

Effects of partial rootzone drying on grapevine physiology and fruit quality

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Declaration

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

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Summary

Growth, productivity and fruit quality of grapevines are closely linked to soil water availability. Withholding of water for any length of time results in slowed growth. If drought continues yield may be lost. Vines can be manipulated to stimulate early defence mechanisms by decreasing soil water availability. By using an irrigation technique, which allows for separate zones with different soil moisture status, it is possible to stimulate response mechanisms of the root system which are normally related to water stress. The difficulty of separating 'wet' and 'dry' zones was initially overcome by using split-root plants with root systems divided between two containers. Such experiments on split-root model plants resulted in the development of an irrigation technique termed *partial rootzone drying* (PRD). Results from irrigation experiments using PRD have shown that changes in stomatal conductance and shoot growth are some of the major components affected (Dry *et al.*, 1996). The idea of using irrigation as a tool to manipulate stress responses in this way had its origin in the concept that root-derived abscisic acid (ABA) was important in determining stomatal conductance (Loveys, 1984). Later experiments on split-root plants have demonstrated that many effects of water stress can be explained in terms of transport of chemical signals from roots to shoots without changes in plant water status (Gowing *et al.*, 1990). The necessary chemical signals are provided by the dry roots, and the wet roots prevent the development of deleterious water deficits.

The general hypothesis tested during this study was that partial drying of the root system gives rise to a change in the supply of root-derived chemical signals which determine changes in grapevine physiology, thereby affecting fruit quality.

Experiments were conducted on split-root vines (*Vitis vinifera* L. cvs. Cabernet Sauvignon and Chardonnay) grown in pots of different sizes, on field-grown vines which had either their root system divided by a plastic membrane (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots or grafted on Ramsey rootstocks) or conventional vines with a non-divided root system (*Vitis vinifera* L. cv. Cabernet Sauvignon, Shiraz and Riesling) with a commercial PRD irrigation design. The irrigation treatments were vines receiving water on both sides (control) and PRD-treated vines, which only received water on one side at any time. The frequency of alternation of 'wet' and 'dry' sides was determined according to soil moisture and other influences such as rainfall and temperature. In most of the experiments the irrigation was alternated from one side to the other every 10 to 15 days.

Chemical signals from roots: the role of ABA and cytokinins

Studies on chemical signals have concentrated on ABA and cytokinins (CK). An improved stable isotope dilution protocol, which enables analysis of ABA and CK from the same tissue sample, was developed. Analysis of cytokinins focused on zeatin (Z), zeatin riboside (ZR), zeatin glucoside (ZG) and iso pentenyl adenine (iP).

Roots are relatively inaccessible, particularly in field situations. To enable easier access to roots of field-grown vines, split-root vines were planted in a trench which was refilled with a sandy soil. This created a homogenous soil substrate and did not restrict root growth while still allowing access to roots under field conditions. Analyses of root samples of field-grown vines have shown that cytokinins and ABA may originate in roots and their concentrations can be substantially altered during an irrigation cycle. Alternating soil water conditions showed that [ABA] in roots on the 'dry' side was significantly higher compared with the 'wet' side. Due to a reduction in CK on the 'dry' side of PRD-treated vines, the ratio between ABA and CK was substantially changed during an irrigation cycle.

The ABA levels in root tissue and in petiole xylem sap were negatively related to stomatal conductance. This further suggests that ABA, mostly synthesized on the 'dry'

side of the root system, might be responsible for a decline in stomatal conductance. Furthermore, a higher pH of petiole xylem sap was observed in PRD-treated vines which may also contribute to the regulation of stomatal conductance. Studies on stomatal patchiness showed that non-uniform stomatal aperture occurred in field-grown vines under natural environmental conditions and was more abundant under PRD conditions. The degree of stomatal opening, determined by using a water infiltration technique, correlated with measurement of stomatal conductance.

Exogenous application of a synthetic cytokinin (benzyl adenine) can override the possible ABA-mediated stomatal closure resulting from PRD treatment, providing further evidence for the *in vivo* role of these growth regulators in the control of stomatal conductance. The effect of benzyl adenine was transient, however, requiring repeated applications to sustain the reversal. In addition, CKs may also be important in influencing grapevine growth. Following several weeks of repeated spray applications with benzyl adenine, it was found that the development of lateral shoots in PRD-treated vines was enhanced compared to PRD-treated vines sprayed with water only. This supports the idea that the reduction in lateral shoot development seen in PRD-treated vines is due to a reduced production of CKs (Dry *et al.*, 2000a). By measuring shoot growth rate it was found that one common feature of PRD-treated vines, which were not sprayed with CK, was a reduction of lateral shoot growth. It can therefore be speculated that the reduction in lateral growth is related to a reduced delivery of cytokinins from the roots. Zeatin and zeatin riboside concentration in shoot tips and prompt buds/young lateral shoots were reduced by the PRD treatment providing further evidence in support of this hypothesis.

Water movement from 'wet' to 'dry' roots

Roots, being a primary sensor of soil drying, play an important role in long- and short-term responses to PRD. Using stable isotopes of water and heat-pulse sap flow sensors water movement was traced from wet to dry roots in response to PRD. The redistribution of water from roots grown in a soil of high water potential to roots growing in a soil of low water potential may be of significance with regard to the movement of chemical signals and the control of water balance of roots. Measurements of the relative water content (RWC) have shown a slower decline of RWC of the 'dry' roots of PRD vines relative to roots of vines which received no water, despite similar water content in soil surrounding those roots. The redistribution of water may help to sustain the response to PRD for longer periods possibly releasing chemical signals and to support the activity of fine roots in drying soil.

Field vines, irrigated with PRD over several growing seasons, altered their root distribution relative to the control vines. PRD caused a greater concentration of fine roots to grow in deeper soil layers and this may contribute to a better water stress avoidance. The effect on root growth may be augmented by the water movement and by the large difference in ABA to cytokinin ratio, which are also known to alter root growth.

PRD makes more efficient use of available water

In experiments where both control and PRD-treated vines received the same amount of water many differences between the vines were demonstrated. Under conditions where water supply was adequate for both treatments, the stomatal conductance and growth of the PRD-treated vines was restricted as has been observed in many previous experiments. As total water input was reduced, however, the stomatal conductance of PRD-treated vines

became greater than control vines, suggesting that the latter were experiencing a degree of water stress, whereas the PRD-treated vines were not. This may have been due to the greater depth of water penetration in the case of the PRD-treated vines, where water was applied to a smaller soil surface area. This distinction between PRD-treated and control vines, at very low water application rates, was also reflected in pruning weights and crop yields which were actually greater in PRD-treated vines. It was concluded that at low water application rates, the PRD-treated vines were more tolerant of water stress and made more efficient use of available water.

Reduction in vigor opens the canopy

The initial aim of the research which led to the development of PRD was to achieve better control of undesirable, excessive shoot and foliage growth which, from a viticultural point of view, has many disadvantages. Grapevine shoot growth rate responds very sensitively to drying soil conditions. The irrigation strategy used in the PRD experiments maintained a reduction of both main shoot and lateral shoot growth. In response to PRD a decrease in shoot growth rate and leaf area was observed. Much of the reduction in canopy biomass was due to a reduced leaf area associated with lateral shoots, thus influencing the canopy structure. This was one major factor improving the light penetration inside the canopy.

Control of vegetative vigour results in a better exposure of the bunch zone to light and, as a consequence, in improved grape quality. It is likely that changes in canopy density, as a result of PRD, is causing changes in fruit quality components. Anthocyanin pigments such as derivatives of delphinidin, cyanidin, petunidin and peonidin were more abundant in berries from PRD vines; by comparison the concentration of the major anthocyanin, malvidin, was reduced. When leaves were deliberately removed from more vigorous control vines, which improved bunch exposure, the differences in fruit composition were much reduced. This further supports the idea that a more open canopy, in response to PRD, improves fruit quality by affecting the canopy structure. Fruit quality consequently determines the quality, style and value of the finished wine. Wines from this study have been produced and data on wine quality from commercial wineries are also available. Sensory evaluations have demonstrated that high wine quality from PRD-treated vineyards can be achieved without any yield-depressing effects.

This study has provided evidence to support the original hypothesis. The major findings were:

- a) Chemical signals, altered under PRD and mostly originating from roots, play an important role in the root to shoot communication in grapevines.
- b) The movement of water from 'wet' to 'dry' soil layers may help to sustain chemical signals as a response of grapevines to PRD and to support the activity of fine roots in drying soil.
- c) A reduction in vegetative growth, in particular of lateral shoots, was sustained using PRD and affected the canopy structure which in turn, due to a better light penetration into the canopy, improved the fruit quality.
- d) The reduction in irrigation water applied did not have a detrimental effect on grape yield and thus the efficiency of water use was improved.
- e) Application of relatively low irrigation rates showed that PRD-treated vines were more tolerant of water stress and made more efficient use of available water.

List of Abbreviations

ABA	abscisic acid
BA	benzyl adenine
b. wt.	berry weight (g)
CK	cytokinins
CP	capacitance probe
°C	degrees Celsius
df	degrees of freedom
FrW	fruit weight (kg)
FrW / PW	ratio of fruit weight to pruning weight
gs	stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$)
iP	iso-pentenyl adenine
LA	leaf area (m^2)
LLA	lateral leaf area (m^2)
MLA	main leaf area (m^2)
n	number of samples
n.d.	not detectable
n.s.	not significant
P	probability for data
PAR	photosynthetically active radiation ($\mu\text{molm}^{-2}\text{s}^{-1}$)
pH	$-\log[\text{H}^+]$
Pn	photosynthetic rate ($\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$)
PRD	partial rootzone drying
PW	pruning weight (kg)
RH	relative humidity (%)
SGR	shoot growth rate (mm/day)
s.e.	standard error of the mean
SWC	soil water content (volumetric: % or mm)
TA	titratable acidity (gL^{-1} as tartaric acid)
TDR	time domain reflectometry
TSS	total soluble solids ($^{\circ}\text{Brix}$)
VPD	vapour pressure deficit (Pa)
VSP	vertical shoot positioning
[x-hormone]	plant hormone concentration
Z	zeatin
[9G] Z	zeatin glucoside
ZR	zeatin riboside
Ψ_L	leaf water potential (MPa)
Ψ_m	soil matric potential (MPa)

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Chapter 1 General Introduction

1.1 Introduction

Grapevines are grown in many countries under a range of natural environments. Ambient conditions may vary considerably, but within any environment there are two major determinants of grape quality. First, the choice of a vineyard location which will be influenced by climatic conditions and soil properties and second, the management of the vineyard (Gladstones, 1992). Vineyard management starts with the choice of grape variety and trellis system and includes all cultural aspects such as pruning, shoot positioning, disease control and irrigation. Once a location is selected, vineyard management plays a key role in the production of valuable fruit. To achieve an optimum in ripeness and fruit composition, all viticultural opportunities need to be taken into account, since any further winemaking processes and the quality of the finished table wine will mostly depend on the composition of the fruit.

Much of the labour expended in vineyard management is a consequence of the inherent tendency of grapevines to vigorous vegetative growth. For the most part, vegetative growth of a vineyard depends on the availability of water and nutrients. Especially in cooler climate regions where water is not limited, excessive vigour may occur. As a consequence, the canopy tends to become dense, bunches more shaded and the vines more susceptible to fungal diseases. This may be overcome by labour-intensive shoot positioning or multiple shoot trimming but the cost of production will consequently be higher, whilst fruit quality may be reduced.

Under hot climatic conditions and in non-irrigated vineyards, shoot growth may be reduced and the canopy may be more open. However, the vines might suffer from water stress resulting in a yield reduction. Although the grape quality tends to be higher, the loss in yield may not be compensated for by the higher unit value of the crop. To maximise the yield, many vineyards in Australia, South Africa, Israel, western USA and South America rely on irrigation. Furthermore, in some of these regions the area of vine plantings is still increasing, and water is becoming an increasingly scarce and valuable resource. An increase in both irrigation efficiency (irrigation required /

irrigation applied) and water use efficiency (yield of fruit / irrigation applied) is desirable.

The potential for a new irrigation technique which withholds water from part of the root system has recently been proposed (Loveys, 1992) and has now been introduced to the Australian wine industry (Dry *et al.*, 1996). The irrigation technique is called: *partial rootzone drying* (PRD).

1.2 The concept of partial rootzone drying

Most physiological processes in plants require water. Plants have evolved different strategies and complex mechanisms for controlling their water loss. One effective barrier is a relatively impermeable cuticle on both sides of the leaves. Transpiration and gas exchange takes place through the stomata, which are mostly located on the leaf-subsurface. Stomata are able to sense and respond to environmental changes. Stomata have two conflicting functions: on the one hand, they must prevent excessive water loss through a reduction in aperture; on the other, they must remain sufficiently open to allow the uptake of carbon dioxide from the atmosphere for photosynthesis. It has been shown that variables such as light, temperature, wind, atmospheric carbon dioxide concentration, atmospheric humidity and soil water availability influence these complex mechanisms (Farquhar and von Cämmerer, 1982). Stimulating these mechanisms through selective withholding of irrigation water may be one strategy to make the vine become more efficient in its water use.

Faced with drying soil a plant's first line of defense is to reduce stomatal aperture. Provided the stomatal closure is not too extreme, so as to detrimentally affect CO₂ uptake, withholding water may form the basis of a useful tool in the control of water loss.

Severe water deficit may result in a decrease in leaf water potential, loss of turgor or wilting which can substantially affect stomatal aperture (Liu *et al.*, 1978). However, as a first response to drying soil conditions the stomatal aperture is more often related to non-hydraulic, chemical signals than to hydraulic changes (Blackman & Davies, 1985;

Zhang *et al.*, 1987). Roots are able to perceive variations in soil water content and send chemical signals to the leaves thereby affecting a stomatal response.

Loveys (1992) proposed that knowledge of root signals and their influence on whole plant physiology has the potential for a wide range of applications in horticultural crops. Applying ideas, which had their origins in a program of basic plant physiology research, a new irrigation technique was initiated for grapevines in 1995 (B. Loveys, pers. com.). By withholding water from half of the root system (Figure 1.1) the soil dries out slowly whilst the other part is kept frequently irrigated. After a certain period of time, ‘wet’ and ‘dry’ zones are alternated and the former ‘wet’ side starts to dry out.

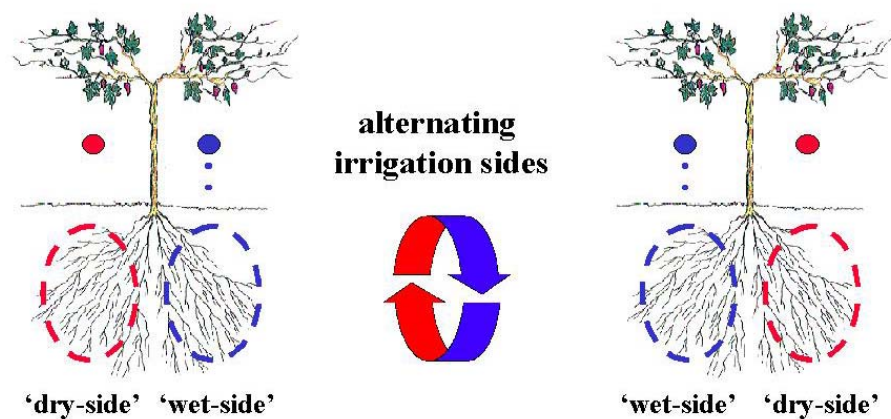


Figure 1.1 Implementation of partial rootzone drying

Why is alternating the ‘dry’ and ‘wet’ side important?

Early experiments on split-root plants of *Commelina communis* and *Zea maize*, in which water was withheld from part of the root system for 6 to 8 days, demonstrated that partial drying of the root system can result in a reduction in stomatal conductance (Blackman & Davies, 1985; Zhang *et al.*, 1987). There was no evidence that effects on stomatal conductance were caused by a reduction of water potential or turgor. Gowing *et al.* (1990) reported that, in apple, a reduction in stomatal conductance also was

concurrent with a reduction in growth rate when water was withheld from part of the root system for a 25 day period. Withholding water from part of the grapevine root system resulted in reduction of stomatal conductance and growth without any changes in plant water status (Düring *et al.*, 1996). After 30 days or less of watering only one side of the root system without alternating of 'wet' and 'dry' sides, however, stomatal conductance and shoot growth rate started to recover (Dry & Loveys, 1999). These findings suggested that a long-term effect on stomatal conductance and shoot growth in grapevines is only possible if the signal from the 'dry' side can be sustained. By alternating the 'dry' and 'wet' sides, it was possible to maintain a long term response (Dry, 1997). It is therefore most likely that a continuous chemical signal or a certain concentration of the signal is necessary to maintain these physiological changes.

1.3 Root to shoot communication and the importance to PRD

The term 'root to shoot communication' refers to the influence on shoot physiology by means of one or more chemical signals synthesised in roots after perturbation of the soil environment. Plant hormones can act as chemical signals. In general plant hormones are "*...organic compounds, synthesised in one part of the plant and translocated to another part where, in very low concentrations, they cause a physiological response*" (Salisbury and Ross, 1985).

In the last decade or so many studies have suggested that roots of plants growing in drying soil sense changes in soil conditions and utilise hormones (Blackman & Davies, 1985; Zhang *et al.*, 1987; Passioura, 1988) and other chemical signals such as changes in xylem sap composition (Meinzer *et al.*, 1991) as a first response to these unfavourable soil conditions. For a long time, regulation of leaf gas exchange in response to soil drying was thought to occur by stimulation of ABA synthesis in leaves in response to a loss of leaf turgor (Morgan and King, 1984). This was not, however, consistent during all observations. It has been found that roots which are in contact with drying soil respond quickly to changes in soil conditions and produce ABA, which can be transported via the xylem to the leaves independently of hydraulic signals (Loveys and Düring, 1984; Blackman & Davies, 1985; Zhang *et al.*, 1987).

To distinguish between effects on plant performance due to hydraulic or chemical signals, experiments were conducted on wheat plants grown with their roots in soil in pressure chambers (Passioura, 1988). Pneumatic pressure was applied to roots in drying soil, keeping the leaves highly turgid. It was found that the leaf elongation rate of these plants was similar to plants grown under drying soil conditions, but in the absence of pressure. Relative to fully irrigated plants, however, both pressurised and non-pressurised plants had reduced growth. It was concluded that roots in drying soil were one source of non-hydraulic signals to the leaves overriding any effects of leaf turgor and reducing growth.

In studying responses to changes in soil water status, hydraulic influences (for example changes in turgor) and chemical signals (for example plant hormones) need to be differentiated. To differentiate between these signals, split-root plants can be used where the root system is equally divided in two pots and water can be withheld from one of the pots. The concept is that the 'wet-side' sustains turgor in the shoot while any effect of soil drying on the metabolism in roots of the 'dry-side' might be transferred as chemical signals to the shoots. By manipulating plant water status in this way, stomatal conductance can be reduced whilst leaf water potential stays high even though only part of the root system is well-watered (Blackman & Davies, 1985; Davies *et al.*, 1986; Zhang *et al.*, 1987). Gowing *et al.* (1990) also observed that when the dried part of a split-root system was removed, leaf growth rate started to recover to the rate of fully irrigated plants. To explain this response, Gowing *et al.* (1990) evoked a mechanism in plants for sensing drying soil conditions in the root system and communicating this information to the shoot. These signals would be lost when the drying half of the root system was removed. There are two conceivable modes of delivery for these chemical signals; an increase or a reduction in supply of the physiologically-active substances. An increased supply of the messenger compound(s) can be considered a 'positive' signal whilst a 'negative' signal is constituted by a reduced supply of active substances (Davies and Zhang, 1991). According to this terminology a negative signal could occur where a signal promotes stomatal opening or growth. The production and transport of this signal would decrease as part of the soil starts to dry out.

Much evidence has been accumulated which links stomatal closure to an increase in endogenous ABA concentration. One possible candidate for a positive signal, therefore,

could be ABA (Zhang and Davies, 1987; Tardieu and Davies, 1992). As opposed to a positive signal, a negative signal may consist of a reduction in a putative plant hormone (Davies *et al.*, 1994); hence the cytokinins may be involved as negative signals in root to shoot communication of soil drying. Itai and Vaadia (1965) suggested that the production and transport of some cytokinins in sunflowers were reduced after water stress.

1.3.1 The role of abscisic acid as a chemical signal

ABA is ubiquitous in higher plants and its concentration in leaves and roots varies in response to environmental stimuli, in particular to water stress. Movement of ABA occurs in both the phloem and the xylem (for a review see Zeevaart & Creelman, 1988). The biologically-active form of ABA is the *cis* (+) isomer shown in Figure 1.2.

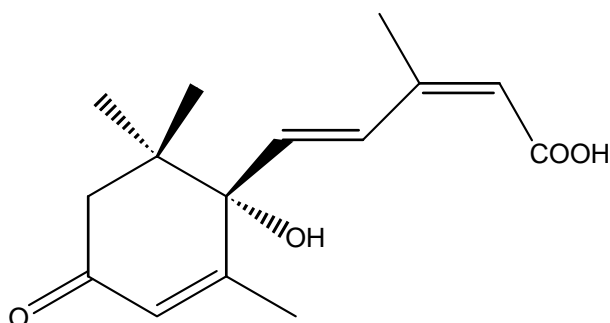


Figure 1.2 Abscisic acid: (+)-s-ABA

ABA, like other plant hormones, has multiple functions in plants. It functions in response to environmental stresses such as drought thereby playing a role in the control of water relations as an endogenous antitranspirant. Kriedemann *et al.* (1972) and Raschke (1975) suggested that ABA probably acts in the control of stomatal aperture in leaves. It is now believed that stomatal aperture is a function of changes in guard cell turgor and volume: an increase in ABA concentration and movement to the guard cells

results in a loss of K^+ from the guard cells to the subsidiary cells, lowering the turgor of the guard cells and therefore causing stomata to close (Atwell *et al.*, 1999).

There is evidence that ABA synthesis in roots in drying soil increases and ABA is transported via xylem vessels to the leaves (Walton *et al.*, 1976; Loveys, 1984a; Zhang *et al.*, 1987). It has been shown in many studies that variations in ABA concentration, due to soil drying, correlate well with the reduction in stomatal conductance (Tardieu *et al.*, 1992; Bano *et al.*, 1993; Dry, 1997). To provide further evidence for the role of ABA as an antitranspirant affecting stomatal aperture, observations on ABA-deficient mutants have shown that such mutants readily wilt (Cornish & Zeevaart, 1988). Furthermore, application of synthetic ABA to different plant organs has also demonstrated a strong negative correlation between stomatal conductance and abscisic acid at concentrations representative of endogenous changes in ABA concentration due to soil drying (Tardieu and Davies, 1993).

From these results it can be concluded that ABA is a potential candidate in the hormone message concept and may play a key role in controlling the stomatal water loss of vines exposed to partial rootzone drying.

However, cases against ABA as a root to shoot signal also exist. Munns & King (1988) found that soil drying in wheat caused an increase of an inhibitory compound in addition to an increase in ABA. They also discovered that by removing ABA from xylem sap using immunoaffinity columns containing monoclonal antibodies to ABA, the inhibitory effects on transpiration still remained. Furthermore, ABA applied in a concentration similar to the endogenous concentration measured did not show responses in transpiration of leaves and a response only occurred when a concentration of 2 orders of magnitude higher than the measured concentration was applied. From these results it was concluded that ABA is not the only inhibitory compound. Munns (1990) reported that 90% of the ABA arriving in the leaf is recirculated in the phloem and that the ABA in xylem sap is unlikely to be the only signal from the roots.

The conclusion that ABA is the sole signal eliciting a stomatal response is only possible if exogenous application of the predicted endogenous stressed levels of ABA to unstressed tissue simulates the stomatal response normally observed in stressed tissue. Such a response was demonstrated for grapevines by Loveys (1984a).

1.3.2 The role of cytokinins as chemical signals

Naturally-occurring cytokinins are adenine molecules, one of the purine bases found in all DNA and RNA, modified by the addition of 5-carbon side chains at the 6 position (x_1 substituent; Figure 1.3). Variations in the 5-carbon side chain form the four main classes of isoprenoid-derived cytokinins, namely *cis*-zeatin, isopentenyl adenine, *trans*-zeatin and dihydrozeatin. Each class of cytokinins exists as a base, riboside and nucleotide form (Hooykaas *et al.*, 1999). Koda and Okazawa (1978) showed in excised tomato root tips that zeatin riboside was the major cytokinin synthesised in root tips. Ribosides are suggested to be the major form of cytokinin transported in xylem and phloem sap (Letham and Palni, 1983). Not all forms of cytokinins are biologically active substances. McGaw (1987) concluded in his review that ribosides and bases are active forms. Glucosides are biologically inactive as growth regulators, but can be converted rapidly to active forms (Incoll and Jewer, 1990).

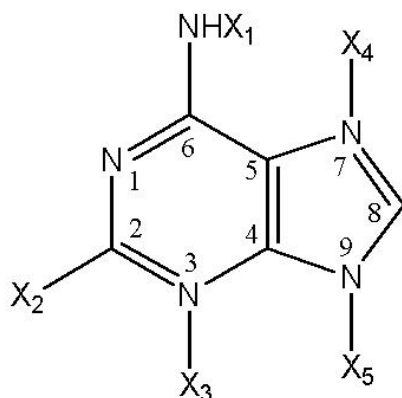


Figure 1.3 Scheme of cytokinin structure: adenine (x_1 - x_5 : substituents)

Cytokinins were originally named due to their ability to promote cell division and their function in the development of shoot structure. Much evidence has now been accumulated that cytokinins constitute a class of plant hormones which stimulate growth (Moore, 1985; Horgan and Scott, 1987).

Much evidence has been accumulated that cytokinins need to be considered as a candidate in the concept of root to shoot communication. First, roots are the main site of cytokinin synthesis (Forsyth *et al.*, 1981; van Staden and Smith, 1978). Second, the synthesis of cytokinins can be influenced by environmental conditions. In response to soil drying it has been reported that the cytokinin concentration in xylem sap of sunflower decreased (Abida *et al.*, 1994). Drying soil conditions can also affect cytokinin concentration in roots of rice causing a significant decrease in levels of zeatin and zeatin riboside (Bano *et al.*, 1993).

It has been reported that cytokinins have the potential to increase stomatal aperture (Incoll and Jewer, 1987); thus it is most likely that they play an antagonistic role to ABA. Füsseder *et al.* (1992) described daily courses of xylem sap cytokinins and ABA relative to stomatal conductance in almond trees. It was shown that during a diurnal cycle the cytokinin concentration, in particular zeatin, increased early in the morning, with the highest concentration around 9 am. In contrast, the ABA concentration at this time was relatively low. At midday and later in the afternoon, the ABA concentration started to increase concurrently with a decrease in cytokinins.

Recent results show that it is most likely that cytokinins have the potential to interact with other hormones such as ABA and thereby affect physiology (Correia *et al.*, 1997; Emery *et al.*, 1998b). Soil drying leads to an increase in ABA and a decrease in cytokinins. This change in abscisic acid/cytokinin ratio may influence the physiological responses which depend on these two classes of plant hormones.

Application of synthetic cytokinins to tomato leaves caused stomata to open (Bradford, 1983; Kumar and Abrol, 1989) and high concentrations of zeatin and kinetin applied to leaf pieces incubated in the light overrode the effect of ABA on maize stomata (Blackman & Davies, 1983).

Their role as growth-stimulating factors as well as their effect on leaf gas exchange make cytokinins interesting candidates as putative root to shoot signals.

1.3.3 Other chemical signals affecting the activity of abscisic acid and cytokinins

There is evidence that chemical signals and plant hormones other than ABA and cytokinins may also be involved in the shoot response to drying soil. Gollan *et al.* (1992) observed changes in pH and ion concentration in xylem sap of different plant species in response to drying soil conditions. Changes in pH of xylem sap may also impact on ABA (Hartung *et al.*, 1990; Slovik *et al.*, 1995). According to these authors pH changes can affect the distribution of ABA in symplastic and apoplastic compartments and could result in the trapping of anionic ABA in different compartments. Changes in pH of the xylem sap alter the availability and distribution of ABA most likely by increasing the ABA concentration of the apoplastic compartment due to a cessation of the normal, rapid sequestration away from the apoplast (Wilkinson & Davies, 1997). It is likely that variation in xylem sap pH induced by drying soil conditions may act as a signal to the leaves and may be supportive in manipulating stomatal aperture.

Access to different nitrogen sources can also affect ABA and cytokinin levels. It was found that, with nitrate as the nitrogen source, a mild water stress resulted in an increase in the long-distance transport of ABA whilst a mild water stress in ammonium-fed plants did not stimulate ABA accumulation (Peuke *et al.*, 1994).

Analysis of the cytokinin concentration in roots of *Urtica dioica* supplied different amounts of nitrogen showed that plants grown with a sufficient supply of nitrogen had a significantly higher cytokinin content and exuded more cytokinins into the shoot than those of plants grown under nitrogen shortage (Beck & Wagner, 1994). Radin *et al.* (1982) argued that the reduction in CK in roots may also be due to a reduction in nitrogenous nutrients and that such low nitrogen nutrient levels may affect stomatal behaviour by altering the balance between ABA and CK.

1.4 Techniques in plant hormone research for hormone signals analysis

The determination of the various endogenous hormones and their distribution among organs is important in understanding their role in 'root to shoot communication'. To

investigate the involvement of chemical signals and to confirm their influence on grapevine physiology, appropriate analytical techniques are required. In any type of plant tissue, hormones represent a very minor component and extensive purification may be required to identify and quantify these substances. For any analysis of plant hormones the basic idea is to maximise the recovery of the compound from a small amount of sample. The low levels of the compounds found in most plant tissues and the limited amount of sample make their quantitative analysis difficult.

Two different strategies are in common use for routine quantification of plant hormones:

- A) Immunoassay methods where plant hormones of a partially purified extract can be measured with monoclonal antibodies that are linked to an enzyme assay (ELISA) (Walker-Simmons, 1987).
- B) Physico-chemical methods (stable isotope dilution analysis) using gas chromatography and mass spectrometry (GC-MS) are very selective but require a highly purified extract (Saunders, 1978; Horgan and Scott, 1987).

Both methods are highly sensitive in their detection. For quantification purposes, however, care needs to be taken in the validation of immunoassays. The reaction of antibody with antigen in immunoassays for cytokinins depends on the structure of the side chain of the molecule, hence significant cross-reactions to other cytokinins may occur (Incoll and Jewer, 1990). In ABA analyses, cross reactivity to ABA metabolites may be possible, depending on the characteristics of the antibody. Cross reactivity to other contaminating compounds unrelated to ABA is also conceivable, and it was suggested that the results need to be verified with physico-chemical methods (Belefant and Fong, 1988).

In contrast, physico-chemical methods provide a highly sensitive and selective technique for identification and quantification of plant hormones (Wang and Horgan, 1986). This method is more time consuming during the purification, but avoids any unforeseen cross reactivity.

To allow analysis by gas chromatography derivatization is necessary to enhance the volatility of a hormonal compound (Hooykaas et al., 1999). A very selective and sensitive derivatization method for measurement of ABA in plant extracts has been

available for a long time (Saunders, 1978). ABA is best analysed as its methyl ester. Methylation can be performed by using ethereal diazomethane. The diazomethane is usually prepared in ether from *N*-methyl-*n*-nitroso-*p*-toluenesulphonamide according to procedures described by Schlenk & Gellerman (1960). Diazomethane is toxic and carcinogenic and needs to be handled with care.

Methods of extraction and identification of cytokinins are reviewed by Horgan and Hillman (1978). Cytokinins are best analysed as their permethyl derivatives. The most common derivatization method used for cytokinins is a permethylation of the hydroxyl groups. It usually involves a reaction of a strong base (methyl sulfoxide) followed by a methyl iodide treatment (Horgan & Scott, 1987).

The quantification with GC/MS for both ABA and cytokinins can be performed by adding a deuterium-labelled internal standard at the beginning of the extraction. Using a mass spectrometer, specific ions derived from the endogenous and the deuterium-labelled compounds can be separated. The ratio of deuterium-labelled and endogenous hormone, which was established at the beginning of the extraction, can be determined in the highly purified extract and the initial hormone concentration can be determined from an appropriate calibration curve. This is known as stable isotope dilution assay.

Since changes in both ABA and cytokinins might be closely related to partial drying of the rootzone, a combined method using stable isotope dilution is desirable.

1.5 Importance of vigour for fruit composition

Fruit composition and consequently wine quality is influenced by macro- and micro climatic factors. The microclimate inside a canopy can vary and dense canopies will lead to a high degree of bunch shading (Smart *et al.*, 1990). Morrison and Noble (1990) found that the rates of berry growth and sugar accumulation were slower in fruit from vines with shaded bunches. In fruit that developed in the shade, anthocyanins and total soluble phenolics were lower (Smart *et al.*, 1985). To maintain bunches in a well exposed state and to improve the microclimate, canopy management plays an important role in viticulture.

It is well known that different trellis systems can produce vines with more or less open canopies (Smart, 1992). Any type of shoot positioning, however, will be labour and cost intensive and not all types of trellis system suit the prevailing climatic conditions or the demands for mechanisation. In vines trained to a restrictive trellis system, excess vegetative growth can have a detrimental effect on canopy density (Reynolds *et al.*, 1996). Controlling the canopy density and vigour by influencing the growth of the vine might be a useful tool in vineyard management.

The term ‘vigour’ was defined by Winkler *et al.* (1974) as “... *the quality or condition that is expressed in rapid growth of part of the vine. It refers essentially to the rate of growth.*” Applied on a single-shoot basis, ‘high vigour’ would imply rapid shoot growth, with long internodes, thick shoot diameter, large leaves and more lateral shoots. The average shoot growth then determines the ‘vigour’ of the vine which then influences the density of the canopy.

Since photosynthetic assimilates are produced in the leaves and transported to a number of sinks, a minimum leaf area is important to maintain a reasonable crop (Koblet, 1969). Excessive growth, however, competes with the fruit crop for assimilates and the ratio of crop to leaf area becomes unbalanced thereby having a detrimental effect on vine biology as well as fruit composition. First, it may reduce fruit initiation (May, 1965) and therefore unbalance the vine. Second, a dense canopy leads to changes in microclimate, resulting in a higher susceptibility of the vines to fungal diseases, e.g. bunch rot (*Botrytis cinerea*) (English *et al.*, 1989), downy mildew (*Plasmopara viticola*) (Pedro *et al.*, 1998) and powdery mildew (*Uncinula necator*) (Willoquet *et al.*, 1996). Third, vigour may influence fruit composition by reducing sugar and tartrate concentration (Reynolds *et al.*, 1996). Phenolic compounds may be reduced and pH increased (Dokoozlian and Kliewer, 1995). According to Reynolds *et al.* (1994) high-density canopies may also change flavour compounds and produce fruit with undesirable aromas and flavours.

Techniques designed to control excess shoot vigour with the consequent improvement of fruit quality, have become a vitally important issue in modern viticulture. The partial rootzone drying irrigation technique has the potential to stimulate hormonal and chemical signals in vines, with an impact on growth physiology (Dry and Loveys, 1998). Medium-term changes in growth are very important for

grapevine canopy management as they can change the balance between vegetative and reproductive development leading to a more open canopy (Smart *et al.*, 1990). In addition, there is a potential for better water use efficiency of the vines and improved irrigation efficiency. For the reasons stated thus far, it is necessary to improve our knowledge of the physiological consequences of *PRD* and to use this information to refine irrigation scheduling, expenditure of water and effects on grapevine physiology and fruit composition.

1.6 Importance of water use for fruit composition

In areas exhibiting low rainfall and a hot climate, successful viticulture relies on irrigation water. Irrigation may be used as a tool to manipulate wine sensory characteristics (Matthews *et al.*, 1990). Bad irrigation management, however, has the potential to overcrop the vine, waste water, or cause environmental problems from the run-off.

An excessive amount of water can enhance berry size and berry weight (Rodrigues, 1987). Berry size, particularly in red varieties where most of the anthocyanins and phenolic pigments are located in the skins, can become crucial for fruit composition. Berries with a higher berry weight and an increased volume have a smaller skin to flesh ratio which may then alter the amount of secondary metabolites. Furthermore, an excessive amount of water can increase the ratio of yield to pruning weight and further influence wine quality due to delayed berry maturation, reduced rates of sugar accumulation or lowered must acid concentrations at comparable sugar contents (Bravdo *et al.*, 1984). To avoid overcropping and to get a higher quality fruit many irrigation trials have been set up during the past three decades. In these trials, water was restricted at different stages of berry development. By restricting the water supply at different periods of berry development Pitts *et al.* (1995) found that the period between flowering and 40 to 50 days after flowering resulted in the greatest reduction in berry weight when compared with well-watered vines. Water deficit after veraison had a minor effect on berry weight at maturity, and berries were insensitive to water deficit just prior to harvest. Observations such as these have led to the idea of regulated deficit

irrigation, whereby water is deliberately withheld for specific periods of time with the aim of influencing berry size and canopy vigour (McCarthy, 1997). Since the exact timing and the degree of water stress necessary is hard to define, deficit irrigation still involves a risk of detrimentally reducing the yield.

Since both a loss in yield and a reduction in fruit quality can influence the economic outcome, a reliable irrigation regime is desirable. Loveys *et al.* (1998) have shown over several growing seasons that by applying a continuous water deficit on alternated halves of the root system whilst the other half is well watered, vegetative growth is restricted while quality and yield is maintained. As a consequence, the water use efficiency is improved.

1.7 General research hypothesis

Growth, productivity and fruit composition of grapevines are closely linked to the soil water availability. By selective withholding of irrigation water, vines can be manipulated to stimulate early defence mechanisms. Using this irrigation technique, the amount of water applied to the vines can be dramatically reduced, thereby improving the water use efficiency. Results from irrigation experiments using partial drying of the rootzone have shown that changes in stomatal conductance and restriction in lateral shoot growth are two of the major components affected in response to PRD. Control of vegetative growth is important for fruit composition as it largely determines the quality of the finished table wine. There is little information available, however, on the influence of chemical signals and their effects on growth of grapevines. This suggests that the role of chemical signals possibly influencing vigour as well as fruit composition of the vines should be investigated.

The general hypothesis to be tested during the study:

'partial drying of the root system gives rise to a change in the supply of root-derived chemical signals which causes changes in grapevine physiology and positively influence fruit composition'.

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 1996 and April 2000.

The potted vines were grown in either temperature controlled greenhouses (*Vitis vinifera* L. cv. Cabernet Sauvignon) or in open shade houses (*Vitis vinifera* L. cv. Chardonnay) at the Waite Campus of The University of Adelaide. All potted plants were on own roots, grown in two pots with standard potting media (Table 2.1) and had a split-root system (Section 2.2). The size of the pots varied and is indicated in the experimental design.

In all experiments irrigation water was applied either to only one side of the root system at any time (PRD; Figure 2.1A) or to both sides (control; Figure 2.1B).

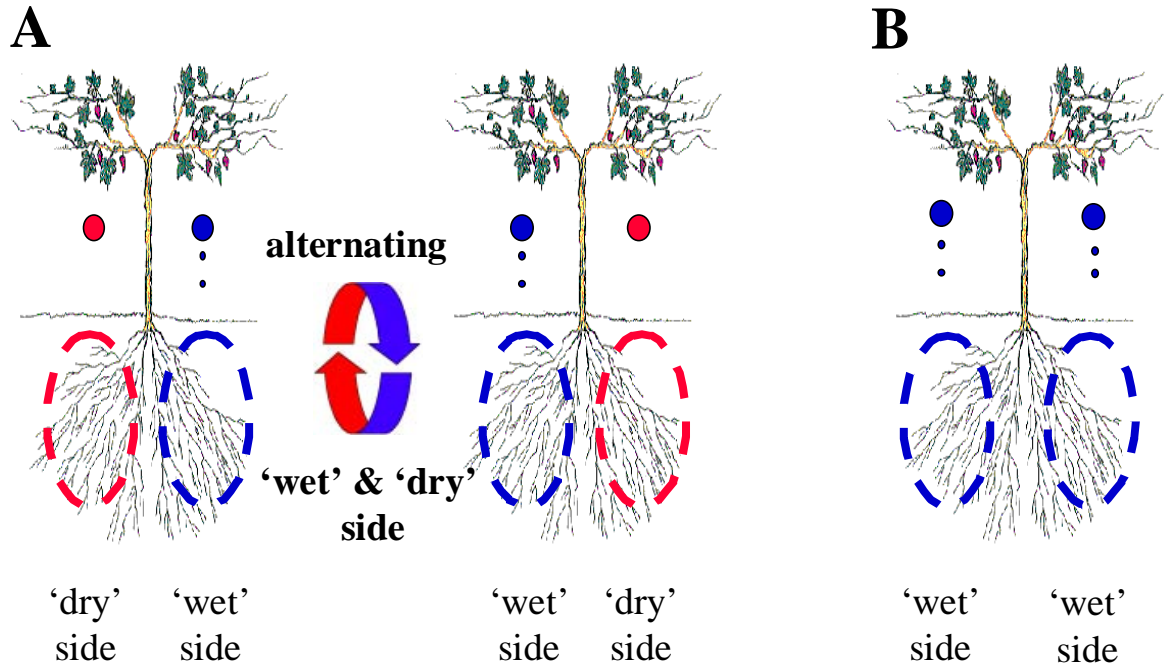


Figure 2.1 Implementation of PRD irrigation set up: A) PRD: at any time water was withheld from one side; B) control: vines received water on both sides.

The experiments on field-grown vines were conducted in the Coombe and Alverstoke vineyards at the Waite campus of the University of Adelaide, and in a commercial vineyard at Waikerie (Riverland, South Australia) on the Oxford Landing property owned by Yalumba Winery (Angaston, South Australia).

The sites located at the Waite Campus were on a relatively sheltered, gentle slope with north-west aspect. Dry and Smart (1988) classified the region as ‘hot, moderately maritime, arid, sunny and not humid’. Meteorological data were recorded at 15 minute intervals with an automatic weather station (Measurement Engineering Ltd., Adelaide, South Australia) which was located 100 metres (m) from the Coombe vineyard and 300 m away from the Alverstoke vineyard (Appendix 1). The mean daily maximum temperature in January for Adelaide (latitude 34.97 S; longitude 138.63 S; elevation 125 m) is 27.8 °C and the mean annual rainfall is 623.7 mm of which 37% (230.2 mm) falls between September and February inclusively. Soils are mostly red loams which are well suited to viticulture. A soil description of the Alverstoke site is in Appendix 2.

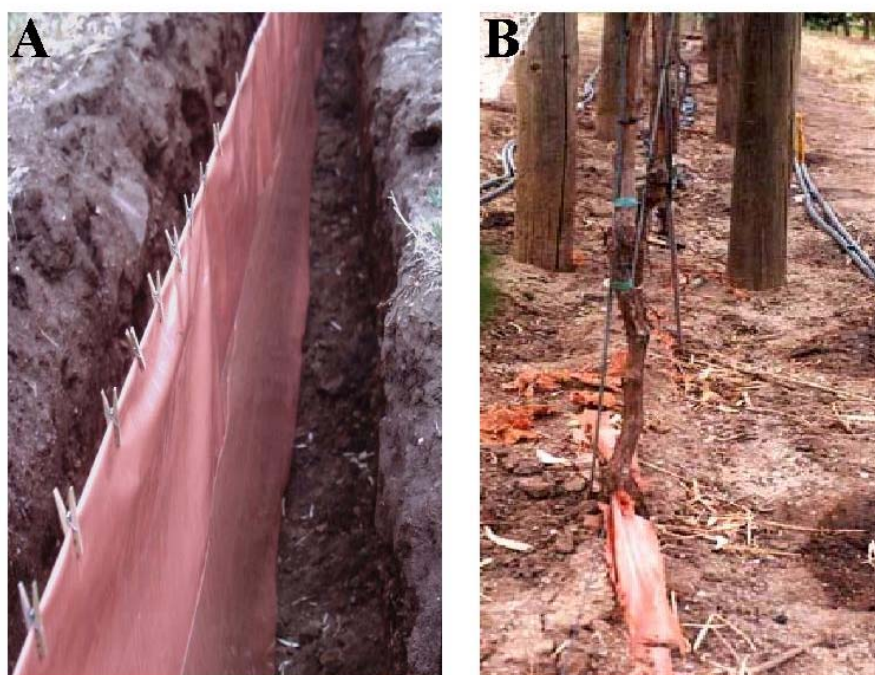


Figure 2.2 Field planting: **A)** trench burying a plastic membrane vertically to a depth of 1.5m **B)** vines planted with half of the root system on either side of the plastic membrane (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots)

The grapevine varieties grown in the Coombe vineyard were *Vitis vinifera* L. cv. Cabernet Sauvignon and Shiraz, both on own roots. The vines were planted in 1992. A drip irrigation system was installed in the planting line 0.4 m from the trunk of each vine on either side. The vineyard had a vertical shoot positioning (VSP) trellis system and were spur pruned. Details of the winter pruning will be provided in Chapter 3.

One site at the Alverstoke vineyard was planted in 1991 with split-root vines (*Vitis vinifera* L. cv. Cabernet Sauvignon (clone LC14) grafted on Ramsey rootstock). The other site had split-roots *Vitis vinifera* L. cv. Cabernet Sauvignon (clone LC14) vines on own roots and was planted in 1997. Both were planted in a trench (1.5 m wide and 1.5 m deep) which had a plastic membrane vertically buried (1.5 m deep) in the center (Figure 2.2). The trench was refilled and the roots were arranged on both sides of the membrane when vines were planted. In 1991 the trench was refilled with the same red and rocky loam (Appendix 2 A). In 1997 the original soil was replaced by a sandy soil (Appendix 2 B) which enabled better access to the root system for collecting root samples. Both treatments had irrigation drip lines 0.4 m away from the planting lines on either side. The vine and row spacing was 2 m and 4 m respectively for the grafted vines and 1.5 m and 3.5 m respectively for the vines which were on own roots. The grafted vines were trained using a Smart-Dyson trellis system (see Figure 3.1 in Chapter 3) with spur pruning. The vines on own roots had a VSP trellis system with cane pruning. Details of the pruning will be provided in Chapter 3.

All replicates of field-grown vines consisted of 3 vines (Figure 2.3); the centre vine, which will hereafter be called the ‘test-vine’, and one buffer vine on either side which were not used for any assessments except leaf sampling to determine leaf area (Section 2.4). For the field-grown split-root vines in the Alverstoke vineyard, the number of replicates (or ‘test-vines’) per treatment was four and in the Coombe vineyard, there were 8 replicates.

