=0.048) accumulation measured on a per berry content basis

were significantly lower in PRD-treated berries by 20% and 19% respectively compared to control (data not shown).

No significant effect of irrigation or pruning level could be found for the NCC concentrations of hydroxy-methyl-proline, methyl proline, glycine betaine or proline. No significant effect of PRD treatment on berry NCC accumulation could also be found on a per berry content basis (data not shown) irrespective of pruning level.

Table 9.8 Sugars and NCCs (μMol/g fresh wt) in berries of field-grown Nuriootpa Shiraz grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 5 ± s.e.)

Sucrose	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	5.5 ± 2.4	13.3 ± 3.4	+ 144	0.376
60 nodes/vine	11.9 ± 1.4	6.7 ± 3.0	- 44	0.199
120 nodes/vine	8.4 ± 2.4	6.7 ± 1.3	- 20	0.461
Glucose	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	619.6 ± 22.8	618.6 ± 7.5	0	0.649
60 nodes/vine	592.8 ± 14.4	628.4 ± 20.5	+ 6	0.049
120 nodes/vine	562.2 ± 21.5	530.0 ± 22.5	- 6	0.464
Fructose	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	558.7 ± 17.6	567.2 ± 6.5	+ 2	0.763
60 nodes/vine	546.4 ± 12.5	570.0 ± 16.8	+ 4	0.044
120 nodes/vine	519.4 ± 20.4	496.7 ± 21.1	- 4	0.580
Hydroxy-Me-Pro	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	12.1 ± 5.7	15.4 ± 4.8	+ 27	0.6900
60 nodes/vine	11.7 ± 2.9	8.8 ± 1.4	- 24	0.5158
120 nodes/vine	6.0 ± 2.2	5.3 ± 3.5	- 11	0.1828
Glycine betaine	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	7.0 ± 1.1	5.3 ± 0.4	- 24	0.3897
60 nodes/vine	5.0 ± 0.6	4.7 ± 0.3	- 6	0.6570
120 nodes/vine	5.8 ± 1.2	4.3 ± 0.6	- 26	0.3421
Proline	**Control**	**PRD**	**% Diff**	**P**
30 nodes/vine	10.4 ± 0.6	9.6 ± 1.4	- 7	0.2328
60 nodes/vine	7.5 ± 0.8	9.3 ± 0.8	+ 23	0.3000
120 nodes/vine	5.7 ± 0.5	6.7 ± 0.9	+ 17	0.7117

b) Amino acids

The evolution in the concentration of free amino acids (nMol/g fresh wt) of Coombe Cabernet Sauvignon berries during the ripening period in 2001 is shown in Table 9.9. There were five major amino acids in Cabernet Sauvignon berries when the grapes were harvested: proline, arginine, histidine, tyrosine, methionine and valine. Between them they accounted for approximately 90% of the total amino acid content. Proline and arginine concentration were the highest, accounting for about 75% of the total free amino acid concentration. PRD treatment of Coombe Cabernet Sauvignon vines had no significant effect on berry arginine concentration at harvest compared to control, however there was a significant increase ($P<0.05$) in proline concentration by 12% compared to control. As the proline and arginine concentration rose during ripening the total concentration of amino acids also steadily increased until harvest. The PRD-treated Cabernet Sauvignon berries at harvest had a significantly higher ($P<0.05$) amino acid concentration compared to control due to higher concentrations in the major amino acids, mainly proline and arginine. The significant difference in proline concentration between PRD and control berries however disappeared when amino acids were calculated on a per berry basis (data not shown) because PRD-treatment significantly reduced the berry size at harvest in 2001. On a per berry basis, PRD-treated Cabernet Sauvignon berries, although significantly smaller at harvest, did not show a significantly reduced per berry content in any individual amino acid compared to control berries except for methionine that was already 34% lower in concentration compared to control.

Table 9.9 The evolution in the concentration of free amino acids (nMol/g fresh wt) in Coombe Cabernet Sauvignon berries during the ripening period in 2001 (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7; [a] = significantly different (P<0.10); * = significantly different (P<0.05)).

Days after veraison	0		12		21		36	
	Control	PRD	Control	PRD	Control	PRD	Control	PRD
Proline	18801	17448	19162	19212	21236	28559 [a]	22120	24849 *
Arginine	1227	1254	1552	1085 [a]	7837	8373	9020	9378
Histidine	1439	1664	1602	1578	2744	2880	2430	2396
Tyrosine	984	1194	1271	951	2092	1856	1834	1863
Methionine	223	301	378	118	869	759	1189	780 *
Glutamic acid	474	509	285	346	759	689	268	218
Valine	297	520	191	73	1347	1381	747	1026
Asparagine	373	379	231	270	227	196	651	556
Threonine	282	335	385	329	426	447	460	435
Leucine	302	267	331	362	383	367	423	467
Aspartic Acid	299	300	100	118	338	298	47	29
Alanine	324	347	365	266 *	219	224	73	76 *
Glycine	50	61	29	0	185	195	194	197
Hydroxy-L-Pro	52	59	56	58	153	126	73	76 *
Glutamine	1458	1294	1445	1424	230	313	147	149
Isoleucine	36	52	52	49	109	105	132	137
Cystine	1213	503	795	367	378	231	74	141
Norleucine	0	6	0	12	0	16	49	27
Serine	5236	3990	6267	6569	53	70	46	43
Tryptophan	5	19	49	0	44	19	46	52
Phenylalanine	3	9	27	7	22	24	36	30
Lysine	14	11	41	13	19	29	23	16
Total	33973	31516	36461	36280	38455	39026	40996	44256 *

The concentrations of free amino acids (nMol/g fresh wt) in Coombe Cabernet Sauvignon berries at veraison and harvest in 2002 are shown in Table 9.10. PRD-treated berries showed significant changes (P<0.10) in only three amino acids during the 2001/2 season. Aspartic acid decreased by 35% compared to control while valine and isoleucine increased by 18% and 52% respectively compared to control. Proline and arginine had the highest concentrations in Cabernet Sauvignon berries and they accounted for approximately 85% of the total amino acid content in control berries and 87% in PRD berries at harvest. There were no significant

differences in the arginine and proline concentrations between PRD-treated and control berries at harvest.

Table 9.10 The concentration of free amino acids (nMol/g fresh wt) in Coombe Cabernet Sauvignon berries during veraison and harvest in 2002 (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7; [a] = significantly different (P<0.10)).

	Veraison		Harvest	
	Control	PRD	Control	PRD
Proline	5053	6565	52647	72612
Arginine	2300	2364	11835	11838
Histidine	611	674	2437	2087
Glycine	1815	1790	1407	1360
Tyrosine	571	492	1214	1170
Glutamine	404	482	1053	950
Serine	464	490	983	885
Threonine	572	597	943	879
Leucine	420	363	886	836
Alanine	1514	1659	906	787
Cystine	242	369	474	628
Aspartic acid	412	411	921	603 [a]
Valine	273	295	511	602 [a]
Norleucine	159	183	564	475
Methionine	187	232	468	447
Tryptophan	179	204	343	325
Glutamic acid	650	620	148	227
Phenylalanine	263	381	158	218
Isoleucine	173	260	124	188 [a]
Hydroxy-L-pro	27	41	129	157
Asparagine	167	190	70	98
Total	16459	18669	75469	97373

The evolution in the concentration of free amino acids (nMol/g fresh wt) of Coombe Shiraz berries during the ripening period in 2001 is shown in Table 9.11. There were four major amino acids in Shiraz berries when the grapes were harvested in 2001: proline, arginine, alanine and tyrosine. Between them they accounted for approximately 82% of the total amino acid content in PRD and control berries. Proline and arginine concentration were the highest, accounting for about 75% of the total free amino acid concentration, however, no significant differences in most individual amino acids or in total amino acid concentrations could be found between PRD

treated and control berries. PRD-treated Shiraz berries showed significant reductions in minor amino acids only for leucine and histidine by 19% and 14% respectively compared to control.

Table 9.11 Amino acid concentration (nMol/g fresh wt) in berries of field-grown Coombe Shiraz grapevines during the 2000/1 season. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different (P<0.05).

Days after veraison	0		19		35	
	Control	PRD	Control	PRD	Control	PRD
Proline	8691	9603	15519	16373	53627	45002
Arginine	5230	4969	5727	5582	5001	5346
Alanine	3820	3782	5185	5079	5883	5839
Tyrosine	2974	3243	3615	3902	4294	4547
Threonine	1411	1371	1435	1428	1559	1507
Leucine	1332	1443	1077	1311	2118	1711 *
Methionine	1112	1255	1753	1520	1503	1471
Serine	822	785	688	729	800	790
Glutamine	809	666	668	651	951	615
Valine	794	912	1580	1390	1005	1145
Aspartic acid	758	667	242	435 *	346	216
Cystine	719	784	1221	919 *	642	762
Histidine	680	652	626	656	1022	879 *
Glycine	329	321	296	297	655	533
Norleucine	327	260	156	195	594	485
Glutamic acid	213	302 *	206	169	0	0
Tryptophan	190	158	111	144	285	250
Isoleucine	142	147	239	207	220	209
Asparagine	95	105	113	121	30	27
Phenylalanine	49	63	97	80	4	8
Lysine	27	46	80	75	2	6
Hydroxy-L-pro	27	26	59	46	68	66
Total	30186	32240	41283	41277	83857	73285

The concentrations of free amino acids (nMol/g fresh wt) in Coombe Shiraz berries at veraison and harvest in 2002 are shown in Table 9.12. PRD-treated berries showed a significant reduction (P<0.10) in only threonine by 16% compared to control at the 2002 harvest. There were five major amino acids in Shiraz berries when the grapes were harvested in 2002: proline, arginine, histidine, glycine and tyrosine. Between them they accounted for approximately 69% and 77% of the total amino acid content in PRD and control berries respectively. Proline and

arginine had the highest concentrations in Shiraz berries and they accounted for approximately 61% of the total amino acid content in control berries and 64% in PRD berries at harvest. There were no significant differences in the arginine and proline concentrations between PRD-treated and control berries at harvest.

Table 9.12 The concentration of free amino acids (nMol/g fresh wt) in Coombe Shiraz berries during veraison and at harvest in 2002. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; [a] = significantly different (P<0.10)).

	Veraison		Harvest	
	Control	PRD	Control	PRD
Proline	1288	1261	19502	19847
Arginine	1272	1184	3352	3170
Histidine	463	374 [a]	2178	2049
Glycine	621	513	1535	1384
Tyrosine	205	193	1306	1335
Threonine	474	455	1181	990 [a]
Leucine	238	225	1168	1198
Serine	356	328	1078	931
Aspartic acid	343	340	1067	926
Glutamine	173	160	977	836
Methionine	88	84	743	592
Alanine	628	623	648	486
Norleucine	116	121	644	564
Cystine	101	89	510	470
Valine	61	76	407	339
Tryptophan	104	98	354	327
Phenylalanine	105	97	286	262
Glutamic acid	253	222	211	150
Hydroxy-L-pro	13	14	126	116
Isoleucine	52	55	104	104
Asparagine	174	92 [a]	99	88
Total	7072	6604	37418	36121

c) Polyamines

The polyamine concentration (μMol/g fresh wt) of field-grown Coombe Cabernet Sauvignon and Shiraz berries at harvest in 2002 is shown in Figure 9.7. PRD treatment did not influence the free polyamine concentration in harvested grapes for either Cabernet Sauvignon that received half the amount of irrigation water as control or Shiraz that received the same amount of irrigation water as control.

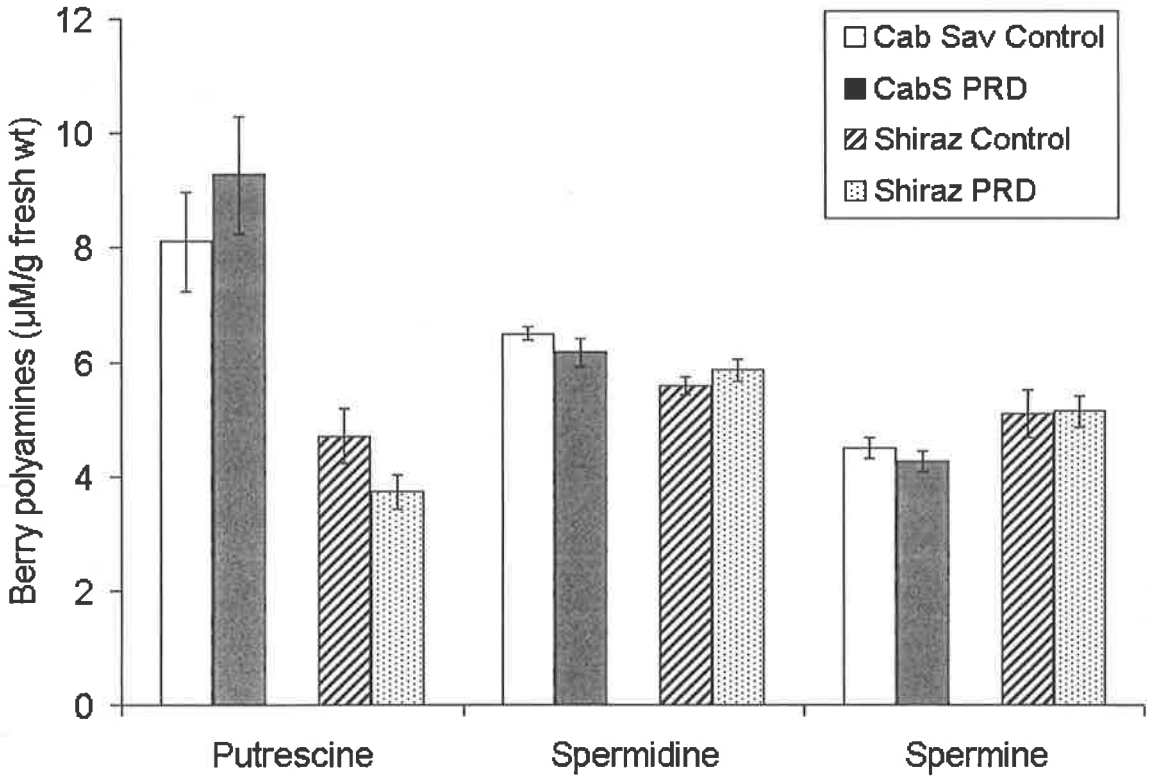


Figure 9.7 The polyamine contents of berries of Coombe Cabernet Sauvignon and Shiraz at harvest in 2002. Cabernet Sauvignon PRD vines received half the amount of irrigation water as control by irrigating on only one side at any time; PRD Shiraz received the same amount of irrigation water as control on only one side at any time; Control vines received water on both sides at the same time; means n = 7 ± s.e.)

9.4 Discussion

Results of experiments in this chapter have provided evidence that PRD affected the accumulation of sugars and nitrogenous compounds in berries by affecting the berry size and the activities of the sucrolytic enzymes that are involved in hexose accumulation. The reduction in irrigation water volume in PRD-treated Cabernet Sauvignon vines compared to control vines had a significant effect on the growth and maturation of berries. It could be concluded from the early increases in berry TSS that maturation rate of PRD-treated berries was faster compared to control during the period shortly after veraison. However, after 4 weeks the growth in PRD-treated berries was significantly lower compared to control and the maturation seemed to be slowed during the last 4 weeks until harvest compared to control. At harvest PRD-treated Cabernet Sauvignon had significantly smaller berries but similar TSS compared to control berries. This difference in berry maturation was not found in PRD Shiraz that received the same amount of irrigation water as control. PRD Shiraz showed no significant differences in berry maturation or growth compared to control in either the 2000/1 or 2001/2 season. It was therefore concluded that the PRD effect on berry maturation was mainly due to the amount of water applied and/or to some degree of water stress during the latter part of the season.

The sink strength of ripening berries can be described by hexose storage activity (Ollat and Gaudillere, 1996). The major hexoses that accumulate in Cabernet Sauvignon and Shiraz berries are glucose and fructose with very little sucrose. It is therefore assumed that although present, the SPS activity in berries would be very low and insignificant in comparison to the SucSy and invertase activities and the role they play in the accumulation of hexose. The enzymes principally responsible for the formation of glucose and fructose by the hydrolysis of sucrose are invertase and SucSy. Sucrose is imported from the phloem and rate at which it can be hydrolyzed will determine sink hexose storage activity and strength (Sturm, 1999). This is the basis of one of three hypotheses proposed to explain the sink strength of developing berries by Coombe (1992) and is supported by the work done by Coombe et al. (1987) and Düring and Alleweldt (1984). However, other work (Hawker, 1969a; Coombe, 1989) suggested that invertase activity exists before the actual commencement of hexose accumulation in berries. Another possible hypothesis is that of Lang et al. (1986) that the movement of phloem sap into the berry is a result of the breakdown apoplast/symplast compartmentation in pericarp cells during ripening.

Hawker (1969a) reported that SucSy activity is substantially lower than the invertases activity in ripening berries and associated with active transport of sugar in the tonoplast (Hawker, 1985). In this study, berries of Cabernet Sauvignon grapevines showed a substantially greater AI activity compared to SucSy and NI activities in both PRD and control treatments. However, the AI activity decreased from veraison to harvest by more than 50% while the SucSy and NI activities at harvest respectively increased or stayed relatively the same compared to veraison activities. PRD treatment of Cabernet Sauvignon vines affected the sucrolytic enzyme activities in berries, as measured shortly after veraison and at harvest, by reducing the AI and SucSy activities on both occasions compared to control. At the same time, the NI activity in PRD-treated berries of Cabernet Sauvignon vines was greatly increased compared to control. The berry sucrolytic enzyme activity in PRD Shiraz berries however showed no significant differences in AI and SucSy activity compared to control, but similar to Cabernet Sauvignon greatly increased the NI activity compared to control. It is therefore concluded that a) the AI and SucSy activities was principally affected by the reduction of irrigation water applied and b) the NI activity was increased by PRD irrespective of the amount of irrigation water applied.

It is expected that the AI and SucSy activity would be correlated with berry growth because AI would hydrolyse sucrose at the same rate as phloem water influx into the berry and SucSy activity would depend on the rate of wall synthesis and the transport of sucrose over the tonoplast, both of which would be decreased with a decrease in berry growth rate. The function of NI activity is still unclear, but reported to be localized in the cytosol, highly substrate specific for sucrose (Sturm *et al.*, 1999) and proposed to channel sucrose into metabolism (Sturm and Tang, 1999; Winter and Huber, 2000). Furthermore, in conditions where AI and SucSy activities are low, the NI is considered to be a 'maintenance' enzyme that is involved in sucrose degradation. It is therefore hypothesized that the increase in NI activity in response to PRD-treatment serves as a way to increase the metabolic function of berries in response to the ABA signal induced in response to PRD (Stoll *et al.*, 2000). Whether or not this increase in NI activity in response to PRD has any effect on berry metabolism and the production of amino acids, phenolics, anthocyanins, etc. is still unknown.

Whether the principal factor that determines berry sink strength is a breakdown of apoplast/symplast compartmentation or acid invertase activity, sugar accumulation is still ultimately the measure of berry development and sink strength. PRD treatment of Cabernet

Sauvignon that received half the amount of irrigation as control significantly increased the hexose and NCC concentrations during the ripening period of 2001 compared to control. Glucose and fructose concentrations were significantly higher in PRD-treated berries until before harvest. However, at harvest there were no significant differences in hexose concentration, and on a per berry basis the hexose content was actually significantly reduced compared to control on average by 8%. The same response in berry hexose accumulation was found during the PRD treatment of Cabernet Sauvignon in 2002. Although the differences in glucose and fructose concentrations were insignificant the PRD-treated berries had significantly lower hexose content per berry at harvest in 2002. The implication is that PRD-treated vines that received half the amount of irrigation water as control vines reduced the sink strength of their fruit as measured in the accumulation of sugars at harvest. This may be evidence of reduced sink strength in PRD-treated Cabernet Sauvignon berries compared to control. PRD-treated Cabernet Sauvignon berries in 2001 also showed an increased proline and glycine betaine concentrations compared to control at harvest, but on per berry basis were not significantly different. In 2002 however, the concentrations of proline and glycine betaine in PRD berries were lower on average and on a per berry basis the contents were significantly lower compared to control.

PRD-treated Shiraz vines received the same amount of irrigation water as control and as the evolution in TSS indicated, PRD treatment did not significantly affect the hexose concentration compared to control during the ripening period between veraison and harvest in either the 2000/1 or 2001/2 season. Because PRD-treatment did not affect final berry size at harvest, the hexose per berry content was also unaffected. This may indicate that the reduction in hexose accumulation in Cabernet Sauvignon berries compared to control was due to the reduction in irrigation water not due to PRD. However, PRD treatment of Nuriootpa Shiraz that also received half the amount of irrigation water showed that PRD-treatment in vines pruned to 60 nodes/vine (a pruning level typical for this region) significantly increased the hexose accumulation on a concentration and on a per berry basis compared to control. However, pruning to a higher number of nodes per vine increased the sink demand in shoots and reduced the berry size under PRD conditions. Consequently the amount of hexose accumulated per berry in PRD-treated vines pruned to 120 nodes/vine was significantly less compared to control. It is therefore concluded that environmental factors and cultural practices may have a

strong influence over the PRD effect on berry size and sink strength. This may be an area of future research to determine the effect PRD under different terroir and cultural practices.

Nitrogenous compounds are second only to sugars in importance as a nutrient for yeast. Of the 22 free amino acids measured in this study during the period between veraison and harvest in 2001 there were 3 groups of amino acids in Cabernet Sauvignon berries that could be classified by their behaviour: Group 1, amino acids that increased towards the end of the ripening period, and although some had ups and downs, they constituted the largest part of the total amino acid profile (proline, arginine, histidine, tyrosine, methionine and valine); Group 2, amino acids that decreased toward the end of ripening (alanine, glutamine, cysteine and serine); and Group 3, the remaining amino acids the concentration of which remained more or less stable throughout ripening. The predominant amino acids found in Cabernet Sauvignon berries were proline and arginine. Stines *et al.* (2000) found that PRD in Cabernet Sauvignon vines had no significant effect on free proline in the berries compared to control. Their result supported the notion that water stress and salt stress do not influence proline content in grape berries (Downton and Loveys, 1978; Coombe and Monk, 1979). Results from this study however showed that PRD-treated Cabernet Sauvignon berries accumulated proline to significantly higher concentrations compared to control in the later stages of ripening. Together with slightly higher arginine concentrations the PRD-treated berries had a significantly higher total amino acid concentration at harvest in 2001 compared to control. The same response in amino acid accumulation in berries to PRD treatment was found during the 2001/2 season, but greater variation in data reduced the significance levels. Calculated on a per berry basis the amino acid content of PRD-treated berries showed no significant differences compared to control in either the 2000/1 or the 2001/2 season. However, irrespective of berry size, the final must concentration of PRD Cabernet Sauvignon berries would have a higher concentration of amino acids and no danger of stuck fermentations. The question of how the changes in amino acid profile would change the fermentation bouquet of PRD musts compared to control fell beyond the scope of this study.

Similar to results for Cabernet Sauvignon berries, there were 3 groups of amino acids in Shiraz berries that could be classified by their behaviour: Group 1, amino acids that increased towards the end of the ripening period (proline, arginine, tyrosine, glycine, histidine, methionine and valine); Group 2, amino acids that decreased toward the end of ripening (phenylalanine, asparagine and glutamine); and Group 3, the remaining amino acids for which concentration

remained more or less stable throughout ripening. PRD Shiraz vines that received the same amount of irrigation water as control vines showed no significant differences in the berry concentrations of the major amino acids at harvest in either the 2001 or the 2001/2 season compared to control. Although there were some significant decreases in minor amino acids the amino acid concentrations in total showed no significant differences.

In conclusion, the significant increase in berry amino acid concentration in response to PRD treatment may be solely due to the reduced amount of water applied compared to control and the reducing effect on berry size that probably caused increased concentration of amino acids. PRD had therefore no significant effect on the amino acid production and accumulation per berry compared to control.

Investigation into berry free polyamine concentration showed that there were no significant differences between PRD-treated and control berries irrespective of the amount of irrigation water applied.

9.5 Conclusions

The experiments conducted in this chapter were conducted to test the hypothesis that PRD increases the sugar and amino acid content due its effects on sucrolytic enzyme activity and/or berry size. Not enough evidence existed to support this hypothesis. The major conclusions were:

1) Irrigating PRD-treated grapevines with half the amount of irrigation as control advanced maturity but significantly reduced berry growth and maturation later in the ripening period.

2) PRD treatment of Cabernet Sauvignon vines reduced the AI and SucSy activities shortly after veraison and at harvest compared to control – reduced sink strength in PRD berries compared to control.

3) At the same time, the NI activity in PRD-treated berries of Cabernet Sauvignon vines was greatly increased compared to control.

4) PRD Shiraz berries showed no significant differences in AI and SucSy activity compared to control, but greatly increased the NI activity compared to control. It is therefore concluded that the AI and SucSy activities was principally affected by the reduction of irrigation water applied and the NI activity was increased by PRD irrespective of the amount of irrigation water applied.

5) Whether or not this increase in NI activity in response to PRD has any effect on berry metabolism and the production of phenolics, anthocyanins, etc. to advance maturity is still unknown.

6) The hexose concentration in PRD Cabernet Sauvignon berries was significantly higher compared to control during the period between veraison and harvest.

7) However, at harvest PRD berries were smaller compared to control, but there were no significant differences in hexose concentration. Therefore, on a per berry basis the hexose content was significantly reduced in PRD-treated berries compared to control which may be an indication that berry sink strength was reduced in PRD berries compared to control.

8) PRD treatment of Shiraz with the same amount of water as control did not significantly affect sink strength as measured in the accumulation of hexose at harvest compared to control.

9) However, the results of PRD Nuriootpa Shiraz, indicated that hexose accumulation in the berries can be increased by PRD in situations where the appropriate cultural practices is used to balance vegetative growth with crop level.

10) PRD-treated Cabernet Sauvignon berries accumulated proline to significantly higher concentrations compared to control in the later stages of ripening. Together with slightly higher arginine concentrations the PRD-treated berries had a significantly higher total amino acid concentration at harvest compared to control.

11) Although there were some significant decreases in minor amino acids, the total amino acid concentration in PRD Shiraz berries showed no significant differences.

12) PRD had no significant effect on amino acid accumulation per berry compared to control, but may have increased the berry amino acid concentration by reducing berry size.

13) There were no significant differences in polyamine concentration between PRD-treated and control berries irrespective of the amount of irrigation water applied.

Chapter 10: PRD effects on berry inorganic ion accumulation, especially potassium, and the effect of berry size.

10.1 Introduction

In dry climates, irrigation is often used in viticulture to increase vine vigor, berry size and yield (Spayd and Morris, 1978; Freeman *et al.*, 1979; Kliewer *et al.*, 1983). In order to increase berry size there has to be an increase in cell turgor to drive cell wall extension and loosening (Salisbury and Ross, 1992). To maintain an influx of water into the berry, soluble sugars and inorganic ions accumulate to maintain the water potential gradient. Sugar is the major solute that accumulates in ripening berries, but minerals such as potassium (K), calcium (Ca), sodium (Na), copper (Cu), magnesium (Mg), manganese (Mn) and phosphorus (P) can contribute significantly as osmotic components (Mpelasoka *et al.*, 2003).

K is one of the most mobile elements in grapevines due to its size, small molecular charge and unhydrated character. Consequently, K is by far the major cation in ripe berries (Mpelasoka *et al.*, 2003) and plays the most important role in osmoregulation and plant water relations compared to other inorganic ions, especially under conditions of low sugar accumulation. Other minerals contained in the berry include Ca, Na, Mg, P, Cu and Mn, but have a small contribution due to their low concentrations and because of their lower mobility and/or toxicity at higher concentrations (Mpelasoka *et al.*, 2003). P is needed by yeast during must fermentation (Markham and Byrne, 1967) and the content of the fruit increases during ripening due to remobilisation from leaves. Ca accumulation in berries occurs when xylem influx is high and almost completely stops after veraison. Ca is considered to be phloem immobile and very little accumulates in berries after veraison (Conradie, 1981) or continues at rates much lower than K in Cabernet Sauvignon (Ollat and Gaudillere, 1996) and Shiraz (Rogiers *et al.*, 2001). Similarly, very little Cu, Mg and Na accumulates in berries under normal circumstances.

In warm regions, high pH of grapes at harvest can be a problem for wine making. High pH in grapes reduces color and stability of the processed product and excessive K can contribute to the problem by decreasing free acids and increasing overall pH. Morris *et al.* (1983) have shown that excessive K fertilization caused high juice pH in Concord berries that produced problems in color stability during storage. During wine making, high K increases the precipitation of tartrate in salt form and therefore decreases free tartrate. This may cause a

reduced tartrate:malate ratio that is undesirable for high quality wines (Mpelasoka *et al.*, 2003). A significant amount of K that accumulates in the berries from veraison to harvest is translocated from the trunk, shoots and leaves (Conradie, 1981; Williams and Biscay, 1991). Ollat and Gaudillere (1996) found that K uptake by Cabernet Sauvignon berries is slow before veraison and increases strongly when ripening starts in the same proportion as sink strength and phloem water influx. K is the predominant cation in sieve tube sap in many species and most of the potassium in fruit is delivered by the phloem (Ziegler, 1975).

In Australia, high K status in fruit is common in most vineyards that necessitate pH adjustments during the vinification process. Many factors may affect the accumulation of K by berries and these include soil, plant, vine microclimate and cultural practices (for review see Mpelasoka (2003)). Increased irrigation generally increases shoot growth (Klein *et al.*, 2000) and therefore increases canopy density and shading within the canopy. Findings of Jackson *et al.* (1993) suggested that wines with high pH and K exhibited poorer characteristics and were generally associated with fruit from internally shaded canopies. PRD may have positive effects on canopy microclimate by regulating vine vigor and allowing higher light interception into the bunch zone (dos Santos *et al.*, 2003). Dry *et al.* (2001) have found positive effects of better bunch exposure due to PRD treatment that included a reduced pH and higher berry anthocyanins and phenolics. The reduction in juice pH due to PRD treatment may be associated with lower K concentration.

Experiments in this chapter were conducted to test the hypothesis that *PRD decreases the accumulation of potassium and other inorganic ions.*

10.2 Materials and methods

Field-grown Cabernet Sauvignon and Shiraz vines in the Coombe Vineyard were used that was trained, irrigated and managed in the way already described in Section 8.2. PRD Cabernet Sauvignon was irrigated with half the amount of irrigation water as control. For Shiraz, PRD received the same amount of irrigation water as control, but only on one side at any given time while water was withheld from the other side. Experimental design of both cultivars consisted of a randomized treatment layout in one row with two treatments, PRD and control, and 7 replicates.

Mature Shiraz vines were used at the Nuriootpa Research Station (Barossa Valley, South Australia). The way the vines were treated and managed was described in Section 8.2. Treatments consisted of two irrigation treatments, PRD and control, and three pruning levels (30, 60 and 120 nodes/vine) with 5 repetitions of each treatment. PRD received half the amount of irrigation water as control.

The inorganic mineral analyses were done by optical emission spectrometry (CSIRO Div. Soils) as described in Section 2.13. Berries were frozen in liquid nitrogen and powdered in a commercial coffee grinder. Thereafter the homogenates were dried in a forced air oven for a minimum of 2 weeks at 60°C and 0.25 g per sample was used for the analysis of inorganic ions that included calcium (Ca), potassium (P), magnesium (Mg), phosphorus (P) and sulfur (S).

10.3 Results

10.3.1 Coombe Vineyard experiments in 2000/1

The changes in the K and Ca ion concentration (mg/g dry wt) and content (mg/berry) of Coombe Cabernet Sauvignon berries during the period between veraison and harvest in 2001 are shown in Figure 10.1. The concentration of inorganic ions in berries may be greatly influenced by the increase or decrease in berry weight. It is therefore useful to also evaluate berry content (mg/berry) that would indicate actual influx or efflux of solutes by integrating the changes in berry size. K was the most dominant inorganic ion measured in the berries of Cabernet Sauvignon berries both at veraison and at harvest, and it was more than ten fold higher concentration than any other ion measured, followed by Ca, P and then Mg and S.

