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).
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):

$$\frac{dW_s}{dt} = Y_g \left( P - \frac{dW_n}{dt} - mW_s \right) - L$$

- $W_s =$ 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):

\[
\text{NO}_3^- + 8\text{e}^- + 10\text{H}^+ \rightarrow \text{NH}_4^+ + 3 \text{H}_2\text{O} \quad (1.1)
\]

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:

\[
\text{NR} \quad \text{NO}_3^- + \text{NADH} + \text{H}^+ \rightarrow \text{NO}_2^- + \text{NAD}^+ + \text{H}_2\text{O} \quad (1.2)
\]

\[
\text{NO}_2^- + 3 \text{H}_2\text{O} + 2 \text{H}^+ + \text{light} \rightarrow \text{NH}_4^+ + 1.5 \text{O}_2 + 2 \text{H}_2\text{O} \quad (1.3)
\]

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\(_2\)O 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 (Mackintosh 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).](image)

### 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).
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\textsuperscript{2+}. Both Ca\textsuperscript{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).](image)

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).

![Diagram of PRD irrigation setup](image)

**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.

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

<sup>a</sup> Bureau of Meteorology (2004).

<sup>b</sup> 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

<table>
<thead>
<tr>
<th>Golden grove sand. Sterilized at 100°C for 30min</th>
<th>Peat moss</th>
<th>Calcium Hydroxide</th>
<th>Calcium Carbonate</th>
<th>Nitrophoska 12-5-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200 L</td>
<td>750 L</td>
<td>1.1 kg</td>
<td>2 kg</td>
<td>2 kg</td>
</tr>
</tbody>
</table>

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 1 m 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 μmolm$^{-2}$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 μmol/mol CO$_2$) was equivalent to ambient air CO$_2$ concentration. Chamber RH
was kept at ambient air levels (8 to 12 mmol H₂O mol⁻¹) by manually controlling the amount of air that passed through a desiccant (Drierite, anhydrous CaSO₄). 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 cm²) and the flow of air was set to 500 μmol·s⁻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 CO₂ and H₂O to measure gas exchange. Photosynthesis was measured in μmol·m⁻²·s⁻¹ and stomatal conductance in mol·m⁻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.

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 C₁₈ 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 C₁₈ 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 C₁₈ 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% HClO₄ at a ratio of about 100 mg fresh weight/mL HClO₄ 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 HClO₄ 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 C₁₈ 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 (HNO₃) 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 Na₂HPO₄·H₂O, 1.6 g NaH₂PO₄·H₂O, 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 H₂O 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 A₅₂₀ 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 H2O.

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 H2O.

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 Na2HPO4·2H2O, 1.6g Na2HPO4·H2O, 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 A436 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 (ETo)
Reference crop evapotranspiration (ETo) was estimated on a daily basis using average data inserted into the Penmann-Monteith evapotranspiration equation. ETo 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.

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

where...
ETo = reference crop evapotranspiration [mm/day]
Rn = net radiation at crop surface [MJ/m²/day]
G = soil heat flux [MJ/m²/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.

<table>
<thead>
<tr>
<th></th>
<th>2000/1</th>
<th>2001/2</th>
<th>2002/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>14.6</td>
<td>15.5</td>
<td>14.2</td>
</tr>
<tr>
<td>October</td>
<td>15.7</td>
<td>13.7</td>
<td>16.3</td>
</tr>
<tr>
<td>November</td>
<td>17.9</td>
<td>16.7</td>
<td>20.3</td>
</tr>
<tr>
<td>December</td>
<td>21.7</td>
<td>17.4</td>
<td>22.0</td>
</tr>
<tr>
<td>January</td>
<td>25.4</td>
<td>20.9</td>
<td>25.3</td>
</tr>
<tr>
<td>February</td>
<td>25.1</td>
<td>20.4</td>
<td>22.4</td>
</tr>
<tr>
<td>March</td>
<td>19.8</td>
<td>19.9</td>
<td>18.3</td>
</tr>
<tr>
<td>Average Jan - Mar</td>
<td>23.4</td>
<td>20.4</td>
<td>22.0</td>
</tr>
<tr>
<td>Average Sept - Mar</td>
<td>20</td>
<td>17.8</td>
<td>19.8</td>
</tr>
</tbody>
</table>
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\textsubscript{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\textsubscript{o}(mm) of the Waite campus. Due to instrument failure the 2002/3 season is missing some values.

<table>
<thead>
<tr>
<th></th>
<th>2000/01</th>
<th>2001/02</th>
<th>2002/03</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>29.3</td>
<td>23.0</td>
<td>29.2</td>
</tr>
<tr>
<td>October</td>
<td>56.2</td>
<td>30.1</td>
<td>35.2</td>
</tr>
<tr>
<td>November</td>
<td>85.0</td>
<td>73.5</td>
<td>82.0</td>
</tr>
<tr>
<td>December</td>
<td>135.3</td>
<td>95.8</td>
<td>110.1</td>
</tr>
<tr>
<td>January</td>
<td>125.4</td>
<td>100.9</td>
<td>108.6</td>
</tr>
<tr>
<td>February</td>
<td>122.1</td>
<td>80.3</td>
<td>85.9</td>
</tr>
<tr>
<td>March</td>
<td>127.2</td>
<td>55.3</td>
<td>75.9</td>
</tr>
<tr>
<td>Total</td>
<td>680.3</td>
<td>465.8</td>
<td>526.9</td>
</tr>
</tbody>
</table>
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).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>15</td>
<td>35.6</td>
<td>22.4</td>
<td>51</td>
</tr>
<tr>
<td>November</td>
<td>9.2</td>
<td>23.2</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>December</td>
<td>13.4</td>
<td>7</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>January</td>
<td>18.6</td>
<td>8.8</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>February</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>March</td>
<td>45.2</td>
<td>12.2</td>
<td>7.2</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>109.4</td>
<td>86.8</td>
<td>81.2</td>
<td>182</td>
</tr>
</tbody>
</table>