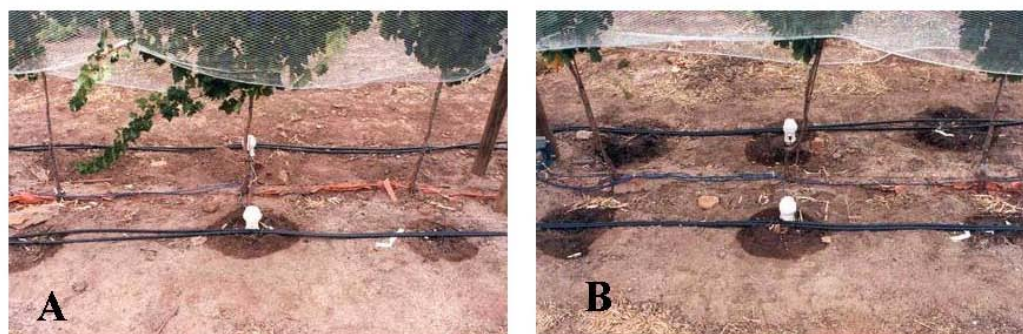


Figure 2.3 Split-root vines with roots arranged on either side of the plastic membrane (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots). Note the ‘wet’ and ‘dry’ patches associated with each dripper and capacitance probes located on either side of the ‘test-vine’. **A)** PRD: at any time water was withheld from one side; **B)** control: vines received water on both sides.

Weeds were controlled under vines either by the use of herbicides (Roundup®, Monsanto, USA) or manual pulling at the Coombe vineyard and the Alverstoke vineyard, respectively. Fertiliser (N-P-K 18+20+2) was applied to the grapevine of the Coombe vineyard in Spring (100 kg/ha). The vines in the Alverstoke vineyard did not receive any fertiliser in the period 1996 to 2000.

The site at Waikerie was planted in 1990 with *Vitis vinifera* L. cv. Riesling grafted on Ramsey rootstocks. This region has a hot and arid climate with extreme temperature variations and relatively low air humidity (Gladstones, 1992). The mean daily maximum temperature in January for Waikerie (latitude 34.18 S; longitude 139.98 S; elevation 25 m) is 33.0 °C and the mean annual rainfall of which 50% (126 mm) falls between September and February inclusively, is 252 mm. The vines were grown on levelled land in deep red sandy soil (depth greater than 1.8 m) over limestone. Soils are well drained and generally favourable for viticulture. The vineyard had a single wire trellis system with minimal pruning (vine x row spacing: 1.3 m x 3.1 m). Irrigation water was applied through subsurface irrigation pipes according to soil moisture measurements (EnviroSCAN®, Adelaide, South Australia) until a set of refill points was reached. The drip lines of both PRD and control treatments were buried at 0.2 m to 0.25 m depth and 0.5 m from the planting line on both sides.

2.2 Production of split-root plants

Split-root vines were propagated from thick cuttings (*Vitis vinifera* L. cv. Cabernet Sauvignon (clone LC14) and Chardonnay). Cuttings (0.35-0.45 m long) were selected in winter and the base of the cutting split for 0.1-0.15 m towards the tip with a bandsaw (Figure 2.4 A), dipped in indole-3-butyric acid (1000 ppm in ethanol) and callused in a heat bed (25 °C) inside a cool-room (2 °C) for 4 weeks. Cuttings with well-developed root systems on both sides were planted so that each half was divided by a plastic card in a single pot (3L volume; Figure 2.4 B) with standard potting media (Table 2.1).

Table 2.1: Standard potting medium

coarse pine bark	sharp, white sand	pH adjustment*	FeSO ₄	Osmocote Plus [®]
20 L	10 L	2 gL ⁻¹	0.6 gL ⁻¹	2 gL ⁻¹

*pH adjustment was: dolomite : gypsum : agricultural lime (2:1:1)

After a shoot with 3 to 5 leaves was established, the vine was transferred so that the root systems were equally divided between two separate pots with standard potting media (Figure 2.4 C).

For the initial several months, the plants were kept in a temperature-controlled greenhouse and thoroughly watered on both sides. The pots were then transferred to a shade house or outdoors. In the winter prior to their use in experiments, the potted plants were cut back to one or two node spurs.

In late spring some of these split-root vines were planted in the Alverstoke vineyard on the Waite Campus of the University of Adelaide in a prepared trench as described in Section 2.1.



Figure 2.4 Propagation of split-root vines: **A)** split winter cutting **B)** split-root vine divided with a plastic sheet **C)** split-root vines into two pots.

2.3 Soil moisture measurements

EnviroSCAN[®] (Adelaide, South Australia) probes were used to measure soil moisture. Each probe consisted of multiple sensors located in an access tube at a range of depths (0.1 m, 0.2 m, 0.3 m, 0.4 m, 0.5 m, 0.7 m and 1 m). Volumetric soil water content was determined by measurements of electrical capacitance of the soil by the sensors and measurements from each probe were expressed as the soil water content (in mm) at each depth. An electrical field is created around each sensor and extends through the access tube. The measured frequency is a function of the soil water content. Installation for the probes was by a proven technique (EnviroSCAN[®], Adelaide, South Australia) which guarantees minimal soil disturbance and preserves the structural integrity of the soil profile. For each treatment, one probe was installed on either side of the membrane to monitor soil moisture.

Vines in field experiments were irrigated either with drip emitters on both sides of the plastic membrane (control) or on one side only at any given time (PRD). By

varying the flow rate of the drip emitters different amounts of water were applied so that the application rate for ‘control’ vines was either the same as, or half that, of the PRD treatment. As a consequence, ‘control’ vines received either the same amount of water, or double the amount of water as PRD-treated vines.

In experiments using potted vines, water was applied once a day until field capacity was reached. Water was withheld from one pot at any time for PRD-treated vines whilst control vines received water in both pots.

2.4 Leaf area and canopy measurements

Leaf area of split-root vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted on Ramsey rootstock) was measured at the Alverstoke vineyard during two growing seasons. The leaf area of the ‘test-vines’ was determined non-destructively by sampling buffer vines as follows. The total number of shoots on the ‘test-vines’ was recorded. A sample of 6 ‘up’ shoots and 6 ‘down’ shoots was selected at random from each ‘test-vine’. The number of leaves on main shoots and the number of lateral shoots was counted on each of the 12 selected shoots. The lateral shoots were classified into lateral shoots with one, two, three or more than three (n) leaves. A sample of 50 leaves of main shoots and 10 lateral shoots with one leaf, 10 lateral shoots with two leaves, 10 lateral shoots with n-leaves were collected from buffer vines. The leaf area of both main shoot (LAMs) and lateral shoot (LAIs) samples were determined using a LI-COR leaf area meter (LI 3000, Lincoln, Nebraska, USA). The mean leaf area per shoot was calculated as the appropriate sum of the mean of LAMs and LAIs. The total leaf area per vine was then calculated as the product of the calculated mean leaf area per shoot and the number of shoots per vine.

2.5 Gas exchange measurements

Stomatal conductance

Stomatal conductance of leaves was measured (in units of $\text{mmolm}^{-2}\text{s}^{-1}$) using an AP4 diffusion porometer (DELTA-T Devices LTD, Cambridge, UK). The diffusion porometer works by measuring the time it takes for the leaf to release a sufficient amount of water vapour to change the relative humidity in the measuring cell by a predetermined amount. The time is then compared with calibration figures which were obtained earlier, by using a calibration plate of a known conductance. The instrument was calibrated with an error tolerance of 5% (as determined by the instrument software) before taking each set of measurements. Between each measurement a desiccant dried the air in the chamber to reduce the relative humidity and hence errors in measurement. The AP4 diffusion porometer instrument is also equipped to measure light intensity and leaf and cup temperature. These measurements are useful in determining the environmental conditions experienced by each leaf and also assisted with the selection of leaves of similar sun exposure for measurements. The instrument measures stomatal conductance on only one side of the leaf surface, so the leaves were positioned with their leaf under surface towards the measuring unit. Four to 6 cycles are normally sufficient to obtain a stable reading and no more than 10 cycles were allowed per reading as the instrument itself alters the behaviour of the stomata. When measurements were taken throughout an irrigation cycle, the measurements were made either between 10 am and noon or 3 pm and 4 pm.

Leaf gas exchange

Assimilation of carbon dioxide and stomatal conductance were measured using a LICOR open photosynthesis system (Li 6400, Lincoln, Nebraska, USA) with an infra red gas analysis instrument (IRGA). This instrument measures differential or absolute changes caused by leaf gas exchange. An open system arrangement of the apparatus allows for a constant air flow through the chamber and minimises effects of the instrument on gas exchange of the leaf. For experiments in this study the reference temperature was set to ambient temperature. An internal light source provided full saturation intensity ($1500 \mu\text{molm}^{-2}\text{s}^{-1}$ for grapevine leaves) which was predetermined

using different light intensities from $2000 \mu\text{molm}^{-2}\text{s}^{-1}$ to $0 \mu\text{molm}^{-2}\text{s}^{-1}$ in steps of $250 \mu\text{molm}^{-2}\text{s}^{-1}$.

The leaves were clamped in a leaf chamber (6 cm^2) and the flow of air was set to 400 mL min^{-1} . For each measurement the instrument was allowed to stabilise as determined by the real-time monitoring within the system. Gas exchange rates were determined by the instrument using the concentration difference between inlet and outlet air. Photosynthesis was measured in units of $\mu\text{molm}^{-2}\text{s}^{-1}$, whilst stomatal conductance was measured as $\text{molm}^{-2}\text{s}^{-1}$.

Measurements were commenced after the leaves had been exposed to bright sunlight ($>1800 \mu\text{molm}^{-2}\text{s}^{-1}$) for at least 3 hours.

2.6 Leaf water potential and xylem sap extraction

Leaf water potential was measured between 11am and 1pm for *Vitis vinifera* L. cvs. Riesling, Chardonnay and Cabernet Sauvignon vines. During diurnal cycles measurements were taken every 2 hours from dawn to dusk.

For each measurement a leaf was wrapped in a polyethylene bag and removed with a single cut across the petiole with a razor blade. Xylem water potential was measured by placing each leaf into a pressure bomb (Scholander *et al.*, 1965) attached to a nitrogen gas cylinder. The pressure was increased slowly until the xylem sap was observed with a magnifying glass being exuded from the leaf petiole. The negative of the pressure (in MPa) required to force sap from the petiole was recorded as the leaf xylem water potential.

After measuring xylem tension the first sap exuded was discarded. Pressurisation was then continued until an additional 0.2 MPa had been applied. Sap exuding from the petiole stump was removed using an Eppendorf pipette and immediately frozen in liquid nitrogen. Between $10 \mu\text{L}$ and $20 \mu\text{L}$ sap was collected from each petiole. The sap of three leaves was combined in one micro tube to form a single sample for further analyses (Section 2.7.2).

2.7 Stable isotope dilution analysis of abscisic acid

2.7.1 Tissue extraction

Due to the light sensitivity of ABA (Parry and Horgan, 1991), exposure of tissue to direct light was avoided wherever possible. Frozen tissue was ground in liquid nitrogen into powder using a mortar and pestle. The tissue weight was recorded after it was transferred into a pre-weighed centrifuge tube (50 mL) and placed in the -20°C freezer until all samples were ground. ABA was extracted in boiling water as described by Loveys and van Dijk (1988).

Adelaide tap water was processed through reverse osmosis, ion exchange and inactivated carbon filtration system (ModulabTM Liquipure, Continental[®] San Antonio, Texas) for production of water of a greater than $15\text{ M}\Omega$ resistivity (ultra pure water). Approximately 10 mL of boiling ultra-pure water was added to the tube containing ground tissue and transferred to a boiling water bath for 5 min. The tubes were rapidly cooled on ice before the internal standard of $[\text{}^2\text{H}_6](\pm)\text{ABA}$ was added. Changes to the concentration of the endogenous amount of ABA was minimised by performing extractions in the same tube and storing tissue extract in the cold and dark. The sample was mixed with a vortex and centrifuged for 10 min at 4°C and $14000 \times g$ (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments). The supernatant was reserved and the pellet re-extracted using 10mL ultra-pure water. The combined supernatants were adjusted to a pH of 2.5 using 1 M HCl.

The aqueous extract was then partitioned with approximately 30 mL ethyl acetate using a separating funnel. The extract was shaken and the lower aqueous phase was collected separately from the upper ethyl acetate phase. The aqueous phase was extracted with ethyl acetate twice more before it was discarded. All ethyl acetate fractions were combined and the remaining water absorbed from that fraction for 1 hour using Na_2SO_4 . The ethyl acetate extract was then transferred to round bottomed flasks and evaporated to dryness using a rotary evaporator (water bath temperature 45°C). The dried extract was resuspended and transferred to a 2 mL microfuge tube using 3 x 500 μL rinses of ethylacetate then dried overnight in a fume cupboard. The extract was further purified using HPLC and quantified as described below (Section 2.8.3 to 2.8.6).

2.7.2 Quantification of ABA in xylem sap

Frozen xylem sap samples from grapevine leaf petioles were thawed. Samples were mixed well with a vortex and centrifuged for 5 min at 5000 x g. For each sample 30 μL of the supernatant was transferred into a new micro tube before internal standard (20 ng $[\text{}^2\text{H}_6](\pm)\text{ABA}$) was added. Samples were mixed well with a vortex, dried in a Speed Vac[®]. The methylating agent ethereal diazomethane was added, the tubes were shut and covered with foil for 20 min before the samples were dried by evaporation in a fume-hood. Methanol was added and samples were transferred to new micro tubes, centrifuged and dried in a Speed Vac[®]. Methanol (20 μL) was added and samples were analysed by GC-MS using SIM as described in Section 2.8.5.

2.8 Combined stable isotope dilution analysis of ABA and CK

2.8.1 Tissue extraction

Both abscisic acid and some cytokinins were extracted from the same leaf or root tissue and quantified by stable isotope dilution analysis. Due to the light sensitivity of ABA (Parry and Horgan, 1991), exposure of samples and extracts to direct light was avoided wherever possible.

The frozen plant tissue sample (0.3-1.5 g) was ground to a slurry in 2mL of cold, modified Bielecki fixative 1 (Bf1) (60:20:15:5 v/v; CH_3OH : H_2O : CHCl_3 : HCOOH ; Bielecki, 1964; Emery *et al.*, 1998). An internal standard mixture, containing 90 ng $[\text{}^2\text{H}_6](\pm)\text{ABA}$ and 25 ng of each of four cytokinins, zeatin ($[\text{}^2\text{H}_5]\text{Z}$), zeatin riboside ($[\text{}^2\text{H}_5][9\text{R}]\text{Z}$), 9-glucosyl zeatin ($[\text{}^9\text{G}]\text{Z}$) and iso pentenyl adenine ($[\text{}^2\text{H}_6]\text{iP}$) was added prior to grinding (all internal standards: Apex Organics Devon, UK). Additional Bf1 solution was added to give a final solvent to sample ratio of 10:1. Samples were thoroughly vortexed, sonicated for 1 min and centrifuged for 10 min at 5000 x g. The supernatant was transferred to a glass round bottomed flask and the pellet was re-extracted twice in Bielecki fixative 2 (60:35:5 v/v; CH_3OH : H_2O : HCOOH ; Emery *et al.*, 1998) by vortexing, sonicating and centrifuging (10 min, 5000 x g). The supernatant was recovered after each extraction. The combined supernatant was dried

at a temperature below 38 °C to 1 mL in a rotor evaporator. The extraction flask was rinsed with 5 mL 0,1 M HOAc. After a freeze (-20 °C) thaw cycle samples were clarified by centrifugation at 5000 x g for 10 min.

2.8.2 Purification

To separate ABA from cytokinins, cation exchange columns (Alltech SCX) preconditioned with 15 mL 0.1 M HOAc were used. The clarified sample was loaded on to the column and washed with 15 mL 1 M HOAc. Eluates from the load and wash steps were retained for ABA analysis. Cytokinins (containing glucosides and ribosides) were then eluted using 20 mL 2 N NH₄OH. Fractions containing either ABA or CK were evaporated to dryness using a rotor evaporator (water bath temperature 38 °C).

2.8.3 HPLC of ABA fraction

Following evaporation the residue was dissolved in methanol, transferred to a 2 mL microfuge tube and dried in a Speed Vac[®] (Savant Instruments Ltd, Farmingdale, NY).

Each sample was re-dissolved in 500 µL 20% methanol and then further purified by High Pressure Liquid Chromatography (HPLC; Hewlett Packard LC1100 series) on a C₁₈ column (Activon Goldpak 10 µm, flow: 1.5 mLmin⁻¹).

The solvent gradient used for separating ABA is shown in Table 2.1. The retention time (RT) under these conditions was determined using an ABA standard and the fraction between 9 and 11 min was collected.

Table 2.2 Solvent gradient for HPLC to separate abscisic acid

time (min)	% solvent A (water)	% solvent B (methanol)	% solvent C (5% acetic acid)
0	75	20	5
11	25	70	5
12	0	100	0
15	0	100	0
17	75	20	5
20	75	20	5

2.8.4 HPLC of cytokinin fraction

Each dried fraction was resuspended in 2 mL 5% acetonitrile transferred to a microfuge tube and dried in a Speed Vac[®]. Samples were re-dissolved in 500 μ L 5% acetonitrile and purified by HPLC on a C₁₈ column (Activon Goldpak 10 μ m, flow: 1.5 mLmin⁻¹) using the solvent gradient as indicated in Table 2.3.

Table 2.3 Solvent gradient for HPLC to separate some cytokinins

time (min)	% solvent A (water)	% solvent B (acetonitrile)	% solvent C (5% TEAB)
0	90	5	5
20	80	15	5
30	35	60	5
32	0	100	0
35	0	100	0
36	90	5	5
37	90	5	5

The retention times under these conditions were determined from standards (Apex Organics, Devon, UK) and the four fractions indicated in Table 2.4 were collected.

Table 2.4 Retention times of some cytokinins and collected fractions

compound	retention time (min)	collected fraction (min)
zeatin glucoside	4.8	4-6
zeatin	14.8	13.8-15.9
zeatin riboside	16.9	15.9-18.0
iso pentenyl adenine	27.1	26.5-28.5

The collected fractions were later combined to one cytokinin sample for GC-MS analysis.

2.8.5 Gas chromatography

Both the ABA fraction and the combined cytokinin fractions were dried in a Speed Vac[®].

The cytokinin sample was permethylated as described by Horgan and Scott (1987) and further steps of the method were based on a modified procedure of Emery *et al.* (1998). To avoid any risk of oxidising the reagents all steps were carried out under argon. DMSA (methyl sulphanyl carbanion, 0.08 M) was generated by mixing 5 mL DMSO (dimethylsulfoxide, Sigma) and 100 mg freshly weighed potassium *tert*-butoxide (Aldrich) thoroughly for 10 min at room temperature. The DMSA was centrifuged for 6min at 4000 g and 50 μ L with 10 μ L methyl iodide was added to a Reacti-vial[®] containing the dried sample under argon. After 30min at room temperature the reaction was quenched using 25 μ L ultra-pure water. The cytokinins were immediately partitioned three times using 100 μ L CHCl₃. The CHCl₃ fractions were combined in a GC-MS vial and dried under a stream of argon.

Solvents with different boiling points were tested on GC-MS to determine which would achieve highest solubility and recovery of cytokinins (Table 2.5).

Table 2.5 Solvents used to test cytokinin sample solubility and recovery for GC-MS

solvent	boiling point (°C)	abundance area ZR (221)	abundance area Z (236)	abundance area iP (237)
dichloromethane	48,9	106856	28615	14194
ethylacetate	77	58191	25032	3433
hexane	69	28140	29357	8891
n-heptane	98.4	71025	40979	10050
methanol	64	67896	19061	4813

Of these solvents it was found that dichloromethane provided the highest and most reproducible recovering of cytokinins in terms of peak size. The dried sample was therefore re-dissolved in 15 μ L dichloromethane.

The GC-MS analysis for permethylated cytokinins was performed using a Hewlett Packard GC System (HP 6890 Series) with a 30 m long and 0.25 mm inner diameter

and a 0.25 micron film thickness silica column (J&W Scientific DB 5MS). The GC-MS was operated in a pulsed splitless mode. The pulse pressure was 172.4 kPa, pulse time 1min at 250 °C, purge flow 29.6 mLmin⁻¹ and post pulse flow 1.5 mLmin⁻¹. The oven temperature ramp was 60°C at the beginning, followed by a fast ramp of 20 °Cmin⁻¹ to 200 °C and a slow ramp of 5 °Cmin⁻¹ to 300 °C. This temperature was held for 10 min.

The GC-MS was run using a selective ion monitoring (*SIM*) program. The compounds, retention times, ions monitored and the dwell per ion are specified in Table 2.6.

Table 2.6 Retention time and selected ions (*m/z*) of some cytokinins using GC-MS (*SIM*)

compound	retention time (min)	mass to charge ratio (m/z)	dwell (µs)
<i>trans</i> -zeatin	14.4	235/230 188 266/261	40
<i>trans</i> -zeatin riboside	25.5	221/216 395/390 426/421	40
zeatin-glucoside	27.4	221/216 439/434	40
iso pentenyl adenine	12	188 219/216 239/231	40

The derivative used most commonly to make ABA sufficiently volatile for GC-MS is a methyl ester formed by a reaction of the free acids with an ethereal solution of diazomethane (Schlenk, 1960). The GC-MS analysis for derivatised ABA was performed using the same instrument and column described above. The GC-MS was operated in a pulsed splitless mode, pulse pressure 82 kPa, pulse time 1min at 220 °C, a purge flow of 18.9 mLmin⁻¹ and a column flow rate of 1.5 mLmin⁻¹. The initial oven temperature was 40 °C, followed by a fast ramp of 12 °Cmin⁻¹ to 240 °C. The total run time was 26.7 min.

The ion pairs monitored using *SIM* mode were 194/190 and 166/162. The *SIM* parameters were on high resolution with dwell 40 giving 4.44 cycles/sec.

2.8.6 Quantification

During a GC-MS *SIM* run certain ions can be monitored separately. These are major ions of an endogenous compound and the corresponding ions from isotopically labelled internal standard. Using *SIM* makes it possible to distinguish between and quantify the relative amounts of both types of ions.

The full quantification protocol is illustrated for one ion pair (216/221) of zeatin riboside (Table 2.7). The endogenous amount of the cytokinins was calculated from a calibration curve which was generated for each ion pair by applying an increasing amount of a cytokinin standard (0 ng to 2000 ng) along with an isotopically labelled internal standard (500 ng). The GC-MS analysis was performed as described above (Section 2.8.5).

Table 2.7 Results of zeatin riboside GS-MS integration analyses of endogenous and internal standard

ratio ZR/[² H]ZR (both in ng)	mass ratio ZR/[² H]ZR	abundance 216 (ZR)	abundance 221 ([² H]ZR)	area ratio (216/221)
0/500	0	611	111071	0.005501
50/500	0.1	6791	114633	0.059241
100/500	0.2	15764	119648	0.131753
200/500	0.4	27115	102863	0.263603
500/500	1	98455	151791	0.648622
1000/500	2	200768	122458	1.639485
2000/500	4	488944	139646	3.50131

Figure 2.5 shows the calibration curve generated for the major ion pair (216/221) of zeatin riboside. The area ratio of the 216 ion and the 221 ion versus the mass ratio of this ion pair from different concentrations of ZR/[²H]ZR is plotted. A regression line, a

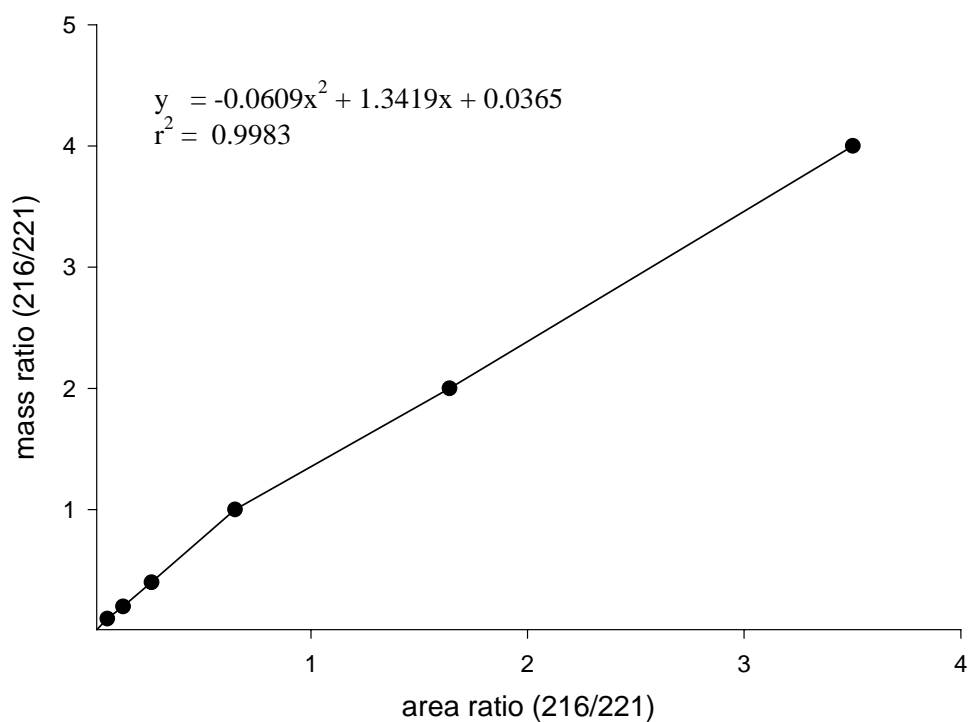


Figure 2.5 Calibration curve of 216/221 ion pair of zeatin riboside to quantify mass ratio.

factor of variance (R^2) and the equation to determine mass ratio given peak areas measured for this ion pair are shown in the figure.

The calibration results for other ion pairs of zeatin riboside, zeatin, zeatin glucoside and iso pentenyl adenine and the equations to calculate the mass ratio from these ion pairs are listed in Table 2.8.

Table 2.8 Cytokinin ions analysed and the equations (generated from standard curves) used to quantify different cytokinins

ions of endogenous compound	ions of internal standard	retention times (min)	relative abundance	equation calculated to determine the mass ratio	r ²
zeatin riboside (ZR)	[² H ₅]ZR	25.9			
216	221		100	$-0.0609x^2+1.3416x+0.0365$	0.9983
390	395		71	$-0.829x^2+1.3915x+0.0433$	0.9984
421	426		6	$-0.0954x^2+1.4048x+0.064$	0.9978
zeatin (Z)	[² H ₅]ZR	15.4			
230	235		100	$-0.0818x^2+1.577x+0.0007$	0.9993
188	188		22	$-0.0019x^2+1.1115x+0.0734$	0.9969
261	266		7	$-0.0825x^2+1.4988x+0.024$	0.9994
zeatin glucoside (ZG)	hexa-me-[9G]-Z	27.4			
216	221			$-0.0194x^2+1.7223x-0.0151$	0.9974
434	439			$-0.0374x^2+1.6522x-0.0075$	0.9976
isopentenyl adenine (iP)	[² H ₆]-isopentenyl adenine	12.67			
188	188		100	$0.0225x^2+1.0212x-0.0152$	0.9972
231	237		70	$-0.0356x^2+1.2443x+0.0002$	0.9997
216	219		57	$-0.0101x^2+0.539x+0.0037$	0.9998

Endogenous ABA was quantified using a previously constructed calibration curve relating peak area ratio (m/z 190/194 and m/z 162/166) to mass ratio (ABA/[²H₄]ABA) (Brian Loveys, pers. comm.).

2.9 Fruit sampling and fruit composition

Berries were sampled once a week from the beginning of veraison (stage 33; Coombe, 1995) for several weeks until harvest. At early stages of berry ripening, 50 berries were randomly chosen from the 'test-vine' at different positions in the canopy

and from as many different bunches as possible. When fruit was more mature (more than 21°Brix) and at harvest, samples of 200 berries were collected once each week.

When the berries of each test vine were collected, they were first stored in plastic bags. The mean berry weight of 50 berries was determined immediately after sampling using an electronic balance. The sample was then used to analyse fruit composition such as total soluble solids (TSS), titratable acidity (TA) and pH. The berries were crushed and pressed with a citrus fruit press, the juice transferred to centrifuge tubes (10 mL) and centrifuged at 1500 g for 5 min. 200 µL of the supernatant was used to measure the total soluble solids (°Brix) using a digital refractometer (BRX 242, Erma Inc., Tokyo, Japan). Before measurements were made, the refractometer was zeroed using distilled water. The pH was measured using a standard pH meter (Activon 110, Thornleigh, NSW).

Samples were further processed and stored for later determination of TA (gL⁻¹) by diluting a 5 mL aliquot of the supernatant with 20mL ultra-pure water and transferred to -20 °C. The TA was later measured on thawed and fully solubilized samples using a Crison-Compact titrator 5202 (Alella, Spain).

At harvest the number of bunches from each ‘test-vine’ was counted (number of bunches per vine) and the fruit weight (g/vine) was measured and recorded. The weight of the rachis was ignored. The fruit weight measured at harvest was corrected to final fruit weight by adding the weights of berry samples harvested previously. The mean bunch weight was calculated using Equation 2-1:

Equation 2-1 Determination of mean bunch weight (g)

$$\text{mean bunch weight} = \frac{\text{final fruit weight (g)}}{\text{number bunches}}$$

A 50 berry sample collected at harvest was used for determination of fruit components and to derive mean berry weight (g). The mean berry number per bunch was calculated by dividing the mean bunch weight by the mean berry weight.

Of the 200 berry samples, 50 berries were used for analyses of fruit composition as described above whilst three lots of 50 berries were stored in plastic containers at -20 °C for later analysis of monomeric ‘free’ anthocyanins and total phenolics.

Total monomeric anthocyanins and total phenolics were determined for the samples of 50 berries using an established method (Patrick Iland, pers. comm.). The partially thawed samples were homogenised in plastic containers using an Ultra-Turrax T 25 (IKA Labortechnik, Staufen, Germany) at 24,000rpm for 30s. After scraping any homogenate from the shaft back into the vessel, the sample was homogenised for another 15s. The homogenate was thoroughly mixed and approximately 1g was transferred to a pre-tared centrifuge tube. The exact weight of the sample was determined using an electronic balance. Ten mL of aqueous ethanol (50% v/v) was added to the homogenate. The sample was periodically inverted over the course of 1 hour before being centrifuged at 1500 g for 10 min. A 0.5 mL portion of the supernatant was acidified using 5 mL 1 M HCl and left to stand for 3 hours before absorbance was measured on a UV/VIS spectrophotometer (PYE Unicam PU 8600, Phillips) at 520 nm and 280 nm. The red colour pigments, expressed as anthocyanin equivalents were calculated using Equation 2-2 and Equation 2-3. The total phenolics were calculated using Equation 2-4 and Equation 2-5 and expressed as absorbance units.

Equation 2-2: mg anthocyanins/ berry =

$$\frac{OD_{520}}{500} \times \frac{DFa}{1} \times \frac{\text{final extract volume (mL)}}{100} \times \frac{50 \text{ berry weight (g)}}{\text{homogenate weight (g)}} \times \frac{1000}{50}$$

Equation 2-3 mg anthocyanins/ g berry weight =

$$\frac{OD_{520}}{500} \times \frac{DFa}{1} \times \frac{\text{finalextractvolume(mL)}}{100} \times \frac{50\text{berryweight(g)}}{\text{homogenateweight(g)}} \times \frac{1000}{50\text{berryweight(g)}}$$

DFa is the dilution factor for the dilution of the portion of the extract into 1 M HCl; for example 0.5 mL extract into 10 mL final volume; DFa = 10/0.5 =20

Equation 2-4 phenolics (in absorbance unit per berry) =

$$\frac{OD_{280}}{1} \times \frac{DF_{ph}}{1} \times \frac{\text{final extract volume (mL)}}{100} \times \frac{50 \text{ berry weight (g)}}{\text{homogenate weight (g)}} \times \frac{1}{50}$$

Equation 2-5 phenolics (in absorbance unit per gram berry weight) =

$$\frac{OD_{280}}{1} \times \frac{DF_{ph}}{1} \times \frac{\text{final extract volume (mL)}}{100} \times \frac{50 \text{ berry weight (g)}}{\text{homogenate weight (g)}} \times \frac{1}{50 \text{ berry weight (g)}}$$

DF_{ph} is the dilution factor of the extract into 1 M HCl; for example 0.5 mL extract in 5 mL final volume; DF_{ph} = 5/0.5 = 10

2.10 Statistics

The statistical analyses were performed using the Microsoft® Excel 97 Data Analysis Toolpack (Excel 97 SR-1). Results comparing multiple groups of data were analysed using ANOVA from the same statistics package. Student T-tests were carried out to determine which groups were different and to identify significant differences between groups. The significance level is indicated by the P-Value. Regression analyses were performed using Sigma-Plot graphics package (Jandel Scientific, version 4.0).

CSIRO Mathematical Information Sciences provided advice on biometrics used in experimental design.

Chapter 3 Partial rootzone drying maintains reduction in vegetative growth and affects canopy density and development.

3.1 Introduction

To produce high quality fruit the vegetative and reproductive growth of a grapevine need to be in balance. The vegetative growth of a vine mainly determines the canopy structure. The architecture and density of a canopy influences light interception and hence carbon assimilation and productivity (Smart *et al.*, 1990). Excessive shoot and foliage growth results in a densely shaded canopy which can result in depression of inflorescence initiation (May, 1965) and a reduction of fruit set and berry growth (Ebadi *et al.*, 1996). In turn, this leads to a reduction in total fruit weight. Reduced fruit weight stimulates vegetative growth due to changes in assimilate allocation (Koblet, 1969) and as a consequence the leaf area increases further and causes an imbalance between vegetative and generative growth. This imbalance in growth is detrimental to the canopy architecture and in many instances this can account for effects on wine quality (Jackson, 1986).

The balance between vegetative and reproductive growth can be influenced at a number of levels. For example, vineyard location, trellis system or choice of variety and rootstock can impact directly on vine development and hence influence the canopy microclimate. Manipulating growth through viticultural practices such as pruning, trimming or irrigation can also improve the balance of a vine and influence canopy development and shoot growth.

Applying water at a level which is less than optimal will stimulate various water deficit responses in the vine. One plant response most likely to be influenced is stomatal conductance which can lead to a decrease in transpiration. This can improve the water use efficiency which can be expressed as the amount of dry matter produced per unit of water transpired (Davies *et al.*, 1978). Under drying soil conditions it has been found in split-root apple trees that shoot growth components such as internode length, leaf area development as well as new leaf initiation and leaf gas exchange are reduced (Gowing *et al.*, 1990). Various other plant species with split-root systems have

exhibited similar effects with respect to shoot growth components (Poni *et al.*, 1992; Kosola and Eissenstat, 1994; Turner *et al.*, 1996) under drying soil conditions. In all cases these changes occurred in the absence of any changes in plant water status.

Manipulating soil water conditions through partial rootzone drying has been proposed as one management strategy for grapevines which rely on supplemental irrigation (Loveys, 1992). Using PRD, a reduction of dry matter production concomitant with a decrease in stomatal conductance can increase the transpiration efficiency of vines (Loveys *et al.*, 1998; Dry & Loveys, 2000a).

In recent work it has been demonstrated that an alternated 'wet' and 'dry' root zone can modify stomatal conductance and shoot growth rate in grapevines (Dry and Loveys, 1998; Dry and Loveys, 1999; Dry & Loveys, 2000a). A reduction in shoot growth rate has the potential to modify canopy structure and hence improve the canopy microclimate.

For this reason, experiments described in this chapter were conducted to test the hypothesis that *PRD exerts long term effects on the shoot vigour of field grown vines and thereby affects canopy density.*

3.2 Materials and Methods

Vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted to *Vitis champini* cv. Ramsey rootstock - split-root) grown at the Alverstoke vineyard of the University of Adelaide and *Vitis vinifera* L. cv. Shiraz and Cabernet Sauvignon on own roots at the Coombe vineyard of the University of Adelaide were used for all experiments in this chapter.

The split-root vines were grown using a 'Smart Dyson' trellis system (Smart and Robinson, 1991). Using this system part of the shoot system on both sides of the planting line is trained downwards and the other part is trained upwards (Figure 3.1).



Figure 3.1 Smart-Dyson trellis system with a divided canopy with both upward and downward trained shoots (field-grown Cabernet Sauvignon vines / Ramsey split-root vines; the picture was taken before winter pruning).

Irrigation in the Alverstoke vineyard was performed using two 2 Lh^{-1} drip emitters per vine, positioned 0.4 m on either side of the vine trunk. The flow rates of the drip emitters were checked twice a year: at the beginning and in the middle of each growing seasons. Drip emitters which deviated by more than 20% from the specified flow rate were replaced. The irrigation was scheduled according to soil moisture measurements (EnviroScan®, Sentek, Adelaide, South Australia). Water was applied when the water content of soil layers between 0.35 and 0.45 m and 0.45 to 0.55 m on the ‘wet’ side

reached a soil water content between 25 and 26 mm. The 'wet'-side and 'dry'-side were alternated when the soil water content on the 'dry'-side dropped to 20 to 22 mm at each sensor depth and did not decline further. The length of one irrigation cycle varied between 11 and 14 days depending on weather conditions and seasonal growth. The soil water content in the deeper soil layers (0.7 m to 1.0 m) on the 'wet'-side was carefully monitored following the first irrigation of each cycle to ensure that soil water content in deeper soil layers was sufficient to refill the soil water content to 20 to 25 mm. With the first irrigation of each cycle 20 to 30% more water was applied compared with other irrigations during a cycle. The amount of water applied with each irrigation was measured using a water flow meter placed in each irrigation line. Appendix 3 lists the date when water was applied, the amount of water applied and the time at which the irrigation sides were changed. The number of irrigations varied due to variations in climatic conditions and 54, 46 and 38 irrigation events were applied in 9, 8 and 8 irrigation cycles in 1997, 1998 and 1999 respectively.

The vines at the Coombe vineyard (*Vitis vinifera* L. cv. Cabernet Sauvignon and Shiraz) were trained using a VSP trellis system. In both seasons the shoots were positioned manually between the foliage wires three weeks after flowering. Five and nine weeks after flowering all vines were trimmed mechanically and netted for bird protection with the onset of veraison.

No means for soil moisture measurement was available in the Coombe vineyard so the amount of water to be used was predetermined from an average commercial water usage for viticulture in this climatic region (Peter Dry, pers. comm.). For 1997/1998 and 1998/1999, 1.4ML/ha and 1.0ML/ha of irrigation water respectively was administered to control vines in addition to annual rainfall. This water was applied over a period of 4.5 months from mid-November to March. Vines were irrigated twice a week on Mondays and Thursdays alternating the sides with every third irrigation. The flow rate of the drip emitters was 2L/h for Cabernet Sauvignon vines in both seasons. Shiraz vines were irrigated with 2L/h drip emitters in the 1997/1998 season for both control and PRD. From the growing season 1998/1999 onwards, the drip emitters were changed to two 1L/h drip emitters on either side of Shiraz control vines and 2L/h drip emitters for Shiraz PRD vines; both treatments received the same amount of water at any irrigation. The flow rate of all drip emitters was monitored at the beginning and

half way through the season. The flow rate of each drip emitter was measured with a measuring cylinder and emitters with greater than 20% inaccuracy were replaced.

3.2.1 Determination of shoot growth rate

To examine the long term-effect of PRD on canopy structure, single shoot growth rate was measured during the 1996/1997 growing season.

The shoot growth rate (SGR) of 6 randomly selected, actively growing upwards trained shoots and 6 downwards trained shoots was measured every 7 days (between 13th Dec 1996 and 9th Jan 1997). All shoots had 2 bunches and were initially of similar length and diameter. The total length of each shoot was measured using a measuring tape subdivided in mm units.

Stomatal conductance was measured daily during the first and second irrigation cycle and every second day during the third irrigation cycle (13th Dec 1996 and 14th Jan 1997). Measurements were conducted between 10 am and 11.30 am on 6 leaves of equal maturity and sun exposure on each test vine as described in Section 2.5.

At winter pruning the total shoot length of main shoots and lateral shoots was measured of four untrimmed downwards trained shoots per 'test-vine'. Upwards trained shoots were not used to determine total shoot growth, since they were trimmed during the season.

3.2.2 Determination of leaf area development and canopy density

The leaf area of each 'test-vine' in the Alverstoke vineyard was determined at two stages of development: 40 days and 110 days after flowering. The experiment was conducted during the 1997-1998 and 1998-1999 growing seasons and the data processed as described in Chapter 2.4.

Both control and PRD-treated vines received the same treatment for shoot positioning and trimming. Before flowering, upwards trained shoots and downwards trained shoots were equally positioned and both separated by different foliage wires. The main shoots of all vines were trimmed 8 weeks after flowering in order to prevent an increase of fungal disease pressure. Twelve and 15 mature leaves per shoot were retained on downwards trained shoots and upwards trained shoots respectively after trimming. The trimming removed all immature leaves on the main shoots of both shoot types. With the beginning of veraison all vines were netted and protected against bird damage.

3.2.3 Determination of canopy density

Light, penetrating to the inside of the canopy, was measured using a ceptometer (DELTA-T Devices, Cambridge, UK) just before harvest during the 1997-1998 and 1998-1999 growing seasons.

For measurements in the Alverstoke vineyard a ceptometer was inserted perpendicular to the planting line at three different positions: a) 0.2 m above bunch zone through upwards trained shoots b) at the centre of the bunch zone c) 0.2 m below bunch zone through downwards trained shoots. Seven readings were taken in 0.2 m intervals at each position. The ceptometer was configured to average readings from all sensors.

Light penetration in the Coombe vineyard was determined with a ceptometer inserted parallel to the planting line 0.2 m above the bunch zone and at the height of the cordon below the bunch zone. Five readings of each test-vine and position were taken.

In 1998 the total volume of the canopy of each panel of three vines was determined. The volume per test-vine was then estimated as 1/3 of the total volume of each panel which included 3 vines. Height (h), length (l) and perpendicular diameter (pd) of each portion of the trellis system (the upwards positioned shoots ('up') and the downwards positioned shoots ('down') on either side of the vine) was measured at 0.5 m intervals and average values (av) calculated. The volume per vine was calculated from the sum

of the volume of upwards trained shoots and downwards trained shoots using Equation 3.1.

Equation 3.1:

$$volume / vine = \frac{l'up' \times av h'up' \times av pd'up'}{3} + 2 \left(\frac{l'down' \times av h'down' \times av pd'down'}{3} \right)$$

3.2.4 Determination of pruning weight

During winter pruning all shoots were cut back to three node spurs. From these shoots the pruning weight of each ‘test-vine’ was determined with a hand-held scale. The shoot weight was calculated by dividing the number of shoots by the total pruning weight. The number of nodes per vine was later adjusted to 35 nodes per kg pruning weight by additional pruning. The nodes retained varied from 70 to 100 and 40 to 60 nodes per vine in the Alverstoke and the Coombe vineyards respectively.

The length between the 5th and 6th internode of each of the same shoots that were used for shoot growth measurements during the season, was measured at pruning.

The fruit weight to pruning weight ratio was determined using yield data as described in Chapter 8.

3.3 Results

3.3.1 Effects of PRD on shoot growth

To determine the effect of PRD on vegetative growth, the shoot growth rate was monitored during 3 PRD irrigation cycles. A reduction in shoot growth rate was observed where PRD was employed compared to fully irrigated control vines (Figure 3.2).

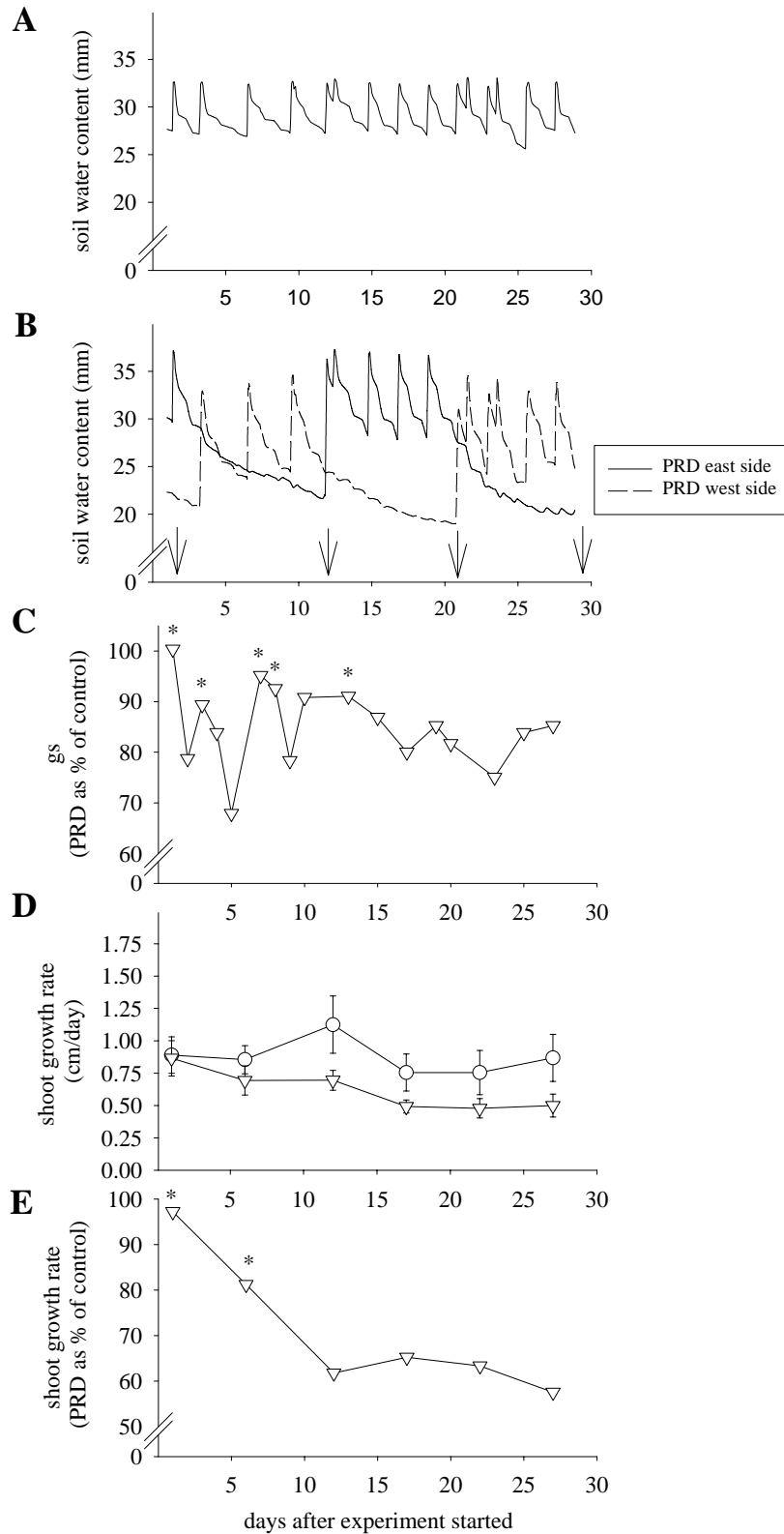


Figure 3.2 Effect of PRD on soil water content (A,B), stomatal conductance (C), shoot growth rate (D,E) for Cabernet Sauvignon / Ramsey rootstock split-root vines (13th Dec. 1996 to 9th Jan. 1997). Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld

A, B: soil water content (in mm); A) on one side of control; B) on both sides of PRD; ↓ indicates when irrigation sides were alternated. Soil water content measures between 0.35m and 0.45m.