The K concentration (mg/g dry wt) in Cabernet Sauvignon berries gradually decreased from veraison until harvest in 2001, but K increased in contents (mg/berry) with berry enlargement and sugar accumulation until 2 weeks before harvest when there was a marked decrease in K contents. It could therefore be concluded that there was a net influx of K in Cabernet Sauvignon berries during the ripening period from veraison up to 2 weeks before harvest, but during the last 2 weeks before harvest there was a net efflux of K. PRD-treated Cabernet Sauvignon berries showed no significant differences in K concentration compared to control during the period between veraison and harvest in 2001. However, a significant reduction in berry weight during the last 2 weeks before harvest (Section 8.3.3) caused PRD-treated berries to have significantly lower ($P<0.05$) berry K content (mg/berry) by 9% at harvest compared to control.

PRD-treated berries showed a significantly lower ($P<0.05$) concentration of Ca shortly after veraison (by 20%). When expressed on Ca per berry content (mg/berry) this related to a significant reduction ($P<0.05$) by 16% compared to control. Two weeks after veraison the PRD-treated and control berries showed similar Ca concentrations until harvest. At harvest, PRD-treated berries had marginally lower Ca concentration (by 7%) that was not significantly different ($P=0.1122$) to control. However, a significantly lower berry weight during the last 2 weeks before harvest (Section 9.3.1) caused PRD-treated berries to have significantly lower ($P<0.05$) berry Ca content (mg/berry) by 15% compared to control.

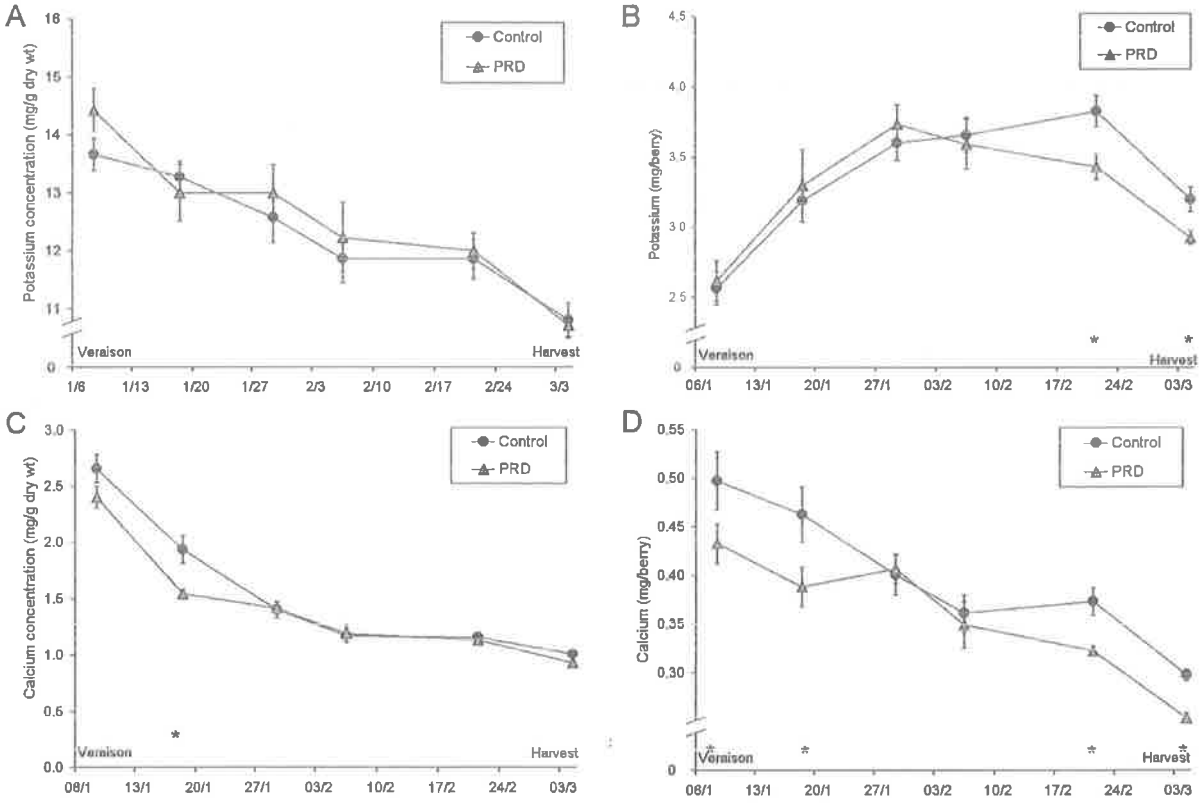


Figure 10.1 The changes in K **(A+C)** and Ca **(C+D)** ion concentration (mg/g dry wt) and ion content (mg/berry) respectively in Coombe Cabernet Sauvignon berries during the ripening period in 2001 between veraison and harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.; * = significantly different ($P<0.05$)).

The changes in the Mg, P and S ion concentrations (mg/g dry wt) and contents (mg/berry) of Coombe Cabernet Sauvignon berries during the period between veraison and harvest in 2001 are shown in Figure 10.2. The Mg, P and S ion concentrations (mg/g dry wt) gradually decreased during the ripening period between veraison and harvest in 2001 in both PRD and control berries that could be attributed to the increase in berry size and the dilution effect. However, the berry ion contents (mg/berry) stayed relatively stable indicating no significant

influx or efflux of ions until a week before harvest when all three ions showed a significant efflux in both PRD and control berries.

Similar to the changes in Ca, Mg in PRD-treated berries showed significantly lower concentrations (on average by 12% compared to control) during the first 2 weeks after veraison. However, after 3 weeks PRD-treated berries had higher Mg concentrations (by 7% compared to control) that were significantly higher ($P<0.05$) on one occasion before harvest. At harvest though, there was no significant difference between PRD and control berries. Similar to the changes in concentration, berry Mg content (mg/berry) of PRD-treated vines was significantly lower during the first 2 weeks after veraison (on average by 12% compared to control). With an increase in concentration the PRD berries showed a significant influx to reach similar berry contents as control after 3 weeks, but closer to harvest the smaller berries of PRD-treated vines had significantly lower Mg content (mg/berry) by 9% compared to control.

This trend in berry Mg accumulation in response to PRD treatment was also found in the changes of P and S concentrations (mg/g dry wt) and content (mg/berry). The berry P and S concentrations (mg/g dry wt) of PRD-treated vines were also significant lower 2 weeks after veraison compared to control and the difference in harvest concentrations of P and S between PRD and control berries at harvest were also insignificant. Furthermore, due to smaller berry sizes at harvest the PRD-treated berries had also significantly lower P and S content by 10% and 12% respectively compared to control.

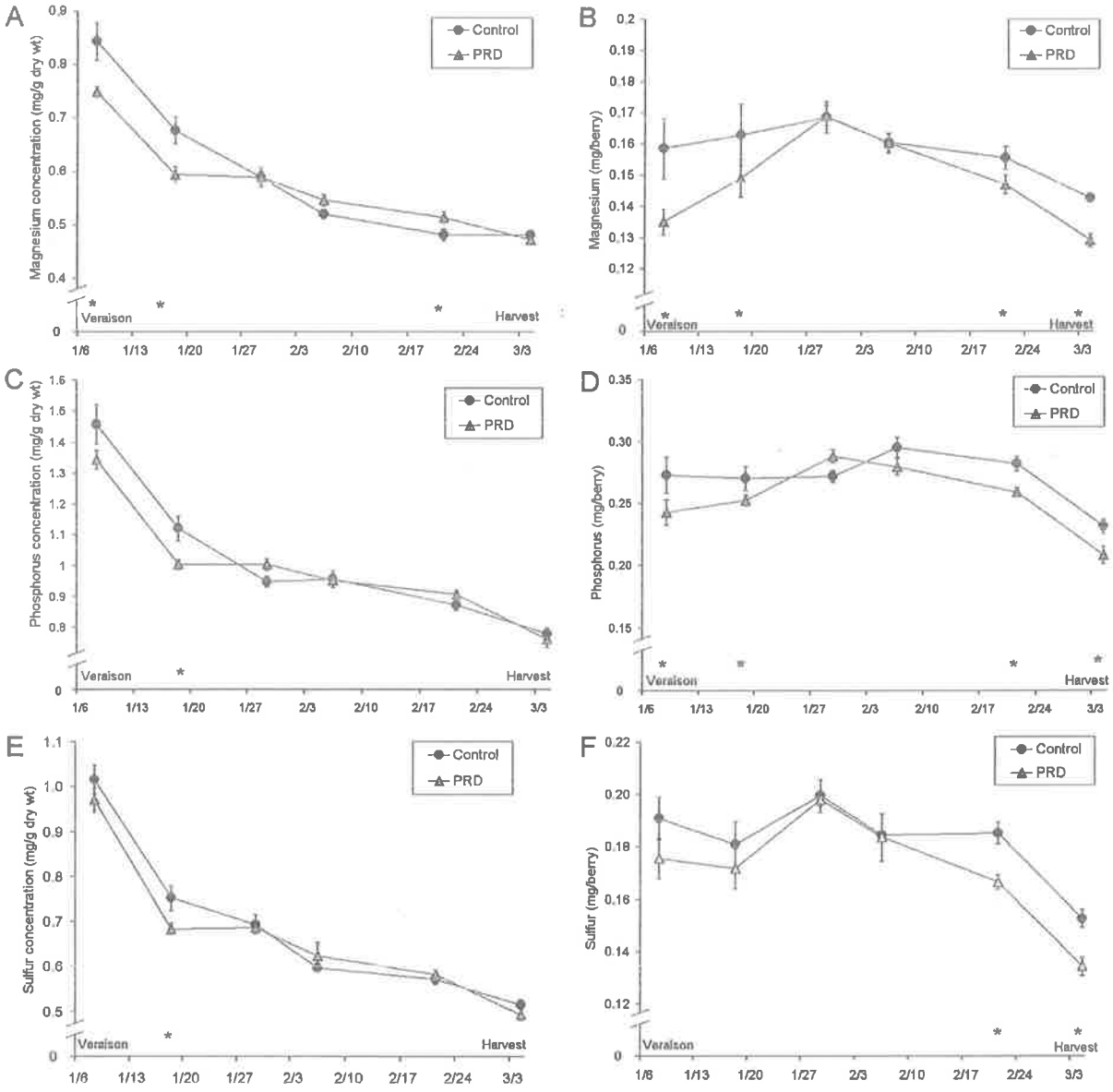


Figure 10.2 The changes in Mg **(A+C)**, P **(C+D)** and S **(E+F)** ion concentration (mg/g dry wt) and ion content (mg/berry) respectively in Coombe Cabernet Sauvignon berries during the ripening period in 2001 between veraison and harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.; * = significantly different (P<0.05)).

The changes in the K and Ca ion concentration (mg/g dry wt) and content (mg/berry) of Coombe Shiraz berries during the last month before harvest in 2001 are shown in Figure 10.3. PRD Shiraz received the same amount of irrigation water as control, but only on one side at any time. Similar to the changes in Cabernet Sauvignon berries, the K concentration in Coombe Shiraz berries decreased during the month before harvest. However, K berry content (mg/berry) increased until 2 weeks before harvest, followed by a marked decrease in content at harvest. It

could therefore be concluded that similar to Cabernet Sauvignon berries that there was a net influx of K into Shiraz berries during ripening until 2 weeks before harvest, but during the last 2 weeks there was a net efflux of K. PRD treatment however had no significant effect on the accumulation pattern of K in Coombe Shiraz berries compared to control.

Contrary to the changes in Cabernet Sauvignon berries, the Ca concentration (mg/g dry wt) in Coombe Shiraz berries did not change significantly during the month before harvest and there was no significant difference between the Ca concentrations for PRD and control berries. The Ca content (mg/berry) consequently increased at a linear rate indicating a net influx of Ca during ripening until harvest. There were no significant differences in Ca content between PRD-treated and control berries.

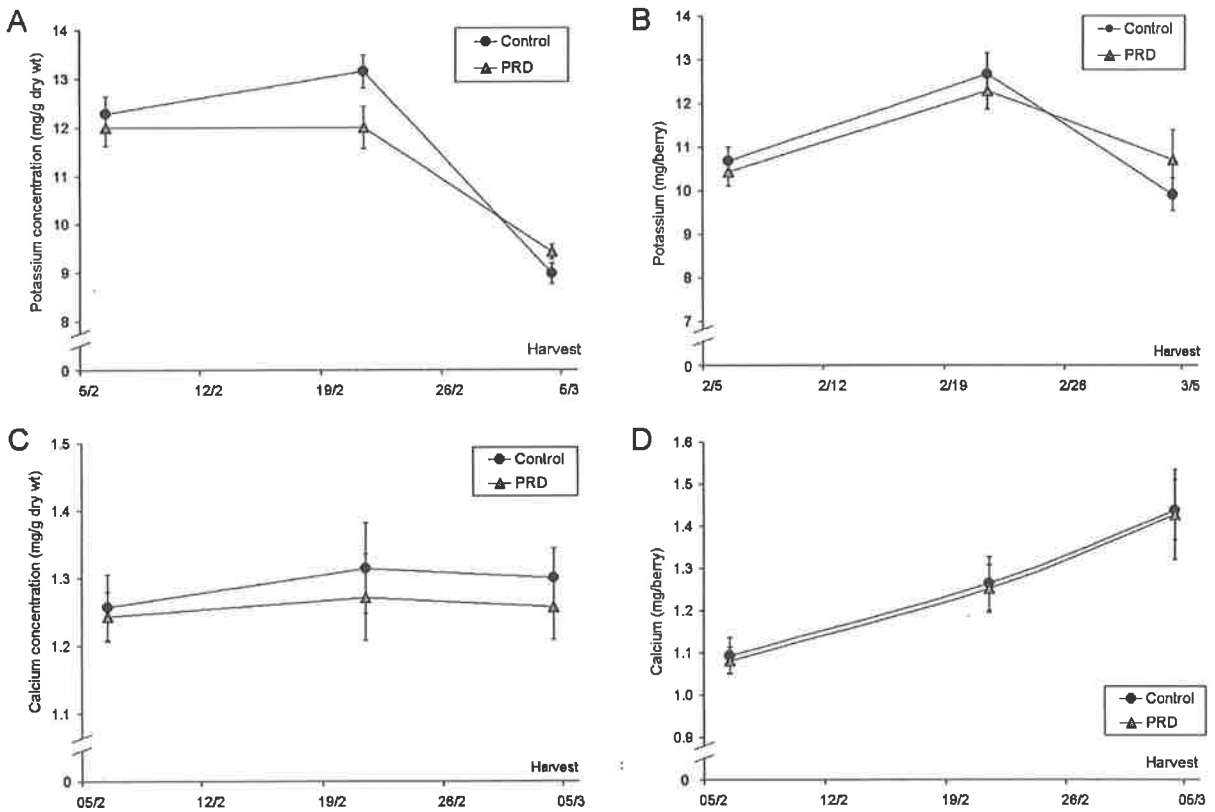


Figure 10.3 The changes in Ca **(A+C)** and K **(C+D)** ion concentration (mg/g dry wt) and ion content (mg/berry) respectively in Coombe Shiraz berries in 2001 during the last month before harvest. (PRD received the same amount of irrigation water as control but only on one side at any time; control received water on both sides; means n=7 ± s.e.)

The changes in the Mg, P and S ion concentrations (mg/g dry wt) and contents (mg/berry) in Coombe Shiraz berries are shown in Figure 10.4 during the month before harvest in 2001. The Mg, P and S ion concentrations (mg/g dry wt) stayed generally stable or slightly increased until 2 weeks before harvest when there was a marked decrease in both PRD and control berries.

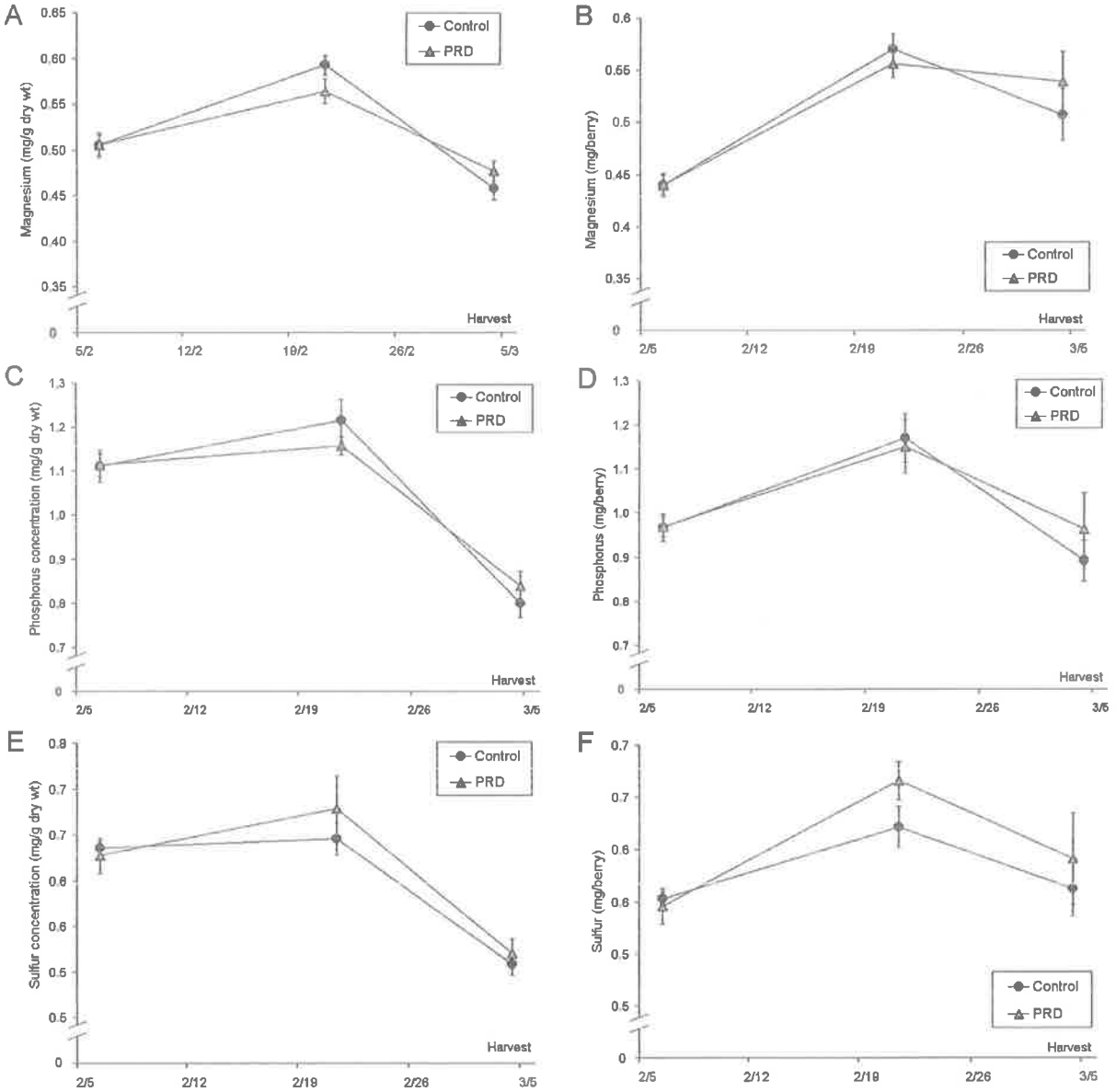


Figure 10.4 The changes in Mg **(A+C)**, P **(C+D)** and S **(E+F)** ion concentration (mg/g dry wt) and ion content (mg/berry) respectively in Coombe Shiraz berries in 2001 during the last month before harvest. (PRD received the same amount of irrigation water as control but on only one side at any time; control received water on both sides; means n=7 ± s.e.)

The berry ion contents (mg/berry) however increased until 2 weeks before harvest indicating a significant influx of ions into Coombe Shiraz berries during this period. However, 2 weeks before harvest Mg, P and S ions showed a significant reduction in berry ion content (mg/berry)

indicating a marked efflux in both PRD and control berries. Although not significant, PRD-treated berries had higher Mg, P and S berry contents by 7% on average compared to control at harvest.

10.3.2 Coombe Vineyard experiments in 2001/2

The inorganic ion concentration (μg/g dry wt) and content (μg/berry) of Coombe Cabernet Sauvignon berries at veraison and harvest in 2002 are shown in Tables 10.1 and 10.2 respectively. Similar to the results of the 2000/1 season, the average concentration of every inorganic ion measured at harvest in 2002 was lower compared to the concentration at veraison. Furthermore, the total Ca ion content (μg/berry) of Cabernet Sauvignon berries was lower at harvest compared to control, indicating a net efflux of Ca from veraison to harvest. In comparison, the K ion content increased from veraison to harvest, indicating a net influx. The ion contents of Mg, P and S stayed relatively the same between veraison and harvest, indicating no significant influx or efflux. K was the most dominant inorganic ion in the berries of Cabernet Sauvignon berries at veraison and at harvest – more than ten fold higher concentration than any other ion measured.

The K concentration at veraison increased by 13% ($P=0.079$) for PRD compared to control, however the concentration at harvest was not significantly different. On average, PRD-treated berries had a 6% higher K concentration at harvest, but a 4% lower K content (μg/berry) compared to control. No significant differences could be found at veraison or at harvest for the concentrations of Ca, Mg, P or S ions in response to PRD treatment. However, similar to the 2000/1 season, the berry Ca content (μg/berry) in PRD berries at harvest in 2002 was significantly ($P<0.05$) reduced by 9% compared to control. The Mg, P and S ion contents in PRD-treated berries were on average also lower compared to control, but the differences were not statistically significant.

Table 10.1 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) in Coombe Cabernet Sauvignon berries at veraison in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.).

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1500 ± 87	1471 ± 81	- 2	0.744
K	10729 ± 334	12143 ± 404	+ 13	0.079
Mg	546 ± 20	537 ± 18	- 2	0.763
P	964 ± 45	997 ± 43	+ 3	0.593
S	644 ± 29	669 ± 28	+ 4	0.539
Content (μg/berry)	Control	PRD	% Diff	P
Ca	435 ± 28	424 ± 22	- 3	0.717
K	3130 ± 188	3501 ± 117	+ 12	0.190
Mg	158 ± 5	155 ± 6	- 2	0.687
P	281 ± 18	288 ± 13	+ 2	0.735
S	187 ± 11	193 ± 8	+ 3	0.664

Table 10.2 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) in Coombe Cabernet Sauvignon berries at harvest in 2002. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.).

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	881 ± 40	883 ± 36	0	0.974
K	10500 ± 323	11143 ± 404	+ 6	0.271
Mg	429 ± 7	457 ± 14	+ 7	0.154
P	771 ± 24	771 ± 28	0	1.000
S	486 ± 6	504 ± 14	+ 4	0.297
Content (μg/berry)	Control	PRD	% Diff	P
Ca	314 ± 15	286 ± 12	- 9	**0.039**
K	3765 ± 225	3632 ± 226	- 4	0.553
Mg	153 ± 5	148 ± 4	- 3	0.313
P	276 ± 15	250 ± 9	- 10	0.112
S	174 ± 7	164 ± 8	- 6	0.117

The inorganic ion concentration (μg/g dry wt) and content (μg/berry) of Coombe Shiraz berries at veraison and harvest in 2002 are shown in Tables 10.3 and 10.4 respectively. PRD Shiraz vines received the same amount of irrigation water as control vines. The average concentration of every inorganic ion measured at harvest was lower compared to the concentration at veraison. Furthermore, the total Ca ion content (μg/berry) of Shiraz berries was lower at harvest compared to veraison, indicating a net efflux of Ca from veraison to harvest. The ion contents of Mg, P and S also reduced slightly between veraison and harvest, indicating a net efflux. In

comparison, the K ion content did not change from veraison to harvest, indicating no net influx or efflux of K. K was the most dominant inorganic ion in the berries of Shiraz berries at veraison and at harvest – more than 6-fold higher concentration than any other ion measured.

There were no significant differences in inorganic ion concentration between PRD-treated and control berries at veraison in Coombe Shiraz vines. However, on a per berry basis PRD significantly increased the total K, P and S ion content (μg/berry) by 16%, 11% and 12% respectively compared to control at veraison. PRD-treated berries at harvest did not have significantly different berry ion concentrations or contents compared to control. However, PRD reduced the S concentration by 14% (P=0.055) compared to control. PRD also reduced the K concentration and total content at harvest by 7% and 8% respectively compared to control, but the difference was not significant (P=0.135).

Table 10.3 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) in Coombe Shiraz berries at veraison in 2002. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n=7 ± s.e.)

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1714 ± 103	1614 ± 59	- 6	0.474
K	10986 ± 317	11771 ± 521	+ 7	0.307
Mg	531 ± 25	514 ± 10	- 3	0.498
P	1080 ± 37	1097 ± 44	+ 2	0.681
S	694 ± 33	711 ± 24	+ 2	0.656
Content (μg/berry)	Control	PRD	% Diff	P
Ca	670 ± 18	692 ± 28	+ 3	0.441
K	4345 ± 194	5052 ± 254	+ 16	0.099
Mg	209 ± 7	222 ± 11	+ 6	0.221
P	426 ± 14	472 ± 25	+ 11	**0.046**
S	272 ± 6	305 ± 10	+ 12	**0.013**

Table 10.4 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) in Coombe Shiraz berries at harvest in 2002. (PRD received the same amount of irrigation water as control on only one side at any time; control received water on both sides; means n=7 ± s.e.)

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1171 ± 61	1126 ± 49	- 4	0.530
K	9829 ± 364	9143 ± 413	- 7	0.216
Mg	427 ± 10	421 ± 6	- 1	0.508
P	820 ± 32	830 ± 29	+ 1	0.802
S	569 ± 28	491 ± 23	- 14	0.055
Content (μg/berry)	Control	PRD	% Diff	P
Ca	518 ± 29	493 ± 19	- 5	0.379
K	4361 ± 227	4000 ± 133	- 8	0.135
Mg	189 ± 7	185 ± 5	- 2	0.506
P	364 ± 20	364 ± 13	0	1.000
S	252 ± 15	215 ± 9	- 15	0.062

10.3.3 Coombe Vineyard experiments in 2002/3

The inorganic ion concentration (μg/g dry wt) and content (μg/berry) of Coombe Cabernet Sauvignon berries at veraison and harvest in 2003 are shown in Tables 10.5 and 10.6 respectively. The average concentration of every inorganic ion measured at harvest was lower compared to their concentration at veraison. However, the ion contents (μg/berry) of Ca, Mg, P and S did not change significantly between veraison and harvest, indicating no significant influx or efflux. In comparison, the K ion content increased from veraison to harvest, indicating a net influx of K. K was the most dominant inorganic ion in the berries of Cabernet Sauvignon berries at veraison and at harvest – more than 7-fold and 9-fold higher concentration at veraison and harvest respectively than any other ion measured.

No significant differences could be found between PRD-treated and control berries in the concentration of ions at veraison, but the total K and Mg ion contents (μg/berry) in PRD-treated berries were significantly reduced ($P<0.05$) by 9% and 13% respectively compared to control. The contents of Ca, P and S were also reduced by PRD treatment at veraison but the differences were not significant. At harvest, there were no significant differences in inorganic ion concentration or content (μg/berry) between PRD and control berries.

Table 10.5 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) of Coombe Cabernet Sauvignon berries at veraison in 2003. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.).

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1729 ± 94	1729 ± 106	0	1.000
K	12714 ± 360	12714 ± 360	0	1.000
Mg	669 ± 18	643 ± 16	- 4	0.445
P	1041 ± 35	990 ± 41	- 5	0.419
S	713 ± 33	741 ± 18	+ 4	0.438
Content (μg/berry)	Control	PRD	% Diff	P
Ca	385 ± 21	348 ± 16	- 10	0.095
K	2842 ± 133	2579 ± 111	- 9	**0.008**
Mg	150 ± 7	130 ± 1	- 13	**0.044**
P	234 ± 13	200 ± 7	- 14	0.087
S	160 ± 10	150 ± 3	-6	0.317

Table 10.6 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) of Coombe Cabernet Sauvignon berries at harvest in 2003. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.).

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1142± 39	1200± 49	+ 5	0.410
K	11003± 216	10829± 352	- 2	0.636
Mg	517± 9	541± 14	+ 5	0.148
P	810± 32	810± 40	0	0.999
S	596± 13	610± 21	+ 2	0.554
Content (μg/berry)	Control	PRD	% Diff	P
Ca	346± 17	356± 10	+ 3	0.629
K	3321± 75	3226± 120	- 3	0.569
Mg	157± 5	161± 2	+ 3	0.486
P	244± 8	241± 10	- 1	0.809
S	180± 5	181± 6	+ 1	0.848

The inorganic ion concentration (μg/g dry wt) and content (μg/berry) of Coombe Shiraz berries at veraison and harvest in 2003 are shown in Tables 10.7 and 10.8 respectively. The average concentration and content of every inorganic ion measured at harvest was lower compared to the concentration and content at veraison. This indicated that there was a net efflux of inorganic ions during the ripening period between veraison and harvest in Shiraz berries in 2003. As usual, K was the most dominant inorganic ion in the Shiraz berries at veraison and at harvest –

more than 6-fold and 7-fold higher concentration at veraison and harvest respectively than any other ion measured.

No significant differences could be found between PRD-treated and control berries in the concentration or content (μg/berry) of ions at veraison. However, at harvest the Ca and Mg concentrations in PRD-treated berries were significantly ($P<0.10$) lower by 10% and 6% respectively compared to control. The inorganic ion contents at harvest of Shiraz berries were however not significantly affected by PRD treatment compared to control.

Table 10.7 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) of Coombe Shiraz berries at veraison in 2003. (PRD received the same amount of irrigation water as control only on one side at any time; control received water on both sides; means n=7 ± s.e.).

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1971 ± 57	2029 ± 64	+ 3	0.569
K	13000 ± 436	12286 ± 286	- 5	0.220
Mg	683 ± 26	674 ± 20	- 1	0.795
P	1329 ± 52	1257 ± 53	- 5	0.253
S	763 ± 19	717 ± 11	- 6	0.124
Content (μg/berry)	Control	PRD	% Diff	P
Ca	755 ± 23	781 ± 39	+ 3	0.549
K	4978 ± 165	4745 ± 261	- 5	0.417
Mg	261 ± 9	259 ± 9	- 1	0.830
P	509 ± 22	485 ± 31	- 5	0.341
S	292 ± 7	276 ± 13	- 5	0.323

Table 10.8 The inorganic ion concentration (μg/g dry wt) and ion content (μg/berry) of Coombe Shiraz berries at harvest in 2003. (PRD received the same amount of irrigation water as control only on one side at any time; control received water on both sides; means n=7 ± s.e.)

Concentration (μg/g dry wt)	Control	PRD	% Diff	P
Ca	1371 ± 42	1229 ± 29	- 10	0.058
K	10343 ± 234	10443 ± 269	+ 1	0.775
Mg	521 ± 7	491 ± 8	- 6	0.065
P	994 ± 31	974 ± 25	- 2	0.474
S	557 ± 9	544 ± 19	- 2	0.488
Content (μg/berry)	Control	PRD	% Diff	P
Ca	576± 26	522± 22	- 9	0.265
K	4335± 119	4438±215	+ 2	0.699
Mg	219± 8	209± 8	- 5	0.484
P	418± 19	413± 17	- 1	0.894
S	234± 7	230± 8	- 1	0.799

10.3.4 Nuriootpa Shiraz in 2000/1

The inorganic ion concentration (μg/g dry wt) and content (μg/berry) of Nuriootpa Shiraz berries at harvest in 2001 are shown in Tables 10.9 and 10.10 respectively. PRD-treated vines received half the amount of irrigation water as control vines. As in other experiments, K was the most dominant inorganic ion in the Shiraz berries at veraison and at harvest – more than 9-fold higher concentration than any other ion measured.

The only significant effect of irrigation treatment on berry ion levels was on the concentration of P at harvest (P=0.0315) with PRD-treated berries having higher P concentrations than control. Pruning level had a significant effect on both Ca (P=0.022) and Mg (P=0.002) concentration: Vines pruned to 120 nodes/vine had significantly higher Ca and Mg concentrations compared to vines with 30 and 60 nodes/vine. There were no significant interactions between pruning level and irrigation treatment on any of the inorganic concentrations at harvest in Nuriootpa Shiraz vines. Analyses on individual pruning levels showed that the vines pruned to 30 nodes/vine had a significant increase in P concentration in PRD berries by 12% compared to control. There was also an average increase of 11% in Ca concentration at all pruning levels in PRD-treated berries compared to control.

Table 10.9 The Ca, K, Mg, P and S concentration (μg/g dry wt) in Nuriootpa Shiraz berries at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=5 ± s.e.)

Ca				
Nodes/vine	Control	PRD	% Diff	Sig.
30	1340 ± 68	1520 ± 102	+ 13	0.104
60	1410 ± 95	1580 ± 97	+ 12	0.313
120	1660 ± 133	1800 ± 126	+ 8	0.578
K				
Nodes/vine	Control	PRD	% Diff	Sig.
30	12200 ± 200	13200 ± 970	+ 8	0.326
60	12754 ± 401	12400 ± 245	- 3	0.441
120	12800 ± 374	11800 ± 583	- 8	0.142.
Mg				
Nodes/vine	Control	PRD	% Diff	Sig.
30	558 ± 17	590 ± 15	+ 6	0.130
60	598 ± 15	616 ± 25	+ 3	0.672
120	662 ± 31	650 ± 17	- 2	0.757
P				
Nodes/vine	Control	PRD	% Diff	Sig.
30	1320 ± 37	1480 ± 66	+ 12	**0.035**
60	1302 ± 34	1420 ± 97	+ 9	0.351
120	1340 ± 68	1400 ± 32	+ 4	0.553
S				
Nodes/vine	Control	PRD	% Diff	Sig.
30	718 ± 20	776 ± 34	+ 8	0.157
60	772 ± 20	766 ± 7	- 1	0.648
120	814 ± 29	792 ± 34	- 3	0.701

The calculated ion contents per berry (μg/berry) showed that irrigation treatment had no significant effect on any of the ion contents of Nuriootpa Shiraz berries at harvest. However, pruning level had a significant effect on both K (P=0.055) and Mg (P=0.012) content: vines pruned to 120 nodes/vine had significantly lower K and Mg per berry contents compared to vines with 30 and 60 nodes/vine. There were no significant interactions between pruning level and irrigation treatment for any of the inorganic ion contents at harvest in Nuriootpa Shiraz vines. Analyses of individual pruning levels however showed that the PRD-treated 120 nodes/vine berries had significantly lower K per berry (by 22% compared to control). The PRD-treated 30 and 60 nodes/vine berries also showed an average reduction in K per berry by

5% compared to control. PRD-treated 120 nodes/vine berries also showed significant reductions in Mg and S per berry by 16% and 18% respectively compared to control.

Table 10.10 The Ca, K, Mg, P and S content (μg/berry) of Nuriootpa Shiraz berries at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=5 ± s.e.)

Ca				
Nodes/vine	Control	PRD	% Diff	Sig.
30	364 ± 22	391 ± 22	+ 7	0.459
60	365 ± 25	402 ± 28	+ 10	0.387
120	390 ± 20	363 ± 17	- 7	0.307
K				
Nodes/vine	Control	PRD	% Diff	Sig.
30	3329 ± 182	3479 ± 449	- 4	0.782
60	3342 ± 283	3175 ± 235	- 5	0.512
120	3073 ± 278	2389 ± 107	- 22	**0.041**
Mg				
Nodes/vine	Control	PRD	% Diff	Sig.
30	151 ± 4	153 ± 9	+ 1	0.905
60	156 ± 10	156 ± 6	0	0.970
120	157 ± 7	132 ± 6	- 16	0.030
P				
Nodes/vine	Control	PRD	% Diff	Sig.
30	360 ± 20	386 ± 36	+ 7	0.586
60	339 ± 20	358 ± 17	+ 6	0.563
120	319 ± 25	284 ± 7	-11	0.250
S				
Nodes/vine	Control	PRD	% Diff	Sig.
30	195 ± 7	203 ± 19	+ 4	0.751
60	202 ± 14	196 ± 13	- 3	0.696
120	194 ± 14	160 ± 4	- 18	**0.050**

10.4 Discussion

Results of experiments in this chapter have provided evidence that PRD affected the inorganic ion accumulation in grapevines when a) less irrigation volume was applied and b) when there were significant effects on berry size.

Because there were significant differences in the berry weights of PRD-treated and control Coombe Cabernet Sauvignon vines (Section 9.3.1), both the berry ion concentration (mg/g dry wt) and the ion content per berry (mg/berry) were investigated. This made it possible to

evaluate the actual influx or efflux of solutes by integrating the changes in berry size (Coombe, 1992). K was by far the major cation measured in the berries of Cabernet Sauvignon and Shiraz vines during the 3 seasons and its concentration gradually decreased during ripening between veraison and harvest in both cultivars. There were no significant differences found between PRD-treated and control vines for berry K concentration at harvest in either cultivar, irrespective of the volume of water applied. The grapevines in the Coombe Vineyard were trained to a VSP system and hedged several times during the growing season in all of the three seasons. This may have produced canopy sizes of similar densities and shading characteristics in both PRD and control vines.

Consequently, the changes in K per berry were greatly influenced by the changes in berry size. The K content per berry in PRD Cabernet Sauvignon vines during the 2000/1 season increased linearly during the first 3 weeks after veraison, slightly more than control berries, indicating a high rate of influx of K and a high sink strength. After 3 weeks PRD-treated berries reached a maximum K content, and thereafter gradually decreased until harvest, indicating a net efflux during the later period of berry ripening. Control berries, however, continued to accumulate K until 2 weeks before harvest, 3 weeks longer than PRD-treated vines and then showed a marked decrease in K content at harvest that corresponded with a decrease in berry weight. The K content per berry was however significantly lower in PRD-treated Cabernet Sauvignon vines relative to control during the last 2 weeks up to and including harvest in 2001. PRD did not significantly affect the K content per berry at harvest in 2002 and 2003, although PRD-treated Cabernet Sauvignon berries had slightly lower K content per berry relative to control in both seasons.

PRD-treated Coombe Shiraz vines received the same volume of water as control vines. The K content per berry in PRD Shiraz during the 2000/1 season increased until 2 weeks before harvest and then showed a marked decrease up to harvest even though berry weight increased significantly. The sharp decrease in K concentration could not be explained by a dilution effect, but by a net efflux of K from the berry during the 2 weeks up to harvest. The K content per berry in PRD-treated berries was however not significantly different to control at the 2001 harvest. PRD also did not significantly affect the K concentration or content per berry at harvest in 2002 or 2003.

PRD-treated Nuriootpa Shiraz vines received half the amount of irrigation water as control. PRD treatment did not significantly affect the berry size (Section 4.3.6) nor the K concentration at harvest compared to control. However, the total amount of K per berry (μg/berry) in PRD-treated vines was slightly lower than control at all pruning levels. PRD treatment in vines pruned to a higher than average number of nodes per vine for the region (i.e. 120 nodes/vine) significantly decreased the K per berry compared to control. This decrease was mainly due to the smaller berry size (by 15%) of PRD vines treated at a higher than the average pruning level (i.e. 60 nodes/vine).

According to Boulton (1980) a significant exchange between K^+ and H^+ ions occurs in berries during ripening, causing an increase in pH as a result of the net loss in H^+ ions. He determined that it was mainly the measure of exchange between K^+ and H^+ ions, and not only the amount of K^+ ions in the juice, that caused the pH increase. This exchange between K^+ and H^+ ions is helped along by dense foliage and/or excessive vigor in vineyards. PRD-treated vines in the Coombe Vineyard had a reduced shoot growth compared to control, but control vines could not be classified as 'excessively vigorous'. Furthermore, the VSP training system and summer hedging produced a canopy of uniform size in both PRD and control vines. The reduction, if any, in canopy shading due to PRD treatment therefore should not have had a major impact on berry K concentrations. Consequently, PRD treatment had no significant effect on berry K concentration at harvest compared to control and this was also associated with no significant differences in pH of berry juice (Section 4.3.6) between PRD and control. The Shiraz vines in the Nuriootpa Vineyard also could not be classified as 'excessively vigorous', but they were allowed to sprawl. PRD-treated vines consequently showed a significant decrease in canopy size (Section 4.3.5) at harvest compared to control. Consequently, PRD vines pruned at highest pruning level (120 nodes/vine) had smaller canopy size than control, with lower K concentrations and content. The effect of PRD treatment on canopy size and berry K was less in vines pruned to lower pruning levels, probably due to lower shoot growth rates at highest pruning level (Section 4.3.1). The lower K content of PRD-treated berries compared to control was also associated with slightly lower juice pH (Section 4.3.6) in vines pruned to high pruning level (120 nodes/vine). Although not the same size, the same effect of PRD treatment on canopy size, berry pH and K content was found in vines pruned to average pruning level (60 nodes/vine) for the region, compared to control. However, vines pruned to higher pruning level had more shoots per vine and therefore a denser canopy with higher total leaf area than vines

pruned to lower nodes/vine. PRD may therefore have a greater effect on berry pH and K accumulation when vines have dense canopies.

Of the lesser minerals measured, Ca is the most abundant cation in berries of Cabernet Sauvignon and Shiraz grapevines. Ca concentration decreased at a constant rate during the ripening period between veraison and harvest in both cultivars, irrespective of the amount of water applied. This can be mainly attributed to the increase in berry size and to a dilution of the berry ion content. However, the amount of Ca per berry (mg/berry) also showed a steady decline during the same period and it is therefore proposed that there was a gradual efflux of Ca out of Cabernet Sauvignon berries after veraison. This supports the hypothesis of Coombe (1992) that as a consequence of xylem embolism, the majority of water that accumulates in berries after veraison is derived from the phloem. Ca accumulation in berries therefore occurs when xylem influx is high and it almost completely stops after veraison (Conradie, 1981) because Ca is considered to be phloem immobile (Lang and Thorpe, 1989). PRD Cabernet Sauvignon vines had significantly lower Ca per berry and lower Ca concentration during the first 2 weeks after veraison compared to control that indicates that PRD-treatment accumulated less Ca than control before veraison in 2001. However, this was not the case in 2002 and 2003 with PRD treatment showing no significant differences in berry Ca concentration or Ca per berry compared to control. The Ca concentration of PRD-treated berries at harvest in all of the 3 years was not significantly different compared to control, however the PRD Cabernet Sauvignon berries had significantly lower Ca per berry at harvest in 2001 and 2002 compared to control. This could either be an indication that PRD-treatment caused a significantly higher efflux of Ca per berry compared to control or that because PRD –treated berries started at veraison with a lower amount of Ca per berry compared to control that both treatments had similar net efflux rates during the period between veraison and harvest. However, in 2002 PRD Cabernet Sauvignon berries had no significant difference in Ca per berry at veraison relative to control but still had significantly lower berry Ca contents at harvest compared to control. This is further confused by PRD-treated Cabernet Sauvignon berries during the 2003 harvest having no significant differences in berry Ca content compared to control and PRD Nuriootpa Shiraz in 2001 having higher average Ca content per berry compared to control. Although it is believed that xylem influx almost completely stops after veraison, the results of this study and others (Ollat and Gaudillere, 1996; Rogiers *et al.*, 2000; Rogiers *et al.*, 2001) have shown that Ca

movement is still possible, although greatly reduced relative to K accumulation, after veraison that may be responsible for Ca efflux or influx.

In comparison to Cabernet Sauvignon, the Ca content per berry in Coombe Shiraz vines increased during the last month of ripening in 2001 while the Ca concentration stayed the same, indicating a net influx of Ca into the berries. This was however not the case in both 2002 and 2003 with veraison Ca concentrations being much higher than concentrations at harvest. There were also significant reductions in Ca content per berry between veraison and harvest in both years, indicating a net efflux of Ca. PRD-treated Shiraz vines received the same amount of irrigation water as control and consequently had no effect on berry size or the efflux of Ca from berries compared to control vines.

The concentrations of Mg, P and S were substantially lower than K and Ca in Coombe Cabernet and Shiraz berries during the three-year experimental period in both PRD and control plants. Similar to the other minerals, the concentration of P, Mg and S decreased gradually in Cabernet Sauvignon berries during the period between veraison and ripening in 2001. Similar reductions in mineral concentration were found between veraison and harvest during the 2001/2 and 2002/3 seasons.

There were no significant differences in mineral concentration between PRD and control berries during most of the ripening period of 2001. However, similar to the changes in K the Mg, P and S contents per berry (mg/berry) increased during the first 3 weeks after veraison. After 3 weeks the minerals reached a maximum content per berry and gradually decreased thereafter until harvest in both PRD and control vines. PRD-treated berries showed significantly lower P and S mineral contents during the early period of ripening compared to control but showed high rates of influx until both PRD and control berries reached a similar maximum for each mineral. Thereafter, PRD showed a significantly higher efflux rate in Mg, P and S mineral content compared to control and eventually the PRD berries had significantly lower Mg, P and S content per berry at harvest. This trend was however not repeated in the 2001/2 and 2002/3 seasons with PRD and control berries having no significant differences in Mg, P or S content per berry.

The same trend in the changes of berry Mg, P and S mineral content of Cabernet Sauvignon could be seen in Coombe Shiraz berries in 2001. Both PRD and control vines increased their berry mineral content by net influx until a maximum and thereafter there was a marked decrease until harvest. PRD had no significant effect on berry Mg, P and S content per berry at harvest in all of the three years of the experiment.

PRD-treated Nuriootpa Shiraz that received half the irrigation water as control showed in most cases no significant effect of PRD on berry Mg, P, S mineral concentration compared to control. Calculated on a per berry basis however, the PRD-treated vines pruned to 120 nodes/vine showed significant reduction in Mg and S compared to control, while there was no significant difference between PRD and control vines pruned to 30 and 60 nodes/vine.

10.5 Conclusions

The experiments conducted in this chapter were conducted to test the hypothesis that PRD reduces the accumulation of potassium and other inorganic ions. Not enough evidence existed to accept this hypothesis. However, the major conclusions were:

1) PRD-treatment of Cabernet Sauvignon and Shiraz vines did not significantly affect the berry K concentration relative to control, irrespective of the amount of water applied.

2) However, in situations where PRD treatment reduced berry size, the K content per berry was reduced by a similar degree.

3) Three weeks after veraison, PRD-treated berries reached a maximum K content per berry and thereafter gradually decreased in K per berry until harvest, indicating a net efflux during the later period of berry ripening. Control berries however continued to accumulate K until 2 weeks before harvest, 3 weeks longer than PRD-treated vines.

4) The K concentration and K content per berry in PRD-treated Coombe Shiraz berries was however not significantly different to control during the three-year experiment. There was therefore a main effect of amount of water applied on K content per berry due to the effect on berry size.

5) PRD-treatment of vines pruned to higher pruning levels had significantly lower berry K content than control vines. PRD treatment may have a greater effect on berry pH and K accumulation when vines are prone to dense canopies.

6) The concentration and amount of Ca content per berry showed a steady decline during the period between veraison and harvest and it is therefore proposed that there was a net efflux of Ca out of Cabernet Sauvignon and Shiraz berries after veraison

7) The Ca concentration of PRD-treated berries at harvest in all of the 3 years was not significantly different to control, however the PRD Cabernet Sauvignon berries had significantly lower Ca content per berry during harvest in 2001 and 2002 compared to control.

8) The concentrations of Mg, P and S were substantially lower than K and Ca in berries of both cultivars during the three-year experiment. Similar to the other minerals, the concentrations of P, Mg and S decreased during the period between veraison and ripening.

9) Similar to the changes in K, the Mg, P and S content per berry (mg/berry) increased during the first 3 weeks after veraison. After 3 weeks the minerals reached a maximum content per berry and gradually decreased thereafter until harvest, indicating a net efflux after a maximum was reached.

10) PRD Cabernet sauvignon berries had significantly lower Mg, P and S contents per berry at harvest in 2001 due to increased efflux rates. This was however not found in the 2001/2 and 2002/3 seasons or in Shiraz berries with PRD and control berries having no significant differences in Mg, P or S contents per berry.

Chapter 11 General discussion and conclusions

11.1 Discussion on PRD research

PRD is being adopted at an increasing rate within the Australian wine industry as a means to improve water use efficiency in grape production. However, the initial aim of PRD was to control excessive shoot growth in irrigated vineyards (Dry *et al.*, 1996). The reduction in shoot growth rate was successfully achieved by drying one part of the root system while irrigating the other, thereby creating two 'zones' that are irrigated in a one to two week rotation. The PRD system relies on hormonal signals originating from the roots in response to low soil water potentials within the 'dry' zone. Much evidence has been accumulated that drying roots are the origin of abscisic acid (ABA), which is involved in regulating stomatal aperture (Düring *et al.*, 1996; Dry *et al.*, 2000b). The observed effects of ABA in aboveground organs due to PRD are a reduction in shoot growth and partial stomatal closure (Dry and Loveys, 1999). Alternating the wet and dry zones was shown to be important to maintain the hormonal signal and the reduction in shoot growth (Dry, 1997; Loveys *et al.*, 1998; Dry and Loveys, 1999).

Although we have now a greater understanding of the physiology behind the PRD system than when first started (Dry *et al.*, 1996), there are still some aspects of its physiology that we do not understand and cannot predict in different environments. There is also a danger that the technique will be inappropriately used and thus lead to negative results. Therefore there were three major focus points of this study into the effect of PRD on grapevine physiology. Firstly, the acquisition of carbon that would include the accumulation of dry weights, sugars and starch and the role that sucrolytic

enzymes play in regulating the source:sink relationship. Secondly, the assimilation of nitrogen and its partitioning into various nitrogen-containing compounds and amino acids, which is highly regulated by specific enzymes. Thirdly, the accumulation and partitioning of inorganic ions in various plant organs, especially K, in the berries of PRD vines that may influence grape composition.

One of the main effects of PRD is a reduction in shoot growth without significant effects on fruit yield. The question arose whether this PRD effect is associated with a change in partitioning of nutrients from vegetative towards reproductive structures. The experiments described in this study were designed to test the general hypothesis that: *'Partial drying of the root system of grapevines will change the partitioning of dry matter, carbon, nitrogen and inorganic ions of grapevines away from shoot growth, towards the permanent structure and fruit by affecting the enzyme activity associated with growth.'*

11.1.1 PRD effects on growth and the accumulation of dry matter

Transient reductions in shoot growth rate in response to soil drying were found in earlier work (Dry *et al.*, 1996) and as a consequence it was proposed that a constant effect on shoot growth could be maintained by alternating the wetting zones. Since then, significant work by Dry and Loveys (1999) and Stoll (2000) has provided evidence that PRD treatment in this way reduces shoot growth rate by 18% to 30% in field-grown grapevines over a whole season. Results in this study support the findings of those authors. PRD decreased grapevine shoot growth when irrigated with either half or the same volume of water and predominantly affected lateral shoot growth. Vigorous lateral shoot growth is undesirable, because this may lead to dense canopies and an imbalance favouring vegetative growth versus fruit production (Smart, 1985).

Furthermore, in crops like tomato the developing side shoots may be a competing sink alongside developing fruit (Davies *et al.*, 2000) and it is speculated that a reduction in the strength of this non-harvestable sink may increase the relative sink strength of the fruit (Dry *et al.*, 1996).

Stoll (2000) observed that PRD-treated vines had canopies with lower leaf area and became more open, resulting in higher light intensities inside the canopy compared to canopies of control vines with a larger leaf area. The exterior leaves of grapevine canopies are exposed to direct sunlight and therefore they are the major contributors in canopy photosynthesis (Smart, 1985). Only a small fraction of total radiation penetrates deep into the canopy and therefore higher light penetration into dense canopies may have significantly positive effects on berry quality. Mabrouk and Sinoquet (1998) suggested that anthocyanin and bunch exposure is not linearly related, but could be described as a "quadratic relationship" with a bell shape. Bunches that received the lowest and highest amounts of solar radiation had the lowest anthocyanin concentration. According to their results, the highest amounts of anthocyanin concentration were achieved with 9% to 11% of ambient solar radiation. In the current study, PRD treatment increased the light penetration into the bunch zone of vines in the Coombe Vineyard (Adelaide, South Australia) irrespective of canopy hedging or the volume of water applied. The reduction in shoot growth caused by PRD also significantly reduced the canopy size of PRD Shiraz vines at Nuriootpa by 26% on average compared to controls. These findings are in agreement with the conclusions of Dry *et al.* (2001) and the findings of dos Santos *et al.* (2003). Those

authors found that the effect of PRD on vegetative growth was characterized by lower total leaf area, lower leaf layer number, decreased canopy wideness and decreased number of water shoots.

Vegetative growth can also be quantified by measuring the pruning weight. Stoll (2000) found that PRD treatment significantly reduced the mean shoot weight and pruning weight in grapevines compared to control, as a consequence of a reduction in shoot growth. In this study, the measurement of the total accumulated seasonal dry matter by winter pruning weight of field-grown vines (Section 4.3.6) were compromised in field-grown vines by mechanical hedging during the growing period, removing more accumulated dry weight from control vines than PRD vines. However, the mean shoot weight of PRD-treated vines was reduced compared to control vines, irrespective of the amount of irrigation water applied. Pot experiments in the current study with both PRD and exogenous ABA treatments reduced the accumulation of dry matter in both leaves and one-year old shoots, the vegetative section of the grapevine, while slightly increasing dry matter of permanent wood compared to control vines (Section 5.3.1). Both treatments however had no effect on the dry weight of roots. According to the 'functional equilibrium' of Porter and Nagel (2000), the physiological reaction of plants to the reduction of shoot growth in response to unfavorable soil moisture conditions, is to partition nutrients to increase root volume in order to overcome the 'most limiting' factor i.e. low soil moisture, by exploring deeper layers of the soil. Mingo *et al.* (2004) found such an increase in the root activity of young tomato subjected to PRD, increasing the root biomass by 55%. In the current study however, older plants were used that already had a well developed root system. Therefore, the volume of soil used in the experiment may have restricted the growth and utilization of the excess nutrients in PRD and exogenous ABA grapevines that would have led to greater root activity in the field environment. Stoll *et al.* (2000a) have shown that PRD-treated grapevines roots grew to greater depths in the field and pot environment while Dry *et al.* (2000a) and Kang *et al.* (2002) found that, in half-dried pot vines, there was a relative increase in root development in the moist soil layers in the 'wet' side as a whole and the deeper layers of the 'dry' side compared to control.

11.1.2 PRD effects on C and N assimilation at the biochemical level

Growth and the accumulation of dry matter are inextricably linked to the regulation of transpirational water loss, as demonstrated in the current study and earlier work by Loveys *et al.* (1998) and Dry *et al.* (2000b). Significant reductions in both shoot growth and stomatal

conductance in response to soil drying took place without any change in shoot water relations, suggesting the involvement of a non-hydraulic signal originating from the roots. Using split-rooted plants, Gowing *et al.* (1990) demonstrated that many effects of water stress could be explained by the transport of chemical signals from the roots to shoots and that the excising of the drying rootzone would eliminate the source, thereby leading to the recovery of water stressed plants. Drying soils have been correlated with increases in root abscisic acid (ABA) and much evidence has been accumulated that drying roots under PRD conditions are the source of ABA, which is involved in reducing stomatal aperture and shoot growth (Loveys, 1984; Düring *et al.*, 1996; Dry *et al.*, 2000b). Stoll *et al.* (2000b) have found that the cytokinin concentrations in roots, shoot tips and buds of PRD-treated vines significantly decreased compared to well-watered vines. This decrease in cytokinins may have contributed to the reduction in shoot growth associated with PRD treatments.

A summary of the effect of PRD treatment on grapevine enzyme activities involved in nitrogen and carbon assimilation and partitioning compared to control is shown in Figure 11.1. The ultimate effect of PRD on the accumulation and partitioning of carbohydrates and nitrogenous compounds in grapevine organs compared to control is summarized in Figure 11.2.

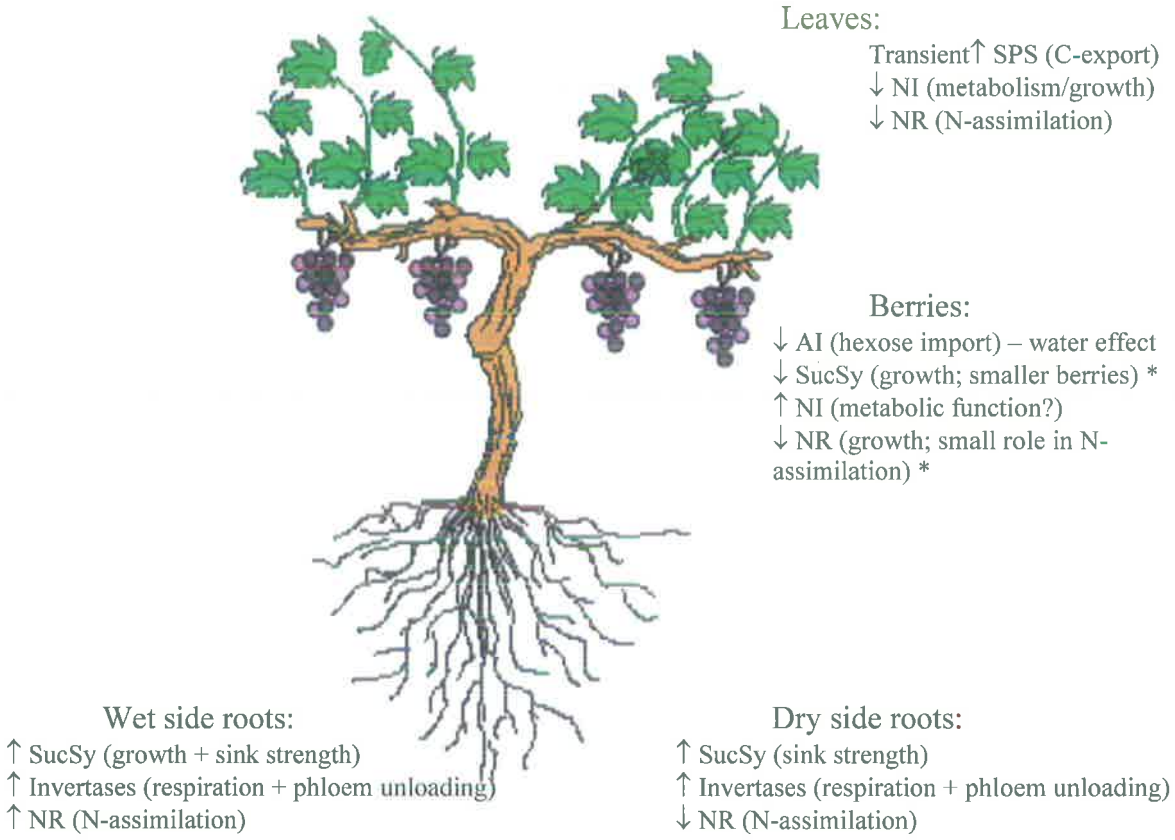


Figure 11.1 Effect of PRD treatment on grapevine enzyme activities related to growth, source:sink relationship, carbon partitioning and nitrogen assimilation (* = may be related to reduced amounts of irrigation water compared to control and/or the effect on berry size; SPS = Sucrose Phosphate Synthase; SucSy = Sucrose Synthase; AI = Acid Invertase; NI = Neutral Invertase; NR = Nitrate Reductase).

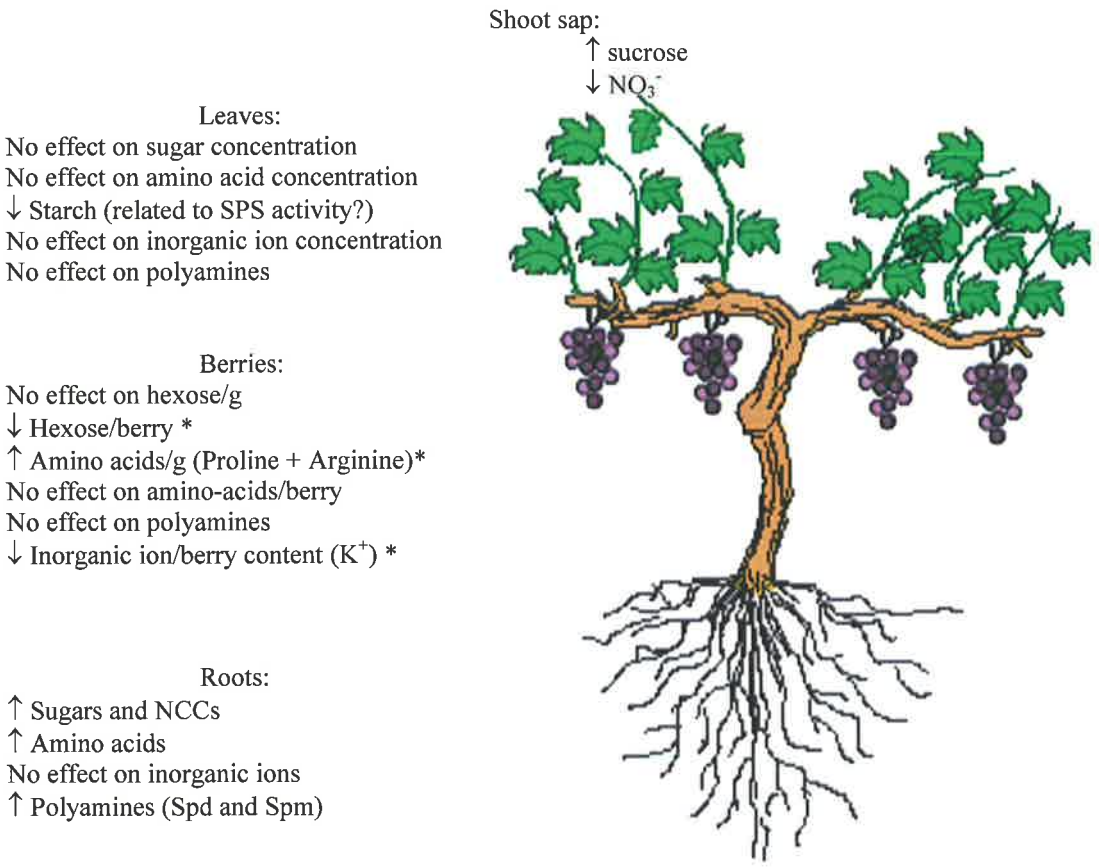


Figure 11.2 Effect of PRD treatment on the accumulation and partitioning of carbohydrates and nitrogenous compounds (* = may be related to reduced amounts of irrigation water compared to control and/or the effect on berry size; Spd = Spermidine; Spm = Spermine).

Leaves are the major factor in determining the bulk of the vine nitrogen content and most fast growing plants reduce nitrate in the leaves where the main reducing power is derived directly from photosynthesis (Huber *et al.*, 1992b; de Cires *et al.*, 1993). Foyer *et al.* (1998) found that water stress caused the inhibition of photosynthesis and was correlated with a marked decrease in total NR activity. In the current study, leaf stomatal conductance and NR activity were significantly reduced in response to PRD treatment in both field-grown Cabernet Sauvignon and Shiraz grapevines regardless of the amount of irrigation water applied (Section 6.3.3). A strong hyperbolic relationship exists between the processes, but PRD may have had independent effects on stomatal conductance and leaf NR activity, because transient increases in NR activity in field vines were not directly associated with increases in stomatal conductance. Exogenous ABA treatment provided further evidence that stomatal conductance and leaf NR activity are independently affected by PRD treatment since ABA action on its own reduced stomatal conductance without influencing leaf NR activity (Section 6.3.4). It is hypothesized that PRD effect on NR activity may be associated with the reduced production of

cytokinin that may start to recover after extended periods of soil drying and cause transient increases in leaf NR activity. Although not measured in this study, Stoll *et al.* (2000) have clearly shown the marked inhibitory effect of PRD on the production of cytokinins in grapevines. Alternatively, a significant decrease in PRD xylem sap NO_3^- concentration may be due to a significant decrease in the 'dry' side root nitrogen assimilation as measured by its NR activity (Section 6.3.6). During periods of extended soil drying, there may be a change in root physiology to increase production of NO_3^- in roots that are well watered, thereby increasing leaf NR activity without affecting stomatal conductance. This could be an area of further research that could have consequences for recommendations on the N fertilizer needs of PRD-treated vines.