C: means of stomatal conductance (gs, $\text{mmolm}^{-2}\text{s}^{-1}$) of PRD vines as % of control vines (*: n.s.; n=24

D: means of shoot growth rate; control (O); PRD (▽); means $n=24 \pm \text{s.e.}$;

E: means of shoot growth rate of PRD-treated vines as % of control; n=24; *: n.s.

Continuous monitoring of shoot growth rate started three weeks after flowering. At that time two PRD irrigation cycles had been already completed. During the first cycle of the experiment only three irrigations were applied, since weather conditions were still mild and the soil water content decreased more slowly than it did in the second and third cycles. During the first two cycles, the soil water content on the 'dry' side between 0.4m to 0.5m depth did not fall below 24.3 and 25.5mm respectively. When soil water content on the 'dry' side decreased further than this in subsequent irrigation cycles, the shoot growth rate decreased in PRD-treated vines compared to control vines.

The average soil water content of the control vines at depth 0.35m to 0.45m was between 26.5mm and 33.5mm both before and after an irrigation (Figure 3.2A). The measurements of soil water content for the 'east' and 'west' sides of PRD differed slightly due to variations in calibration. For example, when the 'east' side of the PRD vines was under irrigation, the soil water content between 0.35m and 0.45m depth varied from 27.0mm to 37.0mm, compared to 24.0mm and 34.0mm for the west side when it was irrigated. Similarly, the 'dry' sides of PRD-treated vines, for the 'east' side and the 'west' side, showed a decrease in soil water content of 22.3mm and 20.0mm respectively (Figure 3.2B).

The soil water content data shows a double peak at the first irrigation of the second, and at the first and second irrigation of the third irrigation cycle. This profile represents the additional amount of water (+30%) applied after switching the 'wet' and 'dry' sides. On the basis of water content in deeper soil layers it was decided that this extra water was necessary to refill soil water content from 21.0mm to 27.0mm in soil layers at 0.7m to 1.0m depths.

On average the shoot growth rate of control vines was 9mm/day compared with an average shoot growth rate of 7mm/day for PRD-treated vines. During the course of the experiment the shoot growth rate of PRD-treated vines was reduced by 27% compared to fully irrigated vines (Figure 3.2D, E).

It was also found that a reduction in stomatal conductance was concurrent with a reduction in shoot growth rate in the PRD-treated vines. On average, stomatal conductance was reduced by 17% compared to control vines. During the first and second irrigation cycle, differences in stomatal conductance on day 1, 3, 7, 8 and 13

were not significant. On all other days a significant reduction ($P < 0.05$) in stomatal conductance was observed (Figure 3.2C).

At the end of the 1996/1997 growing season the total length of lateral and main shoots of non-trimmed downwards trained shoots was measured (Table 3.1). The length of main shoots of PRD-treated vines were reduced by 13% and lateral growth in PRD vines was reduced by 42% compared to control vines.

Table 3.1 Effect of PRD on final shoot length (field grown Cabernet Sauvignon / Ramsey split-root vines; growing season 1996/1997).

variable	control (m)	PRD (m)	% diff. (PRD compared to control)	significance
main shoots	1.77 + 0.26	1.55 + 0.2	- 13	n.s.
lateral shoots	0.67 + 0.2	0.39 + 0.14	- 42	<0.05

Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld; (means \pm s.e.; n=4)

3.3.2 Effects of PRD on canopy development

To investigate the effect of PRD on canopy density, total leaf areas for the 1997/1998 and 1998/1999 seasons were determined (Figure 3.3). In the 1997/1998 season the PRD treatment started 10 days prior to flowering. Forty days after flowering the differences in total leaf area between control and PRD-treated vines were not significant.

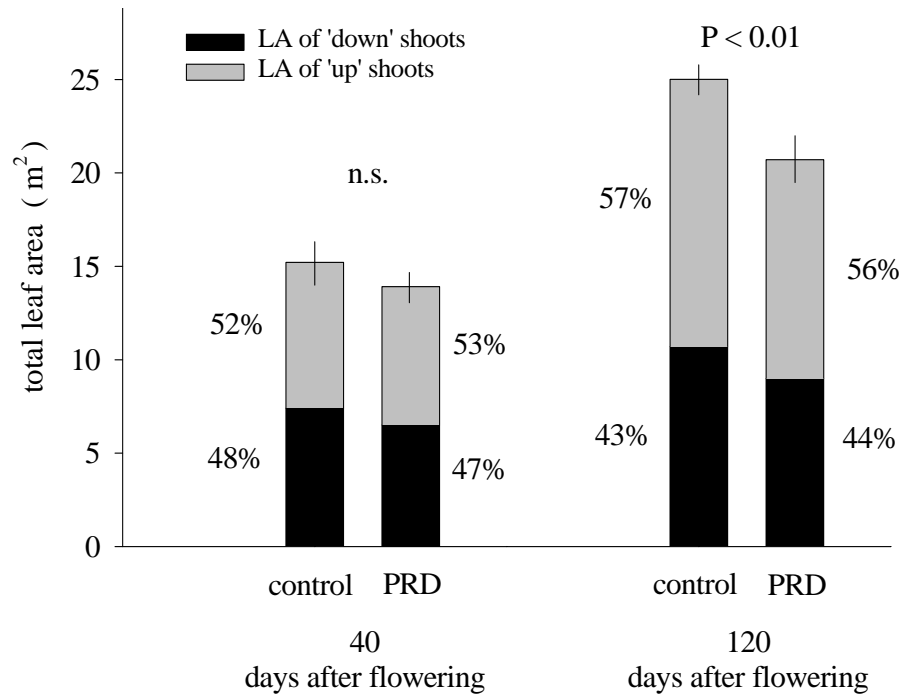


Figure 3.3 Effect of PRD on leaf area (LA, m²). (Cabernet Sauvignon / Ramsey rootstock split-root; season 1997/1998; bars indicate \pm std. dev.). Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld.

The leaf area of upwards trained shoots and downwards trained shoots was evenly distributed showing 52%/48% and 53%/47% for control and PRD-treated vines respectively.

At 120 days, compared to 40 days after flowering, the leaf area for both control and PRD had increased from 13.9m² to 20.7m² (PRD) and 15.2m² to 25m² (control). At 120 days post flowering the leaf area of control vines was 17% greater than that of PRD vines (P<0.01). Both control and PRD-treated vines had a larger leaf area on upwards trained shoots. The ratio upwards /downwards trained shoots, however, stayed the same in both treatments showing that the effect of PRD on leaf area was evenly distributed between both parts of the canopies.

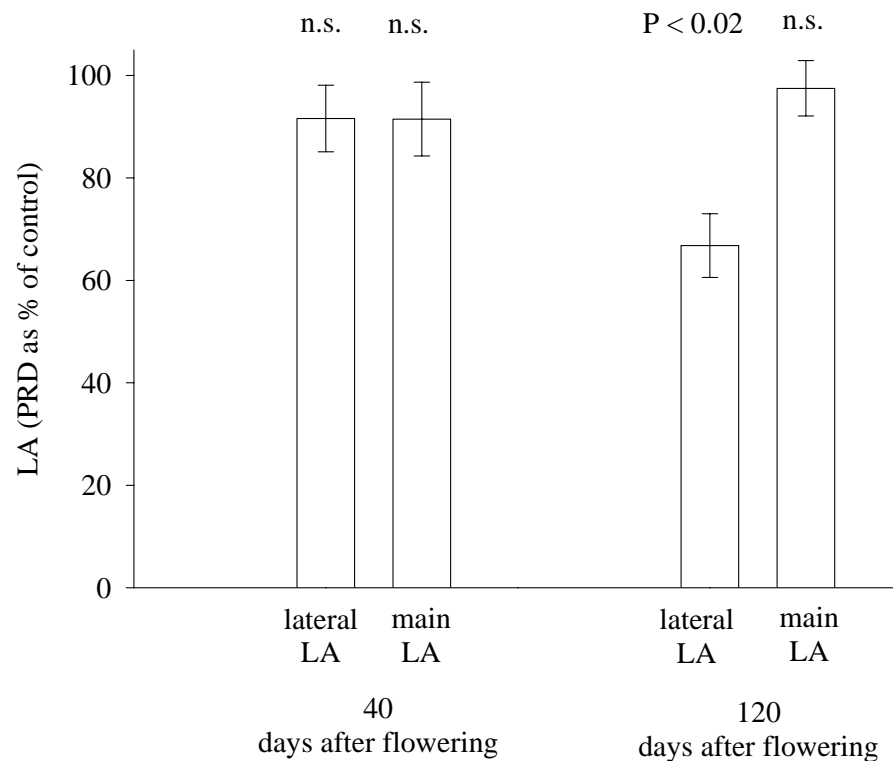


Figure 3.4 Effect of PRD on leaf area (LA) distribution at different times of development (Cabernet Sauvignon / Ramsey rootstock split-root vines; 1997/1998; Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld; means n=4; \pm s.e.).

By 120 days after flowering, the distribution of leaf area on main and lateral shoots had changed compared to that at 40 days after flowering. At 120 days after flowering lateral leaf area contributed more to the whole vine leaf area in control vines than in PRD-treated vines (Figure 3.4). The lateral leaf area of control vines represented 47% of the total leaf area compared to 38% in PRD-treated vines. The lateral leaf area increased from 3.6m^2 (40 days after flowering) to 11.6m^2 (120 days after flowering) in control vines and from 3.3m^2 (40 days) to 7.8m^2 (120 days) in PRD vines.

The results of leaf area measurements in the next season (1998/1999) also demonstrated that an increase in total leaf area of fully irrigated vines was mainly a result of an increase in the area of lateral shoots (Table 3.2). Forty days after flowering,

the ratio of main to lateral leaf area was 2.0 and 2.2 for fully irrigated and PRD-treated vines respectively. During the course of this experiment control vines showed a total increase in leaf area of 32% whilst the leaf area of PRD-treated vines increased by only 12%. The increase in leaf area of control vines was mainly due to a 64% increase in lateral leaf area over time. During the same time period the lateral leaf area of PRD vines increased by only 22%. By 120 days after flowering, control vines had a main to lateral leaf area ratio of 1.42 whilst in PRD-treated vines the ratio was almost unchanged (1.94).

The total leaf area of a vine can be related to the canopy volume as an expression of the surface density of a vine (Smart and Robinson, 1991). The volume of the canopy was measured in the 1997/1998 season with similar volumes observed for control (3.4m^3) and PRD-treated (3.2m^3) vines (difference not significant). Calculation of the leaf surface area density showed that control vines had a higher density ($7.4\text{m}^2\text{m}^{-3}$) than PRD-treated vines ($6.5\text{m}^2\text{m}^{-3}$; $P<0.05$).

Table 3.2 Leaf area development on control and PRD-treated vines during the 1998/1999 growing season on field grown Cabernet Sauvignon / Ramsey split-root vines.

	control (m^2)	PRD (m^2)	% diff. (PRD compared to control)	significance
40 days after flowering				
total leaf area	16.9 ± 0.78	15.5 ± 1.36	91	n.s.
main leaf area	11.3 ± 0.69	10.7 ± 0.93	94	n.s.
lateral leaf area	5.6 ± 0.19	4.8 ± 0.46	86	n.s.
ratio main/lateral LA	66.7 / 33.3	68.7 / 31.3		
120 days after flowering				
total leaf area	22.5 ± 0.94	17.4 ± 0.94	77	<0.01
main leaf area	13.2 ± 0.48	11.5 ± 0.39	87	<0.05
lateral leaf area	9.3 ± 0.51	5.9 ± 0.64	64	<0.01
ratio main/lateral LA	58.7 / 41.3	66 / 34		
increase in % from day40				
total leaf area	32.3	12.2		
main leaf area	16.4	7.9		
lateral leaf area	64.2	21.5		

Leaf area (LA, m^2) means $n=4$; \pm s.e. Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld

3.3.3 Effects of PRD on canopy density

To relate leaf area to light penetration inside a canopy, photosynthetically-active radiation inside the canopy was measured. It was observed that canopies with lower leaf area (PRD) became more open, resulting in higher light intensities inside the canopy compared to canopies with a larger leaf area (control).

Light intensity inside the Smart-Dyson canopies increased by an average of 49% and 45% for 1997/1998 and 1998/1999 respectively for PRD-treated vines compared to control vines (Table 3.3).

Table 3.3 Light intensity inside the canopy of PRD and control vines with a Smart-Dyson trellis system. (Cabernet Sauvignon / Ramsey rootstock split-root vines; ambient radiation: $2050\mu\text{molm}^{-2}\text{s}^{-1}$)

	control ($\mu\text{molm}^{-2}\text{s}^{-1}$)	PRD ($\mu\text{molm}^{-2}\text{s}^{-1}$)	% diff. (PRD compared to control)	significance
1997/1998 ^a				
above bunch zone	119.9 ± 33.9	174.8 ± 23.0	+ 34	n.s.
inside bunch zone	102 ± 17.7	170.5 ± 10.5	+ 67	<0.01
below bunch zone	92.5 ± 22.5	68.6 ± 16.4	+ 45	n.s.
1998/1999 ^b				
above bunch zone	106.7 ± 16.7	165.6 ± 18	+55	n.s.
inside bunch zone	80 ± 25.9	127.2 ± 15.7	+59	n.s.
below bunch zone	91 ± 18.35	110.8 ± 14.8	+21	n.s.

^a means; n=20 ± s.e.; ^b 1998/1999: means n=24 ± s.e.; Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld

Such increases in light intensity inside the canopy have been a consistent feature of PRD experiments. Light intensity measurements taken inside canopies of PRD vines using vertical shoot positioning showed an overall increase of 46% (Cabernet Sauvignon) and 16% (Shiraz) in light intensity above the bunch zone, compared to fully irrigated control vines (Table 3.4). During the 1998/1999 season almost no differences in light intensity were observed for the Shiraz vines, but the PRD and control vines received the same amount of water during that season .

For the growing seasons over which the experiment was conducted, the Smart-Dyson trellis system, with a bigger leaf area and canopy volume, had a lower light intensity inside the canopy compared to a VSP trellis system.

Table 3.4 Light intensity inside the canopy with vertical shoot positioning (VSP). Cabernet Sauvignon and Shiraz / own roots; ambient radiation: 2000-2100 $\mu\text{molm}^{-2}\text{s}^{-1}$
If not specially indicated: PRD: at any time water was withheld from one side of the vines; control: vines received water on both sides; (means \pm s.e.)

variable	control ($\mu\text{molm}^{-2}\text{s}^{-1}$)	PRD ($\mu\text{molm}^{-2}\text{s}^{-1}$)	% diff. (PRD compared to control)	significance
Cabernet Sauvignon				
above bunch zone ^a	158.7 \pm 12.5	295.0 \pm 28.9	46	<0.05
above bunch zone ^b	332 \pm 22.9	391 \pm 24.3	17	<0.05
Shiraz				
above bunch zone ^a	157.3 \pm 14.3	186.2 \pm 26.08	16	n.s.
above bunch zone ^{b,x}	191.5 \pm 13.7	208 \pm 17.3	9	n.s.

^a 1997/1998: means n=40 ^b: 1998/1999: means n=42. Control: vines received water on both sides of the vine (^x: PRD and control received same amount of water); PRD: at any time to one side of the vine water was withheld

Vegetative growth can also be quantified by measuring pruning weight. As a consequence of a reduction in shoot growth, both the mean shoot weight and pruning weight of PRD-treated vines was reduced by 13% and 14% respectively compared to control vines in the Alverstoke vineyard during three growing seasons (Table 3.5). Furthermore, in the 1996/1997 and 1998/1999 seasons, the fruit weight to pruning weight ratio was increased in PRD-treated vines by 9% compared to control. PRD-treated vines also showed a reduction in length of the 5th to 6th internode of 7% during the 1997/1998 and 1998/1999 season.

Table 3.5 Shoot growth components and canopy measurements; Smart-Dyson trellis system (Cabernet Sauvignon / Ramsey split-root vines).

	control	PRD	% diff. PRD compared to control	significance
pruning weight (kg/vine)				
1996/1997	5.2 ± 0.53	4.1 ± 0.31	- 19	<0.05
1997/1998	5.6 ± 0.22	5 ± 0.14	- 11	<0.05
1998/1999	7.1 ± 0.48	6.1 ± 0.29	- 13	n.s.
yield (kg)/pruning weight (kg)				
1996/1997	4.5 ± 0.42	5.3 ± 0.25	+ 19	n.s.
1997/1998	4.2 ± 0.22	4.2 ± 0.14		n.s.
1998/1999	3.3 ± 0.11	3.7 ± 0.22	+ 10	n.s.
shoot number per vine				
1996/1997	72 ± 3.1	72 ± 1.8		n.s.
1997/1998	74 ± 1.7	72 ± 1.2	- 4	n.s.
1998/1999	86 ± 1.4	85 ± 1.8	- 3	n.s.
shoot weight (g)				
1996/1997	72 ± 5	58 ± 3	- 18	<0.05
1997/1998	76 ± 2	70 ± 2	- 7	n.s.
1998/1999	82 ± 2	72 ± 3	- 13	n.s.
length of 5th internode (cm)				
1997/1998	8.9 ± 0.1	8.2 ± 0.1	- 7	<0.05
1998/1999	10.0 ± 0.3	9.4 ± 0.2	- 7	n.s.
leaf area / fruit weight ratio				
97/98	9.6 ± 0.6	9.1 ± 0.5	- 5	n.s.
98/99	8.4 ± 0.7	9.6 ± 0.6	+ 14	n.s.

means n=4 ± s.e.; Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld

With the Shiraz vines of the Coombe vineyard, differences in pruning weight between control and PRD-treated vines were less pronounced and despite receiving only half the amount of water, PRD-treated Shiraz vines had a 10% higher pruning weight compared to control vines during the 1997/1998 season (Table 3.6). During the 1998/1999 season both PRD and control vines received the same amount of water. Measurements of pruning weight during this season have also shown a 13% higher pruning weight of PRD-treated vines compared to control vines. The fruit weight to pruning weight ratio during the two seasons was reduced by 8% when PRD-treated vines were compared to control vines.

For the Cabernet Sauvignon vines from the same vineyard, the pruning weight was increased by 13% in 1997/1998 and decreased by 13% in the 1998/1999 season for

PRD-treated vines (Table 3.6). The fruit weight to pruning weight ratio in the 1997/1998 season was 15% higher for PRD than for control vines. In the 1998/1999 season, when PRD-treated vines had a lower pruning weight, the fruit weight to pruning weight ratio was 4% higher for PRD-treated vines than control vines.

Table 3.6 Shoot growth components and canopy measurements with vertical shoot positioning (VSP) (Shiraz and Cabernet Sauvignon; both own roots).

	control	PRD	% diff. (PRD compared to control)	significance
Shiraz				
pruning weight (kg/vine)				
1997/1998	2.37 ± 0.17	2.64 ± 0.15	+ 10	n.s.
1998/1999 ^x	1.85 ± 0.11	2.13 ± 0.08	+ 13	<0.05
yield (kg)/pruning weight (kg)				
1997/1998	5.2 ± 0.2	4.4 ± 0.2	- 15	n.s.
1998/1999 ^x	5.36 ± 0.1	5.28 ± 0.13	- 1	n.s.
Cabernet Sauvignon				
pruning weight (kg/vine)				
1997/1998	2.46 ± 0.18	2.78 ± 0.16	+ 13	n.s.
1998/1999	1.71 ± 0.18	1.52 ± 0.1	- 13	<0.05
yield (kg)/pruning weight (kg)				
1997/1998	3.71 ± 0.3	3.12 ± 0.3	- 15	n.s.
1998/1999	3.62 ± 0.31	3.78 ± 0.2	+ 4	n.s.

means n=7 ± s.e.; Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld ^x: PRD and control received same amount of water

3.4 Discussion

Using field-grown grapevines it has been demonstrated that PRD has an impact on biomass production and canopy structure. This study has shown that PRD can lead to a long term reduction in shoot growth rate and leaf area per vine.

One aim of the preliminary experiments was to monitor the effect of PRD on shoot growth rate over many irrigation cycles. This was considered important to confirm earlier studies which suggested that continuous application of water to only one side of

the root system caused a transient reduction in shoot growth rate (Dry *et al.*, 1996; Dry and Loveys, 1999). As a consequence these authors proposed that effects on shoot growth rate could only be maintained when the irrigation sides were alternated from side to side. Dry (1997) has shown that manipulation of soil water conditions in this way reduces shoot growth rate by 18 to 30% in field grown vines. The results presented in this study support these findings.

In the period between flowering and veraison the shoot growth rate of main upwards trained shoots was reduced by 20%. The total length of downwards trained shoots at winter pruning was reduced by 13%. This may reflect differences in growth which occur when shoots are oriented in different directions. Indeed, it has been shown that downward trained shoots have reduced growth compared to upwards growing shoots (Kliewer *et al.*, 1989). In another pot experiment on grapevines bearing a single shoot, Schubert *et al.* (1999) showed recently that vegetative growth in downwards trained shoots was reduced compared to upwards trained shoots. The same authors proposed that this effect might be a result of changes in hydraulic conductivity of shoots and suggested that these changes are due to a reduction in xylem vessel diameter of the downwards trained shoots.

The lateral growth of downwards trained shoots was significantly reduced (by 41%) in PRD-treated vines compared to control vines. It was consistently found that the reduction in shoot growth in response to PRD was greater for lateral shoots than for main shoots. The effect of PRD on lateral shoot growth is of particular importance to the canopy microclimate and will be discussed later in this chapter.

A response of shoot growth rate to PRD did not occur prior to flowering. Due to mild weather conditions and high soil water content after winter it was not possible to dry the soil on the 'dry'-side of PRD vines adequately enough within a 12 day cycle. At this early stage the soil water content of the 'dry' side reached a minimum of only 24.0 mm to 25.0 mm compared to 20.0 mm during later drying cycles. The higher soil water content during the irrigation cycles prior to flowering might have lessened the effect of PRD on growth reduction at that time. The higher soil water content at the beginning of the growing season may also account for the relatively minor differences in leaf area between PRD and control vines measured 40 days after flowering compared to comparatively larger differences in leaf area prior to harvest. If lower soil water

contents on the non-irrigated side of the vine occur earlier in the growing season under different soil or climatic conditions, it is possible that the early effects of PRD on shoot growth rate and leaf area may be greater. The potential for a PRD-induced reduction in growth would be greater early in the season, since it is known that maximum shoot growth rate occurs before flowering (Smart and Coombe, 1983). The shoot growth rate declines after flowering.

The maximum shoot growth rate per day for main shoots during the experiment was 12.5 mm/d and 8.5 mm/d for control and PRD vines respectively. During the duration of the experiment the shoot growth rate, however, did not change appreciably and was on average 9 mm/day for control vines and 7 mm/day for PRD vines. Matthews *et al.* (1987) found much higher maximum shoot growth rates in Cabernet Franc during their experiments at the same phenological stages. These authors reported maximum daily rates of 38 mm/day and 44 mm/day for non-irrigated and fully-watered field grown vines respectively, with a tendency to rapid decline as the season progressed. In their experiment, the surface area of soil per vine was much smaller with 4.5 m²/vine compared to 8 m²/vine in this study. Furthermore, the pruning levels and crop load in this study were different. In this study 90 nodes per vine were retained compared to 24 nodes per vine in experiments by Matthews *et al.* (1987). This may further explain differences in growth rate, since shoot vigour is inversely proportional to the shoot number per vine and the number of shoots per vine is a function of the number of nodes retained at pruning (Clingeffer and Sommer, 1995).

A low shoot growth rate does not necessarily indicate that the capacity of the vine is low. Dry & Loveys (1998) stated in their review that "...the capacity of a vine refers to the total crop production of a vine rather than to the rate of activity such as total growth...". By selecting a Smart-Dyson trellis system, using grafted vines and a wide vine spacing it was possible to maintain, on average, a high leaf area per vine of 23.5 m² and 18.7 m², a high pruning weight per vine of 5.9 kg and 5.0 kg and a high crop load per vine of 21.2 kg and 19.6 kg for control and PRD-treated vines respectively. One reason why these vines were selected for these studies was that it was known from previous experiments that they had the potential to grow vigorously (Dry, 1997) and it was considered that grapevines with a high shoot growth rate may be more sensitive to soil drying conditions than those with lower shoot vigour. The effects of PRD on

growth, therefore, may be more pronounced on vines with a large canopy size. Some evidence supporting this hypothesis can be drawn from the experiments with Shiraz vines of the Coombe vineyard. In these studies the differences in pruning weight were less pronounced than in the case of the Alverstoke vineyard with its much larger canopy and wider vine density. This has relevance to South Australian climatic conditions and viticultural practices because large canopy sizes are not unusual.

Fruit production in irrigated vineyards with vigorous vines, however, can become limited, since a large canopy may have a detrimental effect on fruitfulness and therefore vines may become unbalanced (Gao and Cahoon, 1994). Certain measures (Table 3.5) such as the ratio of leaf area to fruit weight or the ratio of fruit weight to pruning weight may be used as indicators of vine performance.

Smart and Robinson (1991) defined the canopy of a single vine by the leaf area and the number of shoots. Both of these parameters can show considerable variation, so there is not likely to be one ideal canopy, but rather a range will exist. Thus, to ripen a certain amount of fruit and to achieve a certain wine quality, the canopy and fruit ratio has to be in balance. A well-balanced canopy, therefore, will be of importance for the subsequent wine quality. According to the standards of Smart *et al.* (1990) both the control and PRD-treated vines were in the recommended range with respect to the ratio of leaf area to fruit weight (Table 3.5).

Differences in light exposure may also have influenced both vegetative and reproductive development in the Cabernet Sauvignon of the Alverstoke vineyard. Relative to the previous year, the 1998/1999 season yield of control vines was reduced to a greater extent than PRD-treated vines (28% and 20% respectively) whilst the pruning weight increased by 26% and 21% for control and PRD respectively. The increase in pruning weight was accompanied by an increase in leaf area and as a consequence there was a lower light penetration into the canopy for control vines. Similar effects on vegetative and reproductive development of Cabernet Sauvignon vines in California have been reported by Dokoozlian and Kliewer (1995). Same authors found a close relationship of high pruning weight and leaf area reducing light interception into the canopy. This indicates that the balance of a vine can be altered in response to management or environmental circumstances which favour vegetative development. With a more open canopy, PRD vines appeared to be less responsive to

these negative influences from management. For example, in PRD-treated vines the reduction in yield and the increase in pruning weight was smaller compared to the fully irrigated control vines in the season 1998/1999.

In unbalanced vigorous vines, a low yield leads to stimulation of vegetative growth (Eibach and Alleweldt, 1983). This leads to increased canopy density as a consequence of a higher leaf area, which may be due to increased lateral shoot growth as has been shown in this chapter. One effect of a more shaded bunch zone is a depression of inflorescence initiation (May, 1965) which then reduces the yield in the following season. It is therefore important to avoid an imbalance of vegetative to reproductive growth and a high canopy density. The Cabernet Sauvignon vines at the Alverstoke vineyard have been maintained as control and PRD treatments for 7 seasons without any apparent detrimental effect on inflorescence initiation.

Stimulated growth of lateral shoots will increase the total leaf area within a given volume and therefore reduce light penetration into the canopy. For Shiraz vines, Smart (1985) reported that less than 9% of the incident photosynthetically-active radiation is transmitted to the interior of the canopy whilst approximately 85% is absorbed by the canopy surface. Those results are in good agreement with figures presented here where it was found that between 5 and 7% of the incident PAR was transmitted to the interior of the canopy. PRD-treated vines showed an increased light penetration into the bunch zone relative to control vines. Measurements in a VSP trellis system have further confirmed that PRD improves light penetration to the bunch zone. This highlights the potential of a well-managed canopy to improve the canopy microclimate. Apart from improving bunch exposure and yield, a well-maintained and open canopy can reduce incidence of some diseases and reduce production costs (Coombe and Dry, 1992).

The variability of light penetration into the canopy can be quite large. A more open canopy can influence the light environment in the canopy interior in several ways. There may be a higher incidence of sunflecks, that is an increased incident of patches of unfiltered sunlight and filtered sunlight due to a reduction in the average number of leaf layers (Dokoozlian and Kliewer, 1995). In a canopy interior with extended dark periods the photosynthetic yield declines progressively whilst inside a canopy with intermittent irradiation the photosynthetic utilisation can improve (Kriedemann *et al.*, 1973). The

contribution of sunflecks to the final light environment in the canopy interior was not monitored during this study and this is an area for further study.

To interpret the results of canopy density of this study, leaf area measurements may therefore be a more valuable indicator than light intensity measurements. During both seasons the lateral leaf area was reduced whilst the light penetration was improved in PRD-treated vines compared to fully irrigated vines. Dokoozlian and Kliewer (1995) reported that the total canopy leaf area strongly correlates with the photosynthetically-active radiation levels inside the canopy. With an increase in leaf area during a growing season the light intensity inside the canopy lessens dramatically after berry set and reaches a minimum from veraison onwards to harvest. Chapter 8 will focus on the influence of bunch exposure on fruit quality.

Relative changes in leaf area can either be due to a reduction in total leaf number or to a reduction of leaf expansion or both. No differences in individual leaf area for either main and lateral shoots were shown due to PRD. However, shoots of PRD-treated vines had fewer leaves and a reduced leaf initiation rather than a reduction in leaf expansion. By examination of the elongation of specific internodes of mature shoots at pruning it has been found that the internode length between the 5th and 6th internode was reduced on average by 11%. In apple trees, growing under half drying soil conditions, Gowing *et al.* (1990) have shown that the decline in growth rate was a combination of a decreased rate of leaf initiation and a decrease in final leaf expansion. By excising the dried part of their root system they observed a recovery in growth rate. These authors explained this phenomenon by proposing that a chemical signal from dried roots was causing a reduction in shoot growth. This signal disappeared after excising the dried part of the root system so that the plant was able to recover. A reduction in stomatal conductance during their soil drying experiment was concurrent with the reduction in shoot growth.

Beside the effects on growth using PRD, another consistent feature in my studies has been a reduction in stomatal conductance. Stomatal conductance was consistently reduced in all PRD experiments in this study. During the experiment where shoot growth rate was reduced by 20% with PRD, stomatal conductance of PRD-treated vines was reduced by 17% on average. Evidence for a strong correlation between stomatal conductance and a reduction in shoot growth rate has now been accumulated from some

studies (Dry, 1997), and signals synthesised in roots of the drying soil portion have been proposed (Zhang *et al.*, 1987; Davies and Zhang, 1991; Dry and Loveys, 1999). The involvement of such chemical signals on stomatal conductance and growth will be discussed further in the following chapters.

3.5 Conclusion

The experiments in this chapter were conducted to test the hypothesis that PRD maintains a long term effect on shoot growth rate and biomass production of field grown vines. In conclusion the following observations were made:

- 1) Grapevine shoot growth rate responded very sensitively to drying soil conditions. The irrigation strategy used in the PRD experiments maintained a lower growth rate of both main and lateral shoots.
- 2) A reduction in total leaf area was mainly due to a reduction in lateral leaf area.
- 3) Lateral shoot growth was found to influence canopy structure and its reduction was one of the major factors in improving the light penetration inside the canopy of PRD vines.
- 4) A higher light penetration inside the canopy of PRD vines was observed using different trellis systems.
- 5) Biomass production expressed as pruning weight was reduced with PRD.
- 6) At very low water application for both control and PRD-treated vines, the pruning weights were actually greater for PRD-treated vines indicating that PRD made more efficient use of available water.
- 7) A reduction in stomatal conductance occurred concurrently with the reduction in shoot growth under PRD.

Chapter 4 Stomatal control of leaf gas exchange under PRD

4.1 Introduction

The majority of a plant's gas exchange occurs through its stomata. Stomata are essential for both controlling water loss and allowing carbon dioxide uptake for assimilation. To co-ordinate and adjust their aperture, stomata respond rapidly to several stimuli which are coupled to changes in soil water conditions or directly to atmospheric changes (Jones & Tardieu, 1998).

In the field, atmospheric conditions are hard to manipulate, however the soil water content can be modified with cultural practices. When irrigation is required, soil water conditions can be manipulated by different irrigation strategies. It has been proposed that stimulating biochemical and physiological processes involved in plant responses to water stress may be a useful tool for reducing transpirational water loss (Loveys, 1992).

The amount of water lost via transpiration and gas exchange can be measured with various gas exchange monitoring systems. The calculation of photosynthesis rate and stomatal conductance from gas exchange measurements assumes that stomatal aperture over a leaf surface is homogeneously distributed (Downton *et al.*, 1988a). This is not always the case; indeed Laisk *et al.* (1980) and Downton *et al.* (1988a) presented results showing that stomata across a leaf surface were open to varying degrees. The phenomenon of non-uniform stomatal aperture between adjacent regions results in non-uniform photosynthesis across the leaf surface (Downton *et al.*, 1988b; Terashima, 1992; Düring & Loveys, 1996).

Depending on the degree of non-homogeneous stomatal aperture the effective leaf area may differ when a proportion of the stomata are fully closed compared to when they are fully open. The actual degree of stomatal closure within the "patches", however, cannot be determined by gas exchange instruments. The resulting errors in leaf gas exchange data will lead to an underestimation of stomatal conductance, photosynthesis and internal partial pressure of CO₂ since existing formulae for calculating these variables all assume a fixed leaf area in their calculations (Downton *et al.*, 1988a; Terashima *et al.*, 1988).

It was shown in the previous chapter that applying more water to a vine increases the transpirational water loss and stimulates vegetative growth. One could consider that application of less water to both sides of a vine's root system may cause the same effect, as seen in PRD-treated vines with alternated 'wet' and 'dry' sides. Thus an alternation of irrigated and non-irrigated sides may not be necessary. Alternation of 'wet' and 'dry' sides, however, restricts the soil surface area of the root zone being watered and may therefore lead to a prolonged saturation of deeper soil layers. Differences in vertical water distribution may cause different stress responses of PRD-treated vines compared to vines that received the same amount of water over a larger soil surface area.

The aim of this chapter is to test the hypothesis *that manipulating soil water conditions with PRD affects the uniformity of stomatal aperture in grapevines.*

4.2 Material and Methods

4.2.1. Determination of effects of PRD on stomatal conductance and vertical distribution of water within the soil profile

Split-root grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots) grown in a sandy soil (Appendix 2) at the Alverstoke vineyard of the University of Adelaide were used to determine effects on stomatal conductance under variations in soil water penetration within the vertical soil profile. This experiment was conducted during the period 18th of January to the 13th of February 1999.

Throughout the experiment the same total volume of water was applied to PRD and control vines. The soil water content of both treatments was monitored using an EnviroScan® (Sentek, Adelaide, South Australia) soil moisture instrument with sensors at 0.1 m, 0.2 m, 0.3 m, 0.4 m, 0.5 m, 0.7 m and 1.0 m depths. The amount of water used was determined such that PRD-treated vines received enough water to refill the soil water content to 15 to 20 mm at a depth of 0.65 m to 0.75 m. To equalise the amount of water applied to both treatments, drip emitters with different flow rates were employed.

PRD-treated vines had drip emitters with a 2 Lh^{-1} output whilst control vines were irrigated with drip emitters of 1 Lh^{-1} on both sides at the same time. The total amount of water per vine was gradually reduced from 9.5 L per irrigation at the beginning of the experiment to 4.7 L per irrigation at the end of the experiment.

Stomatal conductance was determined using a portable porometer (AP4, Delta-T, Cambridge, UK). On cloudless days, 6 fully sun exposed leaves of each 'test-vine' were used to determine the stomatal conductance. Measurements were taken between 10 am and 12 pm.

Leaf water potential was measured from day 5 to day 22 every third day between 2 pm and 4 pm as described in Section 2.6.

4.2.2. Determination of the effect of PRD on stomatal aperture when control vines receive twice as much water as the PRD vines

Vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted to *Vitis champini* cv. Ramsey rootstock) grown at the Alverstoke vineyard of the University of Adelaide were used. Control vines received twice the amount of water as PRD-treated vines during the whole season using drip emitters with equal flow rate (2 Lh^{-1}) for both treatments. The measurements of stomatal patchiness were conducted on the 2nd and 5th of January 1998 between 9am and 1pm. The last alternation of 'wet' and 'dry' sides prior to the 2nd of January 1998 was the 25th of December 1997. After that time each vine received 3 irrigations of 16 and 8L per irrigation for control and PRD-treated vines respectively. This was equivalent to 0.02 and 0.01 MLha^{-1} respectively

Prior to the leaves being detached from the shoots, stomatal conductance was measured at three different positions near the terminal vein of each leaf blade using a porometer as described in Section 4.2.1. Three fully sun exposed leaves of same size were chosen on each 'test-vine' for these measurements.

A simple water infiltration technique was used to determine the extent to which stomatal patchiness occurred (Beyschlag & Pfan, 1990). Leaf samples were taken immediately after stomatal conductance measurements were performed. An area (approximately 4 cm^2 to 5 cm^2) of the blade along the central vein of the terminal lobe,

was cut out with a razor blade and immediately inserted into a 50 mL plastic syringe filled with water. To eliminate the remaining air from the leaf surface, the piston of the syringe was first pulled outward to produce a partial vacuum before pressure was applied to the piston to encourage water infiltration into the leaf blade. The leaf blade was removed from the syringe, blotted dry with paper tissue and was then ready for photography.

Images were taken with a digital camera (Minolta (RD175)) with a shutter speed of 1/125 sec and aperture setting of $f22$. The camera was mounted on a tripod. The leaf blade, together with a ruler scale, was placed on top of a light box with a built in flash light. Images were taken in the dark with back light illumination from the flash (Figure 4.1). Immediately after the photograph was taken, leaf segments were transferred into plastic bags, frozen in liquid nitrogen and stored at -40°C ready for measurements of endogenous abscisic acid concentration, as described in Section 2.7.

The image analysis was performed using several image analysis programmes.

Infiltrated, light green areas with open stomata could be clearly distinguished from dark green areas with closed stomata (Figure 4.1 A). The coloured image was converted to a grey-scale image using Adobe Photoshop® (Figure 4.1 B). Using SigmaScanPro® (Jandel Scientific) a threshold level was determined below which an area on the leaf was considered dark or non-infiltrated. Since all images were taken using the same image-capturing arrangement the same threshold was applied to all images. The dark green regions were then highlighted green (Figure 4.1 C) and the highlighted areas of all images were quantified using area integration (SigmaScanPro®). To calculate the infiltrated area, the area of the dark green non-infiltrated portions of the leaf was subtracted from the area of the whole leaf segment.

Results of the integration of the infiltrated area were related to previous measurements on stomatal conductance. Leaves were classified according to their stomatal conductance, using increments of $50\text{ mmolm}^{-2}\text{s}^{-1}$. For example, leaves with stomatal conductance of 75 to $125\text{ mmolm}^{-2}\text{s}^{-1}$, were combined to a single group and 125 to $175\text{ mmolm}^{-2}\text{s}^{-1}$ to the next *et cetera*.

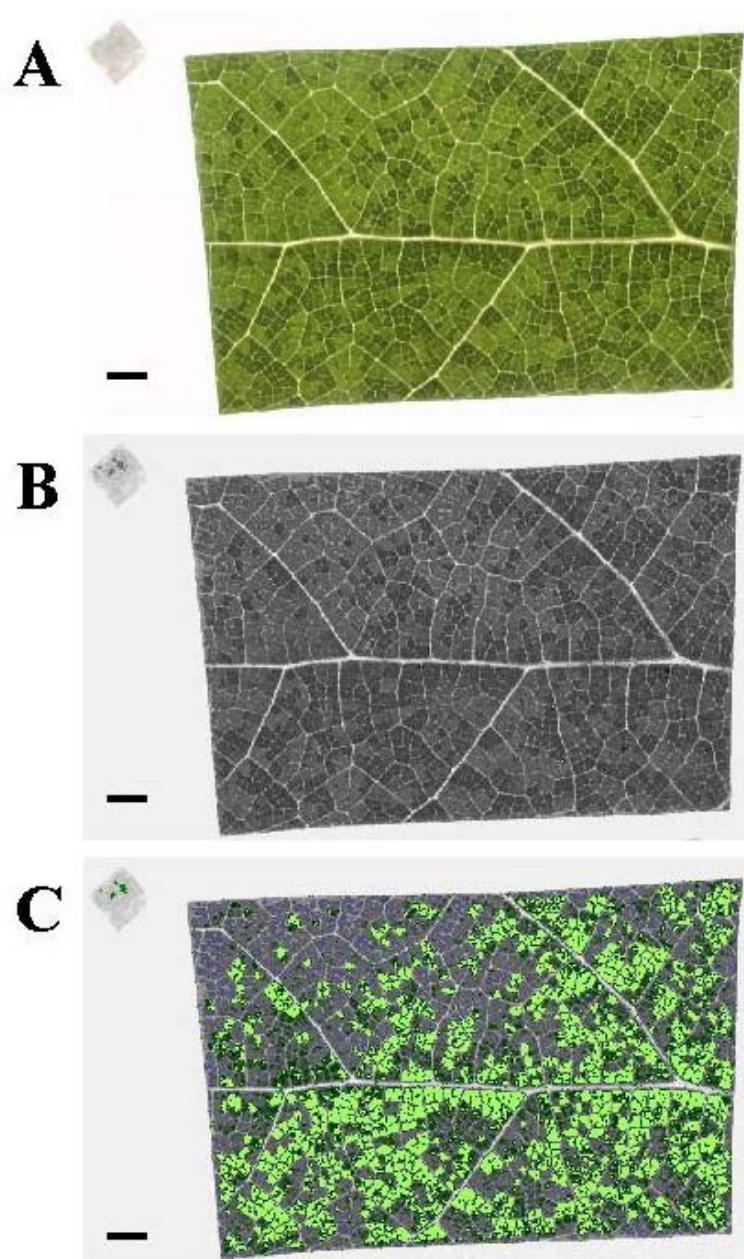


Figure 4.1 Image analyses of stomatal patchiness. Back light photograph of leaf segments (Cabernet Sauvignon / Ramsey rootstock split-root vines). Dark areas: non-infiltrated parts; light areas: infiltrated parts (bars =2.5mm) **A:** original image taken in the field **B:** converted greyscale image **C:** threshold applied to dark areas

4.2.3. Measurement of abscisic acid in leaf segments

In the 1999/2000 season, leaf samples from vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted to *Vitis champini* cv. Ramsey rootstock) were taken for measurements of abscisic acid concentration. The samples were taken on the 25th of February 2000 at the end of an irrigation cycle.

Stomatal conductance was measured on 3 leaves of each ‘test vine’. To determine whether ABA distributes uniformly over the entire leaf surface ABA analysis was performed on leaf segments cut from different positions. Strips of 3 mm were cut along either side of the terminal vein (Figure 4.2 A) and between lateral veins (Figure 4.2 C).

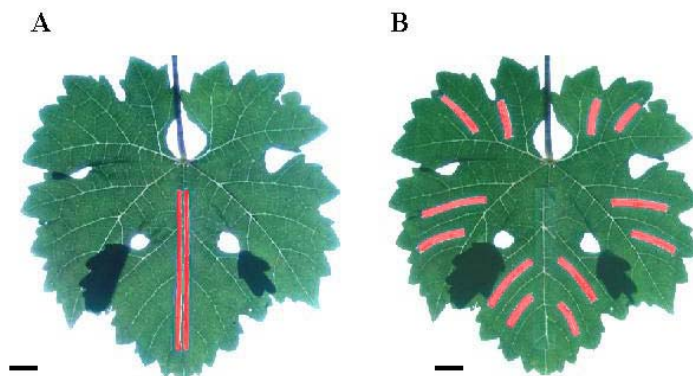


Figure 4.2 Leaf segment for ABA analysis (Cabernet Sauvignon / Ramsey rootstock split-root vine (bars: 1cm). Red areas inside the leaf blade indicate the positions where leaf segments were taken. **A:** segments close to terminal vein **B:** segment between lateral veins

To collect samples quickly, two razor blades, joined together but separated by a distance of 3 mm, were used to cut the section immediately after measuring stomatal conductance. The samples were transferred into a plastic bag, frozen in liquid nitrogen and stored at -40°C until further analysis.

4.2.4. Determination of effects of PRD on photosynthesis

Measurements were conducted between the 9th and 18th of February 2000. Both control and PRD-treated vines received different amounts of water. Control vines were irrigated on both sides of the planting line using drip emitters with a flow rate of 2 Lh⁻¹ whilst PRD-treated vines received only half the amount of water by using 2 Lh⁻¹ drip emitters on only one side of the planting line.

Rates of gas exchange were determined using an open photosynthesis system (LI-COR Li 6400, Lincoln, Nebraska, USA) as described in Section 2.5. Because the measurements were conducted using the internal light source of the system, the light saturation for grapevine leaves had to be pre-determined. A light response curve (assimilation rate vs PAR) was determined using light from 0 to 2000 $\mu\text{molm}^{-2}\text{s}^{-1}$ in 250 $\mu\text{molm}^{-2}\text{s}^{-1}$ increments. According to this saturation curve, a light intensity of 1250 $\mu\text{molm}^{-2}\text{s}^{-1}$ was used for all measurements.

During measurements the cuvette temperature was adjusted to ambient temperature conditions. The cuvette relative humidity was set to approximate ambient air humidity. On each measuring date a set of three readings was taken for each of three replications and of each 'test-vine' between 10 am and 2 pm. Data were stored when the photosynthetic rate reached a steady state which normally took about five minutes per leaf.

The days on which gas exchange measurements were conducted were cloudless, warm and dry with an average maximum air temperature of 30 °C, an average relative air humidity of 38% and an average maximum solar radiation of 27.6 MJm⁻².