It is therefore hypothesized that the inhibition of NR in PRD vines may be due to: 1) because half of the root system is faced with a diminishing soil water content, the nitrogen absorption of the roots may be decreased, thereby reducing NR activity because of its ability to be substrate inducible (Gojon *et al.*, 1991). In terms of root to shoot communication, nitrate itself is the primary signal molecule triggering the activation of transcription of nitrate assimilation and related genes (Takei *et al.*, 2002). Furthermore, nitrogen availability could commonly modulate cytokinin metabolism and translocation in higher plants (Takei *et al.*, 2002). Thus in addition to nitrate, cytokinin could be a root to shoot signal communicating nitrogen availability. 2) It is possible that NR activity may be directly influenced by the change in the ABA/cytokinin balance in PRD vines. The major phytohormone that influences NR is cytokinin (for review see Gaudinova (1990)). NR activity is greatly increased in leaves in response to the treatment with the cytokinin benzyladenine (BA) (Kende *et al.*, 1971; Yu *et al.*, 1998) and suppressed by ABA (Lu *et al.*, 1992). The NR mRNA levels are influenced by the BA/ABA concentration ratio and the inhibition of ABA can only be partially reversed by equal concentrations of BA (Lu *et al.*, 1992). ABA treatment elicits a variety of responses on NR activity in plant systems and may be related to why shoot growth is more sensitive than root growth to soil drying (Sharp and Davies, 1989). At relatively high concentrations it reduces NRA in etiolated leaves of barley (Lu *et al.*, 1992), potato (Palmer, 1985) and in *Agrostemma githago* (Kende *et al.*, 1971). Conversely, ABA was shown to stimulate NRA in root systems (Palmer, 1981; Chraibi *et al.*, 1995; Goupil *et al.*, 1998). This may be due to ABA increasing radial growth of roots under stress conditions, i.e. drought, compacted soil (Hartung and Davies, 1991; Vartanian *et al.*, 1994). Goupil et al. (1998) and Chraibi et al. (1995) found that NR activity in roots opposed to

shoots was not related to intracellular NO_3 concentration and not modulated by a phosphorylation/dephosphorylation mechanism. Palmer (1981) however, found that ABA stimulated root NR activity at low NO_3 levels while inhibiting NR activity at high NO_3 levels. Application of ABA (Chraibi *et al.*, 1995; Goupil *et al.*, 1998) to the roots increased sucrose hydrolysis and the levels of glucose and fructose both decreased dramatically. Chraibi et al. (1995) concluded that the increased NR activity may be due to an increased availability of reductants which are less diverted to growth. The accumulation of ABA in drying roots is found to be necessary for root growth maintenance during water stress (Sharp and LeNoble, 2002) and to prevent excess ethylene production (Saab *et al.*, 1990; Spollen *et al.*, 2000; Sharp and LeNoble, 2002).

In the current study, PRD treatment significantly increased root osmolality in both the PRD 'wet'- and 'dry'-side roots compared to control roots (Section 7.4.1). The increased osmolality was associated with significant increases in sugars, nitrogen-containing compounds and amino acids that indicated an active osmoregulation of roots in response to lowered soil moisture conditions (Section 7.4.2 and 7.4.3). PRD 'dry' side roots also significantly accumulated higher amounts of sugars and osmolytes than its own 'wet' side, indicating a concentration gradient for water towards the 'dry' roots. Stoll *et al.* (2000b) have shown that water moves from the 'wet' side roots towards the 'dry' side in PRD-treated grapevines to maintain the hormonal signal under low soil moisture conditions. It was also found that very little water is absorbed by 'dry' side roots at low water potentials and that the dry roots are mostly reliant on the water derived from the wet side. It is therefore proposed that the increase in root osmolality by the accumulation of solutes in the 'dry' side roots of PRD-treated plants serves as a water potential gradient that facilitates the water movement from wet to dry roots. Solute accumulation and the increases in root osmolality may be related to observed increases in sucrolytic enzyme activities of PRD-treated roots. Responses in root SucSy activity indicated that both the PRD 'wet' and 'dry' side roots increased their sink strength and/or growth rate compared to control roots within 2 days of treatment; PRD 'wet' side roots substantially more so than the 'dry' side. This may be an important finding that supports the reports of increased root growth in response to PRD by a number of authors (Dry *et al.*, 2000a; Kang *et al.*, 2002; Mingo *et al.*, 2004). PRD 'wet' side roots may increase growth rate to compensate for the 'dry' side that showed reduced rates of growth compared to control, thereby causing a more exploratory root system. Enhanced rooting, especially adventitious root formation, is associated

with the accumulation of polyamines, especially spermine and spermidine (Jarvis *et al.*, 1985; Shyr and Kao, 1985; Kakkar and Rai, 1987; Faust and Wang, 1992). In the current study, PRD roots accumulated significantly higher amounts of spermine and spermidine compared to control roots (Section 7.4.4). Spermine and spermidine are synthesized from putrescine by the addition of one or two aminopropyl groups respectively from S-adenosylmethionine, an intermediate of ethylene biosynthesis (Faust and Wang, 1992). It could therefore be speculated by its association with polyamine production, that ethylene biosynthesis in PRD roots may be involved in the conditioning of roots to increase density in order absorb more water per volume of soil. This hypothesis supports the findings of Dry *et al.* (2001) who reported a significantly higher abundance of roots of the 1 mm to 3 mm diameter at the 0.4 m to 0.7 m depth in PRD roots of field-grown grapevines compared to control vines. The implication is that a more exploratory root system of PRD-treated vines may contribute to greater water stress tolerance and a better soil environment for root growth. Stoll *et al.* (2000a) found evidence of a more exploratory root system in field-grown PRD-treated vines with roots that grew to greater depths than control.

The soluble invertases are proposed to have limited involvement in root growth, but serve a function in osmoregulation, cell enlargement and channeling sucrose into metabolism (Sturm and Tang, 1999). In the current study, the response of acid invertase (AI) and neutral invertase (NI) to soil drying in PRD 'dry' side roots was similar to water stressed conditions to some degree but delayed by 2 days and amplified (Section 7.4.5). PRD 'dry' side roots showed significant increases in both AI and NI activity compared to control that may be involved in osmoregulation by regulating sugar accumulation or an increased sugar flow to respiration in order to tolerate the stressful conditions. Alternatively the increased invertase activity in the PRD 'wet' side roots may be associated with increased growth rates and hence the required increase in phloem unloading compared to control roots.

Hunter and Ruffner (1997) showed that the effect of canopy management that caused an increased light penetration into the bunch zone had only a minor effect on berry NR activity compared to control. Berry NR activity was expected to be higher in response to the canopy manipulation. They concluded that the increase in leaf amino acid formation in response to treatment increased amino acid transport from leaves to berries and may have reduced the induction of nitrate reductase. In this study, PRD marginally decreased berry NR activity when

PRD vines received half the amount of irrigation water as control (Section 6.3.2). This supports the observed reduction of PRD berry sizes measured in the 2000/1and 2001/2 seasons compared to control (Section 9.3.1). However, the NR activity in berries may only have a small contribution to berry amino acid content because the major source of amino acids is the leaves.

Huber *et al.* (1992a) demonstrated that the enzymes of nitrate reduction (NR) and sucrose synthesis (sucrose phosphate synthase; SPS) share common features and are most likely coordinated with one another and the rate of photosynthesis. However, in this study the SPS activity in pot and field-grown experiments was not significantly affected on most days and, on occasion, the leaf SPS activity was actually significantly increased in response to PRD (Section 8.3.2). Exogenous ABA application to the roots of pot-grown vines significantly reduced stomatal conductance, but caused a 4-fold increase in leaf SPS activity compared to control vines (Section 8.3.1). Therefore, it may be possible that the transient increases found in SPS activity and associated decreases in leaf starch in the leaves of field-grown PRD-treated vines were caused by increased ABA and associated with improved photosynthetic capacity in response to soil drying conditions. A PRD-induced increase in leaf sucrose efflux may be caused by a shift in the sucrose:starch fraction by an increase in leaf SPS activity. Mild water stress in spinach (Quick *et al.*, 1989) has been shown to cause a marked change in sucrose:starch partitioning and the authors consequently proposed that the first site at which leaves respond to a rising water deficit is the activation of SPS. The partitioning of photosynthates responds very sensitively to water stress and there is a progressive stimulation of sucrose synthesis and inhibition of starch synthesis when the leaf fresh weight is decreased by 8% to 10%. Quick *et al.* (1989) also noticed that the shift occurred in a region where there was no marked change in the CO_2-saturated photosynthesis. Galtier *et al.* (1995) similarly showed that the over-expression of SPS activity in tomato leaves decreased leaf starch content, but resulted in a 2 to 3-fold increase in sucrose level.

PRD treatment in the current study had no effect on the sugar levels of grapevine leaves throughout the growing season from veraison until harvest compared to control (Section 8.3.3). The amount of sucrose in leaves is regulated by the sucrolytic enzyme acid invertases (AI) (Hammond *et al.*, 1984; Huber, 1989) and it is proposed that high vacuolar AI activity would decrease the amount of sucrose available for both export and storage, while low AI activity would favor sucrose accumulation. In the current study, the AI activity in leaves was unaffected

by PRD treatment (Section 8.3.2). Because AI is located exclusively in the vacuole it is concluded that AI activity did not contribute to the turnover of sucrose in grapevine leaves in response to PRD treatment but rather played a role in maintaining glucose and fructose concentrations.

However, the neutral invertase (NI) activity in both the basal and apical leaves of field-grown PRD-treated vines was significantly reduced compared to control (Section 8.3.2). NI is located in the cytosol and probably involved in the channeling of sucrose into metabolism for growth (Winter and Huber, 2000). A reduction in leaf metabolism of sucrose in response to PRD treatment supports the observed reductions in vegetative growth in PRD-treated grapevines in the current study (Section 4.3.1) and by several authors in earlier work (Dry *et al.*, 1996; Dry and Loveys, 1999; Stoll, 2000). However, whether the reduction of NI is the cause of the reduced growth rate in PRD-treated grapevine shoots or just a consequence is not known. The reduced metabolism of PRD-treated leaves may greatly reduce the overall diurnal demand for sucrose in the grapevine, consequently the amount of stored carbohydrates in leaves as starch was found to be less in response to PRD treatment (Section 8.3.3 and 5.3.2).

11.1.3 PRD effects on the partitioning of carbon and nitrogen

Clarkson and Hanson (1980) argued that the quantity of nutrient absorbed by a plant over a given period of time is an integration of the growth that has occurred and that the rate of absorption from a given concentration is determined by the "demand" for nutrients created by growth. In the current study, PRD treatment had no significant effect on the total vine accumulation of C or N compared to control (Section 5.3.5). However, the partitioning of N and C was significantly affected by increasing the accumulation in permanent wood and shoots at the expense of the leaf canopy. The ratio of total N to total C per organ indicated that both PRD and exogenous ABA treatments affected grapevine physiology by significantly increasing the total N/C ratio in berries, leaves, permanent wood and roots. It is clear that PRD and exogenous ABA treatment caused investment of significantly more resources to permanent structures and roots at the expense of vegetative growth. PRD had significantly smaller investment in C storage as starch in leaves that could not be attributed to the hormonal influence of root-borne ABA (Section 5.3.2). Carbohydrate storage in leaves however follows a diurnal pattern and leaf starch builds up in daylight when photosynthesis exceeds the combined rates of respiration and export. To achieve a steady state of sucrose export rate, starch synthesis begins and ends

gradually during the daily light period (Chatterton and Silvius, 1979). The amount of starch that accumulates daily in leaves is regulated by the average rate of photosynthesis and is believed to be just enough to support sucrose export during the night (Sims *et al.*, 1998).

The accumulation of starch in permanent wood may be the most important carbohydrate reserve in the grapevine. Firstly, it is the carbohydrate reserve in the trunk and arms that is rapidly mobilized at the beginning of berry ripening. The stored carbohydrates in permanent wood are utilized for new growth in spring and the amount of available carbohydrates seems to be a significant factor in flower development and fruit set (Winkler *et al.*, 1974). In the current experiment, PRD and exogenous ABA significantly increased the total amount of starch accumulated in the permanent structures compared to control (Section 5.3.5). This partitioning may be explained by the 'functional equilibrium' model of Poorter and Nagel (2000), that under limited soil moisture conditions, the grapevine may "rank" the permanent wood and roots as being the organs most limited and carbohydrates are partitioned to aid in osmotic function and increase vine capacity to overcome times of perceived stress.

The investigation into C and N nutrition in field grown grapevines in the current study provided inconclusive results. No significant effect of PRD treatment on the accumulation of nutrients or in the C/N ratio of all the different plant tissues measured compared too control could be found (Section 5.3.6). It is proposed that, unlike the pot environment where roots have constraints in terms of volume and moisture, the excess nutrients partitioned to permanent structures in field-grown grapevines would be used to enlarge its root structure to exploit the deeper layers of soil.

Labeled nitrogen (^{15}N) measurements are often used to elucidate the absorption and partitioning of newly absorbed N during a certain period of time (Conradie, 1986; Glad *et al.*, 1994). The grapevine's demand for seasonal nitrogen is fairly well known, but by using conventional methods it is not possible to distinguish between nitrogen absorbed during different periods within one growing season. Apart from the permanent structure, leaves and shoots are important transitional reservoirs for absorbed nitrogen and up to harvest nitrogen is turned over at a relatively fast rate in these organs with large contributions to fruit, even though the total nitrogen content does not change (Conradie, 1991). In the current study, PRD-treated vines absorbed significantly less ^{15}N than exogenous ABA-treated and control vines during the ripening season that extended from veraison until harvest (Section 5.3.4). This was manifested

in lower ^{15}N abundances in berries, leaf canopy, one-year old shoots and thin roots. However, the total amount of ^{15}N partitioned to the fruit was the same. As for the rest of the plant, similar to the accumulation of starch and inorganic minerals, PRD and exogenous ABA increased the partitioning of ^{15}N to the permanent structure, specifically to the older wood at the expense of the vegetative structure. It is therefore clear that PRD-treated vines absorb less nitrogen during the ripening period than control and therefore fertilization needs may be lower than control vines. However, the fertilization needs of PRD vines during the pre-veraison and post-harvest period is still unknown and is an area of further research. The effect of PRD treatment on the partitioning of C and N between organs however shows the same trend and is supported by the differences found in enzyme activities involved in the assimilation and partitioning processes at the biochemical level. Vegetative organs of PRD-treated vines that showed significant reductions in growth and accumulation in C and N were associated with significant inhibitions of NR activity and sucrolytic enzyme activity, while organs such as roots and fruit that had favored partitioning of nutrients had increased activities of selected enzymes compared to control vines (see Section 12.1.2).

Soluble sugars (sucrose) and amino acids are formed in photosynthetically active leaves and transported to organs in order of their sink strength. A change in the organ sink strength or production in source leaves may have an effect on the partitioning of C and N in the vine as a whole. In general, soluble sugar content tends to be maintained in the leaves of droughted plants, in spite of lower rates of carbon assimilation (Du *et al.*, 1998; Chaves *et al.*, 2003). This is achieved at the expense of starch, which drastically declines (Chaves, 1991). This response favors osmoregulation and enhances desiccation tolerance. Similarly, PRD treatment in this study had no effect on the evolution of sugars (sucrose, glucose and fructose), sugar-alcohols (pinitol and mannitol) or nitrogen-containing compounds (glycine betaine, methyl proline and proline) in leaves of field-grown vines during the season compared to control, irrespective of the amount of irrigation water applied (Section 8.3.3). The most abundant amino acids were arginine in leaves and proline and arginine in shoots. PRD treatment of Cabernet Sauvignon vines had no significant effect on the amino acid profile nor the total amino acid contents in leaves or shoots compared to control (Section 8.3.3). This indicated that there was no detrimental effect of PRD on the production and accumulation of amino acids in grapevine shoots compared to control even though PRD-treated vines received half the amount of irrigation water as control vines.

11.1.4 PRD effects on berry characteristics and the accumulation of inorganic ions

The ultimate goal of irrigation and canopy management is to benefit fruit quality in a sustainable way without disturbing growth and development in other sinks. In the current study, the volume of irrigation water applied affected the characteristics and composition of berries more than PRD treatment. Irrigating PRD-treated grapevines with half the volume of irrigation as control advanced maturity but significantly reduced berry growth and maturation later in the ripening period (Section 9.3.1). Other berry characteristics such as juice pH and total soluble solids were unaffected by PRD treatment. However, PRD treatment of Cabernet Sauvignon vines reduced the berry acid invertase (AI) and sucrose synthase (SucSy) activities compared to control when irrigated with half the volume of irrigation water compared to control (Section 9.3.2). This may have been an indication of reduced sink strength in response to a reduction in berry growth. At the same time, the neutral invertase (NI) activity in PRD-treated berries was greatly increased compared to control irrespective of the volume of irrigation water applied.

This PRD effect on berry sucrolytic enzyme activities was also manifested in the accumulation of hexose during the ripening period. With the reduced volume of irrigation water, the hexose content per berry was significantly reduced in response to PRD treatment compared to control (Section 9.3.3). According to Ollat and Gaudillere (1996) the sink strength of the berry may be estimated by carbon import and accordingly it may be deduced that in some cases berry sink strength was reduced in PRD berries compared to control. However, when PRD treatment did not affect berry size no significant effects on hexose accumulation and sink strength could be found compared to control. In fact, the results of PRD Nuriootpa Shiraz indicated that hexose accumulation in the berries can be increased by PRD in situations where the appropriate cultural practices is used to balance vegetative growth with crop level (Section 9.3.3). Average pruning levels showed the potential to increase the accumulation of hexose under PRD conditions while higher than normal pruning levels tended to magnify the effect of PRD treatment on berry size. Consequently the amount of hexose accumulated per berry in PRD-treated vines was significantly less compared to control. It is therefore concluded that environmental factors and cultural practices may have a strong influence over the PRD effect on berry size and sink strength.

Amino acids, the first products of ammonium assimilation, are the building blocks of proteins in grapevines and play a major role in the fermentation of musts (Roubelakis-Angelakis and Kliewer, 1992). Both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine (Stines *et al.*, 2000). Stines *et al.* (2000) concluded that the basic pattern of amino acid composition in mature fruit is determined by genetic factors and that environmental and cultural factors have only a modifying effect. In the current study, the volume of irrigation water had a significant effect on the accumulation of amino-acids in berries. PRD-treated vines that received half the volume of irrigation water accumulated proline in berries to significantly higher concentrations compared to control in the later stages of ripening. Together with slightly higher arginine concentrations the PRD-treated berries had a significantly higher total amino acid concentration at harvest compared to control. In comparison, PRD-treated vines that received the same amount of irrigation water as control showed no significant differences in the total amino acid concentration in berries, although there were some significant decreases in minor amino acids compared to control. In conclusion, the significant increase in berry amino acid concentration in response to PRD treatment may be solely due to the reduced volume of water applied compared to control and the reducing effect on berry size that probably caused increased concentration of amino acids.

Sugar is the major solute that accumulates in ripening berries, but minerals such as potassium (K), calcium (Ca), sodium (Na), copper (Cu), magnesium (Mg), manganese (Mn) and phosphate (P) can contribute significantly as osmotic components. K is by far the major cation in ripe berries (Mpelasoka *et al.*, 2003) and plays the most important role in osmoregulation and plant water relations compared to other inorganic ions, especially under conditions of low sugar accumulation. Excessive K in berries can cause problems in the vinification process by decreasing free acids by precipitation of tartrate and increasing overall pH that may cause problems in color stability during storage (Morris *et al.*, 1983). Increased shoot growth and dense canopies caused by high soil moisture conditions would generally increase bunch shading. Jackson *et al.* (1993) found that fruit from internally shaded canopies produced wines with high pH and K that exhibited poorer characteristics. Reduced shoot growth and better light penetration in response to PRD treatment may have positive effects on berry pH and K content. In the current study, PRD-treatment did not significantly affect the berry K concentration relative to control, irrespective of the volume of water applied (Section 10.3) . However, in

situations where PRD treatment reduced berry size, the K content per berry was reduced by a similar degree. Therefore, the main effect of PRD treatment on berry K was due to the effect on berry size in response to the amount of water applied. Furthermore, PRD-treatment of vines pruned to higher pruning levels had significantly lower berry K content than control vines (Section 10.3.4). PRD treatment may have a greater effect on berry pH and K accumulation when vines are grown with dense canopies. The PRD effect on the minor inorganic ions that included Ca, P, S and Mg was irregular over the 3 year study and mostly insignificant compared to control.

11.2 Practical implications

This study has broadened our knowledge of the physiological aspects of the PRD irrigation system. It was shown that not only does PRD affect the vegetative growth of grapevines, but also the biochemical processes that influence the assimilation and partitioning of C and N. Enough evidence was collected to conclude that PRD partitioned nutrients and dry matter away from the vegetative structure towards the permanent structure in order to explore the soil environment by investing C and N into root growth and osmoregulation. Consequently, PRD vines would be more tolerant of stressful environments, such as transient periods of drought. However in the current study, it was shown in labeled nitrogen experiments that the amount of nitrogen absorbed during the season would be less in PRD-treated grapevines. This may be due to the reduced growth rate and lowered NR activity in leaves. It is possible that the fertilization needs of PRD vines may be lower than control vines that would mean a saving in the production costs to the producer. A smaller area of irrigation under PRD vines would also mean that fertilizers would be lost more slowly than control vines.

One of the main questions been asked of the PRD irrigation system is why an alteration of wetting pattern is necessary. Would a simple reduction in irrigation water volume not have the same effect? The main differences that were encountered in the current study between PRD treatments that received half and the same volume of irrigation water as control respectively, are summarized in Table 11.1. This will help us understand the effects attributable to water volume as opposed to a 'real' PRD effect.

Many of the vine characteristics remained unaffected by PRD treatment irrespective of the amount of irrigation water applied compared to control. This in itself seems to be significant,

because sub-optimal soil moisture conditions would normally have a negative impact on vine physiology by reducing the photosynthetic apparatus and challenging the vine's ability to assimilate carbon and nitrogen for growth. Rabe (1990) concluded in his review that water stress usually causes the accumulation of nitrogen containing compounds (NCCs) in plant tissues. The NCCs that most commonly accumulate in response to water stress are proline, glycine betaine and putrescine.

Table 11.1 Various physiological responses of grapevines on PRD treatment (% change compared to control). (n.s. = not significant). Shaded areas = PRD effects that may be related to a degree of water stress.

Response	**PRD gets 50% water of control**	**PRD gets 100% water of control**
Shoot growth rate (Main)	Reduced 12% to 34%	Reduced by 8% to 18%
Shoot growth rate (Lateral)	Reduced 22% to 74%	Reduced by 16% to 19%
Winter pruning weight	No significant difference	No significant difference
Winter shoot weight	No significant difference	No significant difference
Harvest leaf area (canopy size)	Reduced by 26%	Reduced by 30%
Light penetration into the bunch zone	Increased by 11%	Increased by 21%
Stomatal conductance	Reduced by 22% to 41%	Reduced by 7% to 16%
Leaf water potential	No significant difference	No significant difference
Leaf starch	Reduced by up to 40%	?
Total leaf sugars and amino acids	No significant difference	No significant difference
Total shoot sugars and amino acids	No significant difference	No significant difference
Berry and leaf polyamines	No significant difference	No significant difference
Shoot polyamines	Increased putrescine	No difference
Yield	Reduced by 5% (n.s.)	Increased by 4% (n.s.)
Berry weight/size	Reduced by 8%	No significant difference
Berry TSS	No significant difference	No significant difference
Berry hexoses concentration	No significant difference	No significant difference
Hexoses/berry	Reduced by 8 – 10%	Increased by 9% (n.s.)
Berry pH	No significant difference	No significant difference
Berry total amino acid concentration	Increased by 8 – 30%	Reduced by 3 – 13%
Berry K concentration	No significant difference	No significant difference
K/berry	Reduced by 7%	No significant difference
WUE (t/100mm)	Increased by 77 - 106%	No significant difference
Berry N/C ratio	Increased by 16.8%	No significant difference
Leaf N/C ratio	No significant difference	No significant difference
Shoot N/C ratio	No significant difference	No significant difference
Berry NR activity	No significant difference	?
Berry AI activity	Reduced 12 - 32%	Increased by 21%
Berry NI activity	Increased by 146%	Increased by 116%
Berry SucSy activity	Reduced 24 - 48%	Increased by 50%
Leaf NR activity	Decreased up to 29%	Decreased up to 9%
Leaf SPS activity	Transient increases up to 48%	Transient increases up to 30%
Leaf NI activity	Reduced by 57%	?
Leaf AI activity	No significant difference	?
Leaf SucSy activity	No significant difference	?

However, PRD-treated vines in the current study showed no reductions in tissue sugars and amino acids or accumulations in NCCs or polyamines compared to control, irrespective of the amount of irrigation water applied. This study and previous work by Stoll (2000) have shown that large changes in stomatal conductance had only minor effects on assimilation rate under PRD. The higher water loss from fully open stomata without an increase in CO_2 assimilation could be considered a luxurious consumption and an inefficient use of water if no other processes were affected. A reduction in stomatal aperture without a concurrent reduction in assimilation rate would greatly increase the grapevine's water use efficiency. Furthermore, it may be possible that the transient increases found in leaf SPS in response to PRD were caused by an ABA-induced effect to improve the photosynthetic capacity in response to soil drying conditions.

The reduction in shoot growth, accumulation of dry matter, stomatal conductance and NR activity is common to the two different rates of irrigation, and similar to many earlier reports (Loveys *et al.*, 2000; Stoll *et al.*, 2000b; de Souza *et al.*, 2003; dos Santos *et al.*, 2003), it is concluded that PRD is mainly responsible for the effect on vegetative growth. Canopies with lower leaf area become more open, resulting in higher light intensities inside the canopy compared to canopies with a larger leaf area.

PRD treatment in my experiments had no significant effect on yield. However, increased light penetration into the fruiting zone may have beneficial effects on berry composition. PRD-treated vines that received half the volume of irrigation water as control had, on occasion, smaller berry sizes that resulted in higher amino acid concentrations and lower K/berry. This may have positive ramifications on the vinification process and the quality of the wine. Lowered amount of K would prevent high pH due to the precipitation of tartrate in salt form during vinification and also enhance color stability during storage (Mpelasoka *et al.*, 2003). Stines *et al.* (2000) reported that both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine. The evaluation of individual amino acids on wine quality is however a highly specialized area of research that falls beyond the scope of the current study. However, this may be an area of future research.

11.3 Future directions

It has been shown, in the current study, that PRD reduced shoot growth by its inhibitory effects on NR activity in grapevine shoots. However, the principle factor responsible for the reduction in NR activity has not been clearly elucidated. The possibility exists that in my experiments there was a recovery in cytokinin production before the switch in PRD irrigation that might have caused an increase in leaf NR activity. On the other hand, soil drying under PRD conditions may have influenced the amount of nitrate absorbed and delivered to shoots. Both explanations are supported by experimental results in the current study and by earlier CK work that was done by Stoll (2000). Further experimentation that should include the manipulation of CK levels and resultant effect on leaf NR activity under PRD conditions. In addition, the analyses of PRD treatment on the concentration of NO_3^- in the xylem sap of field-grown grapevines should be done on a larger scale over a longer duration. This would elucidate the diurnal and long-term effect of PRD on the absorption of nitrogen and the role that nitrate play in root to shoot signaling.

In future investigations into the effect of PRD on the partitioning of C and N, a larger population of fruiting vines should be used for more detailed total C and N analyses. I started this experiment in 2002 with 80 split-rooted Cabernet Sauvignon, but unfortunately the vines did not bear fruit. The effect of PRD treatment on the distribution of these macronutrients can then be elucidated by periodically harvesting smaller samples of vines throughout the growing season. Destructively harvesting vines and measuring all plant weights and nutrient status would show the detailed effect of PRD on the accumulation and partitioning of nutrients during specific periods in the growing season. This will allow a more detailed explanation of the effect of PRD on the growth and development of treated vines compared to control and a more comprehensive recommendation could be given on fertilization needs.

It was concluded that PRD has the potential to reduce the K content of berries in situations of highly vigorous growth that resulted in dense canopies. This may be an area of future research, because the control vines in the current study had moderate shoot vigor. More significant results may be obtained by using excessively vigorous vines under PRD conditions.

Stines *et al.* (2000) reported that both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the

resultant wine. PRD showed significant increases in some amino acids, while reducing others compared to control. There were also very different combinations of amino acids from year to year that may influence wine characteristics. The effect of different combinations and individual amino acids on wine quality may be an area of future research that may differentiate wines made from PRD grapevines from those of control vines.

I conclude that environmental factors and cultural practices may have a strong influence on the PRD effect on berry size and sink strength. Higher pruning levels that influence canopy architecture and increase sink demand from growing shoots have negative effects on berry growth. This may be an area of future research to determine the PRD effect under different conditions of site and cultural practices. PRD may possibly modulate rootstock and scion interactions to form unique interactions with the soil environment.

Literature cited

Adams, D. O., Bates, D. J., Adams, D. F. and Franke, K. E. 1992. The effect of agmatine and other precursors on the accumulation of putrescine in grape leaves. *Am. J. Enol. Vitic.* **43**(3): 239-243.

Amthor, J. S. and McCree, K. J. 1990. Carbon balance of stressed plants: A conceptual model for integrating research results. Wiley-Liss, Inc.

Aruajo, F. J. and Williams, L. E. 1988. Dry matter and nitrogen partitioning and root growth of young field-grown Thompson Seedless grapevines. *Vitis* **27**: 21-32.

Aspinall, D. and Paleg, L. G. 1981. Proline accumulation: Physiological aspects. In: "The physiology and biochemistry of drought resistance in plants". (eds. Paleg, L. G. and Aspinall, D.) New York, Academic Press: 205-241.

Atkinson, D. E. 1977. Cellular energy metabolism and its regulation. New York, Academic Press.

Barradas, V. and Jones, H. G. 1996. Responses to CO_2 assimilation to changes in irradiance: laboratory and field data and a model for beans (*Phaseolus vulgaris* L.). *J. Exp. Bot.* **47**: 639-645.

Bates, T. R., Dunst, R. M. and Joy, P. 2002. Seasonal dry matter, starch and nutrient distribution in 'Concord' grapevine roots. *HortScience* **37**(2): 313-316.

Bena-Tzourou, I., Lanaridis, P. and Metafa, M. 1999. Influence of cluster thinning on the amino acids concentration of musts and wines of the variety Vilana. Effect on wine volatile compounds. *Journal International des Sciences de la Vigne et du Vin* **33**(3): 111-117.

Boulton, R. B. 1980. The general relationship between potassium, sodium and pH in grape juice and wine. *Am. J. Enol. Vitic.* **31**: 182-186.

Brenner, M. L., Schreiber, B. M. N. and Jones, R. J. 1989. Hormonal control of assimilate partitioning: Regulation in the sink. *Acta Horticulturae* **239**: 141-147.

Bret-Harte, M. S. and Silk, W. K. 1994. Nonvascular, symplastic diffusion of sucrose cannot satisfy the carbon demands of growth in the primary root tip of *Zea mays* (L.). *Plant Physiol.* **105**: 19-33.

Campalans, A., Messeguer, R., Goday, A. and Pages, M. 1999. Plant responses to drought, from ABA signal transduction events to the action of the induced proteins. *Plant Physiol. Biochem.* **37**(5): 327-340.

Chapin, F. S. I. 1991. Integrated responses of plants to stress: a centralized system of physiological responses. *Bioscience* **41**: 29-36.

Chatterton, N. J. and Silvius, J. E. 1979. Photosynthate partitioning into starch in soybean leaves. I. Effects of photoperiod versus photosynthetic period duration. *Plant Physiol.* **64**: 749-753.

Chaves, M. M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.* **42**: 1-16.

Chaves, M. M., Maroco, J. P. and Pereira, J. S. 2003. Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology* **30**: 239-264.

Cheikh, N. and Brenner, M. L. 1992. Regulation of key enzymes of sucrose biosynthesis in soybean leaves. *Plant Physiol.* **100**: 1230-1237.

Cheng, C. L., Acedo, G. N., Christinsin, M. and Conkling, M. A. 1992. Sucrose mimics the light induction of *Aridopsis* nitrate reductase gene transcription. *Proceedings of the National Academy of Science of the USA* **89**: 1861-1864.

Chou, I. T., Chen, C. T. and Kao, C. H. 1991. Characteristics of the induction of the accumulation of proline by abscisic acid and isobutyric acid in detached rice leaves. *Plant Cell Physiol.* **32**: 269-272.

Chraibi, A., Palms, B., Druart, N., Goupil, P., Gojon, A. and Rambour, S. 1995. Influence of abscisic acid on nitrogen partitioning, sucrose metabolism and nitrate reductase activity of chicory suspension cells. *J. Exp. Bot.* **46**(291): 1525-1533.

Claassen, N. and Barber, S. A. 1974. A method of characterizing the relation between nutrient concentration and flux into roots of intact plants. *Plant Physiol.* **54**: 564-568.

Clarkson, D. T. and Hanson, J. B. 1980. The mineral nutrition of higher plants. *Ann. Rev. Plant Physiol.* **31**: 239-298.

Claussen, W., Loveys, B. R. and Hawker, J. S. 1985. Comparative investigations on the distribution of sucrose synthase activity and invertase activity within growing, mature and old leaves of some C_3 and C_4 plant species. *Physiol. Plant.* **65**: 275-280.

Comstock, J. P. 2002. Hydraulic and chemical signaling in the control of stomatal conductance and transpiration. *J. Exp. Bot.* **53**(367): 195-200.

Conradie, W. J. 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. *S. Afr. J. Enol. Vitic.* **1**: 59-65.

Conradie, W. J. 1981. Seasonal uptake of nutrients by Chenin Blanc in sand culture: II. phosphorous, potassium, calcium and magnesium. *S. Afr. J. Enol. Vitic.* **2**: 7-13.

Conradie, W. J. 1986. Utilization of nitrogen by the grapevine as affected by time of application and soil type. *S. Afr. J. Enol. Vitic.* **7**: 76-83.

Conradie, W. J. 1991. Distribution and translocation of nitrogen absorbed during early summer by two-year-old grapevine grown in sand culture. *Am. J. Enol. Vitic.* **42**(3): 180-190.

Coombe, B. G. 1989. The grape berry as a sink. *Acta Horticulturae*(239): 149-158.

Coombe, B. G. 1992. Research on development and ripening of the grape berry. *Am. J. Enol. Vitic.* **43**(1): 101-110.

Coombe, B. G., Bovio, M. and Schneider, A. 1987. Solute accumulation by grape pericarp cells. V. Relationship to berry size and the effects of defoliation. *J. Exp. Bot.* **38**: 1789-1798.

Coombe, B. G. and Monk, P. R. 1979. Proline and abscisic acid content of the juice of ripe Riesling grape berries: effect of irrigation during harvest. *Amer. J. Enol. Vitic.* **30**: 64-67.

Crawford, N. M. 1995. Nitrate: nutrient and signal for plant growth. *Plant Cell* **7**: 859-868.

Crawford, N. M. and Glass, A. D. M. 1998. Molecular and physiological aspects of nitrate uptake in plants. *Trends Plant Sci.* **3**: 389-395.

Davies, C. and Robinson, S. P. 1996. Sugar accumulation in grape berries. *Plant Physiol.* **111**: 275-283.

Davies, W. J., Bacon, M. A., Thompson, D. S., Sobeih, W. and Rodriguez, L. G. 2000. Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signaling system and hydraulic architecture to increase the efficiency of water use in agriculture. *J. Exp. Bot.* **51**(350): 1617-1626.

Davies, W. J., Tardieu, F. and Trejo, C. L. 1994. How do chemical signals work in plants that grow in drying soil? *Plant Physiol.* **104**: 309-314.

Davies, W. J. and Zhang, J. 1991. Root signals and the growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**: 55-76.

de Cires, A., de la Torre, A., Delgado, B. and Lara, C. 1993. Role of light and CO_2 fixation in the control of nitrate reductase activity in barley leaves. *Planta* **190**: 277-283.

de la Hera Orts, M. L., Perez Prieto, L. J., Fernandez, J. L., Martinez Cutillas, A., Lopez Roca, J. M. and Gomez Plaza, E. 2002. Partial rootzone drying. Una experiencia Espanola para la variedad Monastrell. *Nutri-Fitos*: 70-76.

de Souza, C. R., Maroco, J. P., dos Santos, T. P., Rodriques, M. L., Lopes, C. M., Pereira, J. S. and Chaves, M. M. 2003. Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field grown grapevines (*Vitis vinifera* cv. Moscatel). *Functional Plant Biology* **30**: 653-662.

dos Santos, T. P., Lopes, C. M., Rodriques, M. L., de Souza, C. R., Maroco, J. P., Pereira, J. S., Silva, J. R. and Chaves, M. M. 2003. Partial rootzone drying: effects on growth and fruit quality of field-grown grapevines (*Vitis vinifera*). *Functional Plant Biology* **30**: 663-671.

Downton, W. J. S. and Loveys, B. R. 1978. Compositional changes during grape berry development in relation to abscisic acid and salinity. *Aust. J. Plant Physiol.* **5**: 415-423.

Dry, P., Loveys, B., Botting, D. and Düring, H. 1996. Effects of partial root-zone drying on grapevine vigor, yield, composition of fruit and use of water. Proc. 9th Aust. Wine Ind. Tech. Conference.

Dry, P. R. 1997. Response of grapevines to partial root zone drying of the rootzone. Adelaide, South Australia, The University of Adelaide.

Dry, P. R. and Coombe, B. G. 2004. Viticulture Vol 1. Resources. *Winetitles* **(in press)**.

Dry, P. R. and Loveys, B. R. 1999. Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis* **38**(4): 151-156.

Dry, P. R., Loveys, B. R. and During, H. 2000a. Partial drying of the rootzone of grape. II. Changes in the pattern of root development. *Vitis* **39**(1): 9-12.

Dry, P. R., Loveys, B. R. and During, H. 2000b. Partial rootzone drying of grape. I. Transient changes in shoot growth and gas exchange. *Vitis* **39**(1): 3-7.

Dry, P. R., Loveys, B. R., McCarthy, M. G. and Stoll, M. 2001. Strategic irrigation management in Australian vineyards. *J. Int. Sci. Vigne. Vin.* **35**(3): 129-139.

Dry, P. R., Loveys, B. R., Stoll, M., Steward, D. and McCarthy, M. G. 2000c. Partial rootzone drying - an update. *The Australian Grapegrower and Winemaker* (Annual Technical Issue 2000): 35-39.

Dry, P. R. and Smart, R. E. 1988. Grapegrowing regions of Australia. In: "Viticulture:Resources". (eds. Coombe, B. G. and Dry, P. R.) Adelaide, Winetitles. **1**.

Du, Y.-C., Nose, A., Wasano, K. and Uchida, Y. 1998. Responses to water stress of enzyme activities and metabolite levels in relation to sucrose and starch synthesis, the Calvin cycle and the C4 pathway in sugarcane (*Saccharum* sp.) leaves. *Aust. J. Plant Physiol.* **25**: 253-260.

Dundon, C. G. and Smart, R. E. 1984. Effects of water relations on the potassium status of Shiraz vines. *Am. J. Enol. Vitic.* **35**(1): 40-45.

Düring, H. 1978. Studies on the environmentally controlled stomatal transpiration in grapevines. II. Effects of girdling and temperatures. *Vitis* **17**: 1-9.

Düring, H. 1984. Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera* L.). *Vitis* **23**: 1-10.

Düring, H. and Alleweldt, G. 1984. Zur moglichen bedeutung abscisinsaure bei der zuchereinlagerung in die weinbeere. *Ber. Deutsch. Bot. Ges.* **97**: 101-131.

Düring, H., Loveys, B. and Dry, P. 1996. Root signal affect water use efficiency and shoot growth. *Acta Hort.* **427**: 1-13.

Faust, M. and Wang, S. Y. 1992. Polyamines in horticulturally important plants. *Hortic. Rev.* **14**: 333-356.

Fellows, R. J. and Geiger, D. R. 1974. Structural and physiological changes in sugar beet leaves during sink to source conversion. *Plant Physiol.* **54**: 877-885.

Findlay, N. K. J., Oliver, N., Nii, N. and Coombe, B. G. 1987. Solute accumulation by grape pericarp cells. IV. Perfusion of pericarp apoplast via the pedicle and evidence for xylem malfunction in ripening berries. *J. Exp. Bot.* **38**: 668-679.

Finkelstein, R. R. and Somerville, C. R. 1990. Three classes of abscisic acid (ABA)-insensitive mutations of *Arabidopsis* define genes that control overlapping subsets of ABA responses. *Plant Physiol.* **94**: 1172-1179.

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

Flore, J. A. and Lakso, A. N. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *Hortic. Rev.* **11**: 111-146.

Flores, H. E. 1990. Polyamines and plant stress. In: "Stress Responses in Plants: Adaptation and Acclimation Mechanisms", Wiley-Liss, Inc.: 217- 239.

Flores, H. E. and Galston, A. W. 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. *Plant Physiol.* **69**: 701-706.

Foyer, C. H., Valadier, M.-H., Migge, A. and Becker, T. W. 1998. Drought induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Plant Physiol.* **117**: 283-292.

Freeman, B. M., Lee, T. H. and Turkington, C. R. 1979. Interaction of irrigation and pruning level on growth and yield of Shiraz wines. *Am. J. Enol. Vitic.* **30**: 218-223.

Galston, A. W., Dai, Y. R., Flores, H. E. and Young, N. D. 1983. The control of Arginine Decarboxylase activity in higher plants. In: "Advances in Polyamine Research", New York, Raven Press. **4:** 381-393.

Galston, A. W. and Kaur-Sawhney, R. 1987. Polyamines as endogenous growth regulators. In: "Plant hormones and their role in plant growth and development". (ed. Davies, P. J.) Dordrecht, The Netherlands, Martinus Nijhoff Publishers: 280-295.

Galston, A. W. and Kaur-Sawhney, R. 1990. Polyamines in plant physiology. *Plant Physiol.* **94**: 406-410.

Galtier, N., Foyer, C. H., Huber, J., Voelker, T. A. and Huber, S. C. 1993. Effects of elevated sucrose phosphate synthase activity on photosynthesis, assimilate partitioning and growth in tomato (*Lycopersicon esculentum* var. UC82B). *Plant Physiol.* **101**: 535-543.

Galtier, N., Foyer, C. H., Murchie, E., Alred, R., Quick, P., Voelker, T. A., Thepenier, C., Lasceve, G. and Betsche, T. 1995. Effects of light and atmospheric carbon dioxide enrichment on photosynthesis and carbon partitioning in the leaves of tomato (*Lycopersicon esculentum* L.) plants over-expressing sucrose phosphate synthase. *J. Exp. Bot.* **46**: 1335-1344.

Gao, Y. and Cahoon, G. A. 1990. Arginine in Concord grape as affected by high soil nitrogen fertilization and daminozide. *J. Plant Nutrition* **13**(12): 1479-1488.

Gaudinova, A. 1990. Effect of cytokinin on nitrate reductase activity. In: "Molecular aspects of hormonal regulation of plant development". (eds. Kutacek, M., Elliot, M. C. and Machackova, M.) The Hague, The Netherlands, Academic Publishing: 225-231.

Geny, L., Broquedis, M., Martin-Tanguy, J. and Bouard, J. 1997. Free, conjugated, and wall-bound polyamines in various organs of fruiting cuttings of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* **48**(1): 80-84.

Giaquinta, R. T. 1983. Phloem loading of sucrose. *Ann. Rev. Plant Physiol.* **34**: 347-387.

Gifford, R. M. and Evans, L. T. 1981. Photosynthesis, carbon partitioning and yield. *Ann. Rev. Plant Physiol.* **32**: 485-509.

Giraudat, J., Parcy, F., Bertauche, N., Gosti, F., Leung, L., Morris, P. C., Bouvier-Durand, M. and Vartanian, N. 1994. Current advances in abscisic acid and signaling. *Plant Mol. Biol.* **26**: 1557-1577.

Givan, C. V. 1979. Metabolic detoxification of ammonia in tissues of higher plants. *Phytochemistry* **18**: 375-382.

Glad, C., Farineau, J., Regnard, J.-L. and Morot-Guadry, J.-F. 1994. The relative contribution of nitrogen originating from two seasonal ^{15}N supplies to the total nitrogen pool present in the bleeding sap and in whole *Vitis vinifera* cv. Pinot noir grapevines at bloom time. *Am. J. Enol. Vitic.* **45**(3): 328-332.

Gojon, A., Wakrim, R., Passama, L. and Robin, P. 1991. Regulation of NO_3^- assimilation by anion availability in excised soybean leaves. *Plant Physiol.* **96**: 398-405.

Goodwin, I. and Jerie, P. 1992. Regulated deficit irrigation: from concept to practice. Advances in vineyard irrigation. *Aust. NZ Wine Ind. J.* **7**(4): 258-261.

Goupil, P., Loncle, D., Druart, N., Bellettre, A. and Rambour, S. 1998. Influence of ABA on nitrate reductase activity and carbohydrate metabolism in chicory roots (*Cichorium intybus* L.). *J. Exp. Bot.* **49**(328): 1855-1862.

Gowing, D. J. G., Jones, W. J. and Davies, W. 1990. A positive root-sourced signal as an indicator of soil drying in apple (*Malus x domestica* Borkh.). *J. Exp. Bot.* **41**: 1535-1540.

Grayson, R. B., Argent, R. M., Nathan, R. J., McMahon, T. A. and Mein, R. G. 2000. Hydrological Recipes (Estimation Techniques in Australian Hydrology), Measurement Engineering Australia. **2004**.

Hale, C. R. and Weaver, R. J. 1962. The effect of developmental stage on direction of translocation of photosynthates in *Vitis vinifera*. *Hilgardia* **33**: 89-131.

Hammond, J. B. W., Burton, K. S., Shaw, A. F. and Ho, L. C. 1984. Source-sink relationships and carbon metabolism in tomato leaves. 2. Carbohydrate pools and catabolic enzymes. *Ann. Bot.* **53**: 307-314.

Hanson, A. D. and Hitz, W. D. 1982. Metabolic responses of mesophytes to plant water deficits. *Annu. Rev. Plant Physiol.* **33**: 163-203.

Hanson, E. J. and Howell, G. S. 1995. Nitrogen accumulation and fertilizer use efficiency by grapevines in short-season growing areas. *HortScience* **30**(3): 504-507.

Hare, P. D. and Cress, W. A. 1997. Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation* **21**: 79-102.

Hare, P. D., Cress, W. A. and Van Staden, J. 1997. The involvement of cytokinins in plant responses to environmental stress. *Plant Growth Regulation* **23**: 79-103.

Harrell, D. C. and Williams, L. E. 1987. Net CO2 assimilation rate of grapevine leaves in response to trunk girdling and gibberellic acid application. *Plant Physiol.* **83**: 457-459.

Hartung, W. and Davies, W. J. 1991. Drought induced changes in physiology and ABA. In: "Abscisic acid: physiology and biochemistry". (ed. Davies, W. J.) Bios Scientific Publishers Ltd.: 63-75.

Hawker, J. S. 1969a. Changes in the activities of enzymes concerned with sugar metabolism during the development of grape berries. *Phytochemistry* **8**: 9-17.

Hawker, J. S. 1969b. Insoluble invertase from grapes: an artifact of extraction. *Phytochemistry* **8**: 337-344.

Hawker, J. S. 1985. Sucrose. In: "Biochemistry of storage carbohydrate in green plant". (eds. Dey, P. M. and Dixon, R. A.) London, Academic Press. **1-51**.

Hedrich, R., Neimanis, S., Savchenko, G., Felle, H. H., Kaiser, W. M. and Heber, U. 2001. Changes in apoplastic pH and membrane potential in leaves in relation to stomatal responses to CO_2, malate, abscisic acid or interruption of water supply. *Planta* **213**: 594-601.

Helmerhorst, E. and Stokes, G. B. 1980. Microcentrifuge desalting: A rapid quantitative method for desalting small amounts of protein. *Anal. Biochem.* **104**: 130-135.

Hernandez-orte, P., Guitart, A. and Cacho, J. 1997. Amino acid determination in musts and wines by HPLC after derivatization with phenylisothiocyanate. *Am. J. Enol. Vitic.* **48**(2): 229-233.

Hernandez-orte, P., Guitart, A. and Cacho, J. 1999. Changes in the concentration of amino acids during the ripening of Vitis vinifera Tempranillo variety from the denomination d'Origine Somontano (Spain). *Am. J. Enol. Vitic.* **50**(2): 144-154.

Ho, L. C. and Barker, D. A. 1982. Regulation of loading and unloading in long distance transport systems. *Physiol. Plant.* **56**: 225-230.

Ho, L. C., Shaw, A. F., Hammond, J. B. W. and Burton, K. S. 1983. Source to sink relationships and carbon metabolism in tomato leaves I. ^{14}C assimilate compartmentation. *Ann. Bot.* **52**: 365-372.

Hofaecker, W. 1978. Investigation on the photosynthesis of vines. Influence of defoliation, topping, girdling and removal of grapes. *Vitis* **11**: 10-22.

Hoff, T., Truong, H.-N. and Caboche, M. 1994. The use of mutants and transgenic plants to study nitrate metabolism. *Plant, Cell and Environment* **17**: 486-506.

Huber, S. C. 1989. Biochemical mechanism for regulation of sucrose accumulation in leaves during photosynthesis. *Plant Physiol.* **91**: 656-662.

Huber, S. C. and Huber, J. L. 1992. Role of sucrose-phosphate synthase in sucrose metabolism in leaves. *Plant Physiol.* **99**: 1275-1278.

Huber, S. C., Huber, J. L., Cambell, W. H. and Redinbaugh, M. G. 1992a. Comparative studies of the light modulation of nitrate reductase and sucrose phosphate synthase activities in spinach leaves. *Plant Physiol.* **100**: 706-712.

Huber, S. C., Huber, J. L., Campbell, W. H. and Redinbaugh, M. G. 1992b. Apparent dependence of the light activation of nitrate reductase and sucrose phosphate synthase activities in spinach leaves on protein synthesis. *Plant Cell Physiol.* **33**(5): 639-646.

Huber, S. C., Kerr, P. S. and Kalt-Torres, W. 1986. Biochemical control of allocation of carbon for export and storage in source leaves. In: "Phloem transport". (eds. Cronshaw, J., Lucas, W. J. and Giaquinta, R. T.) New York, Alan Liss.

Hunter, J. J. and Ruffner, H. P. 1997. Diurnal and seasonal changes in nitrate reductase activity and nitrogen content of grapevine: Effects of canopy management. *Vitis* **36**(1): 1-6.

Ingledew, W. M. and Kunkee, R. E. 1985. Factors influencing sluggish fermentations of grape juice. *Am. J. Enol. Vitic.* **36**: 65-76.

Ishitani, M., Nakamura, T., Han, S. Y. and Takabe, T. 1995. Expression of betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. *Plant Mol. Biol.* **27**: 307-315.

Itai, C. and Vaadia, Y. 1965. Kinetin-like activity in root exudate of water stressed sunflower plants. *Physiologia Plantarum* **18**: 941-944.

Jackson, D. I. 1986. Factors affecting soluble solids, acid, pH, and color in grapes. *Am. J. Enol. Vitic.*(37): 179-183.

Jackson, D. I. and Lombard, P. B. 1993. Environmental and management practices affecting grape composition and wine quality - a review. *Amer. J. Enol. Vitic.* **44**: 409-430.

Jackson, M. B. 1987. A structural evaluation of the involvement of ethylene and ABA in plant responses to aeration stress. In: "Hormone action in plant development: a critical appraisal". (eds. Hoad, G. V., Lenton, J. R., Jackson, M. B. and Atkin, R. K.) London, Butterworths: 189-200.

Jacobs, W. P. 1959. What substance normally controls a given biological process? Formulation of some rules. *Developmental Biology* **1**(527-533).

Jarvis, B. C., Jasmin, S. and Coleman, M. T. 1985. RNA and protein metabolism during adventitious root formation in stem cuttings of *Phaseolus aureus* cultivar Berkin. *Physiol. Plant.* **64**: 53-59.

Jeschke, W. D. and Hartung, W. 2000. Root-shoot interactions in mineral nutrition. *Plant and Soil* **226**: 57-69.

Jiranek, V., Langridge, P. and Henschke, P. A. 1990. Nitrogen requirement of yeast during wine fermentation. In: "Proceedings of the 7th Australian Wine Industry Conference: 13-17 August 1989". (eds. Williams, P. J., Dadvison, D. and Lee, T. H.) Adelaide, SA, Australian Industrial Publishers. **166-171**.

Jones, H. G. 1992. Plant and microclimate: A quantitative approach to environmental plant physiology. Cambridge, UK, Cambridge University Press.

Jones, H. G. 1998. Stomatal control of photosynthesis and transpiration. *J. Exp. Bot.* **49**: 387-398.

Jones, H. G. and Sutherland, R. A. 1991. Stomatal control of photosynthesis and transpiration. *Plant, Cell and Environment* **11**: 111-121.

Kaiser, W. M. and Förster, J. 1989. Low CO_2 prevents nitrate reduction in leaves. *Plant Physiol.* **91**(970-974).

Kakkar, R. K. and Rai, V. R. 1987. Effects of spermidine and IAA on carbohydrate metabolism during rhizogenesis in *Phaseolus vulgaris*. I. Hypocotyl cuttings. *Indian J. Expt. Biol.* **25**: 476-478.

Kang, S., Hu, X., Goodwin, I. and Jerie, P. 2002. Soil water distribution, water use, and yield response to partial root zone drying under a shallow groundwater table condition in a pear orchard. *Scientia Hort.* **92**: 277-291.

Kang, S., Liang, Z., Pan, A., Shi, P. and Zhang, J. 1998. Water use efficiency of controlled root-division alternate irrigation on maize plants. *Agric. Water Manage.* **45**: 267-274.

Kang, S., Zhang, L., Hu, X., Li, Z. and Jerie, P. 2001. An improved water use efficiency for hot pepper grown under controlled alternate drip irrigation on partial roots. *Scientia Hort.* **89**: 257-267.

Kaur-Sawney, R. a. G., A.W. 1991. Physiological and biochemical studies on the anti-senescence properties of polyamines in plants. In: "Biochemistry and physiology of polyamines in plants". Boca Raton, CRC Press: 201-211.

Keller, F. and Ludlow, M. M. 1993. Carbohydrate metabolism in drought stressed leaves of pigeonpea (*Cajanus cajan*). *J. Exp. Bot.* **44**: 1351-1359.

Kende, H., Hahn, H. and Kays, S. E. 1971. Enhancement of nitrate reductase activity by benzyladenine in *Agrostemma githago*. *Plant Physiol.* **48**: 702-706.

Kerr, P. S., Huber, S. C. and Israel, D. W. 1984. Effect of N-Source on soybean leaf sucrose phosphate synthase, starch formation, and whole plant growth. *Plant Physiol.* **75**: 483-488.

Kerr, P. S., Rufty, T. w. and Huber, S. C. 1985. Endogenous rhythms in photosynthesis, sucrose phosphate synthase activity and stomatal resistance in leaves of soybean (*Glycine max* (L.) Merr.). *Plant Physiol.* **77**: 275-280.

Kingston-Smith, A. H., Galtier, N., Pollock, C. and Foyer, C. H. 1998. Soluble acid invertase activity in leaves is independent of species differences in leaf carbohydrates, diurnal sugar profiles and paths of phloem loading. *New Phytologist* **139**: 283-292.

Klein, I., Strime, M., Fanberstein, L. and Mani, Y. 2000. Irrigation and fertigation effects on phosphorous and potassium nutrition of wine grapes. *Vitis* **39**: 55-62.

Kliewer, W. M. 1969. Changes in the concentration of free amino acids in grape berries during maturation. *Amer. J. Enol. Vitic.* **19**: 166-174.

Kliewer, W. M. and Antcliff, A. J. 1970. Influence of defoliation, leaf darkening, and cluster shading on the growth and composition of Sultana grapes. *Am. J. Enol. Vitic.* **21**: 26-36.

Kliewer, W. M. and Cook, J. 1974. Arginine levels in grape canes and fruits as indicators of nitrogen status of vineyards. *Amer. J. Enol. Vitic.* **25**(2): 111-118.

Kliewer, W. M., Freeman, B. M. and Hosson, C. 1983. Effect of irrigation, crop level and potassium fertilization on Carignane vines. *Am. J. Enol. Vitic.* **34**: 186-196.

Komor, E. 2000. Source physiology and assimilate transport: the interaction of sucrose metabolism, starch storage and phloem export in source leaves and the effects on sugar status in phloem. *Aust. J. Plant Physiol.* **27**: 497-505.

Kuretani, M. 1968. Studies of the defoliation of grape vines in the summer. The effect of soil drying of the rainy season on early defoliation. *Tech. Bull. fac. Kagawa* **19**: 187-192.

Kushad, M. M., Yelenowsky, G. and Knight, R. 1988. Interrelationship of polyamine and ethylene biosynthesis during avocado fruit development and ripening. *Plant Physiol.* **87**: 463-467.

Ladyman, J. A. R., Hitz, W. D. and Hanson, A. D. 1980. Translocation and metabolism of glycine betaine by barley plants in relation to water stress. *Planta* **150**: 191-196.

Lang, A. and Thorpe, M. R. 1989. Xylem, phloem and transpiration flows in grape: application of a technique for measuring the volume of attached fruits to a high resolution using Archimedes principle. *J. Exp. Bot.* **40**: 1069-1078.

Lang, A., Thorpe, M. R. and Edwards, W. R. N. 1986. Plant water potential and translocation. In: "Phloem transport . Proc.Int.Conf. Phloem Transport". (eds. Cronshaw, J., Lucas, W. J. and Giaquinta, R. T.) New York, Alan R. Liss, Inc.: 193-194.

Lawlor, D. W. 1995. The effects of water deficit on photosynthesis. In: "Environment and Plant Metabolism, Flexibility and Acclimation". (ed. Smirnoff, N.) Oxford, BIOS Scientific Publishers: 129-160.

Lewis, C. E., Noctor, G., Causton, D. and Foyer, C. H. 2000. Regulation and assimilate partitioning in leaves. *Aust. J. Plant Physiol.* **27**: 507-519.

Liang, J., Zhang, J. and Wong, M. H. 1996. Effects of air-filled porosity and aeration on the initiation and growth of secondary roots in maize (*Zea mays*). *Plant and Soil* **186**: 245-254.

Lillo, C. 1994. Light/dark regulation of higher plant nitrate reductase related to hysteresis and calcium/magnesium inhibition. *Physiologia Plantarum* **91**: 295-299.

Lin, C. C. and Kao, C. H. 1996. Disturbed ammonium assimilation is associated with growth inhibition of roots in rice seedlings caused by NaCl. *Plant Growth Reg.* **18**: 233-238.

Litchfield, W. H. 1951. Soil survey of the Waite Agricultural Research Institute, Glen Osmond, SA. CSIRO Australia Division of Soils Divisional Report. No. 2/51.

Loveys, B., Stoll, M., Dry, P. and McCarthy, M. 1998. Partial rootzone drying stimulates stress responses in grapevine to improve water use efficiency while maintaining crop yield and quality. *The Australian Grapegrower and Winemaker, Annual Technical Issue*: 108-113.

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

Loveys, B. R., Dry, P. R., Stoll, M. and McCarthy, M. G. 2000. Using plant physiology to improve the water use efficiency of horticultural crops. *Acta Hort.* **537**: 187-199.

Lu, J.-L., Ertl, J. R. and Chen, C.-M. 1992. Transcriptional regulation of nitrate reductase mRNA levels by cytokinin-abscisic acid interactions in etiolated barley leaves. *Plant Physiol.* **98**: 1255-1260.

Mabrouk, H. and Sinoquet, H. 1998. Indices of light microclimate and canopy structure of grapevines determined by 3D digitizing and image analysis and their relationship to grape quality. *Aust. J. Grape and Wine Research* **4**: 2-13.

Mackintosch, C., Douglas, P. and Lillo, C. 1995. Identification of a protein that inhibits the phosphorylated form of nitrate reductase from spinach *(Spinacea oleracea)* leaves. *Plant Physiol.* **107**(451-457).

Markham, E. and Byrne, W. J. 1967. Uptake, storage and utilization of phosphate in yeast. II. Limiting factors of yeast uptake. *J. Inst. Brew.* **73**: 271-273.

Marschner, H. 1986. Mineral nutrition of higher plants. San Diego, Academic Press.

Marschner, H. 1995. Mineral nutrition in higher plants. London, Academic Press: 101-105.

Martin-Tanguy, J. 1997. Conjugated polyamines and reproductive development: biochemical and physiological approaches. *Physiol. Plant.* **100**: 675-688.

Matthews, M. A. and Anderson, M. M. 1988. Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. *Am. J. Enol. Vitic.* **39**: 313-320.

Matthews, M. A. and Anderson, M. M. 1989. Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. *Am. J. Enol. Vitic.* **40**: 52-60.

Meteorology Bureau 2004. Commonwealth Bureau of Meteorology, Commonwealth of Australia. **2004**.

Miflin, B. J. and Lea, P. J. 1980. Ammonium assimilation. In: "The biochemistry of plants". New York, Academic Press. **5:** 169-202.

Minchin, P. E. H., Thorpe, M. R. and Farrar, J. F. 1993. A simple mechanistic model of phloem transport which explains sink priority. *J. Exp. Bot.* **44**: 947-955.

Mingo, D. M., Theobald, J. C., Bacon, M. A., Davies, W. J. and Dodd, I. C. 2004. Biomass allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone drying: enhancement of root growth. *Functional Plant Biology* (In press).

Monteiro, F. F. and Bisson, L. F. 1991. Biological assay of nitrogen content of grape juice and prediction of sluggish fermentations. *Am. J. Enol. Vitic.* **42**(1): 47-57.

Morris, J. R., Sims, C. A. and Cawthon, D. L. 1983. Effects of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. *Am. J. Enol. Vitic.* **34**(1): 35-39.

Mpelasoka, B. S., Schachtman, D. P., Treeby, M. and Thomas, M. 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Aust. J. Grape and Wine Research* **9**: 154-168.

Naidu, B. P. 1995. Method for the treatment of seeds with betaines to increase stress tolerance, seedling vigor and yield. *Australian Patent No. 27071/95 (CSIRO tropical Agriculture: Brisbane).*

Naidu, B. P. 1998. Separation of sugars, polyols, proline analogues, and betaines in stressed plant extracts by high performance liquid chromatography and quantification by ultra violet detection. *Aust. J. Plant Physiol.* **25**: 793-800.

Naidu, B. P., Paleg, L. G. and Jones, G. P. 2000. Accumulation of proline analogues and adaptation of *Melaleuca* species to diverse environments in Australia. *Aust. J. Bot.* **48**: 611-620.

Nissen, P. 1974. Uptake mechanisms: inorganic and organic. *Ann. Rev. Plant Physiol.* **25**: 53-79.

Nonami, H., Wu, Y. J. and Boyer, J. S. 1997. Decreased growth-induced water potential - primary cause of growth inhibition at low water potentials. *Plant Physiol.* **114**: 501-509.

North, G. B. and Nobel, P. S. 1991. Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of *Agave deserti* (Agavaceae). *Am. J. Bot.* **78**: 906-915.

Oaks, A., Stulen, J., Jones, K., Winspear, M. J. and Boosel, I. L. 1980. Enzymes of nitrogen assimilation in maize roots. *Planta* **148**: 477-484.