Analysis of variance (ANOVA) was used to analyse the data as described in Section 2.10.

4.3 Results

4.3.1. Stomatal conductance and soil water conditions under PRD

To determine the effect of depth of soil water penetration into the soil profile on stomatal conductance the same amount of water per vine was either applied to only one side (PRD) or evenly distributed to both sides of the planting line (control). Results of soil water content measurements in two soil layers between 0.35 to 0.45 m and 0.65 to 0.75 m depths are shown in Figure 4.3 (A, B, C). The soil water content for control vines is only shown for one side of the planting line (Figure 4.3 A). The other side of the planting line showed a similar pattern of soil water content. For PRD-treated vines the soil water content is shown for both sides of the planting line (Figure 4.3 B, C).

The distribution of soil water in different soil layers was altered when the total volume of water was applied to only one side of the vine, compared to the situation where the same volume of water was split equally between the two sides. Irrigation water penetrated to deeper soil layers on the 'wet' side of PRD-treated vines compared to control vines where the same amount of water was evenly distributed to both sides. The first and largest irrigation (9.5 L/vine) resulted in an increase in soil water content from 5.5 mm to 9.8 mm in control vines compared to an increase from 5.9 mm to 18.2 mm on the 'wet' side of PRD in the soil layer between 0.65 and 0.75 m. No changes in soil water content at this depth (0.65 to 0.75 m) were detected in control vines after day 5. The soil water content at this soil layer gradually declined to 5 mm by the end of the experiment. For the duration of the experiment the amount of water applied per irrigation was also gradually reduced from 9.5 L/vine to 4.7 L/vine. In contrast to control vines, PRD-treated vines had a higher soil water content in both soil layers after each irrigation and during the course of the experiment. The soil water content during both irrigation cycles increased substantially with every irrigation from 10 to 26 mm on average at the 0.35 to 0.45 m soil layers. The fourth irrigation was delayed by one day and therefore the soil water content was lower prior to the irrigation, hence the soil water content was not restored to the same extent as in earlier and subsequent irrigations.

Figure 4.3 (D and E) shows that transpirational water loss (indicated as stomatal conductance) responds sensitively to soil water conditions. At the beginning of the first

irrigation cycle, when relatively more water was applied and soil water content at 0.35 to 0.45 m was similar for both control and 'wet' side of PRD, the stomatal conductance of control vines was on average 14% higher than for PRD-treated vines. After 6 days, control vines still had a significantly higher stomatal conductance ($P < 0.05$) compared to PRD-treated vines. A gradual reduction of the total amount of irrigation water not only resulted in a decrease in soil water content of control vines compared to PRD vines, it also resulted in values for stomatal conductance of PRD-treated vines which were equal to or higher than that of control vines (for irrigation volumes of less than 5.3 L/vine) (Figure 4.3 D and E).

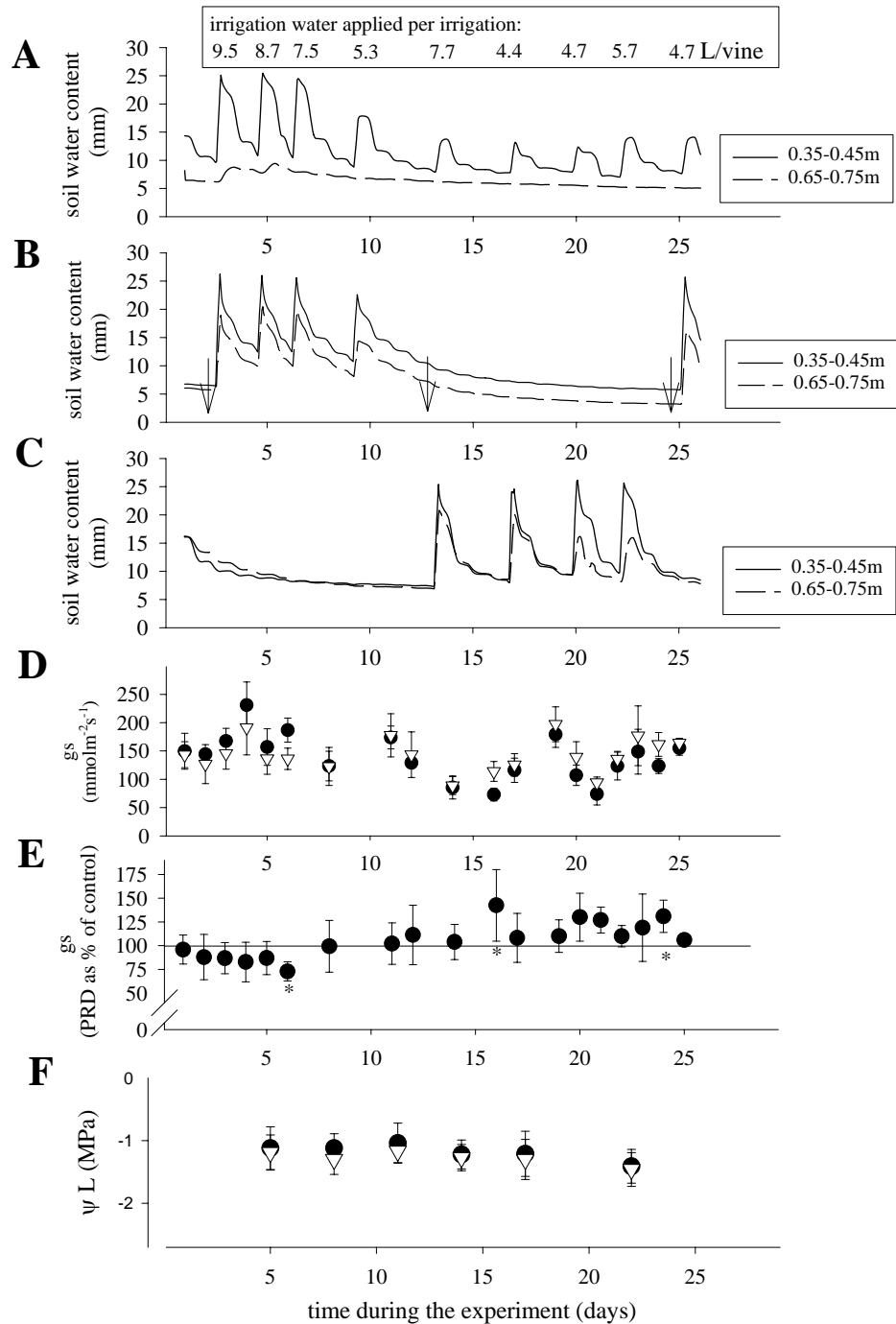


Figure 4.3 Effects of various soil water conditions at different soil layers on stomatal conductance and leaf water potential applying the same amount of water per vine either to only one side (PRD) or evenly distributed to both sides of the vine (control) for Cabernet Sauvignon split-root vines. **A:** soil water content (mm) on one side of the root system of control vines **B, C:** soil water content (mm) on either side of the root system of PRD-treated vines (↓ alternating irrigation sides) **D:** means of stomatal conductance ($gs, \text{mmolm}^{-2}\text{s}^{-1}$; PRD (∇); control (\bullet); mean value of 6 measurements on each replicate \pm s.e.) **E:** means of stomatal conductance of PRD vines as % of control vines (*: $P < 0.05$) **F:** means of leaf water potential (ψ_L , MPa; PRD (∇); control (\bullet); mean value of 3 measurements on each replicate \pm s.e.)

Results of measurements of stomatal conductance for control and PRD-treated vines were divided into two groups: measurements taken before alternating the irrigation sides (days 1 to 14) and measurements taken after alternating the irrigation sides (days 15 to 25, Figure 4.3 E). Both groups were found to be significantly different by ANOVA ($P < 0.05$). The first group of data showed, on average, a 9% reduction in stomatal conductance of PRD-treated vines relative to control vines. In the second group the stomatal conductance of PRD-treated vines was, on average, 19% higher than control vines.

Stomatal conductance of PRD-treated vines started to exceed that of control vines when less irrigation water penetrated soil layers to a depth of 0.35 to 0.45 m around the time of the fourth irrigation. The fourth irrigation refilled the soil water content of control vines from 8.3 mm to 17.5 mm (Figure 4.3 A) compared to an increase from 10.3 to 23 mm for the 'wet' side of PRD-treated vines (Figure 4.3 B). After day 12, when soil water content of soil layers between 0.35 to 0.45 m decreased further, stomatal conductance of control vines started to decline (Figure 4.3 D). The difference between stomatal conductance of control and PRD-treated vines was significant on days 16 ($P < 0.05$) and 24 ($P < 0.05$), which were both days immediately prior to an irrigation event. On these days the soil water content between 0.35 and 0.45 m on either side of control vines was 7.5 mm and 8.4 mm compared to 7.4 mm and 6.9 mm on the 'dry' side of PRD-treated vines for day 16 and 24 respectively. At the same time, the soil water content on the 'wet' side of PRD vines was higher at 10.6 mm on day 16 and 13.4 mm on day 24.

Throughout the experiment, control and PRD-treated vines had similar leaf water potential (Figure 4.3 F). Afternoon leaf water potential for control and PRD-treated vines was on average -1.1 MPa and -1.2 MPa respectively ($P > 0.05$).

4.3.2. PRD and stomatal aperture

The infiltration patterns of leaf segments demonstrated a non-uniform distribution of stomatal aperture in field-grown grapevine leaves (Figure 4.1). This phenomenon was apparent in fully irrigated control and PRD-treated vines. It was found that the area of leaf infiltration increased with increasing stomatal conductance (Figure 4.4). Stomatal conductance of PRD-treated vines was reduced by 18% on average compared to control vines. For that reason, leaves with a stomatal conductance lower than $125 \text{ mmolm}^{-2}\text{s}^{-1}$

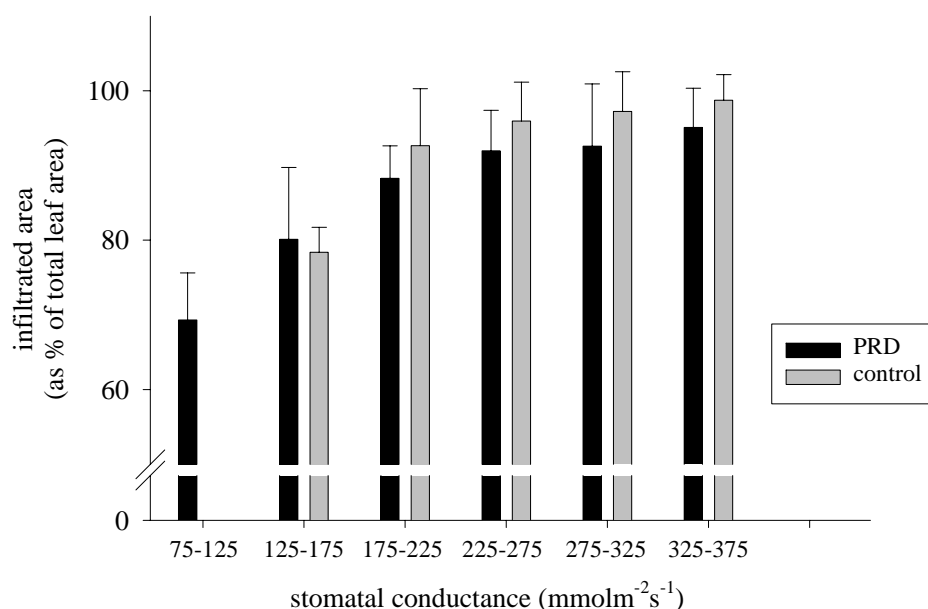


Figure 4.4 Relationship between stomatal conductance (gs ; $\text{mmolm}^{-2}\text{s}^{-1}$) and stomatal patchiness calculated from the leaf area infiltrated with water (Cabernet Sauvignon / Ramsey rootstock split-root vines; mean value of 3 measurements on each replicate; \pm s.e.)

were only found in PRD-treated vines. When stomatal conductance in PRD-treated vines was between 75 and $125 \text{ mmolm}^{-2}\text{s}^{-1}$, 31% of the leaf area was not infiltrated and this figure fell to less than 10% as stomatal conductance rose above $225 \text{ mmolm}^{-2}\text{s}^{-1}$.

The average stomatal conductance was, on most occasions, slightly higher for control vines compared to PRD-treated vines. This corresponded to the general measurement

of larger infiltrated areas for controls compared to PRD-treated vines, although the differences were not found to be significant ($P>0.05$).

Measurements of abscisic acid in leaf segments were conducted to test whether the total ABA concentration was related to the proportion of the leaf segment able to be infiltrated (Figure 4.5). It was found that the concentration of ABA declined when a higher percentage of leaf area was infiltrated. Thus, leaves with a higher stomatal conductance had a smaller bulk leaf ABA concentration. There was no significant difference ($P>0.05$) between ABA concentrations in PRD compared to control vines at any level of infiltration.

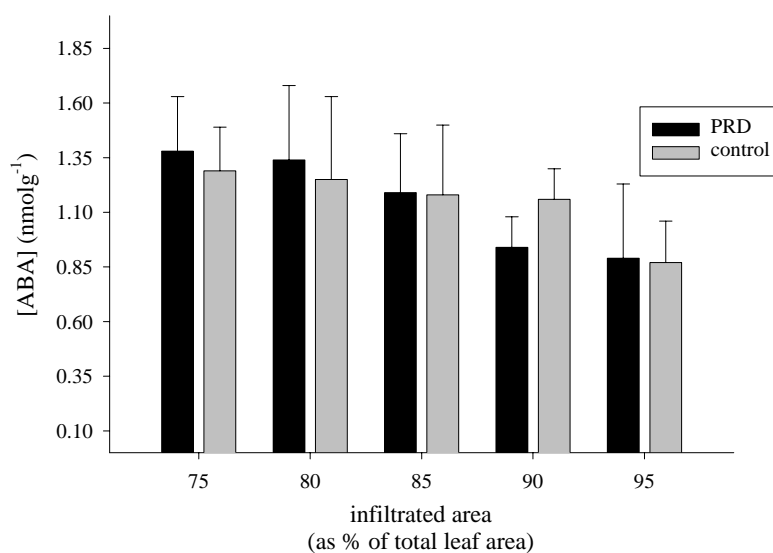


Figure 4.5 Relationship between ABA concentration ($[ABA]$, nmol g^{-1}) in leaf segments and stomatal patchiness calculated from the leaf area infiltrated with water (Cabernet Sauvignon /Ramset rootstock split-root vines; mean value of 3 leaf samples on each replicate; \pm s.e.)

4.3.3. ABA distribution in leaves

To test whether the concentration of ABA over the leaf blade was uniformly distributed, ABA was measured from different leaf segments, which were either close to the terminal vein or in-between lateral veins as outlined in Figure 4.2. ABA concentrations in segments closer to the terminal veins were compared to ABA concentrations in leaf segments between lateral veins (Table 4.1).

Table 4.1 Effect of PRD on ABA distribution in different leaf segments (Cabernet Sauvignon / Ramsey rootstock split-root vines)

origin of leaf segment	control [ABA] (nmolg ⁻¹)	PRD [ABA] (nmolg ⁻¹)	significance
between lateral veins	1.26 ± 0.15	1.39 ± 0.13	P>0.05
close to terminal vein	1.25 ± 0.08	1.32 ± 0.07	P>0.05

(means ±s.e.; n=12) Control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld

Comparing the bulk leaf ABA concentration of both segments it was found that bulk leaf ABA concentration was almost evenly distributed over the leaf area for both control and PRD-treated vines. Concentration differences between treatments were minor and were not significantly different (P>0.05).

Figure 4.6 shows the relationship between bulk leaf ABA in leaf segments from different positions along the leaf blade and the stomatal conductance for both treatments. For control and PRD-treated vines, leaves with a lower stomatal conductance tended to have a higher bulk leaf ABA in both segments close to the terminal vein and segments between lateral veins. For all measurements the variability in stomatal conductance and ABA levels were high.

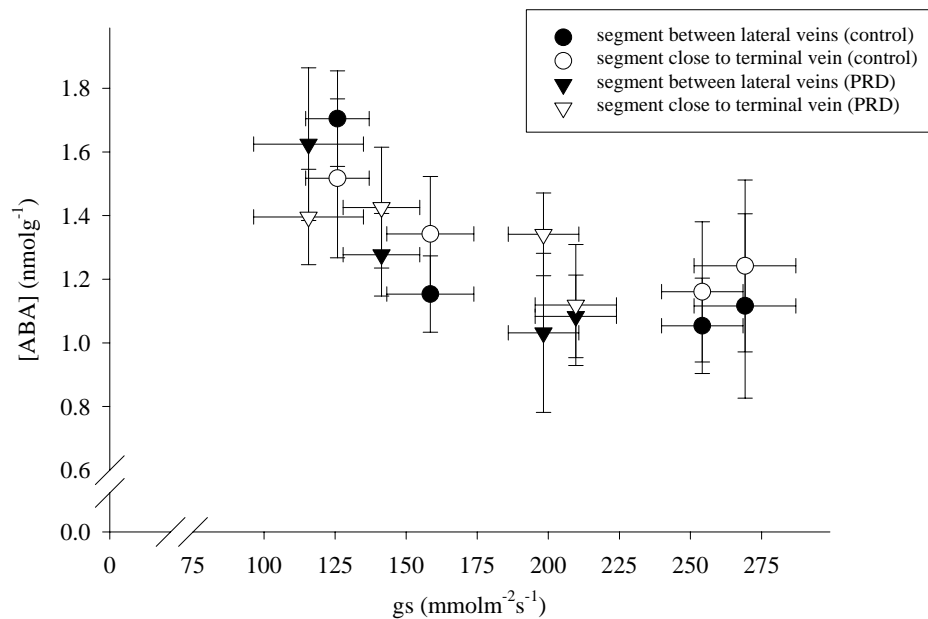


Figure 4.6 Bulk leaf ABA ([ABA], nmol g^{-1}) distribution in leaves in relation to stomatal conductance (gs , $\text{mmol m}^{-2} \text{s}^{-1}$) Cabernet Sauvignon / Ramsey rootstock split root vines (mean value of 6 measurements on each replicate; \pm s.e. bi-directional)

4.3.4. PRD and photosynthesis

To determine the effect of PRD on leaf gas exchange, an experiment in which control vines received twice as much water as PRD vines, was conducted and the influence of stomatal conductance on photosynthesis was measured. Stomatal conductance was found to be higher in control vines than PRD-treated vines. Large changes in stomatal conductance in control vines, however, had little influence on the assimilation rate. Comparison of the photosynthetic rate for both treatments found no substantial reduction in assimilation rate for PRD-treated vines nor a substantial increase in assimilation rate for control vines. An ANOVA, applied to assimilation rate versus conductance for both control and PRD vines, however, showed that a significant

difference ($P < 0.01$) existed between the regression lines fitted for the two treatments (Figure 4.7).

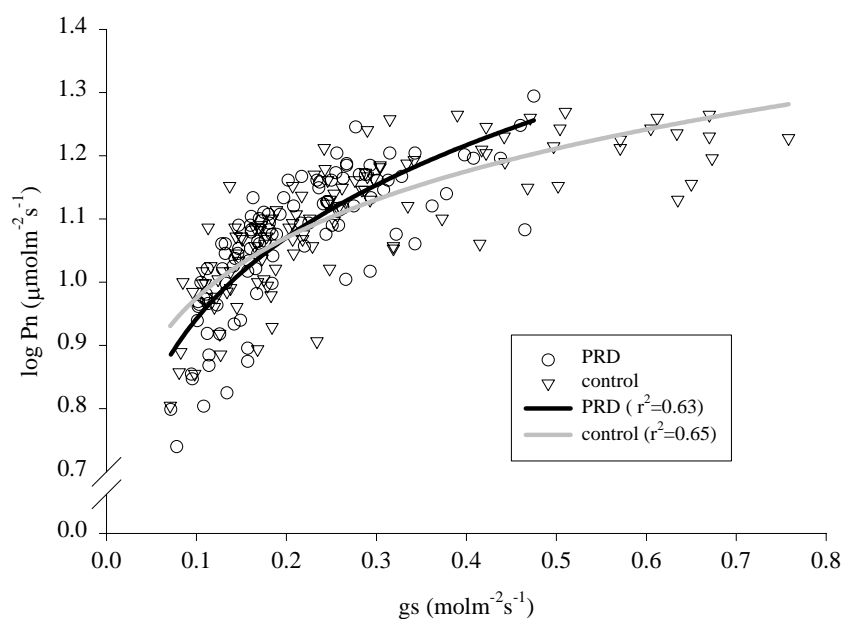


Figure 4.7 Effect of PRD on the relationship between stomatal conductance (gs , $\text{molm}^{-2}\text{s}^{-1}$) and assimilation rate ($\log Pn$, $\mu\text{molm}^{-2}\text{s}^{-1}$; Cabernet Sauvignon / Ramsey rootstock split-root vines; mean value of 9 measurements on each replicate)

4.4 Discussion

Results of experiments in this chapter have provided evidence that stomata of field-grown vines can respond very sensitively to variations in soil water conditions, thereby controlling evaporative water loss from the vine. Consequently, manipulation of stomatal aperture has the potential to reduce transpiration in grapevines.

A non-uniform distribution of stomatal apertures in grapevines has generally been associated with water stress (Downton et al., 1988a), low air humidity (Düring, 1992) and an increase in xylem ABA concentration (Downton et al., 1988b). The infiltration technique used either shows infiltrated or non-infiltrated areas. Using this leaf

infiltration technique, it was demonstrated in this study that stomatal aperture across a leaf is not uniform for either control or PRD-treated vines and that the degree of 'stomatal patchiness' correlates with stomatal conductance.

Cardon *et al.* (1994) have reported that a non-uniform stomatal closure determines the photosynthetic activity through a restriction of CO₂ diffusion into the leaf. They also found that a very dynamic activity in stomatal action in leaves can occur thereby locally alternating the internal CO₂ concentration producing incongruent patterns of photosynthetic activities. It is believed that, even under mild stress conditions, a non-uniform stomatal aperture plays a role in controlling excessive water loss restricting stomatal conductance in field-grown vines. PRD may stimulate such dynamic processes thereby influencing stomatal conductance. Results in this study support recent findings that stomatal conductance is reduced in grapevines exposed to PRD (Dry & Loveys, 1999), and it follows that patches with lower stomatal aperture occur more frequently in PRD vines. By varying stomatal aperture over the leaf blade, CO₂ uptake and transpirational water loss can be delicately manipulated. However, the extent to which this occurs was not determined in this study. A method enabling the researcher to monitor leaf gas exchange and stomatal aperture simultaneously would be required to quantify this relationship. One recent approach used for estimating stomatal conductance on small patches of the leaf is the use of infra-red thermography which has broad application to the detection of stomatal behaviour (Jones, 1999). Images of chlorophyll fluorescence also provide a technique to measure the photosynthetic rate on a small scale in different parts of the leaf (Genty & Meyer, 1995) indicating stomatal conductance of small patches (Mott & Buckley, 2000).

Using the infiltration technique, the degree of infiltration depends on the surface tension of the liquid for infiltration (Beyschlag & Pfanz, 1990) and the pressure applied (Düring & Stoll, 1996). As a consequence, infiltration of areoles of a leaf blade at a given infiltration pressure will require a certain minimum threshold stomatal aperture. In this study, while the pressure applied was not measured, every effort was made to apply a constant force to all leaf segments. There is a possibility that variations in infiltration pressure could have influenced the degree of infiltration. At high stomatal conductance, a low pressure is sufficient to infiltrate a high percentage of the leaf blade. If however, the pressure was relatively high, patches with a low mean stomatal

conductance may also have become infiltrated. To better quantify and compare results to those published in the literature a standardisation of the infiltration technique used for leaf infiltration is essential. Despite the use of a relatively crude method in this study, however, there was a clear trend in the relationship between infiltrated area and stomatal conductance.

In addition to the ecological significance of non-uniform stomatal aperture, this phenomenon must also be an important consideration for interpretation of any calculations made from leaf gas exchange measurements. Gas exchange systems assume in their calculation programs that the stomatal aperture is homogeneously distributed over the leaf area and use a specific leaf area to calculate assimilation and transpiration data. Thus, when stomatal aperture is non-uniform, the values generated may be misleading. Calculation of stomatal conductance, internal CO₂ partial pressure and photosynthetic rate may be underestimated if the area used for the calculations is bigger than the actual area with open stomata (Terashima et al., 1988; Mott, 1995). Thus far, however, there is no method to monitor both non-uniform photosynthesis and non-uniform stomatal aperture at the same time. In this study, leaf gas exchange measurements and the infiltration technique were applied to the same leaf to characterise effects of soil drying on stomatal aperture.

Apart from non-uniform stomatal aperture, leaf gas exchange measurements have shown that PRD makes more efficient use of water available. The experiment where soil water penetration was manipulated will be discussed below. The experiment where twice the amount of water was applied to control vines showed that the stomatal conductance under PRD was reduced relatively to control. This confirms recent observations of transpirational water loss from PRD-treated grapevines (Dry & Loveys, 1999). Furthermore, it was found here that changes in stomatal conductance had only minor effects on assimilation rate under PRD and this result concurs with a recent demonstration that application of a mild water stress only leads to a minor decrease in assimilation rate (Flexas *et al.*, 1999). In that case a substantial reduction in assimilation rate was measured when grapevines were exposed to a severe drought. In the current study a detrimental reduction in assimilation rate under PRD was not observed.

Minor changes in assimilation rate in association with substantial changes in stomatal conductance may be explained by the fact that the dynamics of the assimilation rate and stomatal conductance are different. Barradas & Jones (1996) reported that the assimilation rate can increase faster than the rate of increase of stomatal conductance when beans were exposed to different light conditions. After the assimilation rate reached saturation a further increase in stomatal conductance had no effect on assimilation rate. A higher water loss by the vine via stomata without an associated improved rate of CO₂ uptake could then be considered a luxurious consumption if no other physiological processes were affected.

It was proposed more recently that stomata in well adapted plants could play a major role in limiting the plant water loss but play a relatively small role in determining the rate of photosynthesis (Jones, 1998). If, by using PRD, the stomatal conductance can be significantly reduced with only minor effects on photosynthesis, then PRD will improve the transpiration efficiency of grapevines without detrimentally changing the assimilation rate. For this reason, it is of particular importance to focus on mechanisms controlling stomatal aperture in response to soil drying.

Manipulating soil water content at various soil depths affected stomatal conductance to a greater extent when the same amount of water was applied to a larger soil surface area (control vines), compared to the PRD-treated vines where the same amount of water was applied to a relatively smaller soil surface area. Stomatal conductance was used as an indicator of plant water stress. Earlier reports demonstrated that water stress in grapevines occurs at a fairly low soil water content and that vines can withstand a considerable amount of soil desiccation (Kriedemann & Smart, 1971; van Zyl, 1987). If the available amount of water allocation is limited, however, the use of PRD will result in a smaller proportion of the root system being exposed to drying soil conditions. Hence, it can be speculated that yield is more likely to be maintained or improved by a more efficient water use under PRD compared to conventional irrigation practices. The implications of this will be discussed later in Chapter 8.

One consistent observation during this study was that changes in stomatal conductance were not associated with changes in shoot water status, indicated as leaf water potential. Under conditions of water stress the leaf water potential in grapevines remains relatively high in a range of -0.6 to -1.8 MPa (Winkel & Rambal, 1993)

compared to other species where much lower leaf water potentials are reported; e.g. apricots (-2.0 to -2.5 MPa; Torrecillas *et al.* 1999) or almonds (-2.8 to -4.7 MPa; Germana, 1997). Matthews *et al.* (1987) reported that leaf water potential in *Vitis vinifera* L. cv. Cabernet Franc is only affected under severe water stress. However, the control and PRD-treated vines showed constant leaf water potentials throughout the experiment ($P>0.05$). This suggests that the stomatal control is very active in preserving a high degree of hydration in grapevine leaves.

Since roots are the first plant organs exposed to changes in soil water status, considerable emphasis has been placed in recent years on root-shoot signalling as a mechanism to explain how plants detect drying soil conditions and subsequently communicate this message to shoots. ABA is a strong candidate as a signalling molecule, since it is believed not only to affect stomatal closure (Tardieu *et al.*, 1992) but it is also present in roots and indeed, it has been found that soil drying causes an increase in root [ABA] (Davies *et al.*, 1989) and xylem sap [ABA] (Loveys, 1984a).

The amount of root area exposed to drying soil conditions, therefore, may influence the amount of any signal that is synthesised. There are few studies, however, that have investigated the root distribution of field-grown grapevines and these have shown that the distribution of grapevine roots is not uniform throughout the soil profile and that root density declines with depth (Penkov, 1965; Stoll *et al.*, 2000a). If such chemical signals, in particular ABA, are synthesised in root tips (Zhang & Davies, 1987), then the ABA concentration might be dependent on the number of root tips exposed to drying soil conditions. In the current study, a higher proportion of roots would have been exposed to low soil water content in control vines, due to a substantial decrease of soil water content in the soil layers between 0.65 to 0.75 m which was observed when the same amount of water was applied over a bigger soil surface area. Correspondingly, it was found that stomatal conductance was lower in control vines compared to PRD-treated vines where a smaller part of the root system was exposed to soil desiccation whilst the soil water content on the other side was fully restored at soil layers deeper than 0.65 to 0.75 m. These observations are consistent with an important role of root signals in the regulation of stomatal conductance.

Measurements of ABA concentration in leaves showed only a poor relationship between bulk leaf [ABA] and stomatal conductance when [ABA] was measured in

either total leaves or leaf segments. It was thought that by analysing only certain segments of leaves, the [ABA] distribution over the leaf surface may be better described. Such leaf segments are still large enough, however, to represent bulk leaf concentrations.

Individual guard cells are the primary site of stomatal response and thus, localisation and compartmentation of bulk leaf [ABA] is essential to interpret stomatal responses to ABA (Slovik *et al.*, 1995; Hartung *et al.*, 1998). Popova *et al.* (2000) has recently discussed the importance of foliar compartmentation and liberation of compartmentalised ABA which cannot be detected on crude bulk leaf samples used in my study.

Experiments described in this chapter support the ideas of the importance of root-derived chemical signals manipulating stomatal conductance. Although the nature of the signal may not be completely resolved, ABA appears to be a strong candidate. There is however some evidence that compounds other than ABA, that is variations in xylem sap pH or cytokinins, may also play a role as signals in root to shoot communication (Blackman & Davies, 1985; Gollan *et al.*, 1992). Chapter 5 will focus on the occurrence of some other chemical signals and their role in the root to shoot communication.

4.5 Conclusions

Experiments in this chapter examined the effect of manipulating soil water conditions on transpirational water loss. The following may be concluded:

- 1) Applying the same amount of water to a larger soil surface area resulted in reduced soil water content in soil layers at greater depth. Under these conditions stomatal conductance was lower than when the same amount of water was applied to a smaller soil surface area and the soil water consequently reached deeper soil layers. Sensitive manipulation of stomatal conductance by the soil water status becomes important when only a limited amount of water is available.

- 2) Non-uniform stomatal aperture across a given leaf occurred in field-grown grapevines under natural environmental conditions in both control and PRD-treated vines.
- 3) The proportion of an infiltrated leaf segment was represented by leaf patches with more open stomata, and this correlates with stomatal conductance measurements taken by a porometer.
- 4) Total bulk leaf ABA was weakly related to stomatal conductance and to the degree of infiltrated areas.
- 5) Bulk leaf ABA concentrations were the same in leaf segments taken from close to terminal veins or between lateral veins.

Chapter 5 Changes in chemical signals induced by PRD

5.1 Introduction

In previous chapters it has been shown that by manipulating soil water content thereby exposing part of the grapevine root system to drying soil conditions, a reduction in shoot growth and stomatal aperture occurred. These results support some of the recent findings on grapevine response to PRD (Loveys *et al.*, 1997; Dry *et al.*, 2000a).

It was thought for a long time that when grapevine roots were exposed to drying soil conditions, the restricted access to water resulted in a change in plant water status, leading to a reduction in leaf water potential or turgor, thereby affecting gas exchange and growth (Matthews & Anderson, 1989).

There is now substantial evidence in the literature that chemical regulation of shoot physiology occurs in field-grown plants before there are measurable changes in plant water status (Davies *et al.*, 1994; Dodd *et al.*, 1996; Jackson, 1997). Roots must therefore be able to sense changes in soil water status, which is communicated to the shoots, eliciting a response. It has been proposed that roots mediate the gas exchange characteristics of shoots in response to drying soil by transferring the information via the xylem to the shoot (Jones, 1980).

Using plants with a split-root system Gowing *et al.* (1990) demonstrated that many effects of water stress could be explained in terms of the transport of chemical signals from roots to shoots and showed that by excising the dried part of the root system, where chemical signals are proposed to be synthesised, water stressed plants fully recovered. ABA is a strong candidate as one positive root-sourced messenger which is possibly involved in the response of shoots to drying soil conditions. It has been found that drying soil conditions can be correlated with an increase in root ABA and it has been suggested that ABA moving from roots to shoots could be one effective way for plants to regulate stomatal behaviour (Loveys, 1984b). Much evidence which suggests that ABA can influence stomatal behaviour in many plant species has now been accumulated (Loveys, 1984a; Waringer *et al.*, 1990; Tardieu *et al.*, 1992).

If an increase in xylem sap ABA concentration can be described as a 'positive' signal from the roots (Davies & Zhang, 1991) it follows then that a different sort of signal

involving a decrease in synthesis or signal transport could then be acting as a 'negative' signal (Jackson & Kowalewska, 1983). Studies by Blackman & Davies, (1985) on plants growing in drying soil suggested that a continuous supply of cytokinins from roots was necessary to sustain stomatal aperture and suggested that cytokinins provide a 'negative' signal in root to shoot communication. If that was the case then roots may experience initially reduced cytokinin synthesis in response to drying soils. Fusseder *et al.*, (1992) found, however, that even when cytokinin concentrations in leaves were high, stomata still did not open fully if the ABA concentration in xylem sap was high at the same time.

Apart from their controversial role in regulating stomatal aperture, cytokinins (CK) are also known to act as growth promoters. Mullins *et al.* (1992) characterised growth of grapevines by a dominant apex and weaker lateral bud growth which is supportive of results presented in Chapter 3. Considerable differences in the growth of the apical shoot and lateral shoots were interpreted as a response to differences in cytokinin levels in each tissue (Wilson, 1981; Bollmark *et al.*, 1995). Roots are thought to be the major source of cytokinins, which are translocated to aerial tissues (Letham, 1994), but due to the difficulties of accessing roots of field-grown plants, few studies have attempted to measure hormonal concentration close to the site of synthesis. If root cytokinin levels are affected by drying soil conditions (Blackman & Davies, 1985), there may be important implications for the PRD irrigation technique.

Experiments described in this chapter focus on measurements of both ABA and cytokinins in roots and xylem sap. The experiments described in this chapter tested the hypothesis *that changes in stomatal conductance of grapevines in response to PRD are associated with changes in chemical signals in roots and xylem sap.*

5.2 Material and Methods

5.2.1 Measurement of xylem sap pH and ABA concentration during a diurnal cycle

For this experiment vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted to *Vitis champini* cv. Ramsey rootstock) were grown in the Alverstoke vineyard of the University of Adelaide.

Control vines received twice the amount of water as PRD-treated vines during the whole season using drip emitters with equal flow rate (2 Lh^{-1}) for both treatments. Soil water content was monitored using an EnviroScan® (Sentek, Adelaide, South Australia) soil moisture instrument at various depths.

Measurements during a diurnal cycle were conducted on the 2nd of February 1997 from sunrise to sunset. The maximum temperature on this day was $32.2 \text{ }^{\circ}\text{C}$ with an average relative humidity of 58%. The last alternation of ‘wet’ and ‘dry’ sides prior to the 2nd of February 1997 was the 21st of January 1997, after which the vines had received 4 irrigations of 18.2 and 9.1 L/vine each for control and PRD-treated vines respectively.

Stomatal conductance was determined using a portable porometer (AP4, Delta-T, Cambridge, UK). The instrument was calibrated using the supplied calibration plate every 2 hours. Five fully sun exposed leaves were used to determine the stomatal conductance of each ‘test-vine’. Measurements were taken at hourly intervals.

In conjunction with stomatal conductance measurements, leaf water potential was measured every second hour using a pressure bomb and xylem sap was sampled for later ABA analysis (Section 2.6.). Xylem sap was collected from the petioles of 9 leaves from each ‘test-vine’. Three samples were then combined to form one replicate so that, in total, there were 3 replicates per vine.

Xylem sap pH was determined from thawed samples using a cyberscan® pH meter (Model 2500, Eutech Instruments, Singapore) attached to a microelectrode (Mi 415; Microelectrode INC., Bedford, USA) which was capable of measuring volumes less than $20 \text{ } \mu\text{L}$.

ABA concentration in xylem sap was determined from $30 \text{ } \mu\text{L}$ of each thawed sample. An internal standard of [$^2\text{H}_6$]ABA (20 ng) was added to each sample before they were

dried, methylated with ethereal diazomethane, dried and redissolved in methanol for GC/MS analysis (Section 2.7.2.).

5.2.2 Determination of ABA in xylem sap during an irrigation cycle

The same vines and conditions as described in the previous experiment (Section 5.2.1) were used to determine the effect of PRD on stomatal conductance and xylem sap ABA concentration.

The 'wet' and 'dry' sides were alternated on the 18th of February 1997 just before starting the experiment, and also on the 28th of February 1997.

The same methods used in Section 5.2.1 for measuring stomatal conductance, collecting xylem sap and quantifying ABA concentrations were used here. Measurements on 'test vines' were taken between 10 am and 1 pm on cloudless days. Three xylem sap samples from the same vine formed one replicate which was immediately frozen in liquid nitrogen. Three replicates per vine were collected from each 'test vine'.

5.2.3 Determination of ABA and Cytokinins in roots

Two year old grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon split-root) grown in the Alverstoke vineyard of the University of Adelaide were used to determine effects of PRD on concentrations of ABA and cytokinins in roots. The total amount of water applied for control and PRD vines was the same for both treatments. Samples were taken at midday (10 am to 1 pm) every third day between the 9th and 20th of January 1999 (Figure 5.1).



Figure 5.1 Root sampling for ABA and cytokinin determination. **A)** steel tube and sledge hammer used for root sampling. **B)** soil and root sample stored in plastic bag.

Soil cores were collected from both sides of control vines, and from ‘wet side’ and ‘dry side’ of PRD-treated vines separately by using a 700 mm steel tube (internal diameter: 55 mm; Figure 5.1 A). The end of the steel tube was sharpened to facilitate easier cutting through the soil and roots. Samples were taken in 4 places on a circle (radius 100 mm) around the drip emitter. Each soil core was transferred to a plastic bag and stored on ice during transport (Figure 5.1 B). The roots were separated from the soil in the laboratory under low light conditions and stored at $-40\text{ }^{\circ}\text{C}$ until required for analysis.

ABA and cytokinins were extracted from tissue as described in Section 2.8. A simplified description of the analytical process is shown in Figure 5.2.

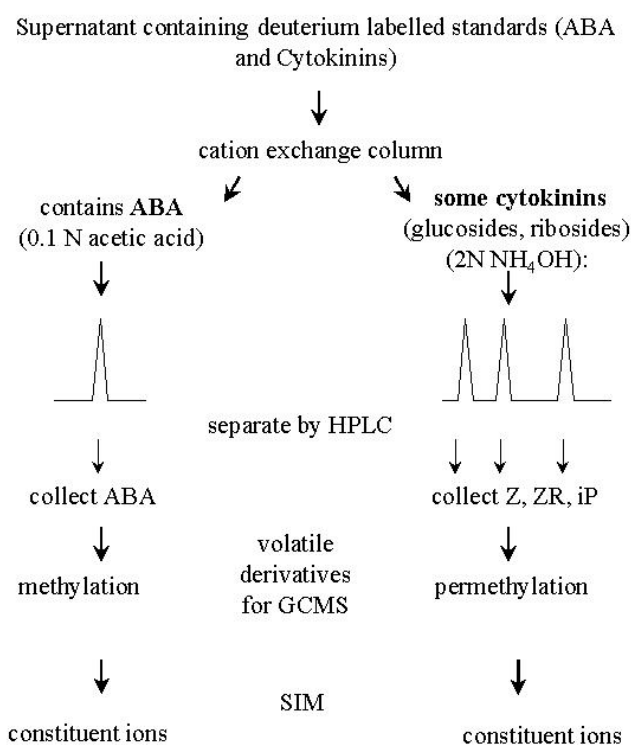


Figure 5.2 Simplified flow diagram for ABA and cytokinin extraction from plant tissue

5.2.4 Determination of cytokinins in different parts of shoots

Vines (*Vitis vinifera* L. cv. Cabernet Sauvignon grafted to *Vitis champini* cv. Ramsey rootstock) grown in the Alverstoke vineyard of the University of Adelaide under the same conditions as described in Section 5.2.1 were used to determine cytokinin concentration in different positions of shoots for both treatments. Furthermore, the effect of releasing the apical dominance after decapitating the shoot tip was tested.

Terminology for the different shoot tissues of the grapevine have been borrowed historically from the French (Mullins *et al.* (1992)). According to the terminology of Mullins *et al.* (1992), the first-formed bud arising in each leaf axil is a prompt bud

(*prompt bourgeon*) which grows out and forms a lateral shoot known as summer lateral (*entre-coeur, rameau anticipé*).

Shoot tips and prompt bud samples were taken towards the end of an irrigation cycle on the 23rd of December 1998, 26th of January 1999 and on the 4th of February 1999 between 8 am and 11 am (Figure 5.3).

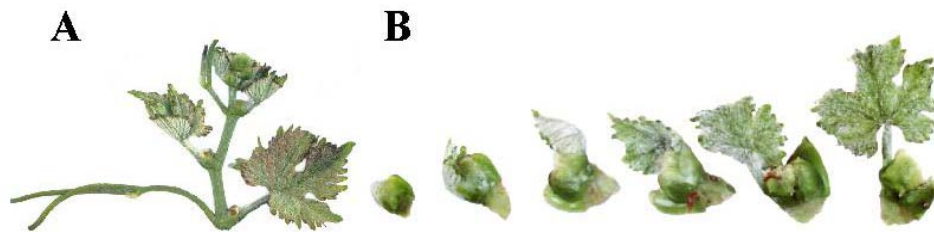


Figure 5.3 Shoots tissue sampled for determination of cytokinins (Cabernet Sauvignon / Ramsey rootstock split-root vines). **A)** shoot tip with one unfolded leaf **B)** prompt buds and developing summer laterals

To determine the effect of decapitating the shoot tip on cytokinin concentration in prompt buds and summer laterals, 9 shoots per ‘test vine’ were selected at random. The shoot tip of each shoot was decapitated below the first unfolded leaf (Figure 5.3 A) and immediately frozen in liquid nitrogen. Prompt buds and summer laterals were cut out using a razor blade. The excised tissue may also have included a very small proportion of tissue of the primordial latent bud, but this was not taken into consideration. The samples were then immediately frozen in liquid nitrogen. Samples of 6 prompt buds and summer laterals, below the first expanded leaf (Figure 5.3 B), were taken at three different times. The first sample was taken at the beginning of the experiment ($t=0$) at the same time that the shoot tips were sampled. The second sample was taken 6 hours after decapitating the shoot tip ($t=6$ h) and the third sample 18 hours after the shoot tip was released ($t=18$ h).

The tissue extraction for cytokinin analysis was performed as described in Section 2.8 except they were only analysed for cytokinins (zeatin, zeatin riboside, zeatin glucoside and iso-pentenyl adenine) so that eluates of the HOAc wash were not used for ABA analyses.

5.3 Results

5.3.1 Diurnal measurements

The soil water content for both control and PRD irrigation regimes on the 2nd of February 1997 are shown in Figure 5.4. The soil water content at most of the soil layers on the ‘wet side’ of PRD-treated vines was similar to that on both sides of control vines. On the ‘dry side’ of PRD-treated vines, the soil water content was substantially reduced in each soil layer relative to control by an average of 30%.

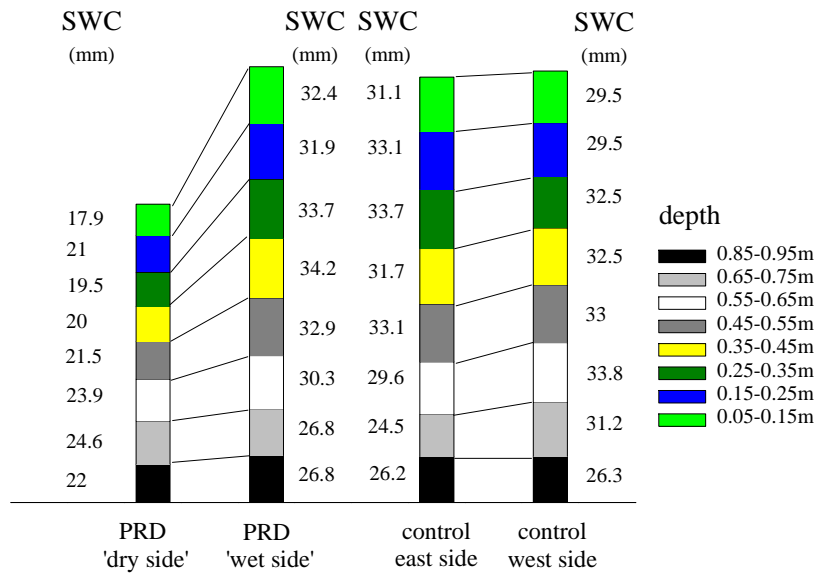


Figure 5.4 Soil water content (SWC; mm) at different soil layers depth classes (depth; m) (Cabernet Sauvignon / Ramsey split-root vines). PRD: at any time water was withheld from one side of the vines; control: vines received water on both sides of the planting line.

Stomatal conductance followed a diurnal rhythm for both treatments with an increase of g_s in the morning, a reduction in the early afternoon followed by a slight recovery before a further reduction towards the end of the day (Figure 5.5 A). Mean stomatal conductance was on average 15% lower for PRD-treated vines than control over the course of the day except for the time between 9 am and 11 am when the conductance of the PRD-treated vines was 23% lower than control.

ABA concentration in xylem sap was significantly higher ($P < 0.05$) in PRD vines than in control vines at 10 am and 12 pm. ABA concentration was negatively associated with stomatal conductance and correspondingly the greatest differences in stomatal conductance between control and PRD vines occurred at the 10 am and 12 pm measurements (Figure 5.5 B). Neither the differences in stomatal conductance, nor the differences in ABA content were associated with any differences in leaf water potential (Figure 5.5 C). Leaf water potential (Ψ_L) for both treatments declined during the course of the day until mid afternoon and then increased towards the end of the day.