Ober, E. S. and Sharp, R. E. 1994. Proline accumulation in maize (*Zea mays* L.) primary roots at low water potentials. I. Requirements for increased levels of abscisic acid. *Plant Physiol.* **105**: 981-987.

Ollat, N. and Gaudillere, J. P. 1996. Investigation of assimilate import mechanisms in berries of *Vitis vinifera* var. 'Cabernet Sauvignon'. *Acta Horticulturae* **427**: 141-146.

Osmond, B., Badger, M., Maxwell, K., Björkman, O. and Leegood, R. C. 1997. Too many photons: photorespiration, photoinhibition and photooxidation. *Trends in Plant Science* **2**: 119-121.

Ough, C. S. 1988. Acids and amino acids in grapes and wine. In: "Modern methods in wine analyses. Volume 6, Wine analyses". (eds. Jackson, J. F. and Liskens, H. F.) Berlin, Springer-Verlag: 92-146.

Paleg, L. G., Stewart, G. R. and Starr, R. 1985. The effect of compatible solutes on proteins. *Plant and Soil* **89**: 83-94.

Palmer, C. E. 1981. Influence of abscisic acid on nitrate accumulation and nitrate reductase activity in potato tuber slices. *Plant Cell Physiol.* **22**: 1541-1551.

Palmer, C. E. 1985. The relationship of abscisic acid on nitrate accumulation and nitrate reductase activity in potato tuber slices. *Plant Cell Physiol.* **26**: 1167-1174.

Patrick, J. W. 1997. Phloem unloading: sieve element unloading and post sieve element transport. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **48**: 191-222.

Pesci, P. 1992. Effect of light on abscisic acid-induced proline accumulation in leaves: comparison between barley and wheat. *Physiol. Plant.* **86**: 209-214.

Pesci, P. 1993. Glucose mimics the enhancing effect of light on ABA induced proline accumulation in hydrated barley and wheat leaves. *J. Plant Physiol.* **142**: 355-359.

Pillay, I. and Beyl, C. 1990. Early responses of drought-resistant and -susceptible tomato plants subjected to water stress. *J. Plant Growth Regul.* **9**: 213-219.

Poorter, H. and Nagel, O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. *Aust. J. Plant Physiol.* **27**: 595-605.

Pouteau, S., Cherel, I., Vaucheret, H. and Caboche, M. 1989. Nitrate reductase mRNA regulation in *Nicotiana plumbaginifolia* nitrate reductase-deficient mutants. *Plant Cell* **1**: 1111-1120.

Pritchard, J. 1994. The control of cell expansion in roots. *New Phytologist* **127**: 3-26.

Pritchard, J., Winch, S. and Gould, N. 2000. Phloem water relations and root growth. *Aust. J. Plant Physiol.* **27**: 539-548.

Quick, P., Siegl, G., Neuhaus, E., Feil, R. and Stitt, M. 1989. Short term water stress leads to a stimulation of sucrose synthesis by activating sucrose-phosphate synthase. *Planta* **177**: 535-546.

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

Rabe, E. 1990. Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. *J. Hort. Sci.* **65**: 231-243.

Rajashekar, C. B., Zhou, H., Marcum, K. B. and Prakash, O. 1999. Glycine betaine accumulation and induction of cold tolerance in strawberry (*Fragaria* x *anassa* Duch.) plants. *Plant Sci.* **148**: 175-183.

Raven, J. A. and Smith, F. A. 1976. Nitrogen assimilation and transport in vascular plants in relation to intracellular pH regulation. *New Phytologist* **76**: 415-431.

Rayment and Higginson 1992. Chapter 6B3. In: "Australian Laboratory Handbook of Soil and Water Chemical Methods".

Rhodes, D. 1987. Metabolic responses to stress. In: "The Biochemistry of Plants". San Diego, Academic Press. **12:** 201-241.

Rhodes, D. and Hanson, A. D. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**: 357-384.

Roberts, D. R., Walker, M. A., Thompson, J. E. and Dumbroff, E. B. 1984. The effects of inhibitors on polyamine levels in cut carnation flowers. *Plant Cell Physiol.* **25**: 315-322.

Roberts, S. K. and Snowman, B. N. 2000. The effects of ABA on channel mediated K^+ transport across higher plant roots. *J. Exp. Bot.* **51**: 1585-1594.

Rodrigues, M. L., Chaves, M. M., Wendler, R., David, M. M., Quick, W. P., Leegood, R. C., Stitt, M. and Pereira, J. S. 1993. Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. *Aust. J. Plant Physiol.* **20**: 309-321.

Rogiers, S. Y., Keller, M., Holzapfel, B. P. and Virgona, J. M. 2000. Accumulation of potassium and calcium by ripening berries on field vines of *Vitis vinifera* (L.) cv. Shiraz. *Aust. J. Grape and Wine Research* **6**(3): 240-243.

Rogiers, S. Y., Smith, J. A., White, R., Keller, M., Holzapfel, B. P. and Virgona, J. M. 2001. Vascular function in berries of *Vitis vinifera* (L.) cv. Shiraz. *Aust. J. Grape and Wine Research* **7**: 47-51.

Roitsch, T. 1999. Source-sink regulation by sugar and stress. *Current Opinion in Plant Biology* **2**: 198-206.

Roper, T. R. and Williams, L. E. 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. *Plant Physiol.* **89**: 1136-1140.

Rose, S. and Botha, F. C. 2000. Distribution patterns of neutral invertase and sugar content in sugarcane internodal tissues. *Plant Physiol. Biochem.* **38**: 819-824.

Roubelakis-Angelakis, K. A. and Kliewer, W. M. 1992. Nitrogen metabolism in grapevine. *Hortic. Rev.* **14**: 407-452.

Saab, I. N., Sharp, R. E., Pritchard, J. and Voetberg, G. S. 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Plant Physiol.* **93**: 1329-1336.

Salisbury, F. B. and Ross, C. W. 1992. Plant physiology. Belmont, California, Wadsworth, Inc.

Salmon, J. M. 1989. Effect of sugar transport inactivation in *Saccharomyces cerevisiae* on sluggish and stuck enological fermentations. *Applied and Environmental Microbiology* **55**: 953-958.

Samuelson, M. E., Ohlen, E., Lind, M. and Larsson, C. M. 1995. The influence of cytokinins in nitrate regulation of nitrate reductase activity and expression in barley. *Physiol. Plant.* **93**: 254-260.

Scheible, W.-R., Gonzales-Fontes, A., Lauerer, M., Muller-Rober, B., Caboche, M. and Stitt, M. 1992. Nitrate acts a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell* **9**: 783-798.

Schmalstig, J. G. and Cosgrove, D. J. 1990. Coupling of solute transport and cell expansion in pea stems. *Plant Physiol.* **94**: 1625-1633.

Scholander, P. F., Hammel, H. T., Bradstreet, D. and Hemmingsen, E. A. 1965. Sap pressure in vascular plants. *Science* **148**: 339-346.

Schultz, A. 1994. Plasmodesmatal widening accompanies the short term increase in symplastic phloem unloading in pea root-tips under osmotic stress. *Protoplasma* **188**: 22-37.

Schultz, H. R. 1996. Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Acta Horticulturae* **427**: 251-266.

Schultz, H. R. 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell and Environment* **26**: 1393-1405

Sharp, R. E. 1990. Comparative sensitivity of root and shoot growth and physiology to low water potentials. In: "Importance of root to shoot communication in the responses to environmental stress". **21:** 29-41.

Sharp, R. E. and Davies, W. J. 1989. Regulation of growth and development of plants growing with a restrictive supply of water. In: "Plants under stress: biochemistry, physiology and ecology and their application to plant improvement". (eds. Jones, H. G., Flowers, T. J. and Jones, M. B.) New York, Cambridge University Press**:** 71-93.

Sharp, R. E. and LeNoble, M. E. 2002. ABA, ethylene and the control of shoot and root growth under water stress. *J. Exp. Bot.* **53**(366): 33-37.

Shyr, Y. Y. and Kao, C. H. 1985. Polyamines and root formation in mung bean hypocotyl cuttings. *Bot. Bul. Acad. Sinica.* **26**: 179-184.

Sims, D. A., Luo, Y. and Seemann, J. R. 1998. Importance of leaf versus whole plant CO_2 environment for photosynthetic acclimation. *Plant Cell and Environ.* **21**: 1189-1196.

Smart, R., Dick, J., Gravett, I. and Fisher, B. 1990. Canopy management to improve grape yield and wine quality - principles and practices. *S. Afr. J. Enol. Vitic.* **11**: 3-17.

Smart, R. E. 1985. Principles of grapevine microclimate manipulation with implications for yield and quality. A review. *Am. J. Enol. Vitic.* **36**: 230-239.

Smart, R. E. and Coombe, B. G. 1983. Water relations of grapevines. In: "Water deficits and plant growth". (ed. Kozlowski, T. T.) London, Academic press. **VII:** 137-196.

Smith, J. A. C. and Milburn, J. A. 1980. Phloem turgor and the regulation of sucrose loading in *Ricinus communis* L. *Planta* **148**: 42-48.

Solomonson, L. P. and Barber, M. J. 1990. Assimilatory nitrate reductase: functional properties and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**: 225-253.

Spayd, S. E. and Morris, J. R. 1978. Influence of irrigation, pruning severity and nitrogen on yield and quality of Concord grapes in Arkansas. *J. Amer. Soc. Hort. Sci.* **103**: 211-216.

Spollen, W. G., LeNoble, M. E., Samuels, T. D., Bernstein, N. and Sharp, R. E. 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiol.* **122**: 967-979.

Steveninck, R. F. M. 1976. Effect of hormones and related substances on ion transport. In: "Encyclopedia of Plant Physiology". (eds. Luttge, U. and Pittman, M. G.) New York, Springer. **2A:** 307-342.

Stewart, C. R. 1978. Role of carbohydrates in proline accumulation in wilted Barley leaves. *Plant Physiol.* **61**: 775-778.

Stewart, C. R. 1980. The mechanism of abscisic acid-induced proline accumulation in barley leaves. *Plant Physiol.* **66**: 230-233.

Stines, A. P. 1999. Expression of the key proline biosynthetic enzymes P5CS and OAT during grape berry development. Adelaide, University of Adelaide.

Stines, A. P., Grubb, J., Gockowiak, H., Henschke, P. A., Høj, P. B. and van Heeswijck, R. 2000. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. *Aus. J. Grape and Wine Research* **6**: 150-158.

Stitt, M., Gerhardt, R., Wilke, I. and Heldt, H. W. 1987a. The contribution of fructose-2,6-bisphosphate to the regulation of sucrose synthesis during photosynthesis. *Physiol. Plant.* **69**: 377-386.

Stitt, M., Huber, S. C. and Kerr, P. S. 1987b. Control of photosynthetic sucrose synthesis. In: "Biochemistry of Plants, Vol 10". (eds. Hatch, M. D. and Boardman, N. K.) London, Academic Press: 327-409.

Stitt, M., Wilke, I., Feil, R. and Heldt, H. W. 1988. Coarse control of sucrose-phosphate synthase in leaves: Alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. *Planta* **174**: 217-230.

Stoll, M. 2000. Effects of partial root zone drying on grapevine physiology and fruit quality. PhD Thesis. The University of Adelaide.

Stoll, M., Dry, P., Loveys, B., Stewart, D. and McCarthy, M. 2000a. Partial root zone drying: Effects on root distribution and commercial application of a new irrigation technique. *Wine Industry Journal* **15**(2): 74-77.

Stoll, M., Loveys, B. and Dry, P. 2000b. Hormonal changes induced by partial rootzone drying of irrigated grapevine. *J. Exp. Bot.* **51**(350): 1627-1634.

Sturm, A. 1999. Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiol.* **121**: 1-7.

Sturm, A., Hess, D., Lee, H.-S. and Lienhard, S. 1999. Neutral invertase is a novel type of sucrose-cleaving enzyme. *Physiol. Plant.* **107**: 159-165.

Sturm, A. and Tang, G. Q. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Sciences* **4**(10): 401-407.

Takei, K., Takahashi, T., Sugiyama, T., Yamaya, T. and Sakakibara, H. 2002. Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. *J. Exp. Bot.* **53**(370): 971-977.

Tiaz, L. and Zeiger, E. 1991. Plant Physiology. California, USA, Benjamin/Cummings.

Tiburco, A. F., Campos, J. L., Figueras, X. and Besford, R. T. 1993. Recent advances in the understanding of polyamine functions during plant development. *Plant Growth Reg.* **12**: 331-340.

Tischner, R. 2000. Nitrate uptake and reduction in higher and lower plants. *Plant, Cell and Environment* **23**: 1005-1024.

Titus, J. S. and Kang, S. M. 1982. Nitrogen metabolism, translocation and recycling in apple trees. *Hortic. Rev.* **4**: 204-246.

Todorov, D., Alexieva, V. and Karanov, E. 1998. Effect of putrescine, 4-PU-30, and abscisic acid on maize plants grown under normal, drought, and re-watering conditions. *J. Plant Growth Regul.* **17**: 197-203.

Turner, N. C. and Jones, M. M. 1980. Turgor maintenance by osmotic adjustment: A review and evaluation. In: "Adaptation of plants to water and high temperature stress". (eds. Turner, N. C. and Kramer, P. J.) New York, Wiley: 87-103.

van Zyl, J. L. 1981. Waterbehoefte en Besproeiing. In: "Wingerdbou in Suid-Afrika". (eds. Burger, J. and Deist, J.) Cape Town, Maskew Miller Bpk.

Vartanian, N., Marcotte, L. and Giraudat, J. 1994. Drought rhizogenesis in *Aridopsis thaliana*. *Plant Physiol.* **104**: 761-767.

Vassey, T. L. and Sharkey, T. D. 1989. Mild water stress of *Phaseolus vulgaris* plants leads to reduced starch synthesis and extractable sucrose phosphate synthase activity. *Plant Physiol.* **89**: 1066-1070.

Wang, S. Y. and Steffens, G. L. 1985. Effect of paclobutrazol on water stress-induced ethylene biosynthesis and polyamine accumulation on apple seedling leaves. *Phytochemistry* **24**: 2185-2190.

Williams, D. W., Andris, H. L., Breede, R. H., Luvisi, D. A., Norton, M. V. K. and Williams, L. E. 1985. Validation of a model for the growth and development of the Thompson seedless grapevine. II Phenology. *Am. J. Enol. Vitic.* **36**(4): 283-289.

Williams, L. E. 1987. Growth of 'Thompson seedless' grapevines: I Leaf area development and dry weight distribution. *J. Amer. Soc. Hort. Sci.* **112**: 325-330.

Williams, L. E. and Biscay, P. J. 1991. Partitioning of dry weight, nitrogen and potassium in Cabernet Sauvignon grapevines from anthesis until harvest. *Am. J. Enol. Vitic.* **42**(2): 113-117.

Winkler, A. J., Cook, J. A., Kliewer, W. M. and Lider, L. A. 1974. General Viticulture. Los Angelas, London, University of California Press Ltd.

Winter, H. and Huber, S. C. 2000. Regulation of Sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes. *Crit. Reviews in Plant Sciences* **19**(1): 31-67.

Wood, A., Paleg, L. G. and Sportswood, T. M. 1974. Hormone-phospholipid interaction:a possible mechanism of action in the control of membrane permeability. *Aust. J. Plant Physiol.* **1**: 167-169.

Wright, S. T. and Hiron, R. W. 1969. [+]-ABA, the growth inhibitor induced in detached wheat leaves by a period of wilting. *Nature* **224**: 719-720.

Xing, W. and Rajashekar, C. B. 2001. Glycine betaine involvement in freezing tolerance and water stress in *Aradopsis thaliana. Environ. Exp. Bot.* **46**: 21-28.

Yang, C. W. and Kao, C. H. 2000. Ammonium in relation to proline accumulation in detached rice leaves. *Plant Growth Regulation* **30**: 139-144.

Yang, J., Zhang, J., Wang, Z., Zhu, Q. and Liu, L. 2002. Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and re-mobilization of carbon reserves in rice subjected to water stress during grain filling. *Planta* **215**: 645-652.

Yu, X., Sukumaran, S. and Marton, L. 1998. Differential expression of the Arabidopsis *Nia1* and *Nia2* genes. *Plant Physiol.* **116**: 1091-1096.

Zarcinas, B. A. and Cartwright, B. 1983. Analysis of soil plant material by inductively coupled plasma - optical emission spectrometry. CSIRO Aust. Div. Soils Tech. Paper. 5.

Zarcinas, B. A., Cartwright, B. and Spouncer, L. R. 1983. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.* **18**(1): 131-146.

Zerihun, A. and Treeby, M. T. 2002. Biomass distribution and nitrate assimilation in response to N supply for *Vitis vinifera* L. cv. Cabernet Sauvignon on five *Vitis* rootstock genotypes. *Aust. J. Grape and Wine Research* **8**(3): 157-162.

Zhou, R. and Quebedeaux, B. 2003. Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. *J. Amer. Soc. Hort. Sci.* **128**(1): 113-119.

Zhu, Y. J., Komor, E. and Moore, P. H. 1997. Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. *Plant Physiol.* **115**(2): 609-616.

Ziegler, H. 1975. Nature of transported substances. In: "Transport in plants I: Phloem transport". (eds. Zimmermann, M. H. and Milburn, J. A.): 59-100.

APPENDIX A: Soil description of the Alverstoke vineyard of the University of Adelaide

Easting 284 175
Northing 6127 675
Mapsheet 6628-3 (Adelaide)
Describer James Hall

General Soil Description
Dark brown clay with shale fragments, grading into red-brown mottled clay; overlying olive-brown mottled cracking clay.

Australian Soil Description
Mottled Eutrophic Red Dermosol / Brown Vertosol; very thick, gravely, clay loamy/clayey, deep. [the "/" indicates one soil overlying another]

Site Description
Geology colluvium: shale fragments & soil; overlying cracky clay
Position mid-slope Slope 6%
Relief/Modal Slope undulating rises Aspect 300°
Landform pediment Elevation 135m
Surface Condition hard
Surface Stone 2-10% coarse gravely shale (2-6 cm)
Drainage well drained

Soil Profile Description

0-10 cm	A11 horizon: dark brown (7.5YR2.5/2), clay loam, with cloddy structure (5-10 cm), pH 6.5 (field test), 10-20% medium gravelly shale fragments (0.6-2cm), and a clean boundary to:
10-35cm	A12 horizon: dark red-brown (5YR2.5/2), light clay, with prismatic structure (5-10cm), pH 6.5 (field test), 2-10% medium gravelly shale fragments (0.6-2cm), and a clean boundary to:
35-60cm	AB horizon: dark red-brown (5YR3/3), clay loam, with weak polyhedral structure (0.5-1cm), pH 7 (field test), 2-10% medium gravelly shale fragments (0.6-2cm), and a clean boundary to:
60-75cm	BA horizon: dark brown (7.5YR4/4), light clay, with a weak polyhedral structure (0.5-1cm), pH 8.0 (field test), 50-9-% coarse gravelly shale fragments (2-6cm), and a gradual boundary to:
75-125cm	Btg horizon: red-brown (5YR4/6), medium clay with some mottles, with weak polyhedral structure (1-2cm), pH 9.0 (field test), and with a horizontal thin band of shale & 20-50% partially weathered rock (0.6-2cm). This B horizon has a wavy upper boundary which varies from 30 to 100 cm depth; and a gradual lower boundary to:
125-140cm	Dsgg horizon: olive-brown (2.5YR44), medium clay with mottles & slickensides, with lenticular structure (1-2cm), and a pH 9.5 (field test). This horizon consists of moist cracking clay with slickensides and seems to be unrelated to soil above. This horizon has a wavy upper boundary.

Notes: This soil profile also includes minor quartz & quartzite fragments. The depth of topsoil above the red clay B horizon varies from 30 to 100 cm. The areas with deeper

topsoil is very stony; for example, the middle of the trench has very gravelly/stony topsoil over red clay B at 100cm.

Samples:

CH117-1	-horizon 1
CH117-2	-horizon 2
CH117-5	-horizon 5
CH117-6	-horizon 6
CH117-X	-20cm to north; sample from 45cm; from red clay B horizon (B horizon upper boundary @ 30cm)
CH117-Z	-20cm to north; sample from 100cm; from red BC horizon with some stony fragments and some weathered rock

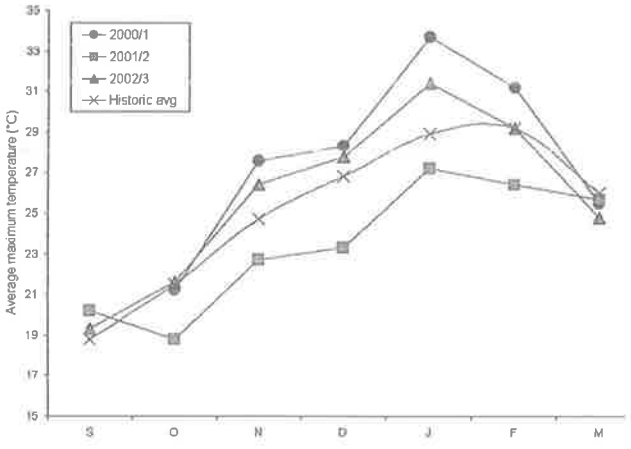
Site – Land Quality Ratings

Note. This site is the land area within a radius of 20m of the described soil profile, and similar in characteristics to the described soil profile. Site ratings are based on a land class system with ratings from 1 to 8 – higher the rating the greater the limitation. The ratings below have mostly been determined using morphological data, and experience with laboratory chemical analyses of similar soils, but are not supported by site specific laboratory chemical analyses.

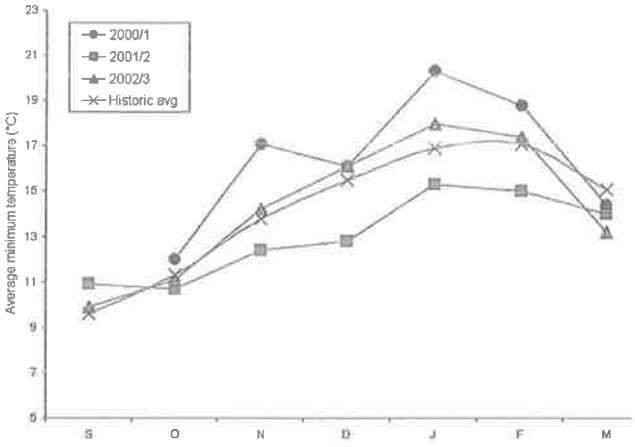
Water logging	2w	moderately well drained
Surface Condition	2c	hard-setting surface
Subsoil Condition	2p	hard & coarsely structured subsoil
Root Zone Water Holding Capacity	2m	moderate
Inherent Fertility	1n	
Subsoil Toxicities	1t	
Acidity	1h	
Alkalinity	1-2I	neutral with alkaline lower subsoil
Salinity	1s	
Scalding	1z	
Water Erosion Potential	2e	moderately low
Wind Erosion Potential	1a	
Water Repellence	1u	
Rockiness	2r	some rocks
Gullying/Tunnelling	1g	
Flooding Potential	1f	

APPENDIX B: Climatological data of the Waite campus of the University of Adelaide

A

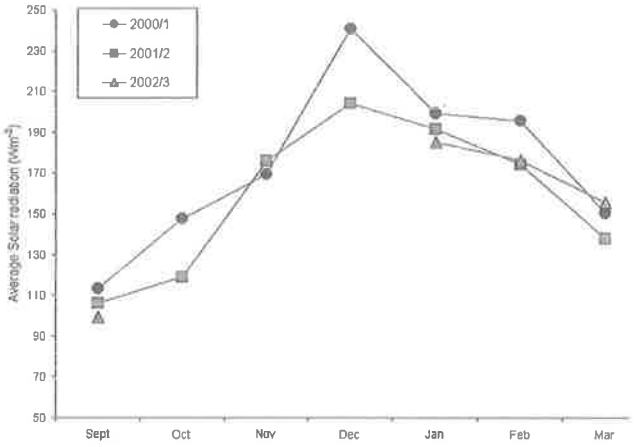


B

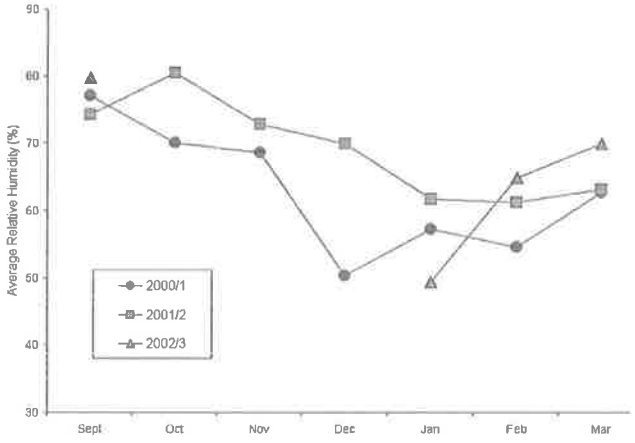


A) Monthly average maximum and B) minimum temperatures for the 2000 to 2003 growing seasons from September to March at the Waite campus.

A



B



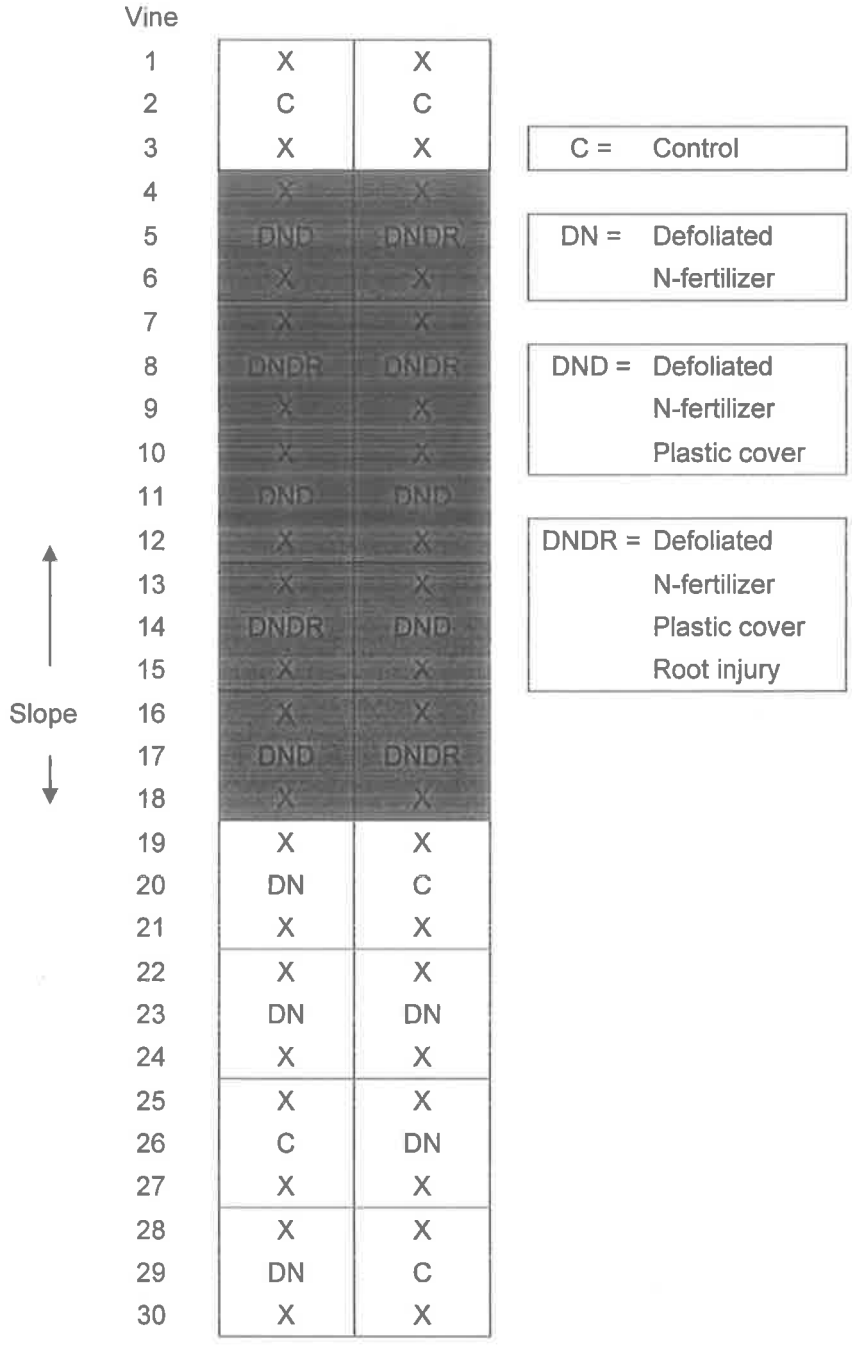
A) Monthly mean solar radiation (Mj/m^2) and B) monthly mean relative humidity (%) for the 2000-2003 growing seasons from September to March at the Waite campus.

APPENDIX C: Experimental design of the Shiraz vineyard at the Nuriootpa research station.

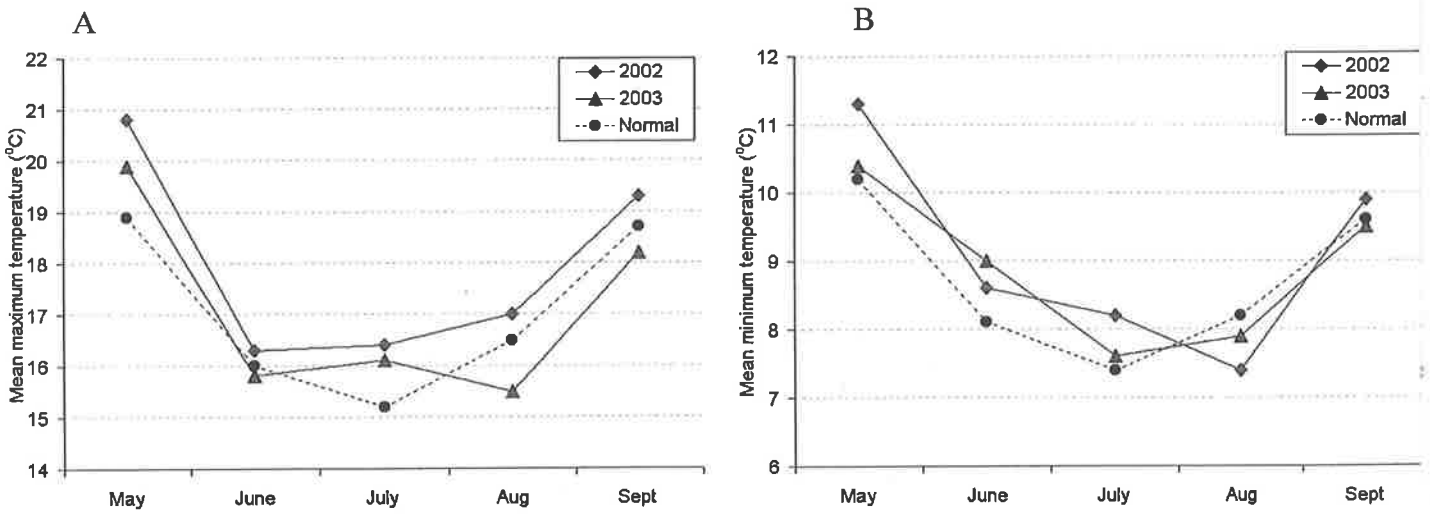
Vine	17	18	19	Row
43	B	B	B	
42	B	B	B	
41	3	2	1	Rep 5
40	3	2	1	
39	3	2	1	
38	3	2	1	
37	1	3	2	
36	1	3	2	
35	1	3	2	
34	1	3	2	
33	1	3	2	Rep 4
32	1	3	2	
31	1	3	2	
30	1	3	2	
29	2	1	3	
28	2	1	3	
27	2	1	3	
26	2	1	3	
25	2	1	3	Rep 3
24	2	1	3	
23	2	1	3	
22	2	1	3	
21	3	2	1	
20	3	2	1	
19	3	2	1	
18	3	2	1	
17	2	3	1	Rep 2
16	2	3	1	
15	2	3	1	
14	2	3	1	
13	2	1	3	
12	2	1	3	
11	2	1	3	
10	2	1	3	
9	3	2	1	Rep 1
8	3	2	1	
7	3	2	1	
6	3	2	1	
5	1	3	2	
4	1	3	2	
3	1	3	2	
2	1	3	2	
1	B	B	B	

Irrigation Main plot		Pruning Sub-plot	
	Single	1	1/2 normal
	PRD	2	normal
		3	2* normal

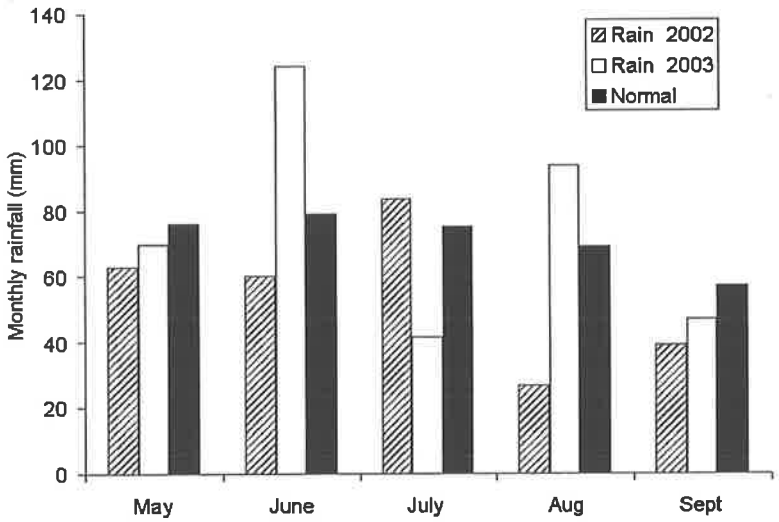
APPENDIX D: Experimental design of the Cabernet Sauvignon vineyard at the Waite campus of the University of Adelaide.



APPENDIX E: Climatological data of the Waite campus of the University of Adelaide



A) Monthly average maximum and B) minimum temperatures for the 2002 and 2003 winter period from May to September at the Waite Campus, Adelaide.



Monthly rainfall for the 2002 and 2003 winter period from May to September at the Waite Campus, Adelaide.

APPENDIX F: Published paper in the South African Journal of Enology and Viticulture.

A PRELIMINARY INVESTIGATION ON PARTIAL ROOTZONE DRYING (PRD) EFFECTS ON GRAPEVINE PERFORMANCE, NITROGEN ASSIMILATION AND BERRY COMPOSITION

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Key words: partial rootzone drying, water use efficiency, berry size, stomatal conductance, nitrate reductase, *Vitis vinifera*

Condensed title: PRD effects on grapevine physiology

Source: South African Journal of Enology and Viticulture (2003) Vol. 24, No.2, pp.43-54

ABSTRACT

Partial rootzone drying (PRD) is an irrigation management technique designed to reduce water use in grapevines without a decline in yield, thereby increasing water use efficiency (WUE). Experiments consisted of field-grown Cabernet Sauvignon where the PRD grapevines were irrigated with half the amount of water as control grapevines and Shiraz where the PRD grapevines received the same amount of water as control grapevines. PRD treatments showed no significant differences in yield or berry composition at harvest, except that PRD grapevines that received half the amount of water had significantly smaller berries than control grapevines.

Cabernet Sauvignon PRD grapevines receiving half the amount of water as control grapevines showed a 34% reduction in main shoot growth and up to a 74% reduction in lateral shoot growth. Shoot growth was inhibited to a lesser extent in Shiraz PRD grapevines receiving the same amount of water with a 20% reduction in main shoot growth and a 33% reduction in lateral shoot growth. PRD also significantly reduced stomatal conductance in Cabernet Sauvignon on average by 31% and 16% in Shiraz. Nitrate reductase (NR) activity in grapevine leaves, which catalyses the first step in the assimilation of nitrate, was significantly lowered in response to PRD, irrespective of the amount of water applied. The reduction in NR activity was closely correlated with the development of the PRD cycle and the associated reduction in stomatal conductance.

INTRODUCTION

Partial rootzone drying (PRD) is an irrigation management technique developed in grapevines with a consistent feature that there is no reduction in yield even though the amount of irrigation water is substantially reduced in comparison to normal irrigation practices (Dry *et al.*, 2001), thereby increasing water use efficiency (WUE). PRD requires the frequent irrigation of approximately half of the root system while the other half is left to dry (Fig. 1). After a certain period of time the 'wet' and 'dry ' zones are alternated, allowing the former 'wet' zone to dry while the 'dry' zone is irrigated (Dry and Loveys, 1999). Two dripper lines per grapevine row with offset drippers that can be operated independently can achieve the desired wetting pattern. PRD irrigation can start when normal irrigation commences and depending on type of soil and climatic conditions, the alternation of 'wet' and 'dry' zones would typically occur on a ten to fifteen day cycle.

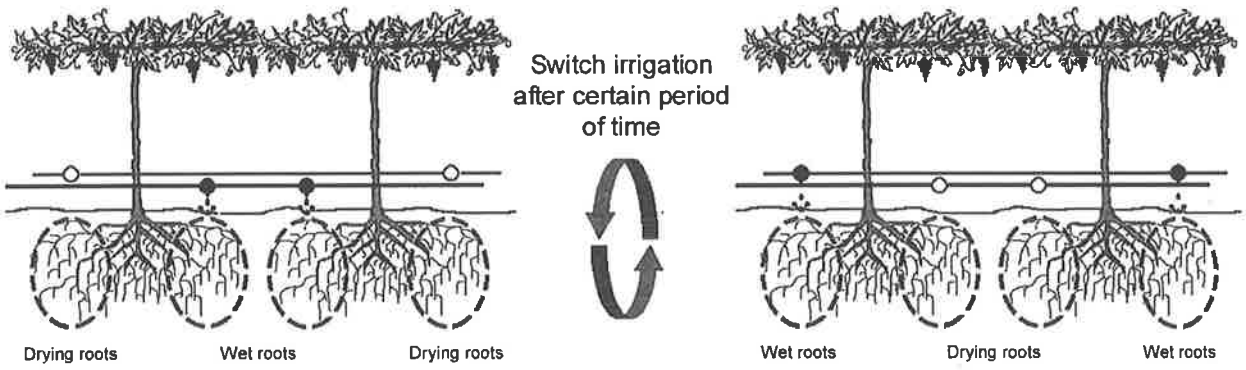

Figure 1: Implementation of partial rootzone drying.

The PRD system probably relies on hormonal signals originating from the roots in response to low soil water potentials within the 'dry' zone. Much evidence has been accumulated that drying roots are the origin of abscisic acid (ABA), which is involved in regulating stomatal aperture (Zhang and Davies, 1990; Davies and Zhang, 1991; Davies *et al.*, 1994; Comstock, 2002). Normally, the closure of stomata in response to drying soil conditions serves to protect leaf tissue from excessive loss of moisture, thereby conserving water by reducing transpiration. In the PRD system the grapevine is given a false sense of water stress, because one root zone is constantly exposed to low soil water potentials, producing ABA and sending a signal to the above ground organs. The observed effects of ABA in above ground organs due to PRD are a reduction in shoot growth and partial stomatal closure (Dry and Loveys, 1999). Without alternating the 'wet' and 'dry' sides, i.e. wetting only one side of the grapevine while the other side continues to dry out, has shown that stomatal conductance and shoot growth rate will start to recover after a certain period of time (Dry and Loveys, 1999). It has been found (Loveys *et al.*, 2000; Stoll *et al.*, 2000b) that this recovery correlated with a reduced production of ABA in the 'dry' roots. It was therefore suggested that a long-term effect on stomatal conductance and shoot growth in grapevines is only possible if the signal originating from the 'dry' side can be sustained. By alternating the 'wet' and 'dry' sides, it was possible to maintain a long-term response (Dry *et al.*, 2001) and it became clear that a continuous chemical signal or a certain concentration of the signal is necessary to maintain a physiological response.

PRD has the effect of controlling vegetative growth in grapevines, which may lead to a reduced canopy density and improved grapevine balance (Dry *et al.*, 2001). While other irrigation management techniques such as regulated deficit irrigation (RDI) may reduce vigor, they are often accompanied by a penalty in yield (Matthews and Anderson, 1988; Matthews and Anderson, 1989; Goodwin and Jerie, 1992; Dry *et al.*, 2001).

Vegetative growth and development are limited by nitrogen availability more than any other nutritional factor (Crawford and Glass, 1998). The absorption of nitrate (NO_3^-) and ammonium (NH_4^+) by plants allows them to form numerous nitrogenous compounds, mainly proteins, essential to growth and metabolism. Central to the assimilation of inorganic N to organic

nitrogenous compounds is the energy dependent and substrate inducible enzyme nitrate reductase (NR) (Gojon *et al.*, 1991; Lewis *et al.*, 2000). However, its activity can be altered by several environmental, hormonal or metabolic factors (Huber *et al.*, 1992; de Cires *et al.*, 1993). Equally important is the glutamine synthase/glutamate synthase (GS/GOGAT) cycle (Givan, 1979; Roubelakis-Angelakis and Kliewer, 1992), thought to be the most important process in the production of amino acids in grapevine leaves and roots. Our study therefore started with an investigation into the activities of NR and glutamine synthase (GS) as a measure of nitrogen assimilation in PRD grapevines. This article reports on the effect of PRD on certain aspects of the nitrogen assimilation process as well as PRD effects on grapevine performance under field conditions and associated effects on berry composition.

MATERIALS AND METHODS

PRD irrigation

To illustrate how the PRD system was maintained, soil water content was monitored by means of the Enviroscan® soil moisture sensor system (Sentek Pty Ltd, Adelaide, South Australia). The irrigation regimes of the control and PRD (PRD received the same amount of water as control) during the 2000/1 season are shown in Figures 2 and 3. Data were summed for the top 700 mm because that is where most of the roots were distributed within the soil profile. Probes were situated on either side of a control grapevine and a PRD grapevine within the wetting zones, 300 mm from the trunk. Measurements were taken every 20 minutes at 100, 200, 300, 400, 500, 700 and 1000 mm depths and automatically recorded by a solar-powered logger. In order to maintain an adequate water supply to both control and PRD grapevines the soil water content of the 'wet' zone was never allowed to fall below a certain soil water content referred to as refill point 1 (Fig. 2 and 3). The PRD cycle was achieved by switching the wetting zones as soon as the soil water content in the 'dry' zone reached refill point 2. Refill point 2 is an arbitrary value where the slope of the graph of the soil water content in the 'dry' zone flattens to indicate a low rate of soil water extraction.

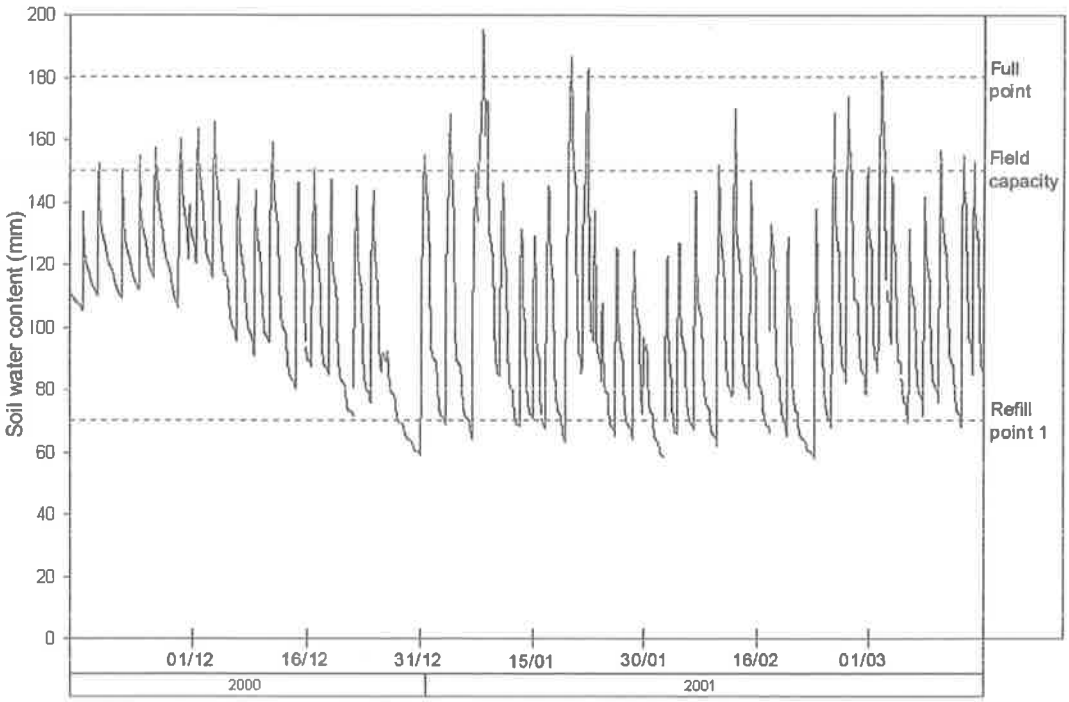

Figure 2: Soil water content (mm) of control irrigation measured at 0–700 mm depth by EnviroSCAN® during the 2000/01 season.

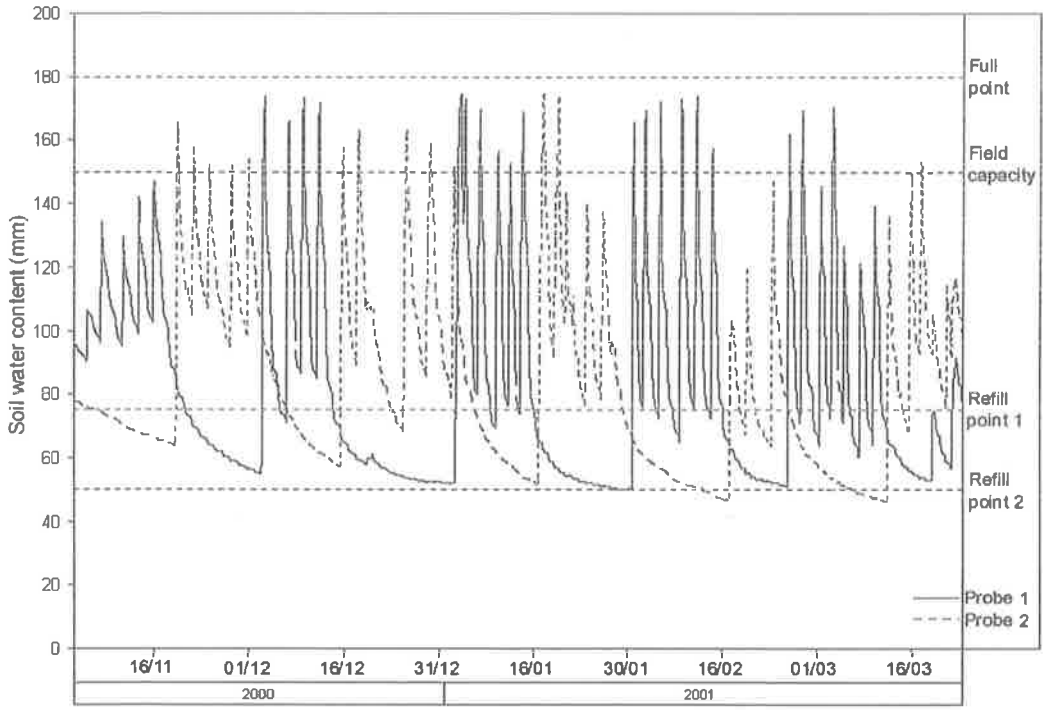

Figure 3: Soil water content (mm) of PRD irrigation measured at 0–700 mm depth by EnviroSCAN® during the 2000/01 season.

As shown in Figure 3, the 'wet' zone of the PRD system was irrigated when the soil water content reached refill point 1. Refill point 1 corresponded roughly to the refill point calculated in a normal irrigation regime and therefore the 'wet' zone constituted a normal irrigation regime. PRD Cabernet Sauvignon grapevines received half the amount of irrigation water of control grapevines and PRD Shiraz received the same amount of irrigation water as control grapevines. Control grapevines received irrigation through one dripper line with two 2 L/h drippers 300 mm on either side of the trunk. PRD grapevines received irrigation from two separate dripper lines with alternating 2 L/h drippers for Cabernet Sauvignon and 4 L/h drippers for Shiraz respectively (Fig. 1). Thereby successfully irrigating both control cultivars and PRD Shiraz grapevines with 4 L/h and PRD Cabernet Sauvignon with 2 L/h irrigation water. Cabernet Sauvignon control grapevines received a total of 107 mm/ha of irrigation, while PRD grapevines received a total of 53 mm/ha. Shiraz control and PRD grapevines both received a total of 107 mm/ha of irrigation. Irrigation amounts are based on total vineyard surface and therefore the amounts in mm applied to the actual wetted zone were considerably higher. Total effective rainfall (above 5 mm/day) for the irrigation period was 62 mm ranging over a total of only 5 days during the PRD irrigation period. Rainfall events were too few to have had any effect on PRD treatments.

Plant material

Experimental grapevines had a vertically shoot positioned (VSP) trellis system and were situated in the Coombe vineyard (Waite campus, Adelaide, South Australia) planted in 1991 to a spacing of 3 m x 1.8 m. The soil type is classified as 'Dr2.23 Hard Pedal Red Duplex' with 8 % clay content at 0-110 mm and 60 % clay content at 300-690 mm (Litchfield, 1951). All grapevines were own-rooted and spur pruned. Experimental design for both cultivars consisted of a randomised block design with two treatments, control and PRD irrigation, and seven replicates within one row. Each plot consisted of three grapevines and data were only collected from the centre grapevine thereby leaving 2 buffer grapevines between each treatment. Grapevines were pruned to leave 30 nodes/kg winter pruning weight and bunch thinning was done in 2000 before flowering aiming for 60 bunches per grapevine.

General methods and calculations

Grapevines were harvested and pruned by hand on the 9th of March and the 20th of July respectively. Bunches and canes were counted and weighed on-site. Berry weight was

calculated for each plot by weighing a random sample of 200 berries. Juice °Brix and pH was assessed after the sample was crushed and filtered. Stomatal conductance of leaves was determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to manufacturer's recommendations. Measurements were conducted on cloudless periods on fully matured and fully sun-exposed leaves selected at random at the same time of the day (12:00 h to 14:00 h).

Shoot measurements started when PRD irrigation commenced at the end of November until active shoot growth stopped in the beginning of January. Shoot growth rate was measured by selecting a reference node at five to seven nodes below the shoot tip. It was labelled and the distance between the reference node and the shoot tip was measured at intervals of seven days. Shoot growth rate (cm/day) was calculated as the average increase in shoot length since the previous measurement. When a shoot stopped growing, that shoot was discarded from the pool and measurements continued on only the remaining shoots. Therefore shoot growth rate was representative of actively growing shoots. In some cases, shoots were replaced after the shoot tip was damaged by wind or machinery.

Leaf water potential was measured on fully matured leaves between 09:00 am and 10:00 am. For each measurement a leaf was wrapped in a polyethylene bag and removed with a single cut across the petiole with a razor blade. Xylem water potential was measured by placing the leaf into a pressure bomb (Scholander *et al.*, 1965) attached to a nitrogen gas cylinder. Pressure was increased slowly until exudation of xylem sap from the cut end of the petiole was observed.
Rainfall aside, WUE is an index of the efficiency with which irrigation water reaches the grapevine and the efficiency with which water is transpired in fixing carbon. In this study WUE will be defined as the amount of crop harvested per unit of irrigation water applied (t/ML). In the Australian environment a WUE for premium red varieties can range between a value of 4 (hot, dry regions e.g. Sunraysia) and 13 (cooler regions e.g. Adelaide and McLarenVale) (Dry *et al.*, 2001).

Statistical analyses were done using both the Microsoft® Excel 2000 and SAS® statistical analysis software. Results comparing multiple groups of data were analysed using ANOVA and Student T-tests were used to determine significant differences between groups. Significance levels are indicated by P-values.

Analytical methods

Soluble sugars, proline, proline analogs and betaines were analysed as described by Naidu (1998). Leaves and berries were frozen in liquid nitrogen and powdered with a mortar and pestle. A sample of 300 mg of powdered tissue was then placed in a centrifuge tube and 3 mL of ice-cold methanol:chloroform:water (MCW; 60:25:15) added. After adding 5 µmol of D-sorbitol as internal standard the contents was inverted for 5 minutes. The MCW emulsion was broken by the addition of 3 mL water and the contents of the tube was centrifuged at 10,000 g for 10 min at 4°C. The clear upper methanol-water (MW) phase was removed and dried. After being redissolved in 500 µL of water the osmolytes were passed through a SepPak C_{18} cartridge (Waters Corporation) and injected into a High Pressure Liquid Chromatography system (Hewlett Packard LC1100) passing through a Waters Sugar-Pak I HPLC column maintained at 80°C. Column eluate passed into a diode array detector scanning every second from 190 to 400 nm at an interval of 1.2 nm. Optimum absorbancy was attained at 192 nm. Standards of soluble sugars (sucrose, glucose, fructose) and other osmolytes (alanine betaine, glycine betaine, hydroxy-N-methyl-proline, methyl proline and proline) were analysed in the same way to generate standard curves over a 10-fold concentration range. The mobile phase was bacteria free water containing 50 mg/L Ca-EDTA. To ensure that the mobile phase is gas free it was passed through an in-line degasser. Flow rate was maintained at 0.6 mL/min.

The activity of glutamine synthase (GS) was determined by the method described by Lin and Kao (1996). Leaves were harvested approximately one month before harvest and consisted of five sun-exposed mature leaves within the first basal five leaves per plot. Plant tissue was homogenized with 10 mM Tris-HCl buffer (pH 7.6), containing 1 mM $MgCl_2$, 1 mM EDTA and 1 mM 2-mercaptoethanol in a chilled pestle and mortar. The homogenate was then centrifuged at 15000 g for 30 min and the supernatant used for the enzyme assay. The whole extraction procedure was carried out at 4°C. GS assay was done on the supernatant by the method described by Oaks *et al.* (1980). The reaction mixture contained in a final volume of 1 mL, 80 µmol Tris-HCL buffer, 40 µmol L-glutamic acid, 8 µmol ATP, 24 µmol $MgSO_4$ and 16 µmol NH_2OH (final pH is 8.0). Reaction was started by the addition of the enzyme extract and after incubation for 30 min at 30°C the reaction was stopped by the addition of 2 mL 2.5% (w/v) $FeCl_3$ and 5% (w/v) trichloroacetic acid in 1.5 M HCl. The mixture was centrifuged at

3000 g and the absorbance of the supernatant was read at 540 nm. One unit of GS activity is defined as 1 μmol L-glutamate γ-monohydroxamate formed per min.

Nitrate reductase (NR) activity was assayed in leaves by the method described by Hunter and Ruffner (1997). Leaves were harvested approximately one month before harvest roughly every second day for seven intervals. Harvests consisted of two samples of each plot and each sample consisted of three fully sun-exposed mature leaves within the first five basal five leaves. After the removal of leaf veins, leaves were cut into 4 mm^2 disks. Representative samples of leaves (0.2 g) were immediately infiltrated under vacuum in pre-cooled 50 mL Erlenmeyer flasks containing 5 mL 0.1M KNO_3 and 5 mL 0.1M phosphate ($Na_2HPO_4.12\ H_2O$-KH_2PO_4) buffer at pH 7.5. In controls, KNO_3 was substituted with water. The infiltration of the tissue comprised repetitive (5 x 30 sec) removal of oxygen by vacuum and replacement with N_2. After infiltration, N_2 was bubbled into the incubation medium for 60 sec. Flasks were then sealed with rubber stoppers, wrapped in aluminium foil and incubated with gentle shaking in a water bath for 1 h at 40°C. After incubation the flasks were vortexed for 10 sec and 1mL aliquots removed for nitrite determination. Nitrite was estimated by the addition of 1 ml 1% (w/v) Sulphanilamide in 1.75 M HCl, 1 mL 0.01% (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride and 5 mL H_2O. Absorbance was read at 540 nm after 30 min. The NRA is expressed as nmol nitrite produced per gram fresh weight per hour.

RESULTS AND DISCUSSION

Grapevine performance affected by PRD

PRD grapevines showed no significant reductions in yield for the season of 200/01 relative to control (Table 1). Bearing in mind that grapevines were bunch-thinned and pruned to a level of 30 nodes/kg pruning weight, WUE for Cabernet Sauvignon and Shiraz at the site was within normal expectations for the region. PRD treatment on Cabernet Sauvignon grapevines (half the mount of irrigation water) increased the WUE by 89% (Table 1) compared to control. PRD Shiraz grapevines irrigated with the same amount of water as control grapevines had higher yields over the two-year period, also increasing WUE (Table 1). However, the increase in yield and WUE in Shiraz may not be attributed to PRD but rather higher bunch numbers per grapevine.

Table 1: Performance data of Cabernet Sauvignon (PRD received half the amount of irrigation water as control) and Shiraz (PRD received the same amount of irrigation water as control). (n.s. = not significant; * = significant ($P \leq 0.05$)

	Cabernet Sauvignon				Shiraz			
	Control	PRD	%Diff.		Control	PRD	%Diff	
Yield (kg/grapevine)	3.94	3.69	-6	n.s.	5.53	6.89	25	*
Juice °Brix	24.4	25.4	4	n.s.	26.8	27.3	2	n.s.
Juice pH	3.53	3.45	-2	n.s.	3.54	3.53	0	n.s.
Main shoot growth (cm/week)	3.16	2.08	-34	*	12.86	10.34	-20	n.s.
Lateral shoot growth (cm/week)	2.70	0.70	-74	n.s.	12.14	8.18	-33	n.s.
Shoot no/grapevine	55	62	13	n.s.	59	75	27	*
Bunch no/grapevine	73	65	-11	n.s.	75	88	18	*
Bunch weight (g)	58.2	58.8	1	n.s.	74.0	78.9	7	n.s.
Berry weight (g)	0.98	0.87	-11	*	1.17	1.16	-1	n.s.
Berry no/grapevine	59	67	14	n.s.	63	68	8	n.s.
Irrigation (ML/ha set to harvest)	1.07	0.53	-50	1.07	1.07	0		
WUE (t/ML)	7.4	13.9	89	10.3	12.9	25		

Differences in bunch counts at harvest (Table 1) may be due to losses incurred with summer hedging or ineffective bunch thinning. A significant difference was found in berry size of Cabernet Sauvignon grapevines receiving half the amount of water. PRD grapevines had significantly ($P \leq 0.05$) smaller berries than control grapevines but more berries per bunch, resulting in comparable bunch weights and yield. It was not clear if the smaller berries on PRD grapevines were a direct consequence of irrigation treatment or an indirect effect of berry number per bunch. Smaller berries may have significantly positive effects on berry and wine quality because the skin surface per unit berry weight or volume would be increased (Singleton, 1972). Singleton (1972) found that even a 10% decrease in average berry size without a change in berry composition produced red wine with recognizable and therefore important increases in aroma, colour, tannin and quality. PRD had no significant influence on berry composition with respect to °Brix, pH (Table 1) or soluble sugars and osmolytes (Table 2).

Table 2: Effect of PRD on berry soluble sugars and osmolytes (µMol/g fresh weight) of field-grown Cabernet Sauvignon and Shiraz (harvest 2001). All comparisons are not significant.

	Cabernet Sauvignon			Shiraz		
	Control	PRD	%Diff.	Control	PRD	%Diff
Sucrose	2.76	2.47	-11	4.01	4.47	11
Glucose	576.5	601.4.	4	823.5	883.1	7
Fructose	514.0	531.2	3	701.8	747.9	7
Alanine betaine	7.01	6.31	-10	9.51	9.39	-1
Hydroxy-N-Me Proline	4.44	3.95	-11	6.76	6.74	-0
Glycine betaine	1.18	1.26	7	5.73	5.90	3
Methyl Proline	0.78	0.70	-10	0.60	1.21	102
DL-Proline	42.8	44.1	3	14.29	15.89	11

PRD-treated Cabernet Sauvignon had significantly higher total soluble solids (°Brix) early in maturity. However, differences disappeared with further berry development until a week before harvest. At this stage no discernable difference could be found between treatments. PRD-treated Cabernet Sauvignon at harvest, however, had slightly higher °Brix. The reasons for this are unclear. For Shiraz juice °Brix where PRD received the same amount of irrigation water as the control there were no significant differences between treatments at any stage from veraison until harvest (Fig. 5).

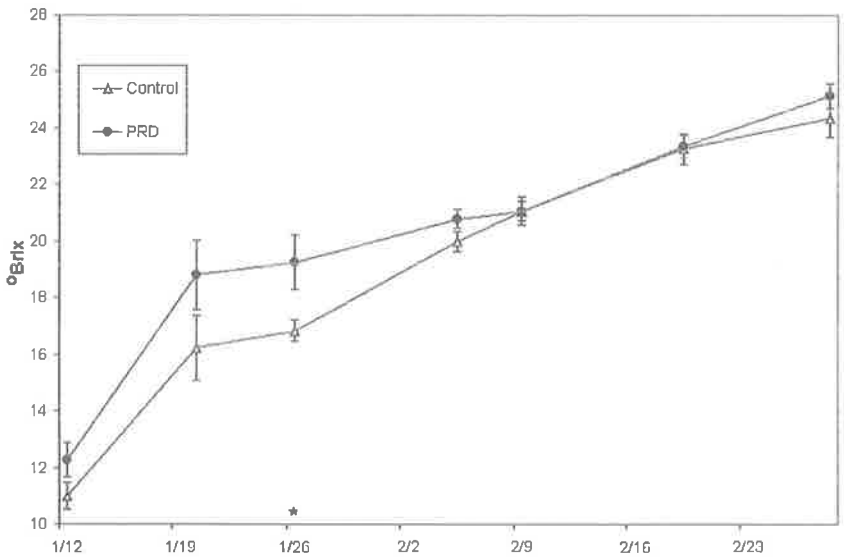

Figure 4: Juice °Brix of Cabernet Sauvignon (2000/01 season). PRD received half the amount of water as control. Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$).

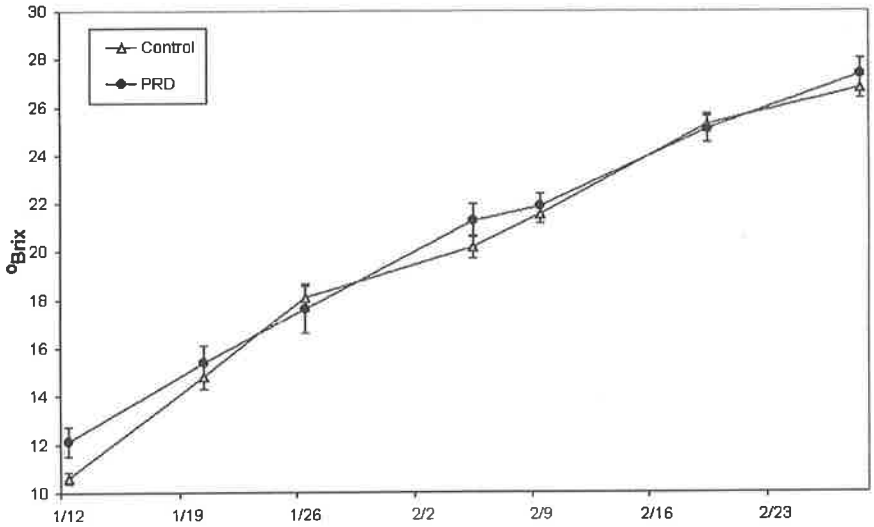

Figure 5: Juice °Brix of Shiraz (2000/01 season). PRD received the same amount of water as control. Vertical bars indicate standard errors of the average.