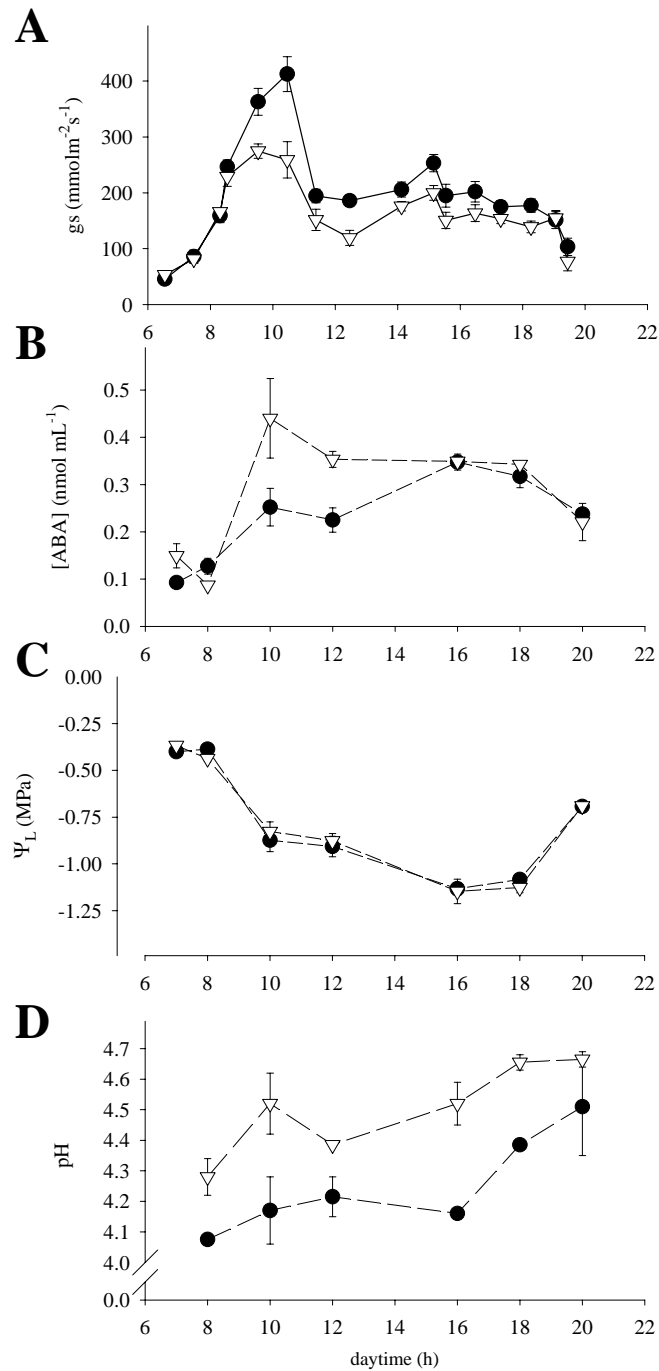


Figure 5.5 Effects of PRD on diurnal changes in stomatal conductance, xylem sap [ABA], leaf water potential and xylem sap pH (Cabernet Sauvignon / Ramsey rootstock split root vines; control (●): vines received water on both sides of the vine; PRD (▽): at any time to one side of the vine water was withheld). **A**) means of stomatal conductance (gs, mmol m⁻² s⁻¹, mean ± s.e.; n=12). **B**) means of xylem sap [ABA] (nmol mL⁻¹; mean ± s.e.; n=4). **C**) means of leaf water potential (Ψ_L, MPa; mean ± s.e.; n=4). **D**) means of pH (mean ± s.e.; n=4).

Figure 5.5 D shows the diurnal change in xylem sap pH. The pH for most of the measurements was significantly higher in PRD vines (0.24 units on average; $P < 0.05$) except for measurements taken at 8 pm, where, due to large variability, the difference was not significant.

5.3.2 Measurements during an irrigation cycle

The soil water content data for the irrigation cycle are shown in Figure 5.6 A,B. Stomatal conductance during the irrigation cycle was consistently lower in PRD-treated vines compared to control vines (Figure 5.6 C). Stomatal conductance was reduced on average by 28% in PRD vines compared to fully irrigated vines ($P < 0.05$). After day 11, stomatal conductance in both treatments increased. This response was also reflected in the ABA xylem sap concentration which was found to be lower when stomatal conductance increased and thus was negatively associated with stomatal conductance measurements (Figure 5.6 D).

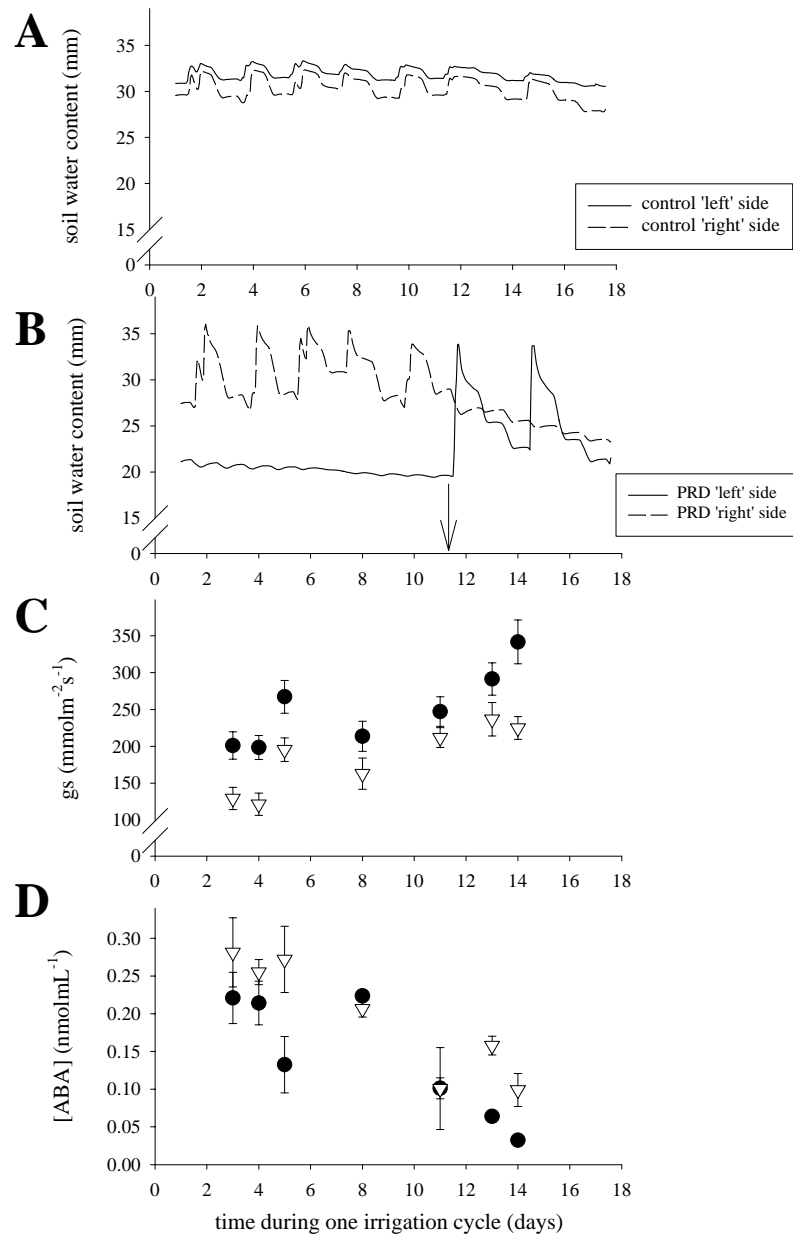


Figure 5.6 Effects of PRD on stomatal conductance and xylem sap [ABA] during an irrigation cycle (Cabernet Sauvignon / Ramsey rootstock split root vines; control (●): vines received water on both sides of the vine; PRD (▽): at any time to one side of the vine water was withheld). **A**) soil water content (mm) on either side of the planting line (control). **B**) soil water content (mm) on either side of PRD-treated vines; (PRD east side: water withheld until day 11; PRD west side: frequently irrigated until day 11; after day 11 alternating of the irrigation (↓)). **C**) mean stomatal conductance (gs, $\text{mmolm}^{-2}\text{s}^{-1}$; means \pm s.e.; n=24). **D**) means xylem sap [ABA] (nmolmL^{-1} ; means \pm s.e.; n=4)

5.3.3 Relationship between stomatal conductance and xylem sap [ABA]

The relationship between stomatal conductance and xylem sap [ABA] is shown in Figure 5.7. The xylem sap ABA concentrations tended to be higher when stomatal conductance was low. Also, PRD-treated vines tended to exhibit lower stomatal conductance than control vines. The variability in xylem sap, however, [ABA] was high and not found to be significantly increased by the PRD treatment ($P>0.05$), even though the stomatal conductance with a lower variability was significantly reduced ($P<0.05$). An ANOVA applied to stomatal conductance rate versus [ABA] for both control and PRD-treated vines showed no significant difference between treatments for the two data sets.

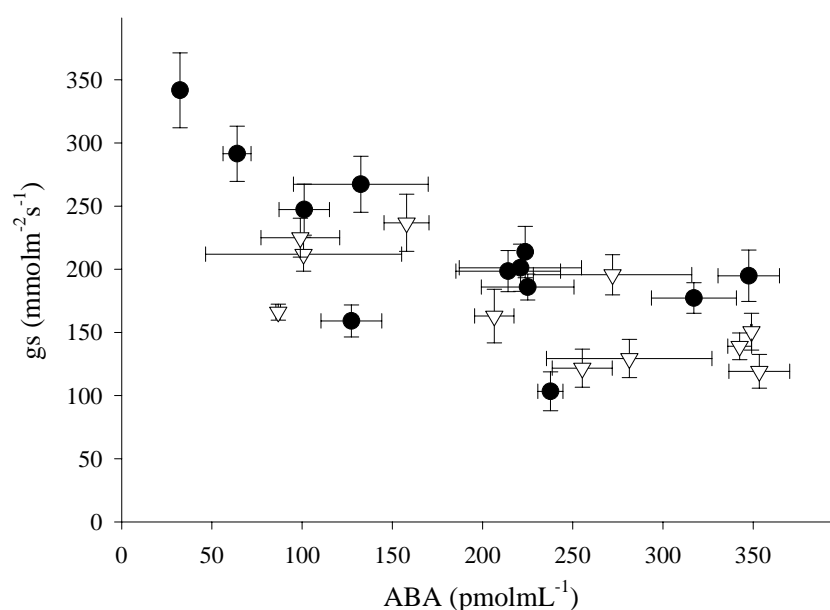


Figure 5.7 Effect of PRD on stomatal conductance (gs , $\text{mmolm}^{-2}\text{s}^{-1}$; $n=12$) as a function of xylem sap ABA ([ABA], pmolmL^{-1} ; $n=4$) in Cabernet Sauvignon / Ramsey split-root vines (control (●): vines received water on both sides of the vine; PRD (▽): at any time to one side of the vine water was withheld; means \pm s.e.; bi-directional).

5.3.4 Concentration of ABA and cytokinins in suberised roots

To determine the level of potential root-sourced signals with PRD, the concentration of ABA and cytokinins was measured in roots collected during an irrigation cycle (Figure 5.8). At this phenological stage all roots sampled were suberised.

It was found that the ABA concentration responded sensitively to soil water conditions (Figure 5.8 C). On day one, the day after alternating irrigation sides, the ABA concentration on the 'wet-side' (which was the 'dry-side' in the previous cycle) was higher than that on the current 'dry-side' (which was the irrigated side during the previous cycle). During the irrigation cycle, however, this changed dramatically: the root [ABA] on the 'wet-side' declined and the concentration on the 'dry-side' increased. With a decline in soil water content on the 'dry-side' to 5.4 and 5.2 mm at soil layers between 0.35 and 0.45 m and 0.65-0.75 m respectively (Figure 5.8 A, B), the ABA concentration in suberised roots gradually increased and had almost doubled (+ 93%) at day 10 compared to the beginning of the experiment.

On the 'wet-side', the ABA concentration declined and showed a similar response to the fully irrigated control. When the last set of samples was taken on day 10, the soil water content for fully irrigated vines and the 'wet-side' of PRD-treated vines had the lowest soil water content of any samples taken throughout the experiment, which may have affected the [ABA] and [CK]. When these last root samples were taken the soil water content had dropped to 14.3 and 16.2 mm for control and the 'wet-side' of PRD-treated vines respectively. The corresponding samples taken during the three previous irrigations had associated soil water content averaging 24.1 mm. Note also that the soil water content in the 0.65 to 0.75 m soil layer of control vines was not affected by any irrigation event except for that on day 1 (Figure 5.8 A, B).

During the irrigation cycle, the suberised roots of the 'dry side' of PRD-treated vines had a significantly lower cytokinin concentration than control vines. The cytokinin concentration in roots of control vines was also significantly higher on day 1 and day 4 ($P < 0.05$) than the 'wet side' of PRD-treated vines, after the irrigation sides were alternated.

As a consequence of a reduction in cytokinins and increase in ABA during one irrigation cycle, the ratio of ABA to CK in roots on the 'wet' and 'dry' sides of PRD-

treated vines changed substantially towards the end of the irrigation cycle whereas this ratio remained constant for control roots ($P < 0.01$; Figure 5.8 E).

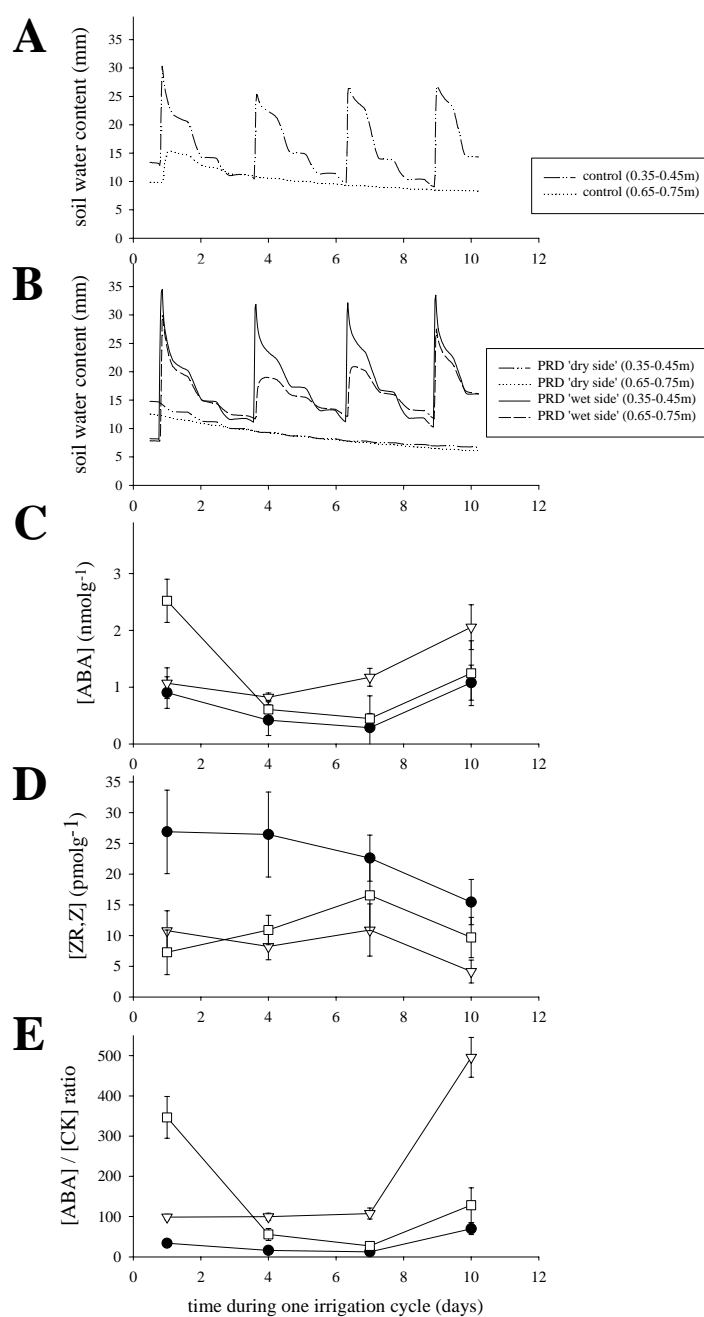


Figure 5.8 Effect of PRD on soil water content, root [ABA], root [cytokinins] and the ratio of [ABA] / [cytokinins] (Cabernet Sauvignon / grafted on Ramsey split-root vines; control (●): vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld (PRD 'dry side' (▽); PRD 'wet side' (□)). **A**) soil water content (mm) on one side of control. **B**) soil water content (mm) on either side of PRD-treated vines. **C**) root [ABA] (nmol g^{-1}) **D**) root [zeatin] and [zeatin riboside] (pmol g^{-1}) **E**) ratio of [ABA] / [cytokinins].

5.3.5 Measurements of cytokinin concentrations in shoots at different positions

Of the four cytokinins analysed, zeatin and zeatin riboside were the two most abundant cytokinins in grapevine shoot tips and prompt buds/young lateral shoots. The concentrations of zeatin glucoside and iso-pentenyl adenine were less than 3 pmol g^{-1} or not detectable for many samples.

Cytokinin concentration in shoot tips was affected in response to PRD: the concentration of zeatin and zeatin riboside was significantly reduced by 50 and 47% respectively in PRD vines relative to controls ($P < 0.05$; Table 5.1).

Zeatin and zeatin riboside concentration of prompt buds and summer laterals was always lower than in shoot tips at all sampling times for both treatments. The cytokinin concentration of prompt buds and summer laterals had a tendency to increase in control vines over the 18 hours following decapitation of the shoot tip, whilst in PRD-treated vines the concentration was unchanged. Eighteen hours after the shoot tip was decapitated, the concentration of zeatin and zeatin riboside in control vines increased by 15% and 28% respectively but did not change in PRD-treated vines.

When the average ratio of zeatin riboside to zeatin in shoot tips was calculated over the whole experiment it was found to be similar, that is 1.46 for control and 1.40 for PRD-treated vines. At $t=0$, the ratio of zeatin to zeatin riboside in prompt buds for both treatments was slightly higher than in shoot tips (control 1.75 and PRD 1.64) but values for control and PRD-treated vines were not significantly different. The ratio of zeatin to zeatin riboside in prompt buds and summer laterals of PRD-treated vines was unchanged during the 18 hours of the experiment whilst in control vines the ratio increased from 1.63 to 2.01 ($P > 0.05$). The minor increase in zeatin to zeatin riboside ratios of prompt buds and summer laterals was due more to changes in zeatin riboside than in zeatin.

Table 5.1 Zeatin and zeatin riboside concentration (pmol/g fresh weight) in shoot tips and prompt buds and summer laterals (Cabernet Sauvignon / Ramsey rootstock split-root vines; means \pm s.e.; n=3)

tissue	cytokinins	control (pmol/g)	PRD (pmol/g)	% diff. (PRD compared to control)	signif.	zeatin rib./zeatin ratio (control)	zeatin rib./zeatin ratio (PRD)
shoot tips						1.5	1.4
	zeatin riboside	31.1 \pm 4.1	15.6 \pm 1.6	49.8	P<0.05		
	zeatin	21.3 \pm 2.74	11.2 \pm 1.13	47.4	P<0.05		
prompt buds and summer laterals (t=0)						1.8	1.6
	zeatin riboside	18.1 \pm 2.3	14.9 \pm 1.2	17.1	n.s.		
	zeatin	10.3 \pm 1.5	9.1 \pm 0.7	12.3	n.s.		
prompt buds and summer laterals (t=6hours)						2.0	1.6
	zeatin riboside	21.9 \pm 3.7	14.2 \pm 1.8	35.1	P<0.05		
	zeatin	10.7 \pm 1.9	8.78 \pm 0.9	17.8	n.s.		
prompt buds and summer laterals (t=18hours)						2.0	1.6
	zeatin riboside	23.2 \pm 5.3	13.8 \pm 2.6	40.5	n.s.		
	zeatin	11.8 \pm 1.8	8.4 \pm 2.5	39.2	n.s.		

5.4 Discussion

Field-grown grapevines (*Vitis vinifera* L. c.v. Cabernet Sauvignon either on own roots or grafted to Ramsey rootstock split-root vines) were used to demonstrate that physiological changes in response to PRD are associated with root-sourced chemical signals which are transported to the shoots.

One major concern in regards to quantifying chemical signals lies in the method of signal analysis. The two most common techniques are physico-chemical or immunological techniques which were recently reviewed by Hooykaas *et al.* (1999). Introducing an improved physico-chemical protocol for measurement of ABA and cytokinins using a combined extraction has the potential to quantify both chemical signals from the same sample. This may be of importance if both are involved in the root to shoot communication during water stress. A potential disadvantage is that the

analytical protocol requires a larger sample volume and the preparation and purification is more time-consuming than immunological techniques (Dodd *et al.*, 1996b). It was found during this study, however, that extensive purification of the crude grapevine samples which frequently contained significant quantities of phenolic compounds was essential for better quantification. The protocol was developed using radioactively labelled ABA and cytokinins which enabled monitoring and optimisation of each purification step to improve the sample recovery at each step and automation of many purification steps.

Although grapevine leaves can be considered to show a high bulk leaf ABA concentration, that of field-grown vines measured in Chapter 4 was not closely correlated to stomatal conductance. A minor increase in bulk leaf ABA in leaves of Sultana under PRD associated with a significant decrease in stomatal conductance has also been reported by Stoll *et al.* (2000b). These studies did not consider where the accumulated ABA was localised. A sequestration has been found to be primarily a function of pH gradients within the leaf which favours retention of ionised ABA within compartments at higher pH (Slovik *et al.*, 1995). It can be speculated that the bulk of accumulated ABA in leaves may not be important in regulation of stomatal conductance. It is more likely that the ABA concentration in roots, where soil water deficit is first sensed and which may then affect the [ABA] in xylem sap, is of greater importance when considering the role of chemical signals in the context of root to shoot communication. Jia & Zhang (1999) interpreted their measurement of ABA accumulation in the absence of an associated further effect on stomatal conductance as the result of xylem derived ABA being rapidly metabolised in the leaves. This was concluded from the observation that applying ABA together with an inhibitor substantially reduces ABA metabolism. The inhibition of ABA metabolism by tetrazolacis did not lead to more stomatal closure, which was still concentration-dependent. Since the accumulation of xylem-derived ABA was enhanced substantially by the presence of tetrazolacis, these results strongly indicate that stomata mainly respond to the prevailing ABA concentration in the xylem stream, rather than to the accumulated amount of xylem-derived ABA in the leaves.

In this study, a much stronger treatment effect was found when ABA levels in roots and xylem sap of control and PRD-treated vines were compared. Results of ABA

measurements in roots during this study showed that in PRD-treated vines the [ABA] was increased on the 'dry-side'. ABA is likely to become available to cause stomatal response through translocation via the xylem. This concurs with the idea of Jia & Zhang (1999) and Tardieu *et al.* (1996) that stomatal closure is positively related to the root-sourced [ABA] in xylem sap which can be stimulated under drying soil conditions.

The quantification of hormonal messages in both roots and xylem sap is, for many reasons, not a simple task. Roots are relatively inaccessible, particularly in a field situation. The variability of root distribution, vine size or soil type in field experiments is much greater compared to vines grown in a controlled glasshouse environment where many of the variables can be better controlled. To enable easier access to the roots of field-grown vines, the split-root vines were planted in a trench which was refilled with a sandy soil (Appendix 2), thus creating a fairly homogenous soil substrate. The soil conditions chosen for this experiment were expected to reduce the impact of soil variability on vine hormonal status, as it had previously been shown that root [ABA] can be altered when soil is compacted (Hartung *et al.*, 1994), or in response to changes in nutritional status (Chapin, 1990). The soil volume per vine in this study can be considered to be large (3.5m^3) and there should not have been any restriction on root growth. This is important because restriction of root growth can affect the synthesis of plant hormones. The problem of root restriction is more likely to occur in potted vines and may result in an increased [ABA] or a reduction in shoot growth (Liu & Latimer, 1995).

Cytokinins are known to increase stomatal conductance (Incoll & Jewer, 1987) and are synthesised in roots (Itai & Vaadia, 1965). The same sphere of activity but with opposing effects on stomatal conductance may also apply to ABA. Roots that are exposed to drying soil conditions can be considered as one important site of ABA synthesis in plants (Loveys, 1984a). Analysis in this study of both ABA and cytokinins in roots from the same tissue samples has highlighted that both groups of plant hormones are present in roots and that their concentrations may become substantially altered during variations in soil water conditions. Due to a reduction in cytokinins and an increase in ABA on the dried side of PRD-treated vines, the ratio of these hormones was substantially changed during an irrigation cycle. Bano *et al.* (1993) found in rice that severe water stress applied to protruding roots resulted in an increase in the ABA to

CK ratio from 15 to 500 fold. Blackman & Davies (1983) have suggested that a continuous supply of cytokinins is important to sustain a high stomatal conductance. Effects of cytokinins on stomatal conductance will be further discussed in Chapter 6.

Most xylem ABA originates in the roots (Wolf *et al.*, 1990). As ABA must be transported to the shoots via the xylem the [ABA] in xylem sap is important in the regulation of stomatal conductance as shown for grapevines by Loveys (1984a) and Correia *et al.* (1995). Further evidence for the role of xylem [ABA] in the regulation of stomatal conductance resulted from experiments where either solutions containing ABA (0.36 μ molar (+)ABA) or ABA-free solutions were fed to excised grapevine leaves (Loveys, 1992). It was found that in leaves fed ABA the transpiration rate could be substantially decreased when the leaf to air vapour pressure gradient was increased. By comparison, in leaves fed ABA-free solutions, the transpiration rate increased significantly. The elevated concentration of ABA found in xylem sap of PRD-treated vines in this study may similarly contribute to a greater reduction in stomatal conductance compared to control vines, allowing the PRD-treated vines to more effectively respond to changes in environmental conditions. When the xylem sap [ABA] was measured over a 15 day period the weather conditions before and after alternating the irrigated side were quite different and this possibly resulted in a continuous decline in the xylem sap [ABA] during the experiment which might be caused by changes in the evaporative demands during this period. When the experiment started and during the first irrigation cycle, the average daily maximum temperature was 36.9 °C and the relative humidity was 38.5%, which resulted in greater transpiration efficiency of PRD-treated vines and higher xylem sap [ABA] compared to controls. During the second irrigation cycle, when the maximum temperature dropped to 25.7 °C and relative humidity increased to 65% stomatal conductance in control vines increased substantially and was negatively related to the xylem sap [ABA]. At the same time PRD-treated vines showed a greater transpiration efficiency with significantly higher xylem sap [ABA].

Jackson (1997) addressed the possibility that ABA flux is more influential on stomatal aperture than ABA concentration and measured the flux of ABA in conifers during a drought cycle. It was found that ABA flux in droughted plants in the middle of the day was usually no higher than that of controls. Conversely, it was found that ABA

flux in droughted plants was higher than in controls in the morning, and it was postulated that stomata are responding throughout the day to these 'morning doses' of ABA (Jackson, 1997). Jia & Zhang (1999) have shown that the higher the [ABA] concentration, the shorter the time until there is a stomatal response. In this study a PRD induced reduction in stomatal conductance occurred early in the day and this was associated with a higher xylem sap ABA concentration. During the day the flux of ABA from roots to shoots was increased only slightly (ca. 30%) and the changes in bulk leaf ABA were relatively small. Although not measured, it can be speculated that the flux of ABA from roots to shoots during the morning was higher in PRD vines causing a faster stomatal response in those vines compared to control vines.

When diurnal measurements were taken it was found that xylem sap [ABA] varied over the course of the day and furthermore, the leaf water potential changed substantially in both control and PRD-treated vines. Changes in leaf water potential are also known to affect the sensitivity of stomatal conductance to ABA and leaves showing a lower Ψ_L have a higher sensitivity to [ABA] than leaves with a higher Ψ_L (Tardieu & Davies, 1992). The interaction between [ABA] and leaf water potential may provide some explanation for the sometimes weak relationship between ABA and stomatal conductance observed during this study. The stomatal response might be further complicated when, apart from variations of [ABA] and Ψ_L , differences in xylem sap pH and cytokinins are also considered. The increase in xylem sap [ABA] in PRD-treated vines was associated with an increase in xylem sap pH in PRD-treated vines and this factor may also have contributed to the stomatal response. Wilkinson & Davies (1997) found that the transpiration of *Commelina communis* leaves was reduced as the pH of xylem sap increased and that this effect was only observed with the presence of ABA in the transpired solution.

When cytokinin concentrations in different parts of the shoots were measured it was found that concentrations in fast-growing shoot tips were higher in control vines than in PRD-treated vines. Growth control in woody plants involves close interdependency between the roots and shoots. Roots depend on leaves for photosynthates and hormonal regulators whilst shoots depend on the supply of water, mineral nutrients and plant hormones such as cytokinins. The stimulating effect of cytokinins on shoot growth has been reviewed by Letham (1994) and it has also been found that the cytokinin

concentration in lateral buds influences bud growth (Turnbull *et al.*, 1997). Results in this study indicate that differences in cytokinin concentration, originating from roots and affected by PRD, play an important role in grapevine main and lateral shoot growth.

When shoot tips are decapitated, prompt buds and summer laterals take over as the dominant sinks for growth and it is suggested that due to the accumulation of cytokinins in axillary buds, growth becomes stimulated (Turnbull *et al.*, 1997). Cytokinins investigated in this study (ZR, Z, iP & ZG) are known to be the major active cytokinin compounds in plants and were detected in shoot tips, prompt buds and summer laterals. The ratio of zeatin riboside to zeatin (Table 5.1) for both shoot tips and prompt buds was similar with the exception of the control vines 6 and 18 h after shoot tips were decapitated. At this time a higher zeatin riboside concentration in control vines was measured. According to Beck & Wagner (1994) trans zeatin riboside is known to be one form of cytokinin transported in the xylem. The higher proportion of zeatin riboside could possibly be indicating a higher rate of transport from roots in control vines. Zeatin riboside concentration in well-watered roots was higher than in roots on the 'dry side' of PRD-treated vines and this may translate to higher lateral shoot growth of fully irrigated vines as described in Chapter 3.

Further support for the involvement of both ABA and cytokinins in root to shoot communication under PRD will be discussed in Chapter 6.

5.5 Conclusion

A new physico-chemical protocol to analyse ABA and some cytokinins from the same tissue sample was developed and applied in experiments to examine the role of chemical signals in response to PRD. It has been found that:

1. Variations in soil water conditions affected the xylem sap [ABA] and this was in turn related to changes in stomatal conductance both during a diurnal cycle and over the 12 days of an irrigation cycle.
2. Xylem sap pH was higher in PRD-treated vines than in fully irrigated vines.

3. The ratio of ABA to cytokinins in roots during an irrigation cycle was substantially increased on the 'dry-side' of PRD-treated vines compared to the 'wet-side' of PRD or fully irrigated vines.
4. Cytokinin concentration in shoot tips of PRD-treated vines was significantly reduced relative to control.
5. Cytokinin concentration in prompt buds and summer laterals was found to be lower than that in shoot tips for all treatments and showed a tendency to increase faster in control vines compared to PRD vines when the shoot tip was excised.

Chapter 6 Externally applied chemical signals mimic or override some effects related to PRD

6.1 Introduction

To test the specificity of any hormonal action in plants, effects which are proposed to be influenced by a single plant hormone should be mimicked either by external hormone application or by manipulation of the endogenous hormone level. These criteria of “correlation and duplication“ and “deletion and reinstatement” for the significance of a hormone effect were first proposed by Jacobs (1959) and later modified by Jackson (1987).

Most investigations of hormonal aspects of plant responses to drought have focussed on abscisic acid (ABA). An accumulation of ABA in wilted leaves was first observed by Wright & Hiron (1969). Since then much evidence has accumulated that ABA plays a central role in regulating stomatal aperture and thereby creating an early plant defence mechanism against water stress.

Different methodologies for the external application of ABA have been used such as applying the hormone through the petiole (Kriedemann *et al.*, 1972), direct application to the central vein of single leaves (Loveys, 1984b), foliar application (Sairam *et al.*, 1989), stem injection (Tardieu & Davies, 1993) or feeding the hormone to roots (Zhang & Davies, 1990). The result of many of these studies has been that ABA reduces water loss by restricting stomatal conductance. These observations, combined with the findings that roots in drying soil have substantially higher ABA concentration compared to these of well watered plants, led to the suggestion that ABA may act as a chemical signal in plants exposed to water stress (Kriedemann *et al.*, 1972).

Suggestions that cytokinins (CK) may also be implicated in response to water stress arose from observations of reduced CK concentration in xylem sap exuded from plants previously exposed to water stress (Itai *et al.*, 1968). Besides many growth-promoting effects on leaves and subtending buds (for review see: van Staden & Davey, 1979), cytokinins have been shown to affect stomatal aperture (Radin *et al.*, 1982; Blackman & Davies, 1985). Studies which have involved application of synthetic cytokinins such as

benzyladenine to leaves have shown that leaf application can stimulate growth (Radin et al., 1982) and support the idea of the possible role of cytokinin in growth regulation.

Both ABA and cytokinins are plant hormones which are defined as compounds synthesised in one part of the plant and translocated to another part where they initiate a physiological response (Salisbury & Ross, 1985). Changes to endogenous levels of both ABA and cytokinins during PRD have been observed and these were discussed in previous chapters. These results supported the original hypothesis that manipulating soil water conditions under PRD may alter the concentration of chemical signals. The aim of work presented in this chapter was to test the hypothesis *that externally applied chemical signals can mimic or override effects normally observed under PRD conditions*.

6.2 Material and Methods

6.2.1 Determination of the effect of benzyladenine application on stomatal conductance and growth

Six year old grapevines (*Vitis vinifera* L. cv. Chardonnay split-root) were grown in 75L pots in the open at CSIRO Plant Industry laboratories (Adelaide, Waite Campus). These plants were used to determine the effect of benzyladenine application on stomatal conductance and shoot growth in response to PRD. The vines were irrigated twice a week applying 4 L per irrigation to each side of the vine (control) or on only one side of the split-root vine (PRD). The irrigated sides of PRD-treated vines were alternated every 11 to 13 days. Soil water content was determined every second day during the experiment using time domain reflectometry (Trase system 6050 X1, Goleta, California, USA) with 0.15 m waveguides. The vines were grown in potting media as described in Section 2.1. Fertiliser (2gL^{-1} ; N-P-K 18+20+2) was applied to the grapevines 4 weeks after bud burst in October 1997. The experiment was conducted between 20th of December 1997 and the 9th of February 1998.

There were 8 vines each of PRD and control treatments. Four vines per treatment were sprayed with benzyladenine (BA; 6.5 μM ; Abbott Australasia) and 4 vines were sprayed with water. The sprays were applied at approximately 6 day intervals from mid November 1997 to mid February 1998 (Figure 6.1).

Stomatal conductance was determined on cloudless days as described in Section 2.5. Measurements were made on 5 leaves per vine between 10 am and 12 pm.



Figure 6.1 Foliar application of benzyladenine to split-root Chardonnay vines (pot volume 75L)

Each vine had 4 main shoots. To determine the shoot growth rate a reference node (5 nodes below the shoot tip) was labelled and the distance of that node to the shoot tip was measured every 3 days (between 20th of December 1997 and the 9th of February 1998). The shoot growth rate (cm/d) was determined and calculated as described in Section 3.2.1. The total length of each of the four main shoots and the total length of lateral shoots of each main shoot was measured at winter pruning in August 1998.

6.2.1 Determination of effects of ABA application on stomatal conductance and hormone concentration in primary roots

Three year old vines (*Vitis vinifera* L. cv. Cabernet Sauvignon, split-root vines) grown under glass-house conditions were used to examine effects of externally applied ABA on grapevine physiology.

Twelve split-root vines grown in standard potting mixture (Section 2.1) in 7 L pots were selected in winter and transferred to modified pots Figure 6.2 A. These modified pots had the original base replaced with a steel mesh (mesh size: 4 mm) and were placed on a second 7 L pot (Figure 6.2 B) which contained perlite (coarse horticulture perlite, Kewarra Lead, QLD, Australia).

For the first 2 months the vines were watered thoroughly on both sides and before the experiment began, all primary roots which had grown into the perlite were removed. The vines were irrigated to field capacity daily. Four of the vines received water to only one pot at any time (PRD); 4 vines received water on both sides (control); the other 4 vines were watered with an ABA solution ($3 \mu\text{M}$ (\pm)ABA (Sigma) in water) on one side and with water on the other (simulated PRD).

Stomatal conductance was determined as described in Section 2.5 for 6 leaves of each vine between 10 am and 12 pm.

Primary roots, which had grown into the perlite, were collected at the end of an irrigation cycle for determination of root ABA and CK concentration (Figure 6.2). The top part of the pot system containing the soil mixture was carefully removed from the second pot containing the perlite. Primary roots which had grown through the mesh were cut off using a razor blade, quickly sorted from remaining perlite and immediately frozen in liquid nitrogen. The amount of primary root material sampled per vine varied between 0.5 and 1.5 g fresh weight. Roots collected from pots of control vines were all combined into a single control sample. Primary roots of all other pots were kept separate. The tissue extraction for ABA and CK analysis was performed as described in Section 2.8.



Figure 6.2 Pot configuration and plant material used for experiments applying ABA. **A)** Pot-system with a steel mesh as bottom of the pot. The red faces have been used to identify the mesh for photographic purposes. The patterns were removed before the pots were used. **B)** Split-root vines (Cabernet Sauvignon) grown in potting mixture and perlite. **C)** Primary root development vine grown into perlite of a PRD-treated (left pot: 'dry side'; right pot: 'wet side').

6.3 Results

6.3.1 Benzyladenine application to the shoot system

To determine the effect of cytokinins on stomatal conductance and shoot growth, benzyladenine (BA) was applied every 6 days to split-root Chardonnay vines.

The effects of spraying PRD-treated vines with either BA or water during one irrigation cycle are shown in Figure 6.3. At the time when stomatal conductance measurements were first taken the vines had been sprayed 5 times.

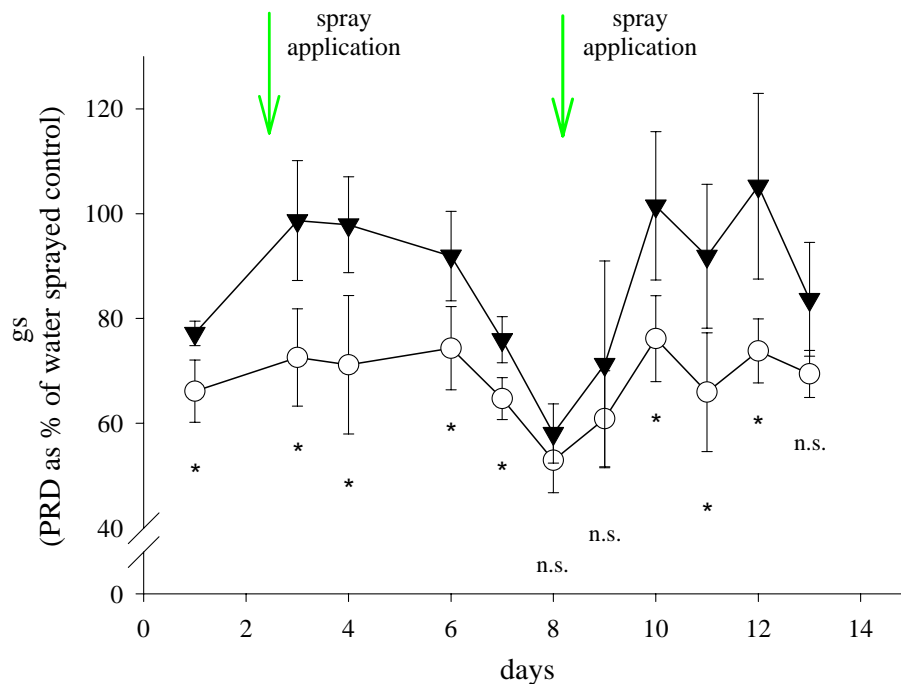


Figure 6.3 Effects of applying benzyladenine (BA) on stomatal conductance (g_s ($\text{mmolm}^{-2}\text{s}^{-1}$); Chardonnay, split-root potted vines). \blacktriangledown : BA sprayed PRD-treated vines; \circ : water sprayed PRD-treated vines; g_s is expressed as a percentage of fully irrigated control vines sprayed with water; control: vines received water on both sides; PRD: at any time to one side of the vines water was withheld (data points represent mean values of 6 measurements on each of the 4 replicates (\pm s.e.); *: $P < 0.05$).

Foliar application of benzyladenine reversed the PRD-induced stomatal closure. During this study, stomatal conductance of PRD split-root vines sprayed with water was significantly reduced by 33% ($P < 0.05$) relative compared to control vines which were also sprayed with water. The overall reduction in stomatal conductance of PRD-treated vines which received foliar application of BA was 13% compared to control vines. In the days immediately following spraying, however, the stomatal conductance of control and BA-sprayed PRD-treated vines did not differ and it was only 3 to 4 days after BA has been applied that stomatal conductance of PRD-treated vines was significantly reduced ($P < 0.05$). Differences between both PRD treatments were greatest 1 to 2 days

after the vines were sprayed and the effect declined over the following days. A second treatment with benzyladenine, however, renewed the effect.

Only minor differences in shoot growth rate of PRD-treated vines were observed

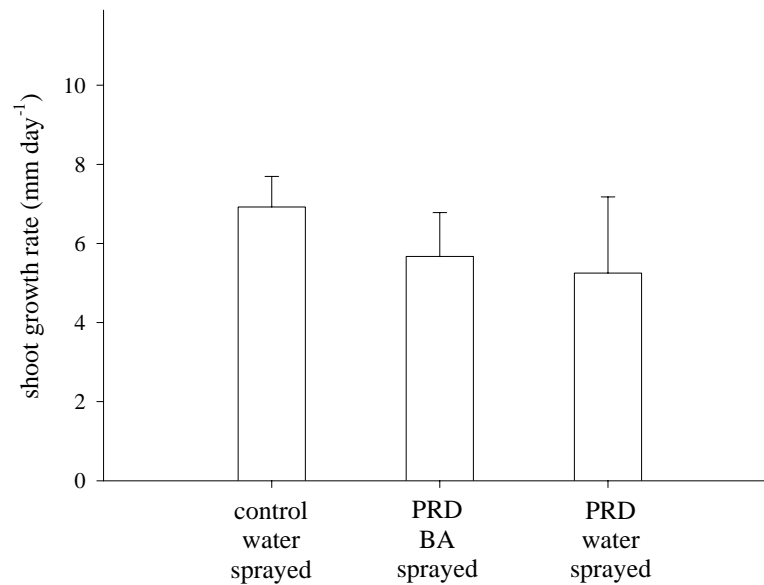


Figure 6.4 Effect of benzyladenine on shoot growth rate (SGR, mm day⁻¹) during one irrigation cycle (Chardonnay, split-root vines; control: vines received water on both sides; PRD: at any time to one side of the vines water was withheld; mean values of 4 measurements on each of the 4 replicates (\pm s.e.)

between sprayed and unsprayed vines during an irrigation cycle (Figure 6.4). PRD-treated vines with and without BA had a lower mean shoot growth rate compared to control vines. The two PRD treatments, BA-sprayed and water-sprayed, did not show significant differences in their shoot growth rate ($P > 0.05$).

When the total length of main shoots at winter pruning of BA-sprayed and water-sprayed PRD-treated vines was compared, no significant differences in shoot length were found ($P > 0.05$) but both BA-sprayed and water-sprayed PRD-treated vines had a significantly lower shoot length than control vines ($P < 0.05$; Table 6.1).

Table 6.1 Effects of spraying potted vines with benzyladenine (BA) on main and lateral shoot growth at winter pruning (Chardonnay split-root vines).

shoot type	control water sprayed	PRD water sprayed	PRD BA sprayed
main shoot (cm)	163 ± 13.4	115.7 ± 9.8	121 ± 6.8
lateral shoot (cm)	25.3 ± 1.3	10.2 ± 0.9	22.0 ± 1.3

Control: vines received water on both sides; PRD: at any time to one side of the vines water was withheld (mean values of measurements on 4 main shoots of each of the 4 replicates (means ± s.e.)

When the total length of lateral shoots per main shoot were compared (Figure 6.5), it was found that the value for water sprayed vines (10.2 cm ± 0.9) was significantly less ($P < 0.01$) than that of BA sprayed vines (22.0 cm ± 1.3; Table 6.1). At winter pruning it was apparent that the reduction in lateral shoot growth of PRD-treated vines relative to control vines had been completely reversed by BA application during the preceding three month period: PRD-treated vines on which BA was applied every 6 days showed only a minor but non-significant reduction in lateral shoot growth relative to control vines ($P > 0.05$).

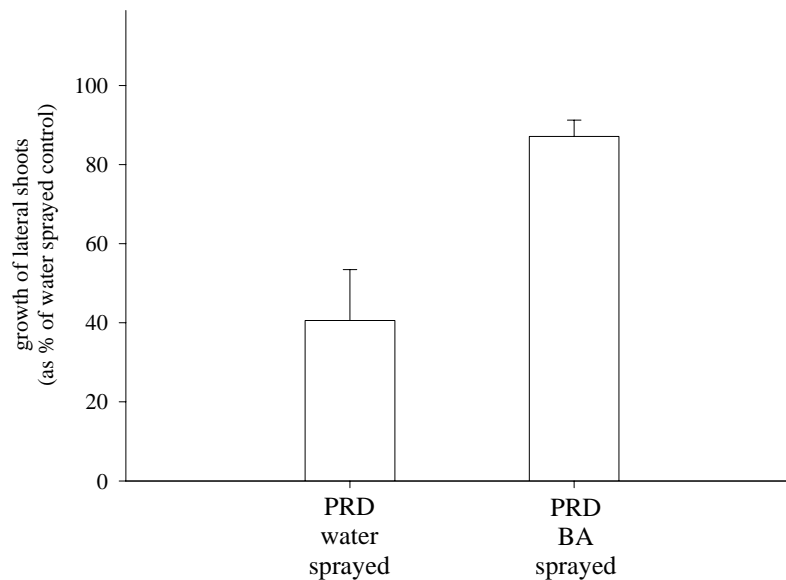


Figure 6.5 Effects of spraying potted vines with benzyladenine (BA) on total length of lateral shoots at winter pruning (Chardonnay split-root vines). Values for lateral shoot growth are expressed as a percentage of fully irrigated control vines sprayed with water. Control: vines received water on both sides; PRD: at any time to one side of the vines water was withheld (n=4; means ± s.e.)

In the following season bud burst in PRD-treated vines occurred 3 weeks earlier in BA-sprayed vines than in water-sprayed. Whether BA application had an effect on berry number or bunch number was not measured in this study.

6.3.2 ABA application to the root system

To test whether externally applied ABA can mimic some of the responses normally observed in response to PRD, a $3\mu\text{M}$ (\pm) ABA solution was applied to one pot of the split-root vine whilst the other side received water (Figure 6.6). The soil moisture in all

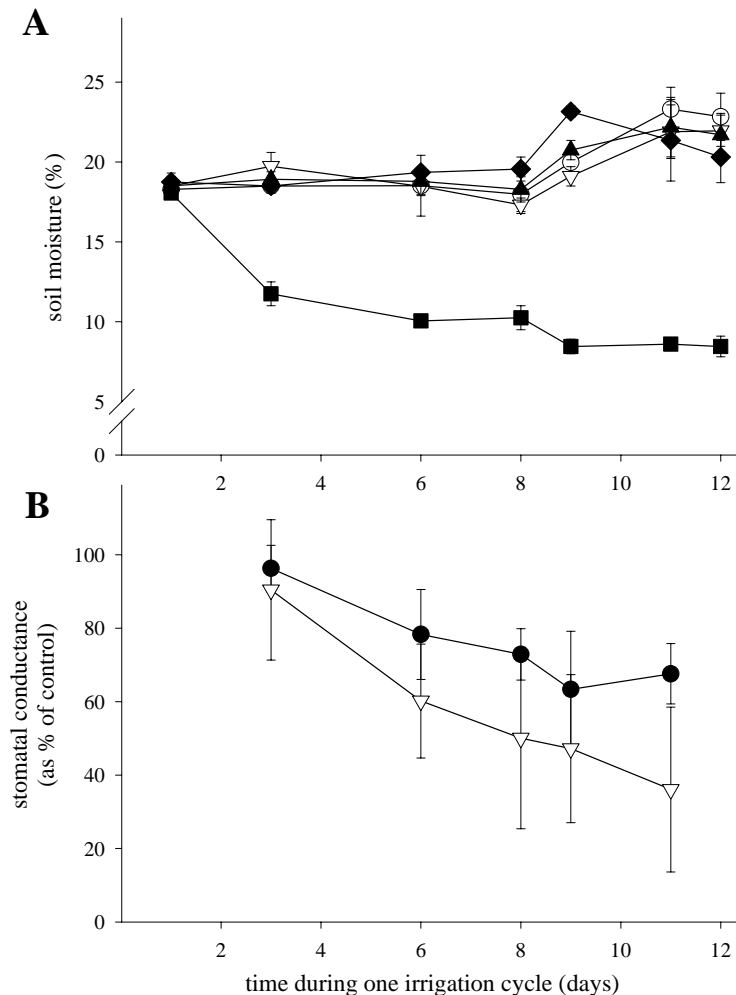


Figure 6.6 Effect of ABA application to one side of a split-root system on stomatal conductance (Cabernet Sauvignon, own roots); control: vines received water on both sides; PRD: at any time to one side of the vines water was withheld; simulated PRD vines: vines received water on one side and $3\mu\text{M}$ +/-ABA solution on the other side A) soil moisture (%); control (▲); PRD: 'dry side' (■), 'wet side' (◆); simulated PRD vine: 'water side' (●), 'ABA side' (▽) B) Stomatal conductance as % of control; simulated PRD vine (●); PRD (▽); data points represent mean values of 6 measurements on each of the 4 replicates (\pm s.e.).

irrigated pots was similar and ranged between 19 and 21% during the experiment. The 'dry side' of the PRD-treated vines, which was not irrigated during the experiment, decreased in soil water content to 8% by day 9 (Figure 6.6 A).

In vines where (\pm) ABA was applied to one side, it was found that even when the soil moisture content in both pots was similar to the soil water content of control vines, the stomatal conductance declined. After day six, stomatal conductance was significantly lower in both PRD-treated and simulated PRD-treated vines than control vines (Figure 6.6 B, $P < 0.05$). PRD-treated vines showed the lowest stomatal conductance of all three treatments with a continuous decline in stomatal conductance during the experiment. In PRD-treated vines, stomatal conductance was reduced by 43%, on average, relative to vines irrigated with water on both sides. When the soil water content on the 'dry side' reached its minimum, the difference between the stomatal conductance of control and PRD-treated vines was found to be the highest, that is stomatal conductance of PRD was reduced by 62% relative to control.

The ABA concentration in primary roots which had grown into the perlite was analysed at the end of the irrigation cycle (Figure 6.7). The [ABA] in primary roots of control vines was significantly lower than [ABA] of either the 'ABA side' of simulated PRD vines or of primary roots which were sampled from the 'dry side' of PRD-treated vines ($P < 0.05$). The [ABA] in primary roots of the 'wet side' of PRD-treated vines was significantly less than the [ABA] of primary roots on the 'dry side' of PRD-treated vines ($P < 0.05$) but not significantly different to the 'ABA side' of the simulated PRD vines. The [ABA] of the 'wet side' of simulated PRD vines did not differ from the [ABA] in roots of either side of control vines.

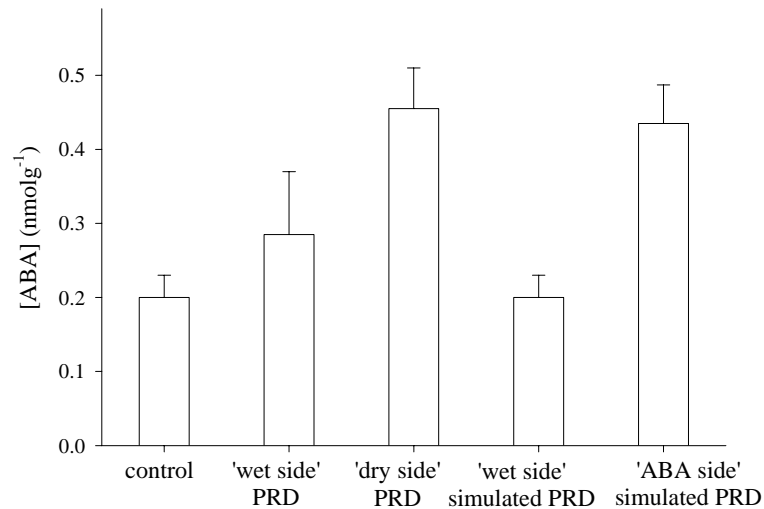


Figure 6.7 Effect of externally applied ABA and PRD on [ABA] ([ABA], nmol g⁻¹) in primary roots at the end of an irrigation cycle (Cabernet Sauvignon, split-root vines; control: vines received water on both sides; PRD: at any time water was withheld from one side of the vine; simulated PRD vine: vines received water on one side and 3 μ M (\pm) ABA solution on the other side; n=4; means \pm s.e.).

Figure 6.8 shows the results of measurements of zeatin and zeatin riboside analysis in primary roots. Primary roots of control vines and the 'wet side' of simulated PRD vines had the lowest cytokinin concentration compared to all other treatments ($P < 0.05$). When the [CK] of primary roots of the 'ABA side' of simulated PRD vines and the 'wet' side and 'dry' side of PRD-treated vines was compared it was found that it was not significantly different ($P > 0.05$) to that of primary roots of control vines and the 'wet side' of simulated PRD vines. The cytokinin concentrations in primary roots of the 'ABA side' of simulated PRD vines and the 'wet side' and 'dry side' of PRD-treated vines were significantly higher than control ($P < 0.05$).

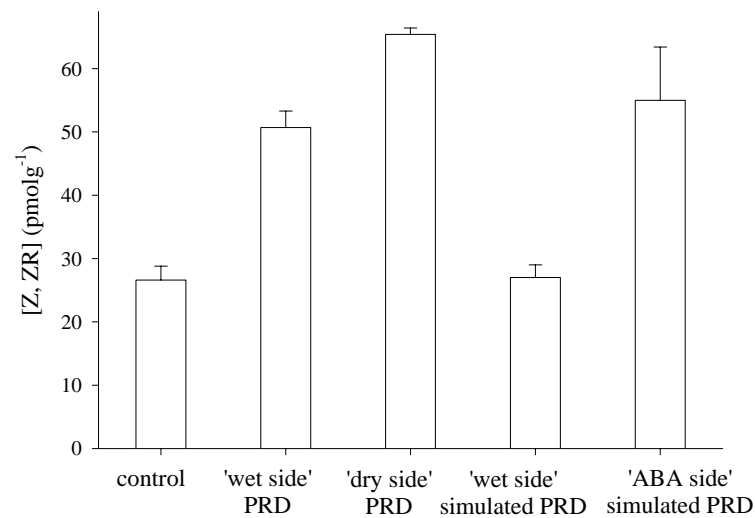


Figure 6.8 Effect of externally applied ABA and PRD on total concentration of zeatin and zeatin riboside ([Z, ZR]; pmol g⁻¹) in primary roots at the end of an irrigation cycle (Cabernet Sauvignon, split-root vines; control: vines received water on both sides; PRD: at any time water was withheld from one side of the vine; simulated PRD vine: vines received water on one side and 3 μM (±) ABA solution on the other side; n=4; means ± s.e.).

The results of zeatin and zeatin riboside analyses and the ratio of zeatin to zeatin riboside are shown in Table 6.2. Compared to results of the same analysis in suberised roots (Chapter 5) the ratio of zeatin riboside to zeatin in primary roots was altered. That is, primary roots had a significantly higher proportion ($P < 0.01$) of zeatin (59% on average) than that in suberised roots (37% on average).

Table 6.2 Zeatin and zeatin riboside concentration (pmol g^{-1} fresh weight) in primary roots (Cabernet Sauvignon split-root vines)

Variable	control	PRD 'wet side'	PRD 'dry side'	simulated PRD 'wet side'	simulated PRD 'ABA side'
zeatin (pmol g^{-1})	14.4 ± 1.9	30.6 ± 3.0	44.7 ± 3.9	14.6 ± 1.8	34.5 ± 5.6
zeatin riboside (pmol g^{-1})	15.7 ± 3.1	20.6 ± 0.9	20.7 ± 4.9	15.4 ± 3.9	15.7 ± 3.1
ratio [ZR]/[Z]	1.090278	0.673203	0.463087	1.054795	0.455072

control: vines received water on both sides; PRD: at any time water was withheld from one side of the vine; simulated PRD vine: vines received water on one side and $3 \mu\text{M}$ (\pm) ABA solution on the other side; $n=4$; means \pm s.e.)

The ratio of [ABA]/[CK] in primary roots showed minor differences between the 'wet side' of PRD-treated vines, 'water side' of simulated PRD vines and control vines i.e. 4.8, 5.7 and 5.7 respectively. Primary roots on the 'dry side' of PRD-treated vines had an [ABA] / [CK] ratio of 5.9 whilst on the 'ABA side' of simulated PRD vines the ratio was 6.7.

6.4 Discussion

Potted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon and Chardonnay, split-root vines) were used to determine the effects of external application of plant growth regulators on physiological responses of vines irrigated under PRD conditions. This study has demonstrated that application of synthetic plant hormones thought to be involved in responses to PRD can mimic some effects in non PRD-treated vines or override some of the effects in PRD-treated vines. Repeated spray applications of

benzyladenine to grapevine leaves resulted in alterations to both stomatal conductance and lateral shoot growth. In the case of ABA it has been shown that externally applied ABA reduces stomatal conductance despite soil water conditions similar to control vines. These results provide additional evidence for the contrasting influence of ABA and CK on stomatal behaviour and suggest that cytokinins might also be involved in influencing lateral shoot growth in response to PRD. Also it supports reports suggesting that stomatal aperture is affected by both groups of hormones as reported by Radin et al. (1982) and Blackman & Davies (1985).

Furthermore, Radin et al. (1982) have suggested that the interaction of cytokinins and ABA must be of a competitive nature since plants respond differently to the same amount of cytokinins applied when the endogenous level of ABA varies. By exogenous application of cytokinins, Radin et al. (1982) concluded that the sensitivity of stomata to ABA and cytokinins may be altered when a plant has a higher endogenous ABA concentration or has previously been exposed to water stress. Davies (1978) demonstrated that stomata of previously water-stressed plants were more sensitive to exogenously applied ABA than stomata of well-watered plants. Previously water-stressed plants closed stomata more rapidly and to a greater degree than well-watered plants. Since part of the root system of PRD-treated vines is exposed to drying soil and therefore has an increased [ABA] it can be speculated that the vines in the present study may have had a higher sensitivity to ABA which might have limited the magnitude of response to BA.

Results presented in Chapter 5 indicate that during PRD irrigation cycles the [ABA] of grapevine xylem sap can show up to 5 fold variation in concentration which may also affect the sensitivity of stomata to ABA. Differences in sensitivity of stomata to various ABA concentrations may explain why the effect of BA on stomatal conductance was only for a short period thus requiring repeated application. Few studies have investigated the degradation of exogenously applied cytokinins. Miernyk & Blaydes (1977) reported that a substantial proportion (50% after 8 h) of kinetin supplied to germinating lettuce seed was degraded by a benzyl cleavage reaction to form AMP (adenosine-5' monophosphate) as the major purine metabolite. A similar degradation of BA via a cleavage reaction was proposed to yield adenine and an unidentified acid

which resembled benzoic acid (Fox *et al.*, 1972). To what degree BA degrades in grapevine leaves was not determined.

Purine metabolites might be of importance in the regulation of ABA biosynthesis. Cowan *et al.* (1999) proposed a model for the regulation of ABA levels in plant tissue, whereby purine metabolisms may affect ABA biosynthesis. In their model of ABA-CK antagonism it is proposed that CK homeostasis, regulated by CK oxidase activity, contributes to production of adenine metabolites which, by positive feedback regulation, enhance the activity of a MoCo (molybdenum-cofactor). MoCo is essential for the activity of aldehyde oxidase, an enzyme converting the ABA precursor (xanthoxal, XAN) into ABA. If BA application provides metabolites which enhance the activity of aldehyde oxidase, additional ABA biosynthesis may occur and this may account for a transient reduction of stomatal conductance.

Whether the actual effect of BA is to inhibit stomatal closure induced by ABA rather than directly promoting stomatal opening has yet to be demonstrated. The experiment described here, however, provides additional evidence that cytokinins can influence stomatal responses of grapevines. Similar effects of BA application on stomatal conductance were found in *Vitis vinifera* L. by Düring & Broquedis (1980) and in *Vigna radiata* by Kumar & Abrol (1989).

Reduced supply of CK was suggested as a response to drying soil by Itai & Vaadia (1965). Pillay & Beyl (1990) reported that a decrease of root cytokinin concentration within the first 24 h of increasing water stress in tomato plants may act as an early response to the induction of water stress. It seems reasonable to propose that stomatal conductance of grapevines may also be affected by a reduced supply of CK from the roots.

After several weeks of repeated BA application, the development of lateral shoots on PRD-treated vines was increased relative to PRD-treated vines which had not been sprayed with BA. Similar results have been reported for apple seedlings where it was shown that lateral shoot development increased after BA application (Richards, 1980). The same author, however, reported that the growth habit of apple seedlings, treated with BA, was dependent on the site of application. BA applied as a foliar spray effectively stimulated shoot development but application of BA to the roots did not show growth stimulating effects on shoots. Despite the difficulty interpreting

experiments involving external application of hormones to plants, it becomes increasingly evident that endogenous hormones play an important role in root to shoot communication.

On the basis of the findings presented here it is reasonable to speculate that the reduced lateral shoot development observed with PRD is due to a reduction in cytokinin availability from drying roots. Measurement of reduced cytokinin concentrations in lateral shoots and prompt buds (Chapter 5) provides further evidence to support this conclusion.

Measurements of shoot growth and stomatal conductance under PRD conditions in this study are in agreement with other studies on split-root plants and provide evidence for the involvement of chemical signals in root to shoot communication (Gowing *et al.*, 1990; Dry & Loveys, 1999). Gowing *et al.* (1990) argued, from their results with apple trees, that growth is controlled by a positive signal, because excising the root system on the 'dry side' caused conductance and leaf growth rate to recover. Similarly, Dry & Loveys (1999) found that after approximately 3 weeks of watering the same side of a split-root grapevine, the effect on stomatal conductance and shoot growth was reduced and vines started to recover.

Results of this study indicate that the reduction in stomatal conductance and lateral shoot growth is not due to a single positive root signal. Rather it is the ratio of ABA to CK that appears to be important to account for the reduction in stomatal conductance and lateral growth. This is because the effect on lateral growth can be completely reversed and the effect on stomatal conductance temporarily reversed when BA is exogenously applied to the shoot system. Alternating 'wet' and 'dry' sides under PRD may therefore stimulate a dynamic process of fluctuating ABA and CK concentrations as discussed in Chapter 5. It is therefore believed that changes in the ratio of ABA to CK control the reduction in stomatal conductance and lateral shoot growth. Gowing *et al.* (1990) may therefore not only have eliminated the positive signal but also altered the ratio of both chemical signals by excising the dried part of the root system.

It has been shown that many responses in plant development usually discussed in relation to water deficit can be induced by an exogenous application of ABA (Trewavas & Jones, 1991). Results of my study provide further evidence that ABA applied

exogenously to the root system induces a reduction in stomatal conductance and also affects the [CK] in primary roots.

There is much controversy over the role that ABA has in determining root growth because there are reports that ABA can stimulate root growth (Saab *et al.*, 1990) or inhibit root elongation (Robertson *et al.*, 1990). Saab *et al.* (1990) showed that endogenous ABA may have a dual role in plants: elevated ABA levels may maintain primary root growth and inhibit shoot growth.

Applying (\pm) ABA to roots of potted vines as was done during this study may not represent the same conditions that a plant experiences in drying soil. Furthermore, naturally-occurring ABA comprises exclusively the (+)-enantiomer which shows a different metabolism compared to the (–)-enantiomer (Loveys & Milborrow, 1984). Since only the + enantiomer is active, the (\pm) ABA was applied at twice the concentration normally expected in grapevine roots (Chapter 5). This did not reduce the growth of primary roots because under both soil drying conditions and under well watered soil conditions, PRD-treated or simulated PRD vines (ABA-watered on one side) maintained primary root growth into the perlite.

The total [CK] in primary roots of potted vines, calculated from [Z] and [ZR], showed a two to threefold higher concentration than suberised roots of field grown vines. This might be due to a higher nitrogen source from the potting mixture of these split root vines compared to field grown vines. Beck & Wagner (1994) reported that roots of plants grown with a sufficient supply of nitrogen had a significantly higher cytokinin content than those plants raised under nitrogen shortage. Radin *et al.* (1982) argued that the reduction in CK in roots may also be due to a reduction in nitrogenous nutrients and that low nitrogen nutrient levels may affect stomatal behaviour by altering the balance between ABA and CK. If this is the case, the availability of nutrients taken up by roots on the ‘dry side’ of PRD-treated vines might explain the reduced supply of CK. Since all cytokinins contain nitrogen in the adenine molecules, the status of nitrogen nutrition can alter the cytokinin concentration (Sattelmacher & Marschner, 1978; Ivanov *et al.*, 1998). The effect of PRD on nitrogen uptake has yet to be investigated.

In Chapter 5 it was shown that [ABA] increased and [CK] decreased in suberised roots on the ‘dry side’. In this experiment, primary roots from the ‘dry side’ of PRD

and the 'ABA side' of simulated PRD-treated vines not only had a higher [CK] but also a higher [ABA] concentration. The white, primary roots might be a source and accumulation site for cytokinins. This was possibly indicated by the observation that the 'dry side' of PRD treated vines had the lowest proportion of zeatin riboside, but corresponding higher levels of zeatin. In contrast, suberised roots had shown a much higher concentration of zeatin riboside compared to zeatin (Chapter 5). Zeatin riboside has been reported to be an important translocation form of cytokinins in the xylem (Letham, 1978). Furthermore, it might be of importance to define whether roots are actively growing, as has been observed in primary roots in this study, or whether the growing activity is reduced. Mullins *et al.* (1992) have shown that after anthesis the growth rate of grapevine roots declines and a second flush of root growth occurs after the fruit has been harvested. The potted vines used for this study were deliberately defruited thereby possibly having an altered root growth compared to roots of field-grown, fruited vines (Chapter 5). The higher [CK] in roots of PRD or simulated PRD vines may therefore be due to actively growing primary roots due to the accumulation of CK and an inhibition of transport of CK to the shoots.

It has been recently reported that water-stressed primary roots of maize showed a high endogenous ABA concentration which limited ethylene production and therefore stimulated root elongation (Spollen *et al.*, 2000). Stimulating primary root elongation may help the plant to overcome water deficit by exploring soil layers faster and thereby accessing soil with a higher water potential. This may result in changes in the pattern of grapevine root development under PRD as has recently been reported for potted (Dry *et al.*, 2000) and field-grown vines (Stoll *et al.*, 2000a).

6.5 Conclusion

Experiments in this chapter examined the effects of externally-applied plant hormones on stomatal conductance, shoot growth and root development. The major conclusions were:

1. Foliar applications of benzyladenine to PRD-treated vines overcame the depressing effect of PRD on stomatal conductance.
2. A reduction of lateral shoot growth, normally observed under PRD, was reversed by exogenous application of benzyladenine.
3. Both PRD and exogenously applied ABA caused an elevation in root ABA and at the same time, reduced stomatal conductance.
4. ABA, either applied exogenously to the root system or manipulated by PRD, increased the cytokinin concentration in primary roots, possibly due to inhibited transport.
5. As a critical feature of this study it is believed that both ABA and cytokinins act in concert as important root-sourced signals in root to shoot communication under PRD conditions.

Chapter 7 Water movement under PRD conditions

7.1 Introduction

One of the essential functions of roots is to supply water and nutrients to the shoots. Water movement through roots is considered passive in response to water potential gradients mainly influenced by transpiration (Steudle & Peterson, 1998). The water budget of a plant is linked to the water potential gradient between the soil and the atmosphere. The gradient is steepest at the interface between the leaf and the atmosphere. The physical properties of water, such as high surface tension and the widely accepted ‘cohesion theory’ are fundamental for long-distance water transport in plants, as they contribute to the pull of water by transpiration from the roots through the xylem to the leaves (Atwell *et al.*, 1999). The complex anatomical structure of xylem vessels limits the spread of embolisms and other forces such as osmotic pressure, capillary and air-water interfacial forces, thereby securing the transport of water in plants (Zimmermann *et al.*, 1993).

The amount of water available to a plant from the soil is heavily influenced by the soil water potential. Soil water potential is affected by soil layer or depth and soil type and structure. Most of the soil to soil water movement follows a gradient from soil layers of high matric potential to soil layers of low matric potential. Such a phenomenon is described as ‘hydraulic lift’ (Richards & Caldwell, 1987; Dawson, 1993), ‘hydraulic redistribution’ (Burgess *et al.*, 1998), ‘downwards siphoning’ (Smith *et al.*, 1997) or ‘inverse hydraulic lift’ (Schulze *et al.*, 1998).

In early experiments, where water transfer between roots in physically separated compartments was observed, roots were described as “equalizers” of soil moisture gradients (Breazeale & Crider, 1934). Evidence has now been accumulated that transport of water in roots can not only be directed from the soil to the plant, but can also occur in the opposite direction. Such processes involving a reversed flow of water, can occur vertically in roots in soil of high water potential to roots in soil layers with low water potential. This is called ‘downward siphoning’ (Smith *et al.*, 1999).

Some studies have recently shown that stable isotope analysis can provide further information to pinpoint exactly where plants obtain their water from. This method has

recently been used to demonstrate hydraulic lift in sugar maples (*Acer saccharum*) during drought periods (Dawson, 1993).

Monitoring sap flow in different plant organs can also be employed to study plant transpiration under field conditions. Using heat as a tracer to detect sap movement was proposed and first used by Huber (1932) and described in Bloodworth *et al.* (1955). The accuracy of the water uptake may be affected by the distribution of conductive elements (Cohen & Li, 1996): hence the anatomical structure of different organs becomes important.

Sap flow sensors have found wide application in irrigation scheduling because it was found that water stress affects sap movement in plants. Hence this technology can provide information about the physiological state of a plant over time (Eastham & Gray, 1998; Braun, 1997).

The PRD irrigation strategy relies on exposing the root system to different soil water gradients at the same time. Manipulating soil water potential under PRD might not only affect chemical signals as has been discussed earlier in this thesis, but may also affect water movement in vines. The phenomenon of passive water movement may therefore have an impact on the water balance of a vine and the transmission of root borne signals. Experiments described in this chapter were conducted to test the hypothesis that *PRD causes movement of water from 'wet' to 'dry' roots.*

7.2 Material and Methods

7.2.1 Determination of soil water movement

A time domain reflectometry (TDR) instrument (Trase[®] system 6050X1, Goleta, California, USA) was employed to determine soil water movement and to decide whether water movement from 'wet' to 'dry' roots can be detected with a conventional soil moisture instrument. Two sets of measurements during soil drying cycles were collected. First, soil water content on the 'dry' side of a two year old split-root vine (*Vitis vinifera* L. cv. Cabernet Sauvignon) grown in standard potting mixture (Section 2.6) during a drying cycle was monitored. Second, soil drying of an identical potting

mixture and pot volume (4.5 L) without a vine was monitored to test whether other factors such as air temperature or soil temperature could affect continuous soil water content monitoring.

Wave guides (0.15 m) were inserted in each pot and for both sets of measurement and soil moisture readings were logged at 15 min intervals. A three point running average was used to smooth the data. Ambient air temperature and the temperature in the potting mixture was measured with thermocouples and recorded at 15 min intervals with a Delta-Datataker[®] (Rowville, Victoria, Australia). Vapour pressure deficit (VPD) was calculated from 15 min averages of air temperature and relative humidity at heights of 2 m (Jones, 1992). The data for relative humidity were collected using a conventional weather station (Measuring Engineering Ltd, Adelaide, South Australia), which was located 200 m away from the potted plants.

7.2.2 Determination of relative water content in grapevine roots in response to different irrigation treatments

Three year old split-root grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) were grown in pots (13 L volume; standard potting mixture (Section 2.2)) in the open at the CSIRO Plant Industry laboratories (Adelaide, Waite Campus). These plants were used to determine the relative water content (RWC) in roots in response to different irrigation treatments. Three different irrigation regimes were applied: 1) control: vines received water on both sides to field capacity once a day; 2) PRD: at any time to one of the pots water was withheld whilst the other pot was watered to field capacity once a day; 3) neither of the pots of the split-root vines were watered. Each treatment was replicated four times.

At the time the experiment was started each vine had 4 shoots with 10 to 12 mature leaves. Root samples from each pot were taken every third day from a soil core obtained using a sharpened steel tube (25 mm diameter; 0.25 m length). The soil samples were collected in plastic bags and roots were separated from the soil immediately after sampling. The fresh weight (fw) was measured before the roots were immersed in water overnight to reach full turgor. After measuring the turgid weight

(tw), roots were dried at 120 °C for 24 h in a vacuum oven and the dry weight (dw) was determined.

The relative water content (RWC) of roots was calculated using Equation 7.1:

Equation 7.1:
$$\text{RWC} = ((\text{fw}-\text{dw}) * (\text{tw}-\text{dw})^{-1})*100$$

The relative water content of soil was determined such that the weight of the soil was recorded immediately after the roots were sorted (fw). The soil was then watered to field capacity (tw), measured and dried at 120 °C in a vacuum oven for 2 days to determine the dry weight (dw). Equation 7.1 was used to determine the relative water content of the soil.

7.2.3 Measurements of sap flow in roots of PRD-treated grapevines

Before any sap flow sensors were installed, conductive elements in roots and trunks of vines of the same age were determined in a preliminary study. An aqueous soluble dye solution (basic fuchsin; 2 gL⁻¹ in water) was used to stain the conductive elements. Before sunrise when stomata were closed and sap flow was at its minimum, roots were cut at 0.3 m below ground and immediately placed in dye solution. The root was allowed to draw up the dye for three hours after sunrise before it was detached from the plant. Photographs of transverse sections from roots, trunk and canes were taken using a spot camera (Diagnostic Instruments Inc., USA) attached to a binocular microscope (Stemi 2000-C, Zeiss, Germany).

Sap flow in sapwood of roots of PRD-treated vines was monitored using heat pulse sap flow sensors (Greenspan[®] Technology, Warwick, Australia). The method used to calculate sap flow was based on the compensation technique (Huber, 1932). Each probe consisted of two temperature sensor pairs (thermistors) spaced at a fixed interval of 5 or 10 mm in a stainless steel tube and a heater with an energy output of 2 Wcm⁻¹ (Figure 7.1).

Two 23 year old vines (*Vitis vinifera* L. cv. Sultana on own roots) grown at the Alverstoke vineyard of the University of Adelaide were selected. Eight weeks before

the probe sets were installed, a trench (3 m x 4 m) was dug to 0.7 m depth and 0.1 m width. The trench was designed to separate the roots close to the surface from the roots of the neighbouring vines. At the same time, the soil around the trunk was removed to excavate suitable roots from each vine. An irrigation system for PRD was then established so that each side of the vine could be irrigated independently. During the experiment, the soil moisture on the dried side was measured with a TDR instrument as described above.

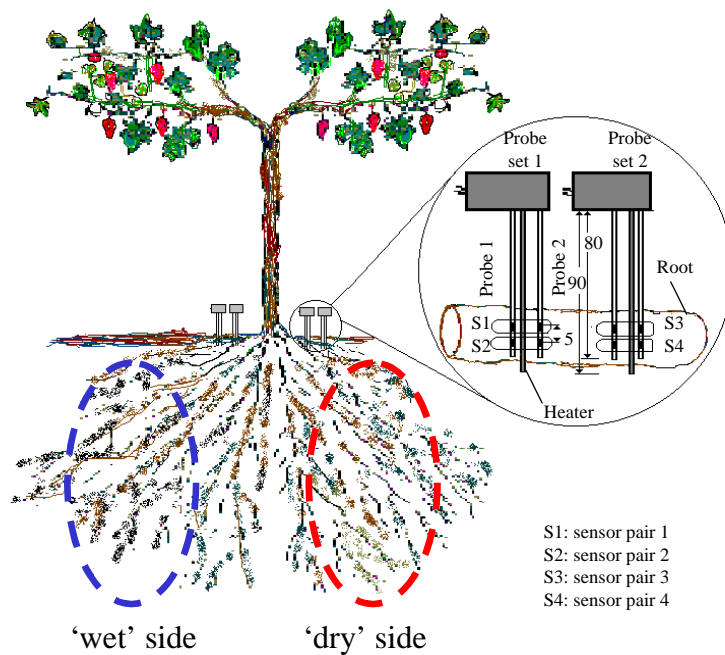


Figure 7.1 Diagrammatic representation of the installation of heat pulse sap flow sensor used for measuring sap flow in acropetal and basipetal directions in vines irrigated using PRD. Probe set 1 was used to measure sap flow in the direction from the trunk to the soil direction and probe set 2 for the direction from roots to the trunk.

The sets of sap flow sensor probes were installed in selected roots, whose orientation suggested that they were associated with either the irrigated or non-irrigated side of the vine. The roots chosen had a minimum diameter of 25 mm.

A total of 4 probe sets per vine were installed, 2 measuring sap flow in the root-to-trunk direction and 2 in the trunk-to-root direction. A set of three holes (2 mm) was required to insert each probe set. The probes were coated with vacuum grease to ensure

an even dissipation of the heat from the probes and their insertion depth was adjusted to obtain minimum compensation times. The probe sets were insulated with polyester and covered with aluminium foil before the excavated soil was replaced. A heat pulse was sent every 30 min and the data were logged at equal intervals. The communication between logger and computer was through SAPCOM2 software (Greenspan[®] Technology, Warwick, Australia). To calculate flux and velocity data SAPCAL software (Greenspan[®] Technology, Warwick, Australia) was used. The ‘time out’ value required by the software to specify the maximum time for both probes to return to the same temperature for a value of sap volume to be registered was set at 100 sec.

The volumetric wood and water content required by the SAPCAL software calculating velocity and flux was determined at the end of the experiment when the parts of the root, accommodating the sensors, were detached. The fresh weight (W_f) and immersed weight in water (W_i) was measured for part of the root which had accommodated the sensors before the samples were dried (W_d) at 80 °C for 48 h in a vacuum oven. The volume fraction of water (V_h) and volume fraction of wood (V_w) were calculated using Equations 7.2 & 7.3 from the ‘heat pulsed sensor technical manual’ (Technical Manual, Greenspan[®] Technology, Australia):

Equation 7.2: $V_h = (W_f - W_d) / W_i$

Equation 7.3: $V_w = W_d / (1.53 * W_i)$

The factor 1.53 corresponds to the specific gravity of wood which is remarkably constant at 1530 kg m⁻³ (Technical Manual, Greenspan[®] Technology, Australia).

7.2.4 Movement of water in grapevines under PRD conditions

Water enriched with the stable isotope deuterium was applied to one pot of two year old split-root vines (*Vitis vinifera* L. cv. Cabernet Sauvignon) to ascertain whether water moved from the ‘wet’ side of the vine to the ‘dry’ side under the influence of different soil water gradients. The soil moisture content was measured on the ‘dry’ side of the split-root vine using a TDR instrument as described in Section 7.2.1. At the start of the

experiment each vine had two shoots with 7 to 8 leaves per shoot. The volume of each pot was 4.5 L. Two different treatments were established. First, PRD-treated vines receiving deuterium-enriched water (250 ml conc. 964.5 ‰) on one pot and second, PRD-treated vines receiving 250 ml of Adelaide tap water on one pot. The irrigated pots were then covered with aluminium foil to prevent evaporation of the water. The extraction method was tested in a preliminary study where leaves were sampled before sunrise, immersed in kerosene and further extracted as for root samples as described below.

Roots of two vines, from the pots of the ‘dry’ side of each treatment, were harvested on days 1,4,7,10 and 12. Samples were collected prior to sunrise, when stomata were still closed and the whole vine transpiration was at its minimum. During root sorting, the roots were kept in plastic bags. The fresh weight of the roots was measured. Immediately after collection the roots were transferred to 250 ml round flasks, immersed in kerosene and sealed with plastic corks.

An azeotropic distillation was used to extract the water from plant or soil material. The samples were distilled over a heating element on a Dean and Stark apparatus (Figure 7.2) for 1 h at 110 °C.

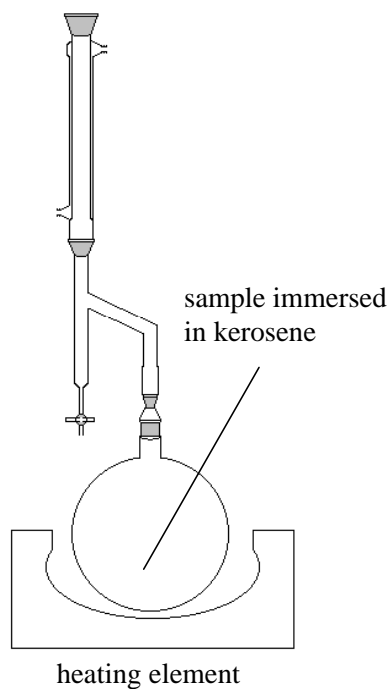


Figure 7.2 Dean and Stark apparatus

At the boiling point of water an azeotrope of kerosene and water is formed and the resultant vapour is then condensed. The water separates from the kerosene in the receiver creating two phases with the heavier water on the bottom. The water fraction was collected in McCartney bottles and 5 g of paraffin wax was added. The sealed bottles were heated for 40 min at 45 °C to melt the paraffin wax to remove contaminants. Analyses for $\delta^2\text{H}$ were performed by reducing 25 μL of sample to H_2 over uranium at 800 °C and further analysed using a PD2 GEO 20-20 stable isotope gas ratio mass spectrometer (Europa Scientific Ltd.). Isotopic concentrations were expressed as delta values ($\delta^2\text{H}$) in units of parts per thousand (per mill; written ‰) relative to V-SMOW (Vienna Standard Mean Ocean Water). The general expression for stable isotope notations is given in Equation 7.4 (Coplen *et al.*, 1999).

$$\text{Equation 7.4: } \delta_x = \delta_{x-\text{std}} = (R_x / R_{\text{std}} - 1) * 1000$$

R_x and R_{std} are $^2\text{H}/^1\text{H}$ ratio of the sample and standard, respectively. Thus a positive value of δ_x indicates that the sample is isotopically ‘heavier’ relative to the standard, whilst a negative value indicates a depletion in the heavy isotope relative to the standard.

7.2.5 Determination of root distribution in vines using PRD irrigation

To determine the root distribution at different soil depths, roots of grapevines (*Vitis vinifera* L. cv. Riesling, grafted on Ramsey rootstock) planted in 1990 and PRD-irrigated since 1995 were used. The vines were grown on levelled land in deep red sandy soil at Waikerie (Riverland, South Australia). The vineyard had a single wire trellis system with minimal pruning (vine x row spacing: 1.3 m x 3.1 m). The vines were irrigated over 4 growing seasons using subsurface drip lines. The drip lines for both treatments were buried at 0.2 m to 0.25 m depth and 0.5 m from the planting line on both sides. Two different irrigation treatments were used during the experiment. Water was either applied to both sides of the vines (control) or, in the other treatment,

vines were irrigated using PRD. Water was applied according to soil moisture measurements using EnviroSCAN® and tensiometers.

Twelve root samples of each irrigation treatment were taken using a PVC cylinder (diameter 0.3 m, length 0.4 m) at 2 different depths (0.1 m to 0.4 m and 0.4 m to 0.7 m). The cylinder was sharpened on one side and pushed through the sandy soil to 0.4 m or 0.7 m depth. According to the diameter of the roots they were sorted into 3 different classes: 1) < 1 mm; 2) 1 mm-3 mm; 3) > 3 mm. After drying the roots at 120 °C for 24 h in a vacuum oven the dry weight of each class per kg soil was measured

7.3 Results

7.3.1 Diurnal fluctuation in soil water content

The purpose of this preliminary experiment was to test whether diurnal fluctuations in soil water content, frequently observed during this study, are related to water movement from roots into the soil or whether other factors are influencing this phenomenon. Volumetric soil water content of the ‘dry’ side of a PRD-irrigated split-root vine and a pot containing the same standard potting mixture but without a vine was monitored over two weeks using a TDR instrument. In both cases it was found that the volumetric soil water content decreased over time (Figure 7.3). The decrease in soil water content without a vine was almost linear and relatively slow (Figure 7.3 A), whilst the soil water content of the pot containing a vine declined rapidly over the first four days. After this rapid decline further water loss of the soil slowed down but the soil water content steadily decreased to 5 % (Figure 7.3 B).

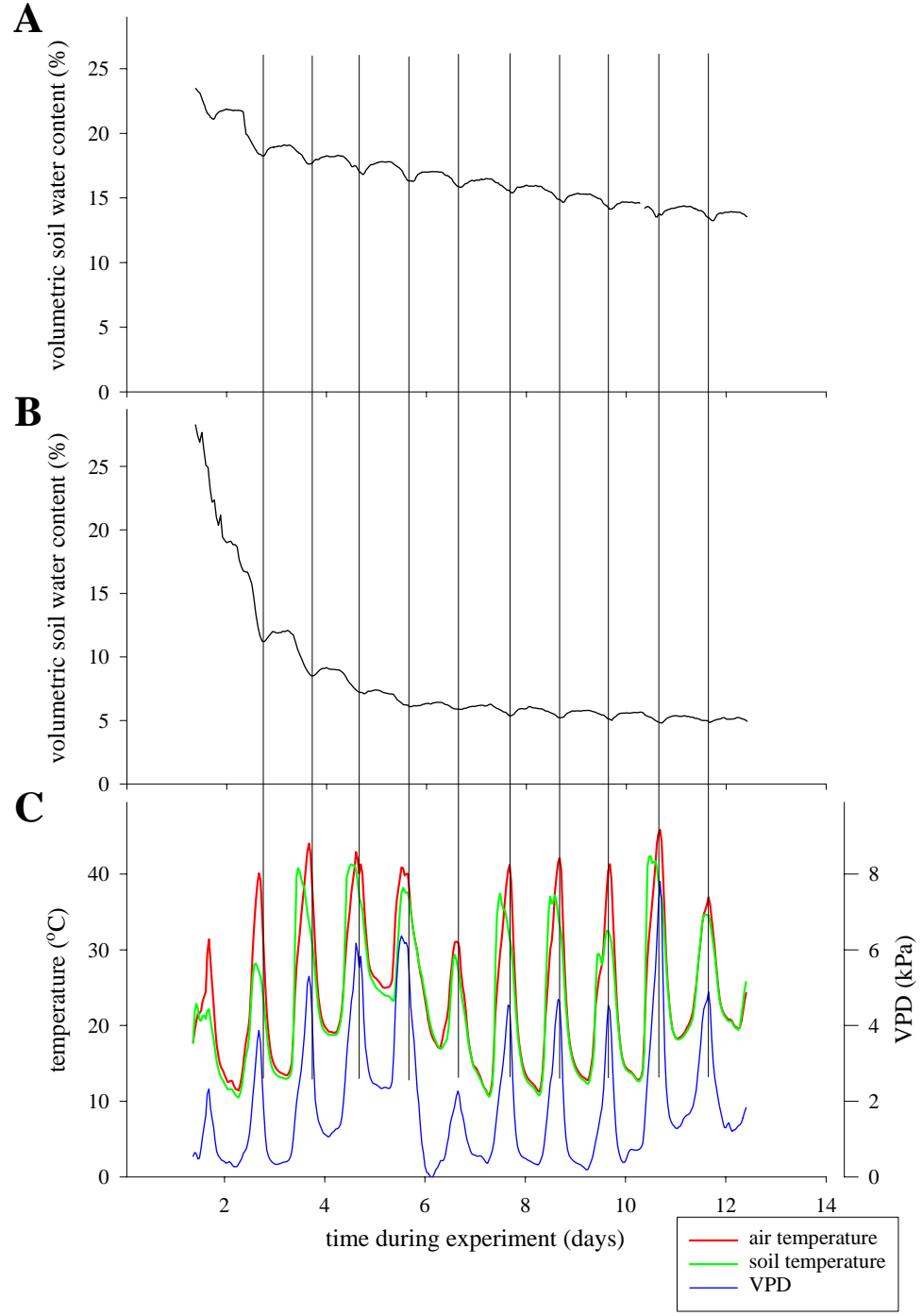


Figure 7.3 Case study of monitoring soil water content (SWC, %). A) standard potting mixture without a vine B) 'dry' side of a PRD irrigated split-root vine (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots; soil: standard potting mixture) C) air and soil temperature (°C) and VPD (kPa).

Both pots, with and without a vine, showed an increase in soil water content overnight. Measurements of ambient temperature and soil temperature showed that these nocturnal changes were closely related to changes in temperature and the

atmospheric water pressure deficit. For example, the relationship between soil water content, measured on the ‘dry’ side of a PRD-treated vine, VPD and ambient air temperature on day 9 is shown in Figure 7.4. The ‘dry’ side had already reached a low soil water content. However, when the temperature in the early evening started to decrease the apparent soil water content increased and reached its maximum overnight value at 6 am when the temperature was lowest. During the day the soil water content was lowest when air and soil temperature were highest, that is at 15:30 h (Figure 7.4 A). A similar diurnal rhythm was found to occur in drying soil without a vine (Figure 7.4 B).

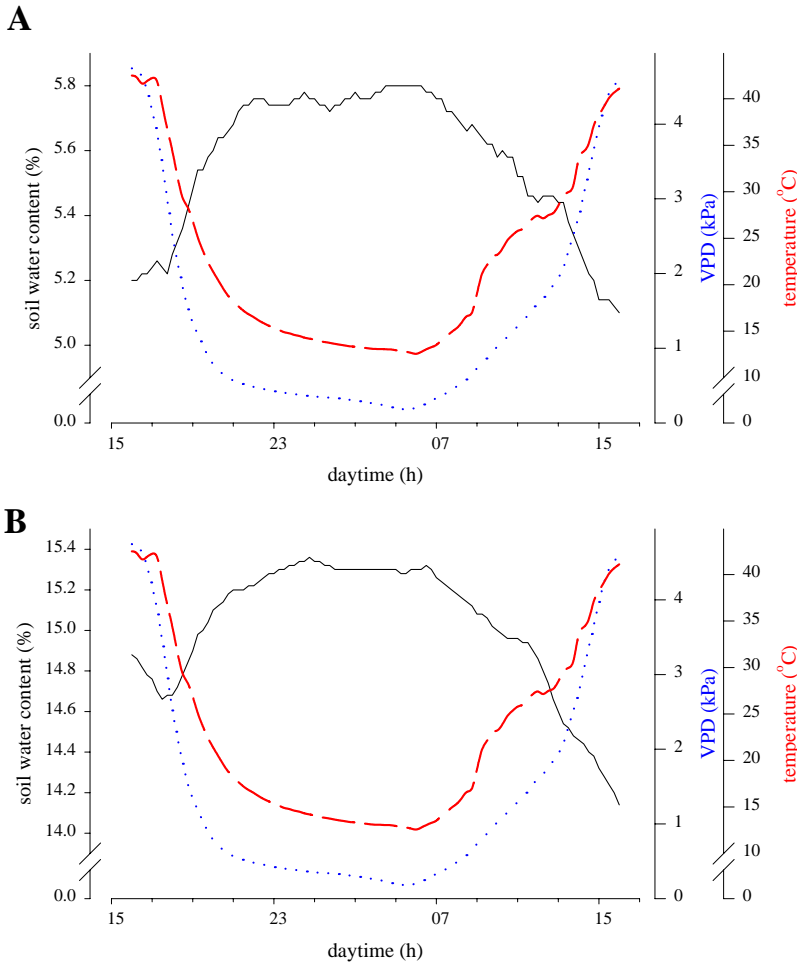


Figure 7.4 Diurnal rhythm of soil water content (%). Solid line: soil water content ; dashed line: ambient air temperature; dotted line: VPD. A) ‘dry’ side of a split-root vine (*Vitis vinifera* L. cv. Cabernet Sauvignon on own roots). B) potting mixture without a vine.