PRD significantly decreased shoot growth rate (Fig. 6) when irrigated with half the amount of water as the control, amounting to a 34% decrease in main shoot growth (Table 1) and a 74% decrease in lateral shoot growth. Although not significant (Fig. 7), Shiraz PRD grapevines receiving the same amount of water as control grapevines showed a 20% decrease (Table 1) in main shoot growth rate and a 33% decrease in lateral shoot growth. These findings are in accordance with earlier reports by Loveys *et al.* (2000). PRD therefore decreased grapevine shoot growth independently of the amount of water applied and predominantly affected lateral shoot growth. Lateral shoot growth plays an important role in increasing canopy density and leaf area. Earlier reports (Loveys *et al.*, 2000; Dry *et al.*, 2001) found significant decreases in leaf area mainly due to a reduction in lateral shoot growth.

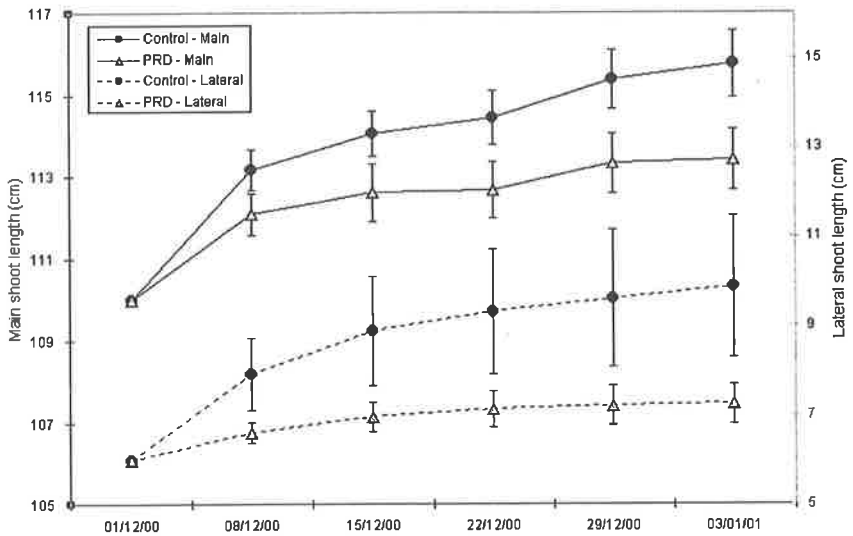

Figure 6: Shoot length of Cabernet Sauvignon (2000/01 season). PRD received half the amount of water as control. Vertical bars indicate standard errors of the average.

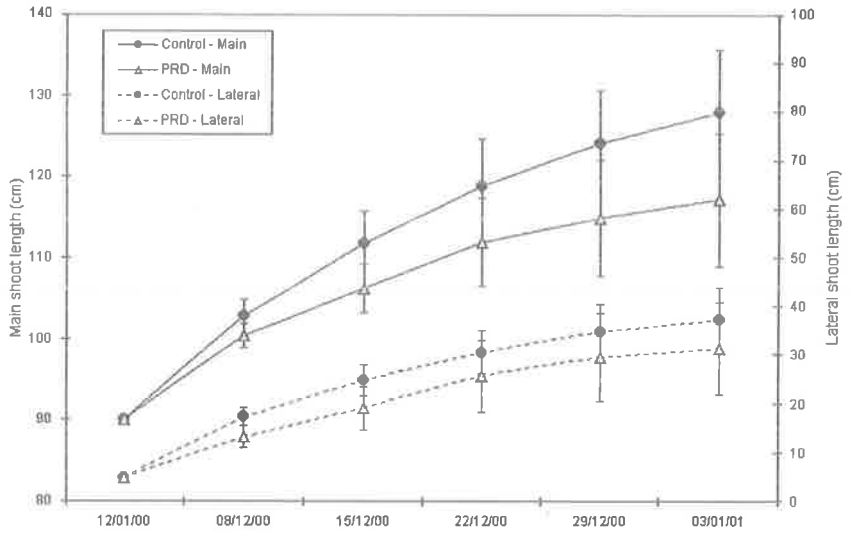

Figure 7: Shoot length of Shiraz (2000/01 season). PRD receiving the same amount of water as control. Vertical bars indicate standard errors of the average.

Grapevine physiology affected by PRD

Earlier PRD experiments with tomato (Davies et al., 2000) and grapevines in pot and field experiments (Loveys et al., 2000; Stoll et al., 2000b) has shown that PRD treatment had no detrimental effect on leaf water potentials. By contrast, deficit irrigation of grapevines may significantly reduce leaf water potential relative to well-watered controls (Matthews and Anderson, 1988). Grapevines exposed to severe water stress may exhibit mid morning leaf water potentials in the order of -1.5 MPa to –2.3 MPa (Dundon and Smart, 1984). Investigations into plant water status during this experiment indicated that PRD had no significant effect on mid-morning leaf water potentials of field-grown Cabernet Sauvignon or Shiraz (data not shown) similar to previous findings. Control leaves of both cultivars had an average of –0.94 MPa while PRD-treated Cabernet Sauvignon and Shiraz leaves averaged –0.98 and –0.92 MPa respectively.

PRD grapevines showed significantly ($P \leq 0.05$) lower stomatal conductance on most sample days when irrigated with half the amount of water (Fig. 8) and with the same amount of water as control (Fig. 9). PRD significantly reduced average stomatal conductance by 31% and 16% in Cabernet Sauvignon and Shiraz respectively. Therefore, the reduced stomatal conductance appears to be mainly due to a PRD effect and not simply a reduction in amount of water applied.

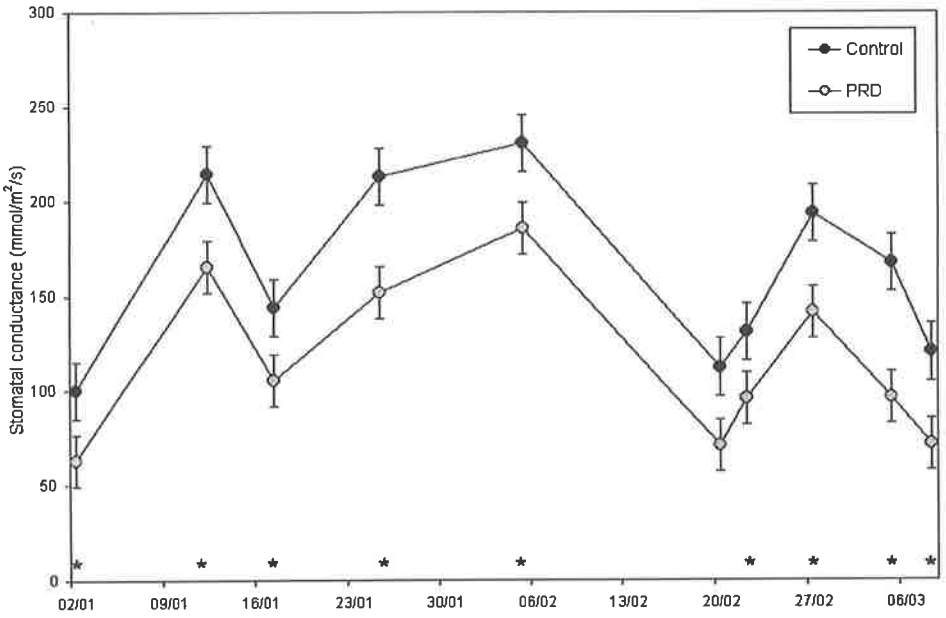

Figure 8: Stomatal conductance of Cabernet Sauvignon 2000/01 season). PRD received half the amount of water as control. Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$).

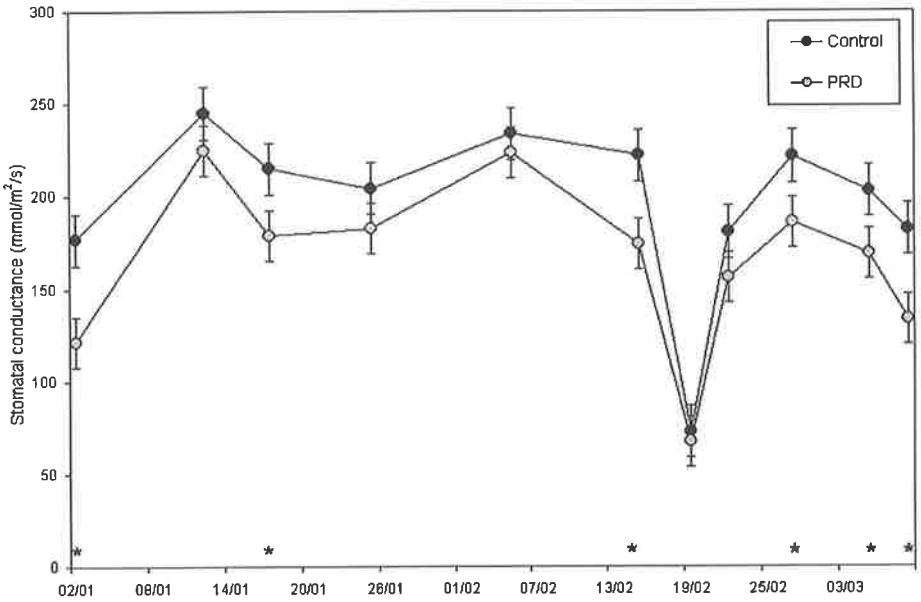

Figure 9: Stomatal conductance of Shiraz (2000/01 season). PRD received the same amount of water as control. Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$).

Investigation into enzyme activity of the GS/GOGAT cycle revealed that GS activity was not significantly influenced in response to PRD (Table 2) even in Cabernet Sauvignon PRD grapevines that received half the amount of irrigation water as control grapevines.

Table 3: GS activity measured in leaves of field-grown Cabernet Sauvignon and Shiraz (2000/01 season). GS activity is defined as μmol L-glutamate γ-monohydroxamate/min.

	Control	PRD	P
Cabernet Sauvignon	0.268	0.233	0.416
Shiraz	0.137	0.123	0.560

NR activity compared closely to values found by Hunter and Ruffner (1997) in basal leaves of Cabernet Sauvignon. Leaf NR in both Cabernet Sauvignon and Shiraz however showed a significant decrease in activity in response to PRD (Figs 10 and 11). The NR activity was investigated over the period of a single PRD cycle. NR activity in response to PRD followed the development of the PRD cycle. Although the effect of PRD was less pronounced in Shiraz (Fig. 11), differences were still significant and the trend was still obvious.

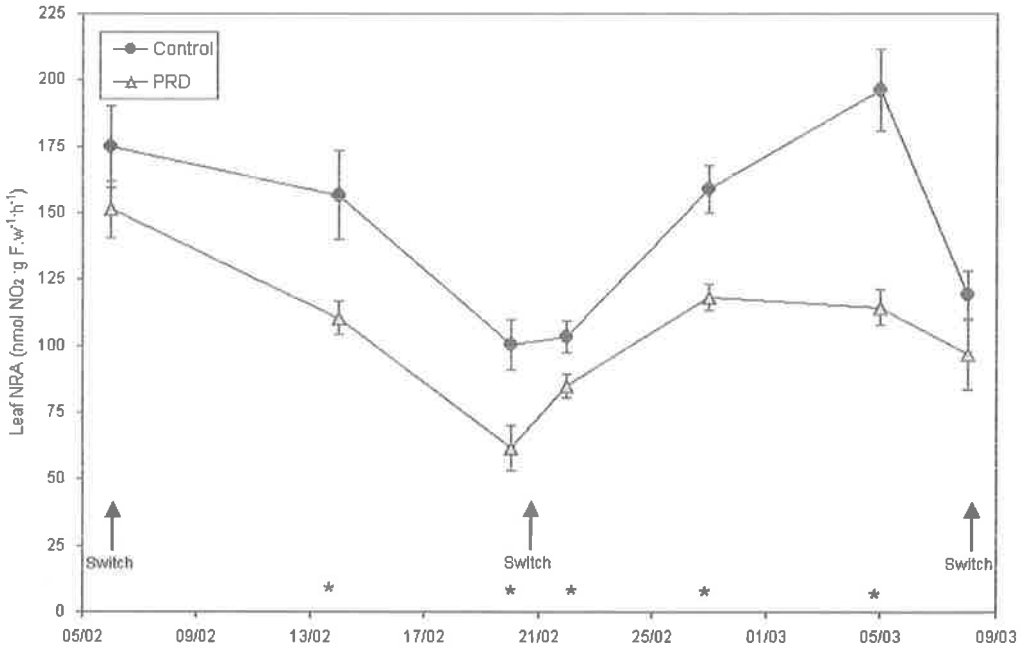

Figure 10: Effect of PRD on NR activity in leaves of field-grown Cabernet Sauvignon over one PRD cycle (PRD received half of control irrigation) measured during the 2000/01 season. Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$).

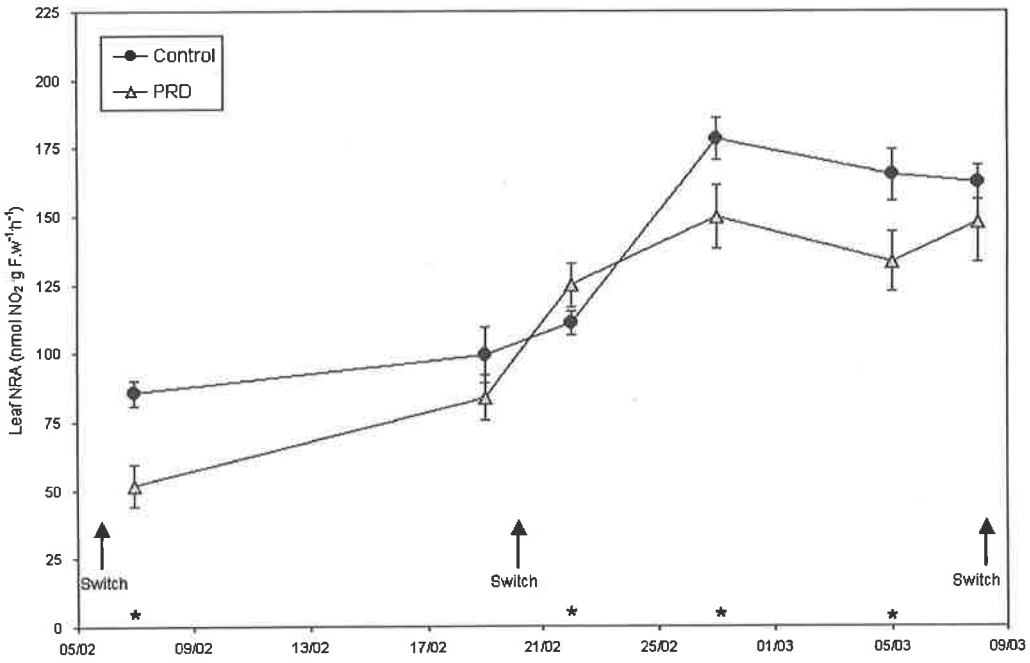

Figure 11: Effect of PRD on NR activity in leaves of field-grown Shiraz over one PRD cycle (PRD received the same amount as control irrigation) measured during the 2000/01 season. Vertical bars indicate standard errors of the average. * = significantly different ($P<0.05$).

At the beginning of the PRD cycle where one rooting zone is kept wet and the 'dry' side had just started to dry, the difference in NR activity between control and PRD grapevines was small but significant. As the PRD cycle continued, the magnitude of the difference in NR activity increased, indicating a growing inhibition of NR in PRD grapevines. During these stages NR activity correlated closely with stomatal conductance (Fig. 12). By the end of the PRD cycle the magnitude of the difference in NR activity between control and PRD grapevines had diminished (Figs 10 and 11), and the correlation between NR and stomatal conductance was not as strong (Fig. 13). Earlier studies by Dry and Loveys (1999) and Dry et al. (2000) showed a PRD induced reduction in both stomatal conductance and assimilation rate (Pn). PRD may, through its effect on stomatal conductance, have a direct effect on NR activity due to lowered Pn.

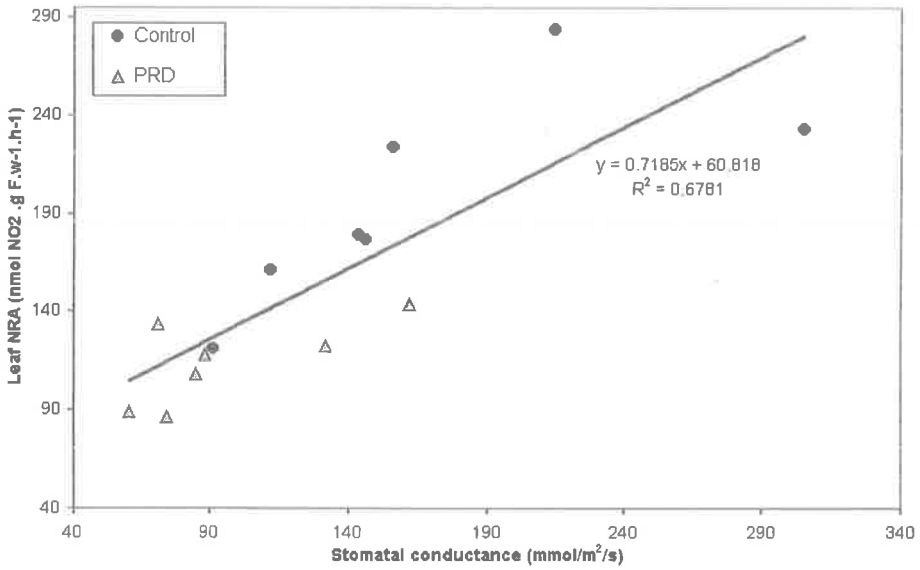

Figure 12: Relationship between leaf NR activity and stomatal conductance of field-grown Cabernet Sauvignon (05/03/01) (P=0.001)

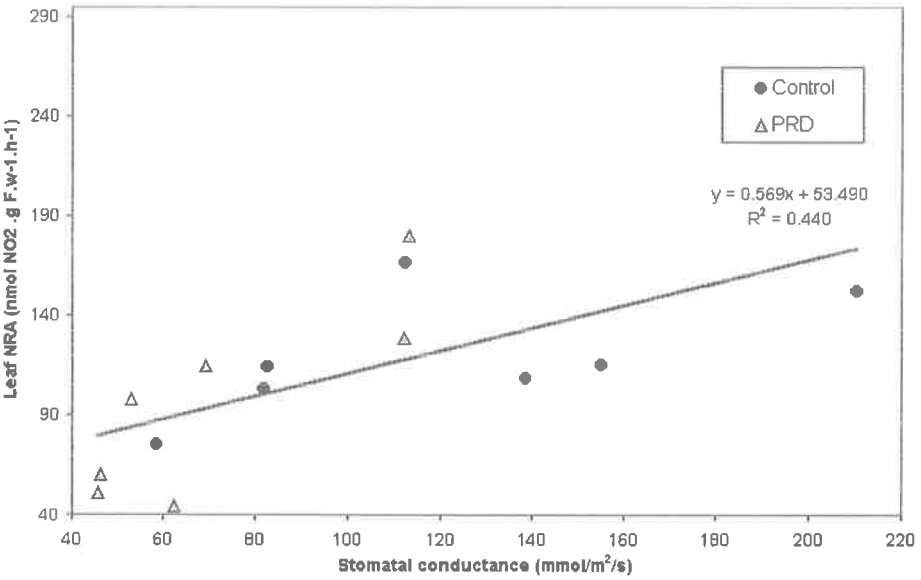

Figure 13: Relationship between leaf NR activity and stomatal conductance of field-grown Cabernet Sauvignon (08/03/01) (P=0.114)

An alteration of NR enzyme activity may be caused by both metabolic and environmental factors. Environmental factors aside, PRD may influence NR activity by changing substrate availability and/or by hormonal influences. It is hypothesized that the inhibition of NR in PRD grapevines may be due to one or more factors. Firstly, a reduction in Pn could have further far-reaching effects on NR activity at the transcriptional and posttranscriptional level. CO_2 removal from the atmosphere or stomatal closure in response to drought causes a rapid inactivation of

leaf NR (Kaiser and Förster, 1989). Secondly, because half of the root system is faced with a diminishing soil water content, nitrogen absorption of the roots may be decreased, thereby reducing NR activity because of its ability to be substrate-inducible (Gojon *et al.*, 1991). In terms of root to shoot communication, nitrate itself is the primary signal molecule triggering the activation of transcription of nitrate assimilation and related genes (Takei *et al.*, 2002). Furthermore, nitrogen availability could modulate cytokinin metabolism and translocation in higher plants (Takei *et al.*, 2002). Therefore, in addition to nitrate, cytokinin could be a root to shoot signal communicating nitrogen availability. Thirdly, it is possible that NR activity may be directly influenced by the change in the ABA/cytokinin balance in PRD grapevines. The major phytohormone that influences NR is cytokinin (for a review see Gaudinova (1990)). NR activity is greatly increased in leaves in response to treatment with the cytokinin benzyladenine (BA) (Kende *et al.*, 1971; Yu *et al.*, 1998) and suppressed by ABA (Lu *et al.*, 1992). The NR mRNA levels are influenced by the BA/ABA concentration ratio and the inhibition of applied ABA can be only partially reversed by the application of equal concentrations of BA (Lu *et al.*, 1992). ABA concentration in roots and xylem sap, and delivery rate of ABA from xylem increases under mild water stress while cytokinin supply from the roots may be significantly reduced by soil drying (Itai and Vaadia, 1965; Blackman and Davies, 1985; Abida *et al.*, 1994; Shashidhar *et al.*, 1996).

ABA elicits a variety of responses on NR activity in plant systems and this may explain why shoot growth is more sensitive to soil drying than root growth (Sharp and Davies, 1989). At relatively high concentrations it reduces NR activity in etiolated leaves of barley (Lu *et al.*, 1992), potato (Palmer, 1985) and in *Agrostemma githago* (Kende *et al.*, 1971). Conversely, ABA stimulated NR activity in root systems (Palmer, 1981; Chraibi *et al.*, 1995; Goupil *et al.*, 1998). This may be due to ABA increasing available reductants (Chraibi *et al.*, 1995) that are less diverted to growth in shoots, favouring radial growth of roots under stress conditions, i.e. drought, compacted soil (Hürtung and Davies, 1991; Vartanian *et al.*, 1994). Goupil *et al.* (1998) and Chraibi *et al.* (1995) found that NR activity in roots, unlike shoots, was not related to intracellular NO_3 concentration and not modulated by a phosphorylation/dephosphorylation mechanism. Palmer (1981) however, found that ABA stimulated root NR activity at low NO_3 levels while inhibiting NR activity at high NO_3 levels. The inhibition of NR in PRD grapevine leaves indicates that the overall nitrogen assimilation process could be decreased and nitrogen

partitioning influenced which is in accordance with earlier findings of Stoll *et al.* (2000a) that PRD grapevines showed more exploratory root systems while shoot growth was reduced.

CONCLUSIONS

The PRD irrigation system is effective in reducing vegetative growth in grapevines while sustaining yield and grapevine health, thereby increasing water use efficiency. PRD affected both main shoot and lateral shoot growth, particularly the latter, irrespective of amount of water applied. Although berry size was not affected in Shiraz grapevines receiving the same amount of water, PRD Cabernet Sauvignon with half the amount of water had significantly smaller berries without a decrease in yield. Although it is uncertain if PRD was the main factor influencing berry size, smaller berries without a change in composition may produce wines with higher quality due to increased skin surface per unit berry weight. Berry composition was not influenced by PRD, suggesting that carbon accumulation or its partitioning towards berries was not detrimentally affected. PRD effects on grapevine shoot growth may be due to decreases in nitrogen assimilation as measured by the activity of NR. The PRD influence on leaf NR activity was found to be independent of amount of water applied. It is hypothesized that the observed reduction in NR activity may be influenced by either a reduced assimilation rate due to stomatal closure, a reduction in nitrogen absorption by roots and/or a hormonal influence.

LITERATURE CITED

Abida, P. S., Sashidhar, V. R., Manju, R. V., Prasad, T. G. and Sudharshana, L. 1994. Root-shoot communication in drying soil is mediated by the stress hormones abscisic acid and cytokinin synthesized in the roots. Current Science 66(9), 668-672.

Blackman, P. G. and Davies, W. J. 1985. Root to shoot communication in maize plants of the effects of soil drying. J. Exp. Bot. 36, 39-48.

Chraibi, A., Palms, B., Druart, N., Goupil, P., Gojon, A. and Rambour, S. 1995. Influence of abscisic acid on nitrogen partitioning, sucrose metabolism and nitrate reductase activity of chicory suspension cells. J. Exp. Bot. 46(291), 1525-1533.

Comstock, J. P. 2002. Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. J. Exp. Bot. 53(367), 195-200.

Crawford, N. M. and Glass, A. D. M. 1998. Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci. 3, 389-395.

Davies, W. J., Bacon, M. A., Thompson, D. S., Sobeih, W. and Rodriguez, L. G. 2000. Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signalling system and hydraulic architecture to increase the efficiency of water use in agriculture. J. Exp. Bot. 51(350), 1617-1626.

Davies, W. J., Tardieu, F. and Trejo, C. L. 1994. How do chemical signals work in plants that grow in drying soil? Plant Physiol. 104, 309-314.

Davies, W. J. and Zhang, J. 1991. Root signals and the growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 55-76.

de Cires, A., de la Torre, A., Delgado, B. and Lara, C. 1993. Role of light and CO2 fixation in the control of nitrate reductase activity in barley leaves. Planta 190, 277-283.

Dry, P. R. and Loveys, B. R. 1999. Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. Vitis 38(4), 151-156.

Dry, P. R., Loveys, B. R. and During, H. 2000. Partial rootzone drying of grape. I. Transient changes in shoot growth and gas exchange. Vitis 39(1), 3-7.

Dry, P. R., Loveys, B. R., McCarthy, M. G. and Stoll, M. 2001. Strategic irrigation management in Australian vineyards. J. Int. Sci. Vigne. Vin. 35(3), 129-139.

Dundon, C. G. and Smart, R. E. 1984. Effects of water relations on the potassium status of Shiraz vines. Am. J. Enol. Vitic. 35(1), 40-45.

Gaudinova, A. 1990. Effect of cytokinin on nitrate reductase activity. In: Kutacek, M., Elliot, M. C. and Machackova, M. (eds). Molecular aspects of hormonal regulation of plant development. Academic Publishing, The Hague. pp. 225-231.

Givan, C. V. 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18, 375-382.

Gojon, A., Wakrim, R., Passama, L. and Robin, P. 1991. Regulation of NO_3^- assimilation by anion availability in excised soybean leaves. Plant Physiology 96, 398-405.

Goodwin, I. and Jerie, P. 1992. Regulated deficit irrigation: from concept to practice. Advances in vineyard irrigation. Aust. NZ Wine Ind. J. 7(4), 258-261.

Goupil, P., Loncle, D., Druart, N., Bellettre, A. and Rambour, S. 1998. Influence of ABA on nitrate reductase activity and carbohydrate metabolism in chicory roots (*Cichorium intybus* L.). J. Exp. Bot. 49(328), 1855-1862.

Hürtung, W. and Davies, W. J. 1991. Drought induced changes in physiology and ABA. In: Davies, W. (ed). Abscisic acid: physiology and biochemistry. Bios Scientific Publishers Ltd. pp. 63-75.

Huber, S. C., Huber, J. L., Campbell, W. H. and Redinbaugh, M. G. 1992. Apparent dependence of the light activation of nitrate reductase and sucrose phosphate synthase activities in spinach leaves on protein synthesis. Plant Cell Physiol. 33(5), 639-646.

Hunter, J. J. and Ruffner, H. P. 1997. Diurnal and seasonal changes in nitrate reductase activity and nitrogen content of grapevine: Effects of canopy management. Vitis 36(1), 1-6.

Itai, C. and Vaadia, Y. 1965. Kinetin-like activity in root exudate of water stressed sunflower plants. Physiologia Plantarum 18, 941-944.

Kaiser, W. M. and Förster, J. 1989. Low CO_2 prevents nitrate reduction in leaves. Plant Physiol. 91, 970-974.

Kende, H., Hahn, H. and Kays, S. E. 1971. Enhancement of nitrate reductase activity by benzyladenine in *Agrostemma githago*. Plant Physiol. 48, 702-706.

Lewis, C. E., Noctor, G., Causton, D. and Foyer, C. H. 2000. Regulation and assimilate partitioning in leaves. Aust. J. Plant Physiol. 27, 507-519.

Lin, C. C. and Kao, C. H. 1996. Disturbed ammonium assimilation is associated with growth inhibition of roots in rice seedlings caused by NaCl. Plant Growth Reg. 18, 233-238.

Litchfield, W. H. 1951. Soil survey of the Waite Agricultural Reseach Institute, Glen Osmond, SA. CSIRO Australia Division of Soils Divisional Report. No. 2/51.

Loveys, B. R., Dry, P. R., Stoll, M. and McCarthy, M. G. 2000. Using plant physiology to improve the water use efficiency of horticultural crops. Acta Hort. 537, 187-199.

Lu, J.-L., Ertl, J. R. and Chen, C.-M. 1992. Transcriptional regulation of nitrate reductase mRNA levels by cytokinin-abscisic acid interactions in etiolated barley leaves. Plant Physiol. 98, 1255-1260.

Matthews, M. A. and Anderson, M. M. 1988. Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. Am. J. Enol. Vitic. 39, 313-320.

Matthews, M. A. and Anderson, M. M. 1989. Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. Am. J. Enol. Vitic. 40, 52-60.

Naidu, B. P. 1998. Separation of sugars, polyols, proline analogues, and betaines in stressed plant extracts by high performance liquid chromatography and quantification by ultra violet detection. Aust. J. Plant Physiol. 25, 793-800.

Oaks, A., Stulen, J., Jones, K., Winspear, M. J. and Boosel, I. L. 1980. Enzymes of nitrogen assimilation in maize roots. Planta 148, 477-484.

Palmer, C. E. 1981. Influence of abscisic acid on nitrate accumulation and nitrate reductase activity in potato tuber slices. Plant Cell Physiol. 22, 1541-1551.

Palmer, C. E. 1985. The relationship of abscisic acid on nitrate accumulation and nitrate reductase activity in potato tuber slices. Plant Cell Physiol. 26, 1167-1174.

Roubelakis-Angelakis, K. A. and Kliewer, W. M. 1992. Nitrogen metabolism in grapevine. Horticultural Reviews 14, 407-452.

Scholander, P. F., Hammel, H. T., Bradstreet, D. and Hemmingsen, E. A. 1965. Sap pressure in vascular plants. Science 148, 339-346.

Sharp, R. E. and Davies, W. J. 1989. Regulation of growth and development of plants growing with a restrictive supply of water. In: Jones, H.G., Flowers, T.J. and Jones, M.B. (eds). Plants under stress: biochemistry, physiology and ecology and their application to plant improvement. Cambridge University Press, New York. pp. 71-93.

Shashidhar, V. R., Prasad, T. G. and Sudharshan, L. 1996. Hormone signals from roots to shoots of sunflower (*Helianthus anuus* L.). Moderate soil drying increases delivery of abscisic acid and depresses delivery of cytokinins in xylem sap. Annals of Botany 78(2), 151-155.

Singleton, V. L. 1972. Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. Am. J. Enol. Vitic. 23(3), 106-113.

Stoll, M., Dry, P., Loveys, B., Stewart, D. and McCarthy, M. 2000a. Partial root zone drying: Effects on root distribution and commercial application of a new irrigation technique. Wine Industry Journal 15(2), 74-77.

Stoll, M., Loveys, B. and Dry, P. 2000b. Hormonal changes induced by partial rootzone drying of irrigated grapevine. J. Exp. Bot. 51(350), 1627-1634.

Takei, K., Takahashi, T., Sugiyama, T., Yamaya, T. and Sakakibara, H. 2002. Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. J. Exp. Bot. 53(370), 971-977.

Vartanian, N., Marcotte, L. and Giraudat, J. 1994. Drought rhizogenesis in *Aridopsis thaliana*. Plant Physiol. 104, 761-767.

Yu, X., Sukumaran, S. and Marton, L. 1998. Differential expression of the Arabidopsis *Nia1* and *Nia2* genes. Plant Physiol. 116, 1091-1096.

Zhang, J. and Davies, W. J. 1990. Changes in the concentration of ABA in the xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant, Cell and Environment 13, 277-285.