7.3.2 Determination of relative water content in grapevine roots using PRD

Relative soil water content and relative root water content were determined for soil and roots of split-root vines, which were either watered on both sides (control), PRD-treated or water was withheld from both sides (Figure 7.5). The soil water content on the ‘dry’ side of both PRD-treated vines and non-irrigated vines declined at a similar rate whilst the soil water content on the ‘wet’ side of PRD-treated vines remained high during the experiment (Figure 7.5 A). The PRD-treated vines showed a slower decrease in relative water content in roots on the ‘dry’ side compared with roots from vines where no water was applied to either pot. The RWC of roots after 16 days is shown in Figure 7.5 B. It was found that the RWC of roots in PRD-treated vines was significantly higher after 16 days compared to the RWC of roots where water was completely withheld during the entire experiment ($P<0.05$).

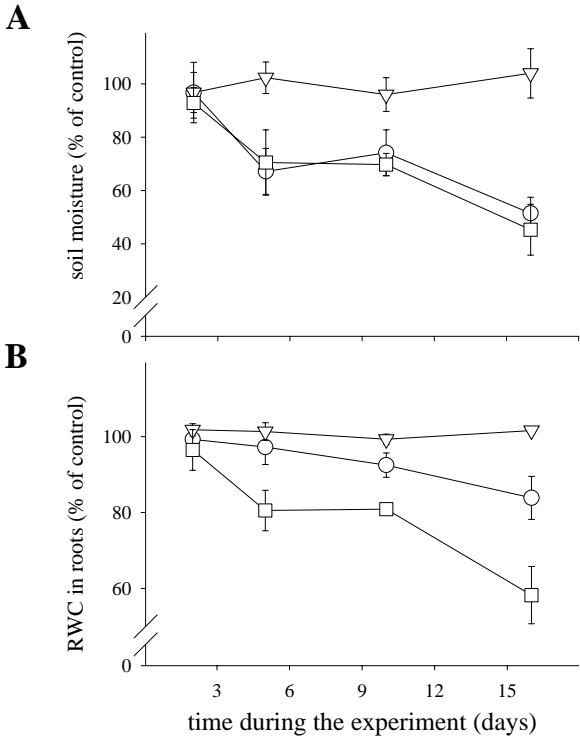


Figure 7.5 Effect of different irrigation regimes on soil moisture content (A) and relative water content of roots (B). (Cabernet Sauvignon split-root vines on own roots; the treatments were: control: vines received water on both sides. PRD: water was withheld from one pot (O) whilst the other pot received water (∇). Water was withheld from both pots (□); n=4; means ± s.e.).

7.3.3 Monitoring sap flow in roots of PRD-treated grapevines

Location of conductive vessels and determination of structural differences of trunks, roots or two year old canes was done by applying a water soluble dye (basic fuchsin) to different organs (Figure 7.6). Information on the structural differences between organs is essential for correct positioning of the heat pulse sap flow sensors in conductive elements.

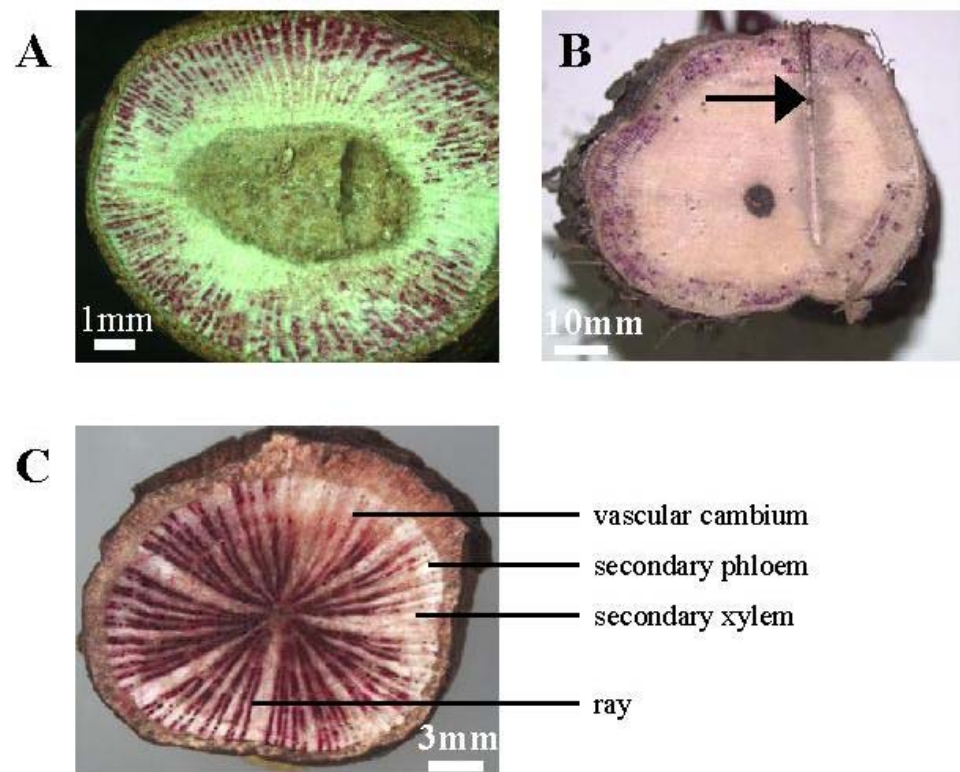


Figure 7.6 Transverse sections of a grapevine stained with basic fuchsin (*Vitis vinifera* L. cv. Sultana, own roots) A) cane in the second growing season B) trunk (arrow indicates one of the holes required to install sap flow sensor) C) root with secondary growth

The dye was readily taken up and stained the conductive elements red in each organ. Application of basic fuchsin to different organs of grapevines highlighted some distinctive characteristics in the differentiation of roots compared to trunks and canes. The secondary tissue of roots have a greater proportion of conducting elements

compared with secondary tissue of trunks or canes. In the second growing season, there is a high proportion of non-conductive elements in the center in both trunks and canes, and the conductive vessels are restricted to a small ring-shaped boundary on the outside. The width of the ring which includes the conductive vessels was variable, but it was found that in a stem of 80 mm diameter the width of the conductive ring was never wider than 6 to 10 mm. The roots showed a large volume of rays with bigger tracheids than shoots.

Daily flux of sap in roots was measured from soil to the trunk and in the opposite direction (Figure 7.7). The daily flux towards the trunk on the ‘wet’ side between 6 am to 6 pm during the whole experiment was on average 1.3 Lh^{-1} compared to 0.9 Lh^{-1} on the ‘dry’ side (Figure 7.7 A, B).

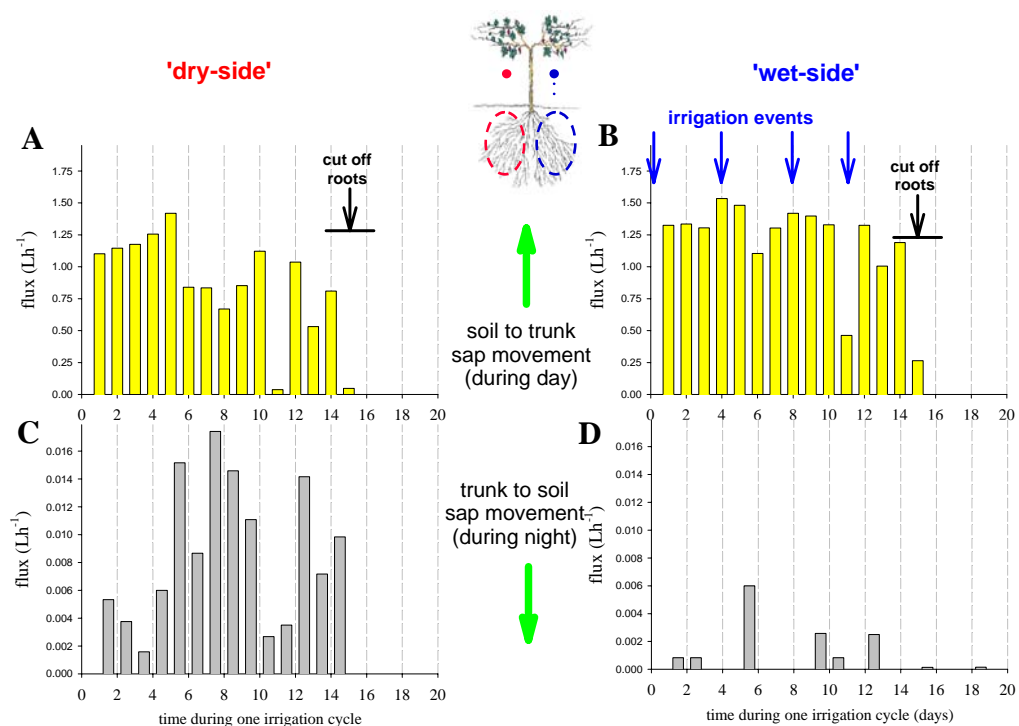


Figure 7.7 Sap flow in grapevine roots using PRD (*Vitis vinifera* L. cv. Sultana on own roots). Sap movement from soil to trunk (sap flow, Lh^{-1} ; average of readings from 6 am to 6 pm) on ‘dry’ side (A) and ‘wet’ side (B). Sap movement from trunk to soil (sap flow, Lh^{-1} ; average of readings from 6 pm to 6 am) on ‘dry’ side (C) and ‘wet’ side (D).

During the experiment, the total daytime sap flow on the ‘dry’ side was on average 27% lower than for the ‘wet’ side. On day 6 the sap flow for both the ‘wet’ and ‘dry’ sides was lower than on previous days. This was concurrent with a substantial drop in temperature (Figure 7.9 B). From this day onwards the sap flow on the ‘dry’ side remained lower as soil drying intensified (Figure 7.8 A).

Day 11 was very overcast with relatively low air temperature which affected the sap flow on both the ‘wet’ and ‘dry’ sides, causing a very low flow on that day (Figure 7.7 A, B). No sap flow was detected in roots on either side of the vine after the roots were detached from the trunk (day 15) showing that external conditions such as changes in temperature did not interfere with the sap flow sensors.

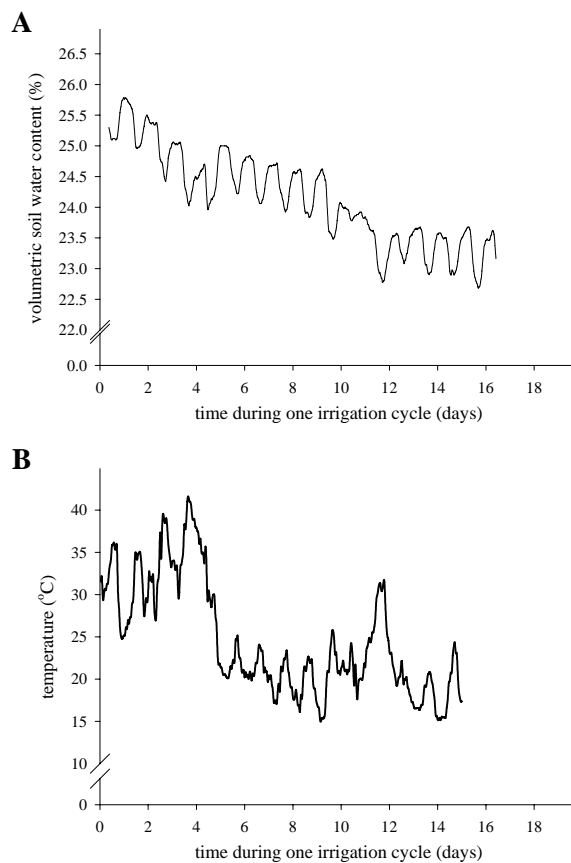


Figure 7.8 Soil moisture content and climatic conditions during the experiment (*Vitis vinifera* L. Sultana on own roots). A: volumetric soil water content (%). B) ambient temperature (°C).

Overnight, when stomata were closed, only sap movement towards the soil was observed (Figure 7.7 C, D). On the 'wet' side, however, sap movement in the same direction was almost not detectable (Figure 7.7 D) whilst, on the 'dry' side, a higher sap flow was monitored and showed a tendency to increase during the experiment (Figure 7.7 C). Sap movement towards the soil on the 'wet' side was only 12% of the flow monitored on the 'dry' side.

Sap velocity measurements for sap movement on the 'wet' and the 'dry' side of the vine are shown for each day of the experiment in Figure 7.9. During the daytime, sap velocity followed a diurnal rhythm showing an increase in velocity during the morning, a substantial sap movement during the day and a reduction of sap movement in the afternoon. On most days maximum sap velocity on 'dry' side almost reached the same level as the 'wet' side. On many days, however, the velocity on the 'dry' side showed a shorter duration during the day. This effect tended to become more pronounced as soil drying intensified.

Because of a failure of the weather station during this period only temperature data were recorded. This does not allow relation of the velocity data to VPD or solar radiation. On hot days (day 1 to 5 [14th Jan. – 18th Jan. 2000]), however, with an average maximum temperature of 36.5 °C and a high solar radiation (average 24.7 MJm⁻²) VPD is expected to be higher than on cooler and cloudier days (day 11 to 14 [24th Jan. – 27th Jan. 2000]) with an average daily maximum of 23.9 °C and average solar radiation (17.6 MJm⁻²). Sap velocity on the first five days exhibited a diurnal rhythm with a slight depression around midday. On cooler days the velocity curve of both treatments was more 'bell shaped'.

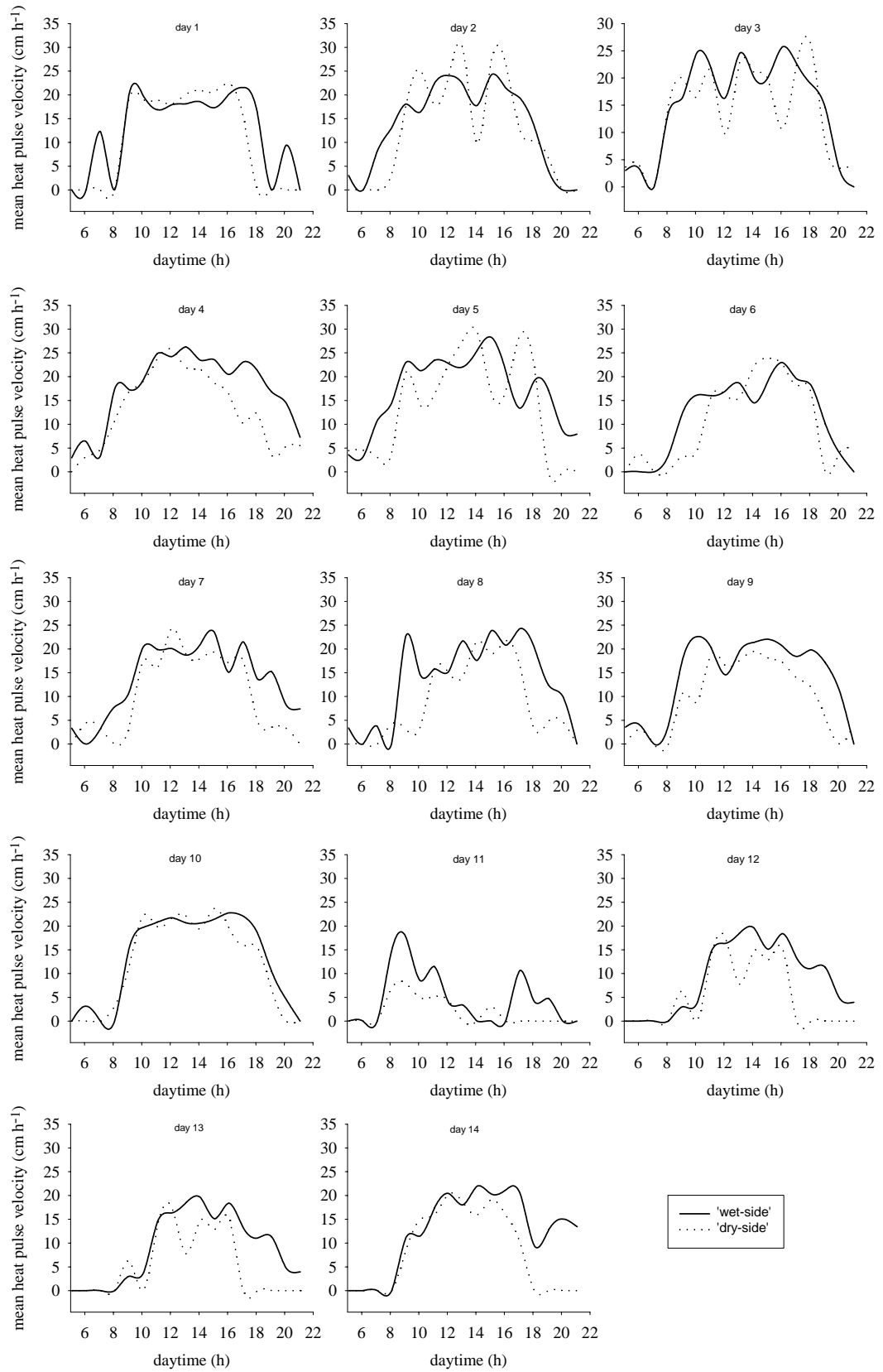


Figure 7.9 Mean heat pulse velocity (*Vitis vinifera* L. cv. Sultana on own roots).

7.3.4 Monitoring water movement in grapevines using PRD

Applying deuterium-enriched water to split-root vines changed the isotope ratio in different organs of the vines. In a preliminary experiment, the deuterium/hydrogen ratio in leaves of a split-root vine, where one pot was dried for several days (soil water content not measured), was measured to test whether any enrichment of the hydrogen was detectable. The day after the ‘wet’ side was watered with deuterium-enriched water the isotopic $^2\text{H}/^1\text{H}$ ratio in leaves was higher than plants which were irrigated with tap water (Table 7-1). Some natural enrichment of deuterium in the leaves of the vines irrigated with tap water was also observed.

Table 7-1 Isotopic $^2\text{H}/^1\text{H}$ ratio in tap water, deuterium enriched water and leaves (*Vitis vinifera* L. cv. Cabernet Sauvignon split-root vines on own roots).

Sample	isotope ratio ($\delta ^2\text{H}$ (‰, V-SMOW))
tap water	-18
deuterium-enriched water	306.5
leaves of plant irrigated with tap water	36.4
leaves of plant irrigated with deuterium-enriched water	87.0

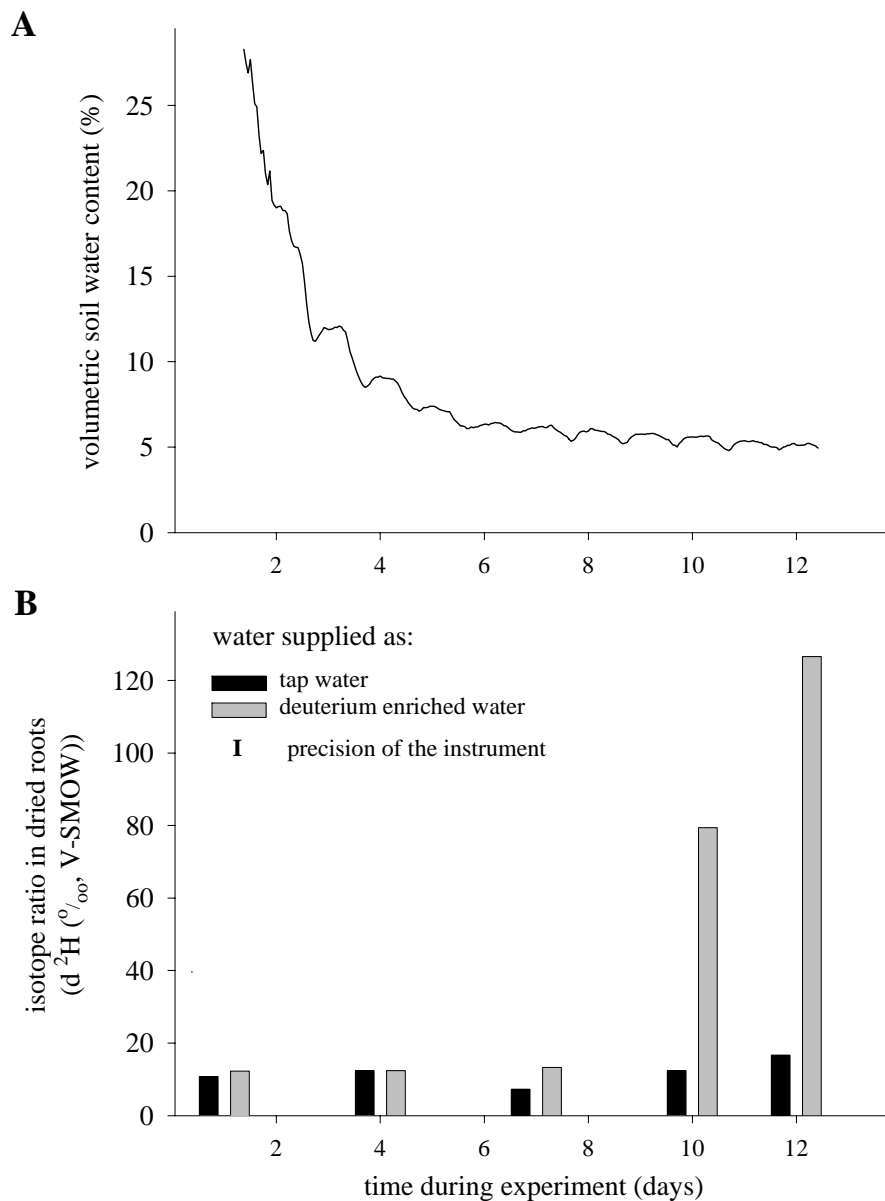


Figure 7.10 Effect on the isotope ratio in roots of the ‘dry’ side after applying deuterium-enriched water to the ‘wet’ side of split-root vines (*Vitis vinifera* L. cv. Cabernet Sauvignon split-root vine on own roots). A) Volumetric soil water content (%) of ‘dry’ side B) isotope ratio in roots on ‘dry’ side of PRD-treated vines (vertical bar indicates the precision of analyses).

The results from measurements of the $^2\text{H}/^1\text{H}$ ratio in roots are shown in Figure 7.10. During the experiment the isotope ratio in roots of PRD-treated vines which received tap water was almost constant. However, in vines receiving deuterium-enriched water on one side the isotope ratio in roots on the ‘dry’ side started to increase over the course of the experiment. An increase in the isotope ratio started to occur as soil drying

intensified. Ten days after the experiment was started the roots on the 'dry' side had a 6-fold "heavier" isotope ratio compared to control vines. On day 12 the isotope ratio in vines receiving deuterium-enriched water on one side was 8 times higher than vines receiving tap water.

7.3.5 Effects of PRD on root distribution

Generally, the majority of grapevine roots are concentrated in the top metre of soil profile and in zones that are favourable for growth. Samples were taken under the vines and from either side of the drip lines where the soil compaction is lowest. Under these conditions we found that withholding water from one side of the root-system changed the distribution of roots and that PRD caused roots to grow to deeper soil layers (Figure 7.11). At depths of 0.4 m to 0.7 m, the roots in the 1 mm to 3 mm diameter class had a significantly higher abundance for PRD vines compared to those which received water on both sides ($P < 0.05$). Also, vines watered on both sides had a higher proportion of roots in soil layers closer to the surface compared to PRD vines. In these samples, the dry weight of roots in the diameter classes smaller than 1 mm and 1 to 3 mm was significantly increased ($P < 0.05$). When the total mass of roots from both soil layers was combined, however, the total dry weight for the control and the PRD vines did not differ ($P > 0.05$).

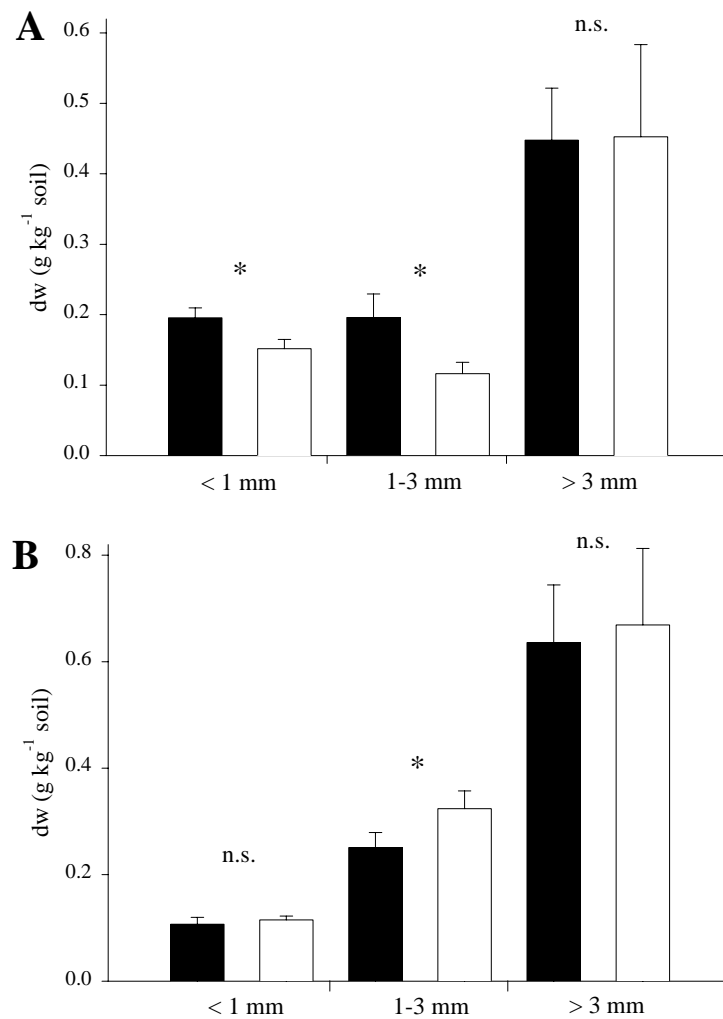


Figure 7.11 Effect of different irrigation treatments on root distribution of various diameter classes at two depths (*Vitis vinifera* L. cv. Riesling grafted on Ramsey rootstock; dw: dry weight (g kg⁻¹ soil)). ■ control: both sides of the vine irrigated; □ PRD: at any time water was withheld from one side of the vine (means, n=12 *P<0.05; ns P>0.05).

7.4 Discussion

Roots, being a primary sensor of soil drying, play an important role in long- and short-term responses to PRD. Using stable isotopes of water and heat-pulse sap flow sensors, water movement was traced from ‘wet’ to ‘dry’ roots in response to PRD.

Stable isotope analysis complements other techniques and provides information which cannot be obtained by other means. In particular, it can identify the source of

water used by a plant and the localisation of that source. This has been particularly problematic where there is more than one water source available to a plant. Stable isotope analysis has been successfully used to trace water use of plants and it was demonstrated that plants can have access to different water sources (Sternberg & Swart, 1987). By using deuterium-enriched irrigation water it was shown that water movement from 'wet' to 'dry' roots occurred even in split root vines, if there was an appropriate difference in soil water gradient. Changes in deuterium to hydrogen ratio in 'dry' roots occurred when differences in soil water content were the highest. This study provided further evidence in support of this phenomenon and demonstrated its occurrence in grapevines which does not appear to have been previously described for grapevines. At night, when stomata are closed, water may flow from roots in a soil of relatively high water potential to roots in soil of relatively low water potential. This may have several implications for root performance: it may prolong activity and growth, or affect signals within the root to shoot communication. Thus, it can be seen as an important component in controlling the water balance of roots and the movement of signalling chemicals around the plant.

The phenomenon of passive water movement along a soil water potential gradient has been described in the literature as 'hydraulic lift' (Richards & Caldwell, 1987) (Caldwell & Richards, 1989). A redistribution of water from roots in a soil of high water potential to roots in a low water potential has recently been described in both *Grevillea robusta* trees (Smith et al., 1997) and orange trees (B. Loveys, pers. comm.), where sap flow from 'wet' to 'dry' roots occurred during the night. The phenomenon of hydraulic lift is not necessarily limited to a particular plant species and has been reported for many other woody species grown in mesic, semi arid and arid environments (Caldwell *et al.*, 1998).

In most studies the amount of water moved counts for a considerable amount of the daily evapotranspiration. In shrub (*Gutierrezia sarothrae*) (Wan *et al.*, 1993) and maple trees (*Acer saccharum*) (Dawson, 1996) the estimated proportion of the water used in daily evapotranspiration contributed by hydraulically-lifted water was 14% and 25% respectively. This temporarily stored water may be used rapidly during the following morning, which is possibly important for the movement of chemical signals and thereby the sap velocity during that day. Caldwell *et al.* (1998) reported that water

redistribution in roots was prevented when, after several normal day-night cycles, plants were illuminated at night, thereby forcing stomata to open. On the day following this nocturnal light treatment, transpiration rate was reduced. The explanation these authors provided for this phenomenon was that on the following day less water was available and hence the reduction in transpiration provided an indirect measure of the quantity of water hydraulically-lifted. This could also be an interesting approach for future research in relation to plant responses to chemical signals. It could be hypothesised that if such water movement from 'wet' to 'dry' soil is of importance in sustaining the effect of chemical signals, an interruption of the normal day-night cycle might prevent water redistribution thereby making less chemical signals available from the 'dry' side.

It has been recently reported that the effects of PRD on shoot growth and gas exchange are transient if water is withheld from one side of the root system for a prolonged time period (Dry *et al.*, 2000a). These observations were interpreted by suggesting that sustained signals from the 'dry' side are important to maintain PRD responses as has been shown in previous chapters of this study. It is possible that maintenance of chemical signals may require water from the 'wet' side.

Results of the relative water content measurements of roots during this study have shown that when using PRD, the 'dry' side of the root system maintained a higher water content over a longer time period. This was relative to roots grown in similar soil water conditions, but where water was withheld from both sides. A slower decline in RWC of non-irrigated roots in response to PRD can also be discussed in the context of the movement of chemical signals. The redistribution of water may help to sustain the response to PRD and support the activity of fine roots in drying soil. Usually, as soil dries, both roots and soil move apart due to shrinkage thereby affecting the hydraulic conductivity of the soil-plant system by increasing the water potential at the interface between the two (Passioura, 1988).

Vines irrigated using the PRD irrigation strategy over several years altered their root distribution relative to non-PRD vines which received a higher amount of water over the same period. The greater abundance of roots in deeper soil layers in PRD-treated vines may contribute to the water stress tolerance of these vines. A similar effect on root distribution and an increase in root density was also observed in pot experiments (Dry *et*

al., 2000b). Forcing root growth in deeper soil layers may improve drought tolerance capabilities.

The effect of PRD on root growth may be augmented by water movement and by the large difference in ABA to cytokinin ratio, which are known to alter root growth as discussed in Chapter 5 and 6. It was observed that even when water was withheld from one side of a potted split-root vine over several months, some primary roots on the 'dry' side were maintained in a healthy condition (B. Loveys, pers. comm.). This also provides evidence for the occurrence of water movement from root to root along water potential gradients.

There are now many different techniques available to determine soil water content. Time domain reflectometry (TDR) and capacitance soil moisture probes (CP) have gained rapid acceptance as a technique for measuring soil moisture. Both instruments are able to continually monitor soil moisture, which is essential for the observation of diurnal trends. Measurements of soil water content in this study have provided evidence that diurnal fluctuations are related to environmental factors. Changes in soil water content caused by water movement within the plant are considered to be very low and would therefore require very sensitive instruments or a high density of the root system close to the sensors for detection (Caldwell *et al.*, 1998). When individual maize roots were placed close to TDR wave guides, a dielectric signal, interpreted as water efflux from roots, was observed (Topp *et al.*, 1996). Using capacitance probes, Dawson & Pate (1996) interpreted fluctuations such as these as a result of changes in root water content without necessarily releasing water into the soil. Another study, however, which used capacitance probes, concluded that soil temperature fluctuations are more likely to explain variations in soil water content and that capacitance probes themselves may be affected by temperature subsequently affecting the soil moisture reading (Mead *et al.*, 1996). Results from my study support this idea. It was demonstrated that diurnal fluctuations apparent in soil water content can also occur in the absence of a plant root system. It can be concluded therefore that differences in environmental conditions such as temperature, relative humidity and vapour pressure deficit are causing fluctuations in soil moisture content, or that the instrument itself has some degree of temperature sensitivity and that environmental changes impact directly on the accuracy of the probes. If the measured change in soil moisture is real it may be caused by capillary

water in the soil rising from deeper soil layers to upper soil layers. This also needs to be considered as a source of water available for the plant as has been discussed in relation to the hydraulic lift of water (Richards & Caldwell, 1987). With a decline of temperature and VPD overnight this capillary water may transfer back to its original position and thus generate a signal in the wave guides, which might then be monitored by the instrument (John Dighton, pers. comm.). It needs to be critically decided therefore, whether the output signal of the soil moisture instrument may come from other variables such as temperature rather than roots. Thus, other methods need to be considered for monitoring plant water movement.

Monitoring sap flow within the plant is one approach which has been widely used to demonstrate water movement in plants and heat pulse sensors have found a wide application. The heat pulse does not only move directly from the heat source to the sensor; there can also be quite a large proportion of heat which is transferred by conduction across the stem (Marshall, 1958). Roots have a high preponderance of conductive elements as demonstrated by the uptake of dye solutions. For this reason roots seem to be amenable for heat pulse sap flow measurements. In contrast, grapevine trunks and canes have a high proportion of non-conductive elements in the centre, and the conductive vessels are restricted to a small ring near the outer surface. When using heat pulse sap flow sensors in trunks or canes of grapevines, the depth of installation of the sensors is crucial, since conductive elements in trunk and canes can be very narrow. Thus heat pulse sap flow sensors might not be ideal and other sap flow systems such as heat balance (Cermak/Granier Type) or radial flow meter may be advantageous to monitor sap flow in these situations (Braun, 1997).

Using field-grown vines, the origin of roots is difficult to determine. One attempt to limit this error and reduce the interface from other vines in a horizontal direction was to dig a trench to a depth of 0.7 m around the vines since most of the roots were located in this soil layer. In this study it was assumed that the direction of the first 0.3 to 0.4 m of roots, which were excavated to install the sap flow sensors, pointed in the direction where the bulk root mass for the vine was located and where the irrigation water was applied.

During the experiment the sap velocity during the daytime from 'wet' and 'dry' sides did not differ greatly in terms of daily maximum. The reduction in total flux was more

a consequence of differences in the diurnal pattern. The velocity, measured from the 'dry' side began to rise later and declined earlier during the day. Since both 'wet' and 'dry' sides of the root system are exposed to different soil water gradients differences in hydraulic conductivity of roots on both sides may explain these diurnal differences. It has been reported that a large variability in hydraulic resistance in roots and variations of hydraulic conductivity are major forces involved in the water movement in plants (Steudle & Peterson, 1998). Specific manipulation of soil water conditions using PRD can therefore be seen as another tool for influencing plant water use. Roots growing under drying soil conditions, however, still had substantial sap flow during the day. This might also be of importance in the discussion of mobilising root-borne chemical signals since they are proposed to be synthesised and transported from the 'dry' side of the plant to the shoot as has been discussed in Chapter 5.

PRD deliberately manipulates drying soil conditions by creating different soil water potential gradients in the rootzone, thereby exposing the root system to high and low soil water potentials at the same time. Water movement from roots of different soil matric potential may become stimulated and possibly sustain signals from the 'dry' side or make them available for root to shoot communication.

7.5 Conclusions

Experiments in this chapter examined the occurrence of water movement in roots growing in soil of different soil water gradients. It can be concluded that:

1. Where part of the rootzone is well-watered, the 'dry' part is able to maintain a higher relative water content than the situation where water is withheld from the entire root system, even though the water content of the soil surrounding the roots may be similarly low.
2. Sap movement from 'wet' to 'dry' roots was observed during the night.
3. Application of deuterium-enriched water to the wet side of the root system of split-root vines caused an accumulation of the heavier isotope of water on the 'dry' side as soil drying intensified.

4. Apparent nocturnal fluctuation in soil water content, observed by time domain reflectometry and capacitance probes, may be related in part to changes in environmental factors rather than to real changes in root water content.
5. Using PRD over several growing seasons caused a greater concentration of fine roots in deep soil layers.

Chapter 8 Fruit composition and wine quality with PRD treatment

8.1 Introduction

It was shown in Chapter 3 that PRD has the potential to affect shoot growth. This can result in better vine balance and a more open canopy which may be important for fruit composition. Many studies have shown that relative to vines with open canopies, those with shaded canopies produce fruit with higher potassium concentration, pH, malic acid concentration and botrytis bunch rot incidence, and reduced levels of sugar, phenols and anthocyanins (reviewed by Smart, 1985; Morrison & Noble, 1990). The use of canopy management to produce a more open canopy is important and has the potential to significantly impact on wine quality.

Shoot growth of grapevines normally continues beyond optimum length unless it is inhibited, for example by summer hedging (Reynolds & Wardle, 1989), light winter pruning (Clingeffer, 1989) or by soil water deficit (Kliewer *et al.*, 1983).

Many previous studies have used irrigation as a tool to manipulate vegetative growth, but typically deficit irrigation has been associated with a reduction in yield (Matthews & Anderson, 1989). More recent research has shown that the impact on yield depends on the strategy by which the soil water deficit is applied and there are now several forms of deficit irrigation which may have the potential to influence vegetative growth, i.e. regulated deficit irrigation (RDI) (Goodwin & Macrae, 1990; McCarthy, 1997) and partial root-zone drying (PRD) (Dry *et al.*, 1996; Loveys *et al.*, 1998).

Most shoot growth in grapevines occurs before veraison but may still overlap the rapid berry growth phase (stage I, Coombe, 1976). The timing and level of stress which will predominantly affect shoot growth but not fruit development is difficult to define. Although it has been reported that grapevine shoot growth is more sensitive to water stress than berry growth (Williams & Matthews, 1990), severe soil water deficits can affect berry development and yield (Matthews & Anderson, 1988).

Phenolic substances are very important to grape composition and wine characteristics. Phenols include red pigments (anthocyanins), brown-forming pigments, astringent flavours and bitter substances (Boulton *et al.*, 1996). One important quality factor in red grapes results from the accumulation of anthocyanins. Levels of

anthocyanins may be correlated with the degree of bunch exposure (Mabrouk *et al.*, 1997). Bunch exposure is negatively related to canopy density and shoot growth, both factors influencing the light penetration into a canopy (Smart *et al.*, 1985). Since PRD has the potential to affect bunch exposure, as discussed in Chapter 3, this may impact on anthocyanins. For many varieties, most of the anthocyanins are located in berry skins and are extracted during fermentation. Each *Vitis* species has a certain profile of anthocyanins. The anthocyanins of *Vitis vinifera* cultivars are found as 3-glucosides and their derivatives (Nagel & Wulf, 1979; Mazza & Miniati, 1993). Mazza (1995) reported that *Vitis vinifera* L. species usually produce 3-monoglucoside, 3-acetylglucoside and 3-*p* coumaroylglucoside derivatives of delphinidin, cyanidin, peonidin, petunidin and malvidin, with malvidin derivatives often being the major forms present. Anthocyanins may also contribute to the wine quality via their interaction with other phenolic substances as well as proteins and polysaccharides (Ribéreau-Gayon, 1982). Red colour pigments are therefore considered to be important determinants of wine quality.

From a grower's perspective, improving water-use efficiency (WUE) is important. WUE can be expressed in many different ways, for example as a factor of the amount of crop harvested per amount of irrigation water applied or as the amount of irrigation water applied per crop value. Both yield and quality affect vineyard returns, therefore viticultural techniques maintaining yield and improving fruit quality by improving the WUE are desirable.

The aim of this chapter is to test the hypothesis *that PRD has a positive influence on fruit and wine quality and at the same time improves water use efficiency.*

8.2 Material and Methods

8.2.1 Determination of Cabernet Sauvignon fruit composition

Split-root vines (*Vitis vinifera* L. cv. Cabernet Sauvignon) grafted to *Vitis champini* cv. Ramsey rootstock grown in the Alverstoke vineyard of the University of Adelaide

were used to determine the effect of PRD on fruit composition. Analyses were conducted during the 1996/1997, 1997/1998 and 1998/1999 seasons. The vines were trained using a Smart-Dyson trellis system (Figure 3.1). Control vines received double the amount of water of PRD-treated vines in all seasons.

From the beginning of veraison, when berry colouring begins, 50 berries of each 'test vine' were collected at different positions in the canopy once a week and used to determine mean berry weight, total soluble solids (TSS, °Brix) and pH. Just after veraison a bird net was applied and was left on the vines until harvest. When fruit was more mature, i.e. more than 21° Brix, 200 berries of each 'test vine' were collected once a week. Each sample was divided into 4 sub-samples. The mean berry weight was determined for each sub-sample, then one sub-sample was used immediately to determine total soluble solids (TSS), titratable acidity (TA) and pH. The other 3 sub-samples were stored at -20 °C until further analysis. These sub-samples were used for further determination of anthocyanins and phenolics as described in Section 2.9.

Yield components were determined at harvest. The total number of bunches per 'test vine' was counted and the total fruit weight was determined per 'test vine' using a hand held balance. In the 1996/1997 season ca. 10% of both control and PRD treatments were infected by oidium (*Uncinula necator*). Single bunches with severe infection were discarded and an equivalent number of uninfected bunches from buffer vines were also removed to correct the yield of the 'test vine'. During the other 1997/1998 and 1998/1999 seasons fungal disease problems were negligible.

8.2.2 Determination of Shiraz fruit composition

To determine the effects of PRD on fruit composition, vines (*Vitis vinifera* L. cv. Shiraz on own roots) grown in the Coombe vineyard of the University of Adelaide were used. Measurements were undertaken during the 1998/1999 and 1999/2000 seasons. The vines were trained on a vertical shoot positioning (VSP) trellis system and equal amounts of water were applied to control and PRD-treated vines.

Measurements of canopy density, fruit sampling and assessment of fruit composition were conducted as described above (Section 8.2.1).

Close to harvest the deformability of 50 berries from each ‘test vine’ was measured between the 3rd and 20th of February 2000. The berry deformability was defined using a Harpenden skin-fold gauge (British Indicator, Burgess Hill, West Sussex, UK) as described by Coombe & Bishop (1980).

8.2.3 Anthocyanin content of berry skins

The effect of bunch exposure on anthocyanins in berry skins was determined using vines (*Vitis vinifera* L. cv. Cabernet Sauvignon split-root vines grafted to *Vitis champini* cv. Ramsey rootstock) grown in the Alverstoke vineyard of the University of Adelaide. The berries were sampled in the 1996/1997 and 1997/1998 seasons.

Different canopy management strategies were used to manipulate the bunch exposure in each season. The idea was to have either the same bunch exposure for both control and PRD or to affect canopy development using PRD. First, manual removal of leaves at the bunch zone of control vines thereby improving the bunch exposure of control vines was used in the 1996/1997 season. Second, the bunch exposure was improved by using PRD, but the control vines were not treated: this resulted in differing degrees of bunch exposure for each treatment.

In the 1996/1997 season leaves were deliberately removed from the bunch zone 8 weeks after flowering to achieve a similar bunch exposure for control and PRD-treated vines. The bunch exposure was measured using a “point quadrat” measurement (Smart & Robinson, 1991) which determines the proportion of leaves and fruit on the exterior or in the interior of the canopy. A rod is passed from the vine exterior into the interior of the canopy to simulate a beam of light and each contact with leaves or bunches is recorded. For this purpose a 2 mm thick, sharpened, metal rod was horizontally inserted through a guiding tube into the canopy. Fifty insertions per ‘test vine’ were performed at different positions that is above and below the bunchzone. The leaf layer number was calculated as the mean number of leaf contacts per insertion and used as an index of canopy density.

During the 1997/1998 season canopy density was determined using a ceptometer as described in Section 3.2.3.

When fruit was more mature, that is more than 21° Brix, 200 berries of each 'test vine' were collected once a week. The mean berry weight was determined for four 50 berry sub-samples. One sub-sample was immediately used to determine total soluble solids (TSS), titratable acidity (TA) and pH. The other 3 sub-samples were stored at –20 °C until further analysis.

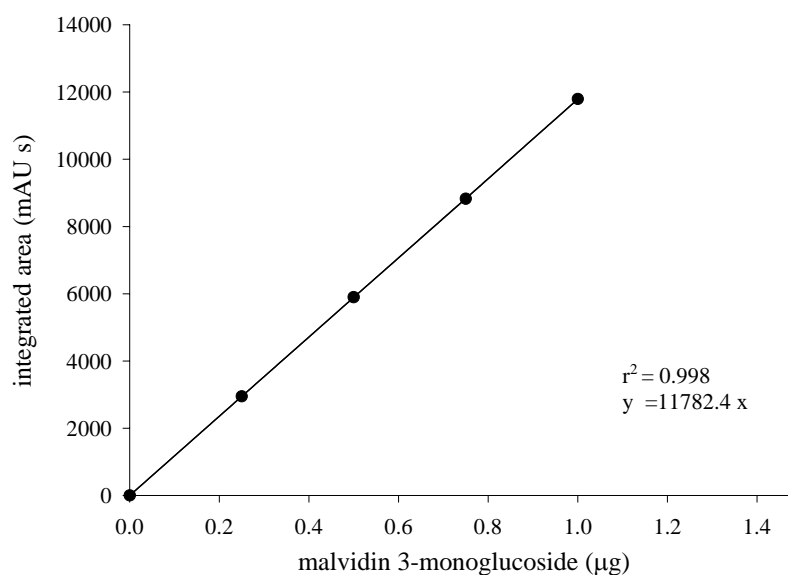
Anthocyanins were extracted from berry skins of 25 frozen berries from 50 berry sub-samples with a TSS concentration of 23° Brix. Peeled berry skin tissue was ground to a fine powder in liquid nitrogen using a pre-chilled mortar and pestle. Anthocyanins were extracted from duplicate 0.5 g ground berry skin samples by thorough mixing in 1.0 mL methanol and storing at –20 °C for 1 h, mixing several times throughout the extraction. Extracted skin tissue was then pelleted via centrifugation at 10,000 g for 15 min at 4 °C and the supernatant was transferred to a new tube. The pellet was re-extracted twice in 0.75 mL methanol, centrifuging and collecting the supernatant after each wash. The final volume was then adjusted to 3 mL with methanol for HPLC analysis.

HPLC analyses were performed using a Gold Pack (Activon, Sydney, Australia) C₁₈ reversed phase column (250 mm x 4.6 mm, 5 µm packing) on a HPLC (Hewlett Packard LC 1100 series) equipped with a diode array detector measuring absorbance at 520 nm. 200 µL of 1% (v/v) perchloric acid was added to 200 µL of each sample. Following centrifugation at 5,000 g each sample was transferred to an injection vial. 100 µL of sample was injected onto the reversed phase C₁₈ column and separated using the method shown in Table 8.1.

Table 8.1 Solvent gradient for the HPLC separation of anthocyanins

Time (min)	% solvent A (water)	% solvent B (methanol)	% solvent C (1.5% (v/v) perchloric acid)	flow rate (mLmin ⁻¹)
0	50	20	30	1
10	40	30	30	1
15	35	35	30	1
30	10	60	30	1
35	0	100	0	1
40	0	100	0	1
42	80	20	0	1
50	80	20	0	1

The different 3-monoglucoside forms of the anthocyanins, delphinidin, cyanidin, peonidin, petunidin and malvidin and their 3-acetylglucosides and 3-*p*-coumaroylglucosides derivatives were determined according to their retention times as described by Wulf & Nagel (1978) and quantified using their integrated peak areas, expressed as malvidin 3-monoglucoside equivalents (Figure 8.1).

**Figure 8.1** Calibration curve of malvidin 3-monoglucosides to quantify anthocyanin concentration in berry skins (s.e. smaller than symbols).

8.2.4 Small scale winemaking and assessment of wine quality

The aim of this experiment was to assess wine quality from control and PRD-treated 'test vines'. Due to the experimental nature of mini-winemaking using 3kg of fruit, minimum handling, no malolactic fermentation and no oak were used. The wine was to reflect the varietal and organoleptic characters of the fruit from different treatments.

All 'test vines' were harvested with the same concentration of total soluble solids (TSS). To determine the fruit composition after veraison, the same berry sampling technique as described in Section 8.2.1 was used. Fruit from each 'test vine' was picked when the TSS of the 50 berry sub-sample reached 24°Brix. At harvest a 200-berry sample was collected to determine fruit composition. During harvest all bunches on each 'test vine' were counted and the total crop level was determined using a hand held balance. All bunches were then frozen at $-20\text{ }^{\circ}\text{C}$ until all 'test vines' were harvested. Six kg of berries for each replicate were picked randomly from the frozen bunches, divided into two containers (volume 3.5 L) and defrosted. When all samples had a temperature of approximately $18\text{ }^{\circ}\text{C}$, TSS was measured and fermentation was initiated. The berries were mashed using a potato masher. All containers were inoculated with yeast (Lallemand ADWY Enoferm M2 *Saccharomyces cerevisiae*) using 4 mL of a yeast solution (10 g yeast in a 10 % glucose solution) for each mini ferment (Patrick Iland, pers. comm.). Two mL of a 4 % potassium metabisulfite solution and 2 mL of a 8% diammonium phosphate solution were added to each container (Patrick Iland, pers. comm.). The maceration technique for each ferment was hand plunging 4 to 5 times a day. Plunging enabled gentle extraction and crushing of any remaining whole berries. Temperature and total soluble sugars were measured daily. The targeted fermentation temperature range was $20\text{-}22\text{ }^{\circ}\text{C}$. This temperature range was chosen to ensure a controlled fermentation reducing the sugar content by 1.5 to 2° Brix per day. Once the fermentation reached dryness, that is a residual sugar content less than 5 gL^{-1} , the crushed must was pressed using a 3 L capacity water bag press at 10 kPa pressure. The pressing was repeated once.

The wine from each replicate was stored in a 2 L flagon. The wines were kept on the lees deposit for 2 weeks in a cold room ($1\text{ }^{\circ}\text{C}$) before they were racked into cleaned

flavons and stored at the same temperature for an extra 6 months. After storing, the wines were carefully raked from the remaining fine lees and each of the two fermentation replicates were combined into a larger flagon to create a single blend for each fruit from each ‘test vine’. The result was 5 to 6 bottles (0.75 L) of wine for each ‘test vine’ which were stored at 1°C until tasting. The cold storage was used to avoid any malolactic fermentation which in red wines often occurs straight after the alcoholic fermentation under warm temperature conditions. Analysis for residual sugar, alcohol content and TA was performed on finished wines using the protocols described by Iland *et al.* (2000).



Figure 8.2 Procedure of mini wine making. A: De-stemmed, crushed berries. B: Temperature control during fermentation. C) Must pressing with a water press. D) Bottles after pressing

To assess the wine quality, a paired bilateral tasting was used. The taster panel consisted of 35 tasters. One third of the panellists were trained, one third had experience in wine tasting and the rest were inexperienced tasters. The tasting was performed in the tasting room of the University of Adelaide, where each taster is separated in a booth which is illuminated with yellow light so that colour perception of the wine is eliminated as a variable. Three pairs of samples consisting of one control and one PRD wine were presented to each taster. The pairs were arranged in a random order. The panelists were instructed to rank the wines in each pair according to the following statements:

- 1) Circle the number of the sample which has the most aroma.
- 2) Circle the number of the sample which has the most intensity in flavour.
- 3) Circle the number of the sample which has the highest astringency.

8.3 Results

8.3.1 Fruit composition of Cabernet Sauvignon

To investigate the effect of PRD on fruit composition, measurements were taken in 3 consecutive seasons (1996/1997; 1997/1998; 1998/1999). No differences in timing of phenological stages (budburst, flowering, fruitset or veraison) were observed in any of the seasons when control and PRD-treated vines were compared. Vines with a smaller crop load and/or better bunch exposure showed a tendency to reach *circa* 23° Brix ripeness level one to two weeks earlier than higher yielding vines or vines with relatively shaded bunches, irrespective of the treatment.

Results of measurements on berry composition during the three seasons are shown in Table 8.2. There was a considerable spread of values for most variables pertaining to fruit composition and high variability occurred between the replicates.

PRD-treated vines tended to have consistently higher TA concentration at the same total soluble solids (TSS). TSS during the 1996 to 1999 seasons were not greatly affected by irrigation treatment. The timing of maturity (ca. 23° Brix) was affected when differences in bunch exposure and crop load occurred.

Measurement of anthocyanins and phenolics in berries in the 1996/1997 season revealed minor differences between control and PRD-treated vines. In the 1997/1998 season PRD-treated vines had a significantly higher anthocyanin concentration per g berry weight and a 15% higher anthocyanin concentration on a per-berry basis compared to control vines. Phenolic concentrations of PRD-treated vines were higher, both on a per-berry and per-berry mass basis, on average 11%.

In each of the three seasons, effects on yield components were minor. The average reduction in yield of PRD-treated vines relative to control vines was 6% ($P > 0.05$). Notably, there was no significant effect of treatment and berry weight in any season. Water-use efficiency of PRD-treated vines, however, was significantly improved by more than 80% for three consecutive seasons.

Table 8.2 Effects of PRD on fruit composition and water use efficiency (WUE) (Cabernet Sauvignon / Ramsey split-root vines)

	variable	control	PRD	diff. (%)	sig.
1996/97	TSS (°Brix)	22.2 ± 0.09	22.1 ± 0.65		n.s.
	TA (g/L)	5.6 ± 0.16	5.8 ± 0.19	4	n.s.
	pH	3.6 ± 0.03	3.6 ± 0.02	-1	n.s.
	anthocyanins (mg/ g berry mass)	1.0 ± 0.02	1.1 ± 0.07	6	n.s.
	anthocyanins (mg/ berry)	1.0 ± 0.12	1.0 ± 0.05	2	n.s.
	phenolics (AU/g)	1.0 ± 0.04	1.1 ± 0.01	5	n.s.
	phenolics (AU/g berry mass)	0.9 ± 0.06	1.0 ± 0.01	9	n.s.
	berry weight (g)	1.0 ± 0.02	0.9 ± 0.03	-4	n.s.
	no. of bunches	159.8 ± 16.9	164.8 ± 6.9	3	n.s.
	yield (t/ha)	23.4 ± 1.24	22.0 ± 1.78	-6	n.s.
	irrigation water applied (ML/ha)	2.4	1.2	-50	
WUE (t/ML)	9.8	18.3	88		
1997/98	TSS (°Brix)	24.3 ± 0.40	24.5 ± 0.26	1	n.s.
	TA (g/L)	6.6 ± 0.19	7.0 ± 0.21	6	n.s.
	pH	3.4 ± 0.03	3.4 ± 0.01	-1	n.s.
	anthocyanins (mg/g berry mass)	1.3 ± 0.12	1.4 ± 0.09	12	P<0.05
	anthocyanins (mg/ berry)	1.3 ± 0.13	1.4 ± 0.05	15	n.s.
	phenolics (AU/g)	1.0 ± 0.12	1.1 ± 0.08	10	n.s.
	phenolics (AU/g berry mass)	1.0 ± 0.07	1.1 ± 0.04	12	P<0.05
	berry weight (g)	1.0 ± 0.04	1.0 ± 0.05	5	n.s.
	no. of bunches	160.3 ± 9.2	143.3 ± 6.7	-11	n.s.
	yield (t/ha)	22.9 ± 1.31	21.0 ± 0.80	-8	n.s.
	irrigation water applied (ML/ha)	1.4	0.7	-51	
WUE (t/ML)	16.6	30.2	82		
1998/99	TSS (°Brix)	22.9 ± 0.56	23.1 ± 0.36	1	n.s.
	TA (g/L)	4.9 ± 0.13	5.2 ± 0.32	6	n.s.
	pH	3.5 ± 0.02	3.5 ± 0.01		n.s.
	berry weight (g)	1.0 ± 0.05	1.0 ± 0.05	-2	n.s.
	no. of bunches	149.3 ± 9.2	156.8 ± 9.3	5	n.s.
	yield (t/ha)	23.6 ± 1.78	22.4 ± 0.58	-5	n.s.
	irrigation water applied (ML/ha)	1	0.5	-50	
WUE (t/ML)	33.6	63.9	90		

(control: vines received water on both sides; PRD: at any time to one side of vines water was withheld; means ± s.e.; n=4)

Table 8.3 is a correlation matrix of fruit composition and physiological measurements for PRD-treated vines in the seasons between 1996 to 1999. These data

show that leaf area was significantly negatively correlated with light penetration into the canopy ($P<0.05$). Leaf area and light penetration were correlated with TSS ($P<0.05$), anthocyanins ($P<0.01$) and phenolics ($P<0.01$). Furthermore, it was found that anthocyanins ($P<0.01$) and phenolics ($P<0.05$) were strongly correlated with TSS .

Table 8.3 Correlation matrix of fruit components and physiological measurements of PRD-treated vines (Cabernet Sauvignon / Ramsey split-root vines; season 1996 to 1999; grey cell: $P<0.05$; dark grey: $P<0.01$)

	berry wt (g)	TSS ($^{\circ}$ Brix)	anth. (mg/g berry mass)*	phen. (mg/g berry mass)*	no of bunches per vine	yield (t/ha)	leaf area (m^2)	light penetration ($\mu\text{molm}^{-2}\text{s}^{-1}$)
berry wt (g)	1							
TSS ($^{\circ}$ Brix)	0.2927	1						
anth. (mg/g berry mass)*	0.5339	0.9499	1					
phen. (mg/g berry mass)*	0.2675	0.7413	0.8820	1				
no of bunches	-0.2116	-0.1182	-0.2820	-0.4710	1			
yield (t/ha)	-0.2213	-0.2788	-0.2069	-0.3138	0.2535	1		
leaf area (m^2)	0.6326	0.5704	0.8537	0.7863	-0.5442	-0.1222	1	
light penetration ($\mu\text{molm}^{-2}\text{s}^{-1}$)	-0.5493	-0.7333	-0.8680	-0.7163	0.1949	0.1985	-0.6775	1

df=10; if $r>0.5760$, $P<0.05$; if $r >0.7079$, $P<0.01$; *: df=6, if $r>0.7067$, $P<0.05$; if $r >0.8343$, $P<0.01$

8.3.2 Fruit composition of Shiraz

To determine the effect of PRD on fruit composition of Shiraz when the same amount of water was applied to both the control and PRD-treated vines, fruit composition was compared over two seasons (Table 8.4).

Table 8.4 Effects of PRD on fruit composition and water use efficiency (WUE) of Shiraz (own roots) when same amount of water was applied to control and PRD-treated vines

	variable	control	PRD	diff. (%)	sig.
1998/99	TSS(°Brix)	23.9 ± 0.32	23.9 ± 0.28		n.s.
	TA (g/L)	3.3 ± 0.06	3.8 ± 0.05	14	P<0.05
	pH	3.6 ± 0.01	3.6 ± 0.01		n.s.
	berry weight (g)	1.0 ± 0.04	1.1 ± 0.03	8	n.s.
	no. of bunches per vine	87.4 ± 3.5	92.7 ± 3.8	6	n.s.
	yield (t/ha)	17.3 ± 0.95	19.7 ± 0.96	13	n.s.
	irrigation water applied (ML)	0.5	0.5		
	WUE (t/ML)	37.7	42.7	13	
1999/00	TSS (°Brix)	24.7 ± 0.39	24.5 ± 0.23	-1	n.s.
	TA (g/L)	5.3 ± 0.06	5.6 ± 0.11	4	n.s.
	pH	3.4 ± 0.01	3.4 ± 0.02	-1	n.s.
	anthocyanins (mg/g berry mass)	1.4 ± 0.05	1.4 ± 0.03	4	n.s.
	anthocyanins (mg/ berry)	1.4 ± 0.05	1.3 ± 0.02	-2	n.s.
	phenolics (AU) / g	1.2 ± 0.04	1.2 ± 0.02	2	n.s.
	phenolics (AU) / g berry mass	1.2 ± 0.05	1.1 ± 0.02	3	n.s.
	berry weight (g)	1.0 ± 0.03	1.0 ± 0.03	-1	n.s.
	no. of bunches	92.1 ± 7.6	109.4 ± 9.5	19	n.s.
	yield (t/ha)	9.2 ± 1.46	11.4 ± 1.50	23	n.s.
	irrigation water applied (ML)	0.5	0.5		
	WUE (t/ML)	20.4	25.1	23	

(control: vines received water on both sides; PRD: at any time to one side water was withheld; means ± s.e.; n=8)

Anthocyanin and phenolic concentration per gram fresh weight were slightly higher in fruit of PRD-treated vines than in fruit of control vines. In addition, it was found that the yield of PRD-treated vines was increased by 13% and 23% in 1998/1999 and 1999/2000 seasons respectively ($P>0.05$). In the 1998/1999 season the increase in yield of PRD-treated vines was due to a combination of larger bunch weight and more bunches per vine. The larger bunch weight was due to an increase in berry weight with no effect on berry number per bunch. In the 1999/2000 season the berry weight was not significantly affected, but the number of bunches was increased by 19%. Changes in crop yield also affected the water use efficiency. During the 1998/1999 and 1999/2000 seasons, WUE of PRD-treated vines improved by 13% and 23% respectively compared to control vines.

Another observation made in the 1999/2000 season was that the deformability of fruit from PRD-treated vines was lower than that from control vines (Figure 8.3). The berry deformability was measured close to harvest when the berries had reached almost full ripeness (Figure 8.3 A). A rainfall event occurred over days 44 and 45 after veraison with a total rainfall of 37.5 mm m⁻² (20th - 21st of Feb. 2000). After the rainfall, which accounted for 100% of the rainfall during February 2000, the berry weight increased and the deformability of both control and PRD-treated vines decreased. At harvest (22th of Feb. 2000) the deformability of berries from control vines was still significantly higher than that from PRD-treated vines (Figure 8.3 C).

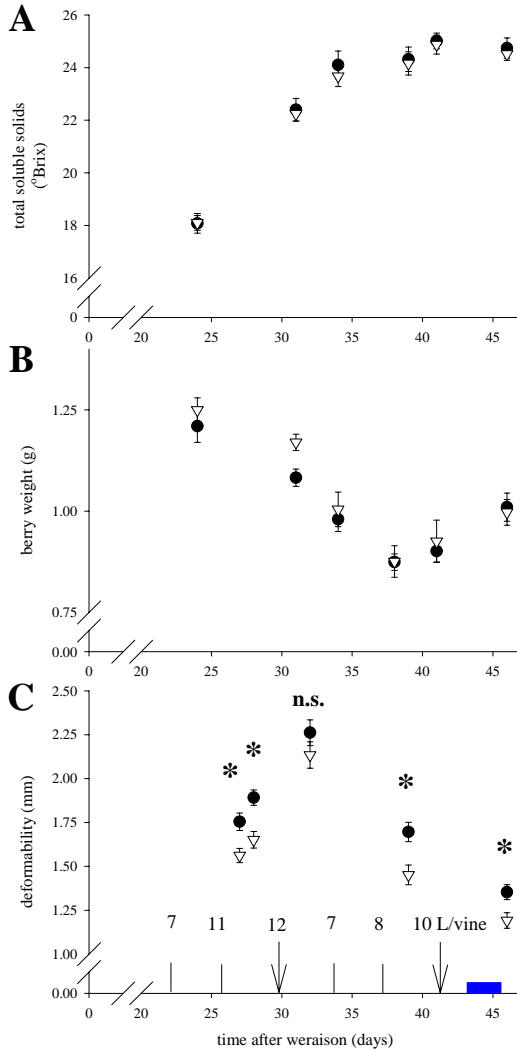


Figure 8.3 Effects of PRD on fruit composition and berry parameters. The same amount of water was applied using two different irrigation regimes; control (●): vines received water on both sides of the vine; PRD-treated vines (▽): at any time to one side of the vine water was withheld. **A**) total soluble solids (TSS, °Brix; means ± s.e.; n=8) **B**) berry weight (bw (g); means ± s.e.; n=8) **C**) berry deformability (mm); | :irrigation; ↓:irrigation plus alternation of the PRD sides, ■ rainfall; means ± s.e.; n=20; * P<0.01.

8.3.3 Anthocyanins in berry skins

To determine the effects of different degrees of light penetration into the canopy on anthocyanin concentration in berry skins, the bunch exposure of control vines was either manipulated by leaf removal in control vines (1996/1997) or differences in light penetration into the canopy in 1997/1998 season between control and PRD-treated vines were achieved by the PRD irrigation treatment as has been demonstrated in Section 3.3. Table 8.5 shows the results of canopy density and light penetration measurements. Control and PRD-treated vines had very similar leaf layer numbers measured at the bunch zone in the 1996/1997 season which indicates a similar level of bunch exposure. In the 1997/1998 season light penetration into the canopy, measured as photosynthetically active radiation at the bunch zone, was significantly higher in PRD-treated vines than control vines.

Table 8.5 Canopy density and light intensity inside the canopy (Cabernet Sauvignon / Ramsey split-root vines).

	variable	control	PRD	diff. (%)	sig.
1996/1997	LLN	1.10 ± 0.11	1.05 ± 0.10	-5	n.s.
1997/1998	PAR ($\mu\text{molm}^{-2}\text{s}^{-1}$)	102.0 ± 17.7	170.5 ± 10.50	67	P<0.05

(control: vines received water on both sides; PRD: at any time to one side of the vine water was withheld; 1996/1997: leaf layer number (LLN); manual leaf removal 8 weeks after flowering; 1997/1998: photosynthetically active radiation (PAR, $\mu\text{molm}^{-2}\text{s}^{-1}$); no leaf removal; means ± s.e.; n=4)

Results comparing 3-monoglucosides, 3-acetylglucosides and 3-p coumaroylglucosides, where canopy density was manipulated are shown in Table 8.6. 3-monoglucosides and 3-acetylglucosides showed the highest abundance and accounted for nearly 90% of the total anthocyanins during both seasons. The ratios for the three groups of anthocyanins were not significantly different for control and PRD-treated vines, even though in the

1997/1998 season the bunch exposure was different. Changes in 3-monoglucosides and 3 acetylglucosides groups were minor for both irrigation treatments. Derivatives of 3-monoglucosides and 3 acetylglucosides increased by 2.0 and 2.8% in control vines and by 5.0 and 2.5% in PRD-treated vines respectively. The 3-*p*-coumaroylglucosides were 8 % in the first season and -4% lower in the second season when control and PRD-treated vines were compared.

Table 8.6 Proportion of 3-monoglucosides, 3-acetylglucosides and 3-*p* coumaroylglucosides as % of the total anthocyanin concentration (Cabernet Sauvignon / Ramsey split-root vines)

	group	control (%)	PRD (%)	diff. (%)	sig.
1996/1997	3-monoglucosides	58.2 ± 2.0	57.5 ± 2.4	-1	n.s.
	3 acetylglucosides	29.2 ± 1.1	28.9 ± 1.1	-1	n.s.
	3- <i>p</i> coumaroylglucosides	12.6 ± 1.0	13.7 ± 1.3	8	n.s.
1997/1998					
	3-monoglucosides	59.6 ± 0.8	60.4 ± 0.9	1	n.s.
	3 acetylglucosides	30.0 ± 0.4	29.6 ± 0.5	-1	n.s.
	3- <i>p</i> coumaroylglucosides	10.4 ± 0.5	10.0 ± 0.4	-4	n.s.

(control: vines received water on both sides; PRD: at any time to one side of the vine water was withheld; 1996/1997: leaf layer number (LLN); manual leaf removal 8 weeks after flowering; 1997/1998: no leaf removal)

The total skin anthocyanin content of PRD-treated vines was 10% higher than control vines in the 1996/1997 season ($P > 0.05$; Table 8.7). The differences were highest for the group of 3-*p* coumaroylglucosides which represents the group with the smallest proportion of the three anthocyanin groups. For each group of anthocyanins, changes in derivatives were minor in the 1996/1997 season compared to the 1997/1998 season.

Table 8.7 Effect of PRD on anthocyanin derivatives in berry skins (mg g^{-1} ; season 1996/1997; Cabernet Sauvignon / Ramsey split-root vines)

	derivatives (mg g^{-1})	control	PRD	diff. (%)	sig.
total anthocyanin		0.47 ± 0.038	0.52 ± 0.036	10	n.s.
group					
3-monoglucosides		0.28 ± 0.023	0.30 ± 0.034	10	n.s.
3 acetylglucosides		0.14 ± 0.014	0.15 ± 0.005	8	n.s.
3- <i>p</i> coumaroylglucosides		0.06 ± 0.006	0.07 ± 0.003	17	n.s.
group					
3-monoglucosides	delphinidin	0.03 ± 0.005	0.04 ± 0.009	6	n.s.
	cyanidin	0.01 ± 0.002	0.01 ± 0.003	8	n.s.
	petunidin	0.03 ± 0.003	0.03 ± 0.005	6	n.s.
	peonidin	0.03 ± 0.003	0.03 ± 0.006 0.006	7	n.s.
	malvidin	0.18 ± 0.014	0.18 ± 0.011	-2	n.s.
group					
3 acetylglucosides	delphinidin	0.01 ± 0.001	0.01 ± 0.002	3	n.s.
	cyanidin	n.d.	n.d.	6	n.s.
	petunidin	0.01 ± 0.001	0.01 ± 0.001	4	n.s.
	peonidin	0.01 ± 0.001	0.01 ± 0.001	5	n.s.
	malvidin	0.11 ± 0.011	0.11 ± 0.002	-1	n.s.
group					
3- <i>p</i> coumaroylglucosides	delphinidin	0.01 ± 0.001	0.01 ± 0.001	3	n.s.
	cyanidin	n.d.	n.d.		
	petunidin	0.01 ± 0.001	0.01 ± 0.001	6	n.s.
	peonidin	n.d.	n.d.		
	malvidin	0.05 ± 0.005	0.05 ± 0.002	0	n.s.

control received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld; means \pm s.e.; n=4)

In the 1997/1998 season, the total anthocyanin content of PRD-treated vines in berry skins was 8% higher than in control vines ($P > 0.05$; Table 8.8.). The different groups of anthocyanins were, on average, 6% higher in PRD-treated vines than control vines. In contrast to the 1996/1997 season, there were significant differences in the distribution of anthocyanin derivatives between control and PRD. In both the 3-monoglucosides and 3 acetylglucosides groups, the major derivative malvidin was reduced with PRD whilst the other derivatives, delphinidin, petunidin and peonidin were significantly increased. In the smallest group, 3-*p* coumaroylglucosides, the abundance of most of the derivatives was very small and undetectable for petunidin and peonidin.

Table 8.8 Effect of PRD on anthocyanin derivatives in berry skins (mg g^{-1} ; Cabernet Sauvignon / Ramsey split-root vines season 1997/1998)

variable	derivatives (mg g^{-1})	control	PRD	diff. (%)	sig.
total anthocyanin		0.84 ± 0.091	0.90 ± 0.063	8	n.s.
group					
3-monoglucosides		0.50 ± 0.054	0.54 ± 0.043	9	n.s.
3 acetylglucosides		0.25 ± 0.028	0.27 ± 0.017	6	n.s.
3- <i>p</i> coumaroylglucosides		0.09 ± 0.011	0.09 ± 0.005	3	n.s.
group					
3-monoglucosides	delphinidin	0.06 ± 0.003	0.07 ± 0.005	17	$P < 0.05$
	cyanidin	0.01 ± 0.001	0.03 ± 0.002	15	n.s.
	petunidin	0.04 ± 0.002	0.06 ± 0.002	12	$P < 0.05$
	peonidin	0.03 ± 0.003	0.04 ± 0.003	6	n.s.
	malvidin	0.35 ± 0.009	0.33 ± 0.012	-5	$P < 0.05$
group					
3 acetylglucosides	delphinidin	0.04 ± 0.002	0.05 ± 0.001	22	$P < 0.05$
	cyanidin	0.01 ± 0.001	0.01 ± 0.001	8	n.s.
	petunidin	0.04 ± 0.001	0.04 ± 0.001	12	n.s.
	peonidin	0.01 ± 0.004	0.02 ± 0.001	40	$P < 0.05$
	malvidin	0.15 ± 0.006	0.14 ± 0.004	-7	$P < 0.05$
group					
3- <i>p</i> coumaroylglucosides	delphinidin	0.01 ± 0.001	0.01 ± 0.00	-2	n.s.
	cyanidin	n.d.	n.d.		
	petunidin	0.01 ± 0.001	0.01 ± 0.001	-1	n.s.
	peonidin	n.d.	n.d.		
	malvidin	0.06 ± 0.001	0.06 ± 0.001	0	n.s.

control: vines received water on both sides of the vine; PRD: at any time to one side of the vine water was withheld; means \pm s.e.; n=4

8.3.4 Assessment of wine quality

The quality of wines made in mini-ferments of fruit from each 'test vine' in the 1998 vintage (Cabernet Sauvignon, Alverstoke vineyard) was assessed in a sensory evaluation. The wine pairs of control and PRD treatment were chosen on the basis of identical TSS content at harvest. Results of fruit composition, wine analysis and sensory evaluation are shown in Table 8.9.

The winemaking from thawed berry samples did not detrimentally affect the wine quality and there were no signs of any off-flavours in the wine. Results of the tasting

showed that differences in wine quality were not associated with the irrigation treatment. It does appear, however, from the results of the sensory evaluation that other factors independent of the irrigation treatment influenced wine ranking. For example, wines with lower yield (control (v1)) and better bunch exposure (PRD (v1)) were rated higher than wines of higher yielding vines (PRD (v4)) or from vines with relatively poor bunch exposure (control (v2)). With respect to aroma, flavour and astringency, control (v1) and PRD (v1) were preferred.

The anthocyanin and phenolic concentration of homogenised berries also seems to be associated with wine quality. Wines produced from fruit with higher anthocyanin and phenolic concentrations (control (v1), PRD (v4 & 3)) were consistently ranked higher in flavour and astringency than wines with a lower concentration (PRD (v4), control (v2 & 3)).

Table 8.9 Effects of PRD on fruit composition, wine analysis and sensory evaluation of fruit of single ‘test-vines’ (Cabernet Sauvignon / Ramsey split-root vines)

variable	con. (v 1)	PRD (v 4)	diff. (% of con.)	con. (v 2)	PRD (v 1)	diff. (% of con.)	con. (v 3)	PRD (v 3)	diff. (% of con)
canopy measurements									
PAR (bunch zone as % of ambient)	8.3	8		4.5	10.1		4.1	8.6	
yield components and fruit composition									
no. of bunches	151	177	17	187	154	-18	119	131	10
berry weight (g)	0.87	1.04	20	0.98	0.91	-7	1.06	1.07	1
no. of berries per bunch	139	110	-21	129	127	-2	125	104	-17
yield (t/ha)	22.8	25.4	11	29.6	22.2	-25	19.7	20	2
harvest date (diff. in days)	08.04	17.04	+9 d	17.04	23.03	-25 d	27.03	14.03.	-13 d
TSS (°Brix)	24.9	24.9		23.9	23.9		25	24.9	
anthocyanins (mg/g b. wt.)	1.42	1.31	-8	1.3	1.58	22	1.38	1.5	9
phenolics (AU/g b. wt.)	1.23	0.95	-23	1.09	1.29	18	0.9	1	11
Wine analysis									
alc. (%)	13.9	13.6	-2	13.2	13.2	0	13.2	13.9	5
residual sugar (g/L)	3.6	4.1	14	4	4.3	15	4.1	3.7	0
TA (g/L)	5.3	6.1	15	6.1	5.2	-15	5.4	5.6	4
sensory evaluation	ranking		sig.	ranking		sig.	ranking		sig.
aroma	26	9	P<0.05	10	25	P<0.05	23	12	P<0.05
flavour	27	8	P<0.05	10	25	P<0.05	15	20	n.s.
astringency	24	11	P<0.05	11	24	P<0.05	14	21	n.s.

(control: vines received water on both sides of the vine; PRD: at any time to one side water was withheld. Abbreviation v # relates to field-vine of each treatment. The sensory evaluation was performed by 35 tasters selecting one wine of each pair which was considered highest in aroma, flavour and astringency)

The criteria used for pairing wines of control and PRD treatments was the level of TSS at harvest. The final alcohol content of the finished wines differed slightly, which

was associated with a higher concentration of residual sugar in the finished wine. These differences in alcohol concentration seem to be associated less with the wine quality than with other fruit parameters such as yield, anthocyanins or phenolics.

The amount of crop yield also affected the date of harvest and it was found that the lower yielding vines (control (v1), PRD (v1)) ripened 9 to 25 days earlier than the higher yielding vines (PRD (v4) and control (v2)).

8.4 Discussion

Experiments determining effects of PRD on yield components during three growing seasons have shown that PRD does not detrimentally reduce yield, even though only half the amount of water commercially used in this growing region was applied. The actual amount of irrigation water applied to control vines was, on average, 1.3 ML/ha during the seasons between 1996 to 1999, which is an amount comparable to that used in the McLaren Vale region of South Australia under similar climatic conditions and trellis systems (Peter Dry, pers. comm.).

When the amount of irrigation water is reduced by 50%, a decrease in yield of the order of 20 to 30% is the typical response (Grimes & Williams, 1990). Using half the amount of irrigation water employing PRD, the crop yield was only reduced by an average of 5 to 7%. Consequently, the water-use efficiency, expressed as tonnes of fruit per ha to ML of irrigation water per ha was substantially improved. A survey of first experiences with PRD on a commercial scale has shown that a substantial increase in water-use efficiency can be achieved by halving the amount of irrigation water (Stoll *et al.*, 2000a). Some respondents to this survey reported only minor reductions in yield of PRD-treated vines of Shiraz, Riesling and Cabernet Sauvignon relative to well-irrigated controls in different regions of South Australia and Victoria.

Williams and Matthews (1990) showed that berry weight is one of the most sensitive yield components when vines are exposed to water stress. In this study, the berry weight was almost unaffected when only half the amount of water was applied to PRD-treated vines. Also the number of bunches, or the number of berries per bunch which

normally affect yield were unchanged. Results of this study on effects of PRD on yield components supports earlier findings of Dry (1997).

The crop yield of a vine is determined by the number of berries per vine and berry weight. By applying the same amount of water to both control and PRD-treated vines, but to a different soil surface area, it was found that the yield of control vines was lower than that of PRD-treated vines. During the first season, the yield reduction of control vines was mainly due to a reduction in berry weight with less effect on bunch number. In the following season more nodes were deliberately retained at winter pruning for PRD-treated vines thereby affecting the number of bunches and both treatments had the same berry weight. Since more nodes were retained on PRD-treated vines, these vines had a higher number of bunches and thus more berries per vine still maintaining the same berry weight. It would be reasonable to expect that the berry weight of PRD vines would have been lower, because there is an inverse relationship between berry number per vine and berry weight. The fact that there was no treatment effect, however, suggests that for control vines, the berry size increase (due to relatively fewer berries per vine) was counteracted by the inhibition of berry size caused by water stress.

Furthermore, berries of control vines showed a higher degree of berry deformability. This further highlights that under the conditions of this experiment the control vines may have experienced hydraulic water stress but leaf water potential was not measured. It can therefore be concluded that at very low water application rates the PRD-treated vines were more resistant to water stress and made more efficient use of available water. The phenomenon of berry deformability is known as 'shriveling' and occurs particularly in ripe Shiraz fruit (McCarthy, 1999). An interesting topic for further research would be an investigation of the cause of berry deformability in Shiraz and the relationship with berry composition and concentration of secondary metabolites.

McCarthy & Coombe (1999) proposed that during berry ripening, continuation of berry transpiration leads to berry shrinkage along with concentration of solutes and increase in total soluble solids. Results of my study using Shiraz support these ideas: a berry weight decrease was accompanied by an increase in TSS and a higher degree of deformability. Towards the end of the berry ripening phase, however, an increase in berry weight occurred. There are two proposed hypotheses for the uptake of water by the berry post veraison. First, water could accompany sugars from the phloem as a

solution, with the flow becoming impeded at maximum berry weight (Coombe, 1992 and McCarthy & Coombe, 1999). Second, the water inflow may occur via the apoplast in response to a decrease in berry water potential due to an accumulation of sugars (Williams, 1995). In my study an increase in berry weight occurred after the rainfall event on 20th/21st of February. At that time, the sugar concentration stayed unchanged or declined even further. The data presented in this study are insufficient to draw any firm conclusions, but there is an indication that at this stage of berry ripening, water and sugar uptake may be independent.

It was noted in chapter 3 that PRD reduces shoot growth. A strong correlation between light penetration into the interior of the canopy and leaf area per vine was demonstrated. It has been shown in this chapter that changes in the canopy microclimate are strongly associated with changes in fruit composition, that is: colour pigments, phenols, titratable acidity and total soluble solids. A very strong positive correlation ($P < 0.01$) was found between berry skin anthocyanin concentration and light interception in the bunch zone and a strong negative correlation ($P < 0.01$) between skin anthocyanin concentration and leaf area.

The amount and compositional profile of anthocyanins present in red grapes varies greatly with the grapevine variety, region, maturity, seasonal or growing conditions and yield of the vine (Mazza, 1995). Anthocyanins tend to have a very close relationship with total soluble solids and treatments should only be compared at the same stage of berry ripening (Gholami & Coombe, 1995).

Changes in anthocyanins during ripening have been described by Mazza & Miniati (1993). From veraison onwards the total amount of anthocyanins per berry steadily increases during the first 35 days before there is a decline (10 to 15% of the maximum concentration). This suggests that the duration of ripening may also determine the amount of anthocyanins per berry. During this study, however, samples were compared at the same total soluble solids (or less than 0.2° Brix difference between samples) even though it was observed that vines with a lower bunch exposure or a higher crop, mainly observed in control vines, often had a longer ripening period than many PRD-treated vines (Table 8.9). The question of whether this also affected the anthocyanin concentration was not explored within the confines of this study.

When anthocyanin concentrations were measured at the same total soluble solids, independent of the duration of ripening, it was found that the total anthocyanins in berry skins of PRD-treated vines was elevated relative to control vines. The total anthocyanin content of PRD-treated vines was 8% higher than in control vines during the season when the leaf layer number in the bunch zone was similar for control and PRD-treated vines. In the 1997/1998 season, when the bunch exposure (expressed as photosynthetically active radiation in the bunch zone) was higher in PRD-treated vines, the total anthocyanin concentration was 10% higher in PRD-treated vines relative to control vines. Since there were no differences in berry weight of PRD-treated vines, it can be concluded that the changes in anthocyanin concentration were due to the treatment and not due to changes in the ratio of berry skin surface to the total berry volume.

The influence of light microclimate and canopy structure on fruit components has recently been determined using a 3D digitising technique to create a virtual canopy of a grapevine (Mabrouk & Sinoquet, 1998). These authors have shown a relationship between anthocyanin content and the transmitted solar radiation into the canopy. When 8 to 11% of the ambient radiation reached the bunch zone the highest anthocyanin concentration was measured relatively to lower or higher solar radiation. In my study, a solar radiation of the same order inside the canopy of PRD-treated vines was found whilst solar radiation in control vines was lower. An increase in anthocyanin concentration in response to reduced canopy density and greater bunch exposure has been previously discussed by Smart *et al.* (1985b) and Dokoozlian & Kliewer (1996). Dokoozlian & Kliewer (1996) found that shading had its biggest impact in the initial stages of berry development thereby delaying veraison and reducing the skin anthocyanin content.

Wulf & Nagel (1978) reported a distribution of 3-monoglucosides, 3 acetylglucosides and 3-*p* coumaroylglucosides in the proportions 65%, 26% and 9% respectively in berry skins of Cabernet Sauvignon. In the current study a similar proportion of these groups of anthocyanins was found in Cabernet Sauvignon fruit which supports the findings of Wulf & Nagel (1978). For example, in the 1996/1997 and 1997/1998 seasons there was, on average, 60% for 3-monoglucosides, 29% for 3 acetylglucosides and 11% for 3-*p* coumaroylglucosides.

In this study it was observed that not all of these groups were equally affected by the PRD treatment. Derivatives most affected in the monoglucosides group were delphinidin, cyanidin and petunidin. These derivatives have been reported to be only minor components in *Vitis vinifera* (Nagel & Wulf, 1979). In the monoglucosides group, malvidin derivatives had the highest proportion (at 61%) of the total monoglucosides. This result agrees with Wulf & Nagel (1978) who found that 65% of the total monoglucoside in Cabernet Sauvignon was malvidin.

In this study, the derivatives that were the most affected by PRD are known as precursors for malvidin, petunidin or peonidin. In the anthocyanin biosynthesis pathway (Figure 8.4) cyanidin, which was found to be increased by 15% by PRD relative to control vines, acts as a precursor for peonidin. Delphinidin, which is the precursor for petunidin and malvidin, was increased by 17% by PRD.

Since most of the anthocyanins are located in berry skins they need to be extracted during fermentation. The extent to which cyanidin and delphinidin were extracted during fermentation was not investigated during this study. Leone *et al.* (1984) reported that the capability of extracting anthocyanins derivatives from berry skins during must fermentation depends on the anthocyanin species, with malvidin showing the highest abundance. During fermentation and wine storage a high proportion of anthocyanin derivatives interact with other phenolic compounds as well as with proteins and polysaccharides forming large polymeric compounds in the finished wine (Ribéreau-Gayon, 1982). As a consequence, in finished wines, the monomeric compounds are difficult to analyse.

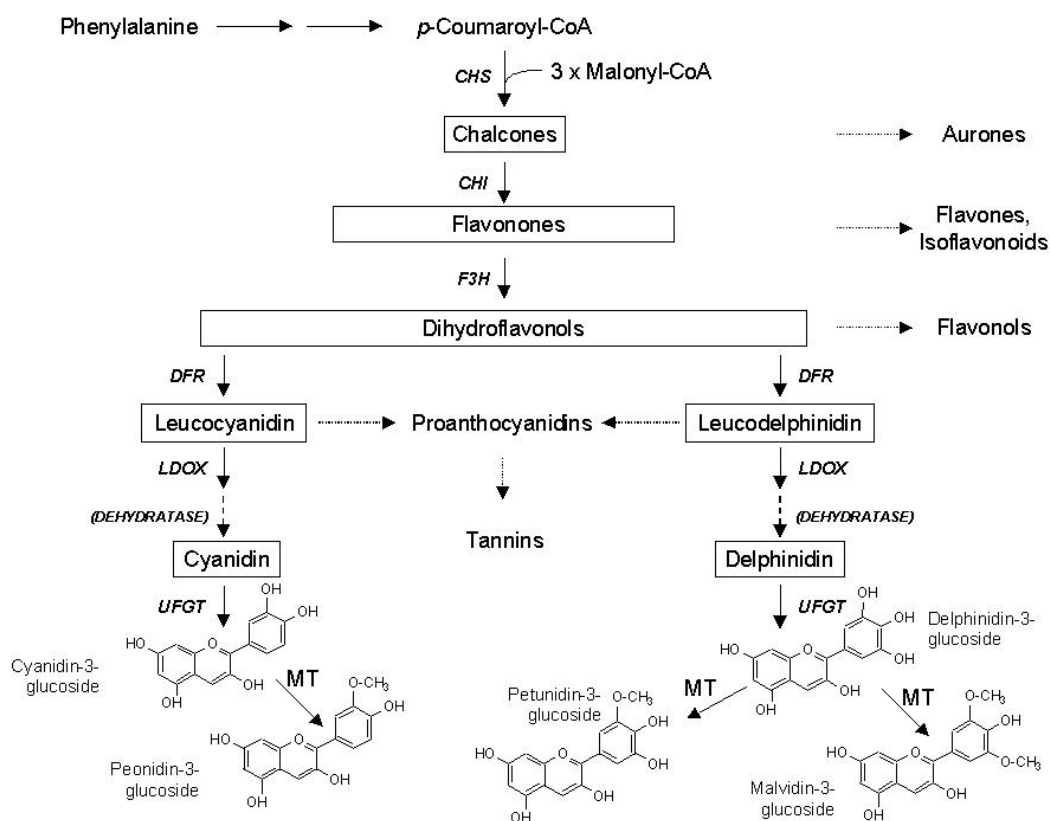


Figure 8.4 Simplified schematic of the anthocyanin biosynthesis pathway. The 3 monoglucosides are further modified in *Vitis vinifera* L. to produce 3-acteylglucosides and 3 coumarylglucosides. (CHS chalcone synthase; CHI: chalcone isomerase; F3H: Flavanone 3-hydroxylase; DFR: dihydroflavanol 4-reductase; LDOX: leucoanthocyanidin dioxygenase; UFGT: UDP glucose-flavonoid 3-o-glucosyl transferase; MT: methyl transferase; redrawn with permission of Boss *et al.* (1996).

If cyanidin or delphinidin were extracted less during fermentation, or influence red pigmentation in finished wines to a lesser extent than, for example, malvidin, it would be important to gain a better knowledge of methyl transferase, the enzyme modifying both precursors (cyanidin and delphinidin). There is no published information to date on the regulation of methyl transferase activity in the anthocyanin biosynthesis pathway nor on the location of the enzyme. It can be speculated that methyl transferase may only be expressed during ripening and may function to create a metabolically-inert compound. If malvidin, peonidin and petunidin are more desirable than their precursors in improving red wine quality it would make methyl transferase an attractive enzyme

for future research, since manipulation of the anthocyanin profile, by stimulating enzyme activity, may then have further benefits for winemaking.

The quality of a wine is obviously linked to its chemical composition. Wine contains a certain concentration of alcohol, sugars, acids and phenolic compounds which all influence the taste. Wine also contains volatile substances which have the potential to evaporate either in the glass or in the mouth. Typically, these substances belong to families of alcohols, esters, aldehydes, acetals, terpenes and so on (Rapp, 1988). One would expect then that the wine quality can be determined as an algebraic sum of these diverse compounds. Many factors, however, may influence wine and the quality may not be predictable by pure analysis. During the last few decades analytical protocols and techniques have improved and many hundreds of compounds have now been described in wine. Without diminishing the importance of chemical analyses, determination of wine quality in this study was assessed by tasting and not by intensively analysing the finished wines. This method was thought to have the potential to link the sensory impression to the chemical composition of the wine.

The term 'wine quality' still remains undefined and because it is not yet measurable, it is subjective. "Quality in wine is easier to recognize than to define" (Peynaud, 1996) although each taster may have a different interpretation of the term 'quality'. From the results in this study it can be concluded that yield components and canopy density are major factors influencing wine quality. Furthermore, anthocyanin and phenolic compounds, which have been related to degree of maturity and canopy density, are also important parameters influencing the overall impression of a wine. Wider commercial application of the PRD irrigation technique will provide further data on the effect of PRD on wine quality. From the experiences of this study, using only a small-scale winemaking technique, it can be concluded that the use of PRD can be seen as a very effective tool for growers to manage their production and provide a high potential to positively influence wine characteristics.

8.5 Conclusions

Experiments in this chapter examined the effect of PRD on fruit components and wine quality. It was found that:

1. Fruit from PRD-treated vines showed a higher titratable acidity and similar total soluble solids to control vines.
2. PRD improved the light penetration into the canopy which was correlated with increased anthocyanin and phenolic content in berries.
3. Greater light penetration into the canopy was associated with an increase in anthocyanin derivatives such as delphinidin, cyanidin, petunidin and peonidin.
4. Water-use efficiency of PRD-treated vines was substantially improved relative to control vines:
 - a) when PRD-treated vines received only half the amount of water, the reduction in yield was minor.
 - b) when both control and PRD treated vines received the same amount of water, PRD-treated vines had a substantially higher yield.
5. Wine quality, determined using sensory evaluation, showed a good correlation between crop yield, light penetration into the canopy and anthocyanins or phenolic compounds of the wines. This was not strictly a consequence of PRD, however, and was observed in some lower yielding control vines.

Chapter 9 Discussion and conclusions

The initial aim of PRD was to develop a strategy to control excessive shoot growth in irrigated vineyards (Loveys, 1992). A reduction in shoot growth was successfully achieved by drying part of the root system while fully irrigating the remainder. Alternating the wet and dry zones was shown to be important to maintain a sustained reduction in shoot growth, thus supporting earlier results of Dry (1997) and Dry & Loveys (1999). Manipulating soil water conditions in this way also affects stomatal conductance. The effects of PRD on shoot growth and stomatal conductance occurred without any changes in plant water status. This suggested that root-derived chemical signals might be involved in the control of these physiological processes as proposed by Loveys (1992).

The experiments described in this study were designed to test the hypothesis that: *'partial drying of the root system gives rise to a change in the supply of root-derived chemical signals which causes changes in grapevine physiology and positively influences fruit quality.'*

Although precise mechanisms still require elucidation, evidence indicates that root to shoot signalling is an important component of the response of plants to drying soil conditions (Davies *et al.*, 1994). This study has focussed on the role of ABA and some cytokinins and provided further evidence for the importance of root-derived chemical signals in vine growth and vineyard water use. Evidence has been accumulated to suggest that these chemical signals are altered independently of hydraulic changes in grapevine plant water status.

9.1 Stomatal sensitivity

The ability to respond to changes in environmental conditions is important for a plant's ability to maximise resource utilisation. Manipulating soil water conditions using PRD, thereby altering chemical signals, is thought to manipulate stomatal conductance. Stomatal

behaviour may therefore be a very sensitive indicator of a grapevine's physiological state and may reflect changes induced by the PRD treatment.

The sensitivity of stomata to environmental inputs is likely to vary amongst plant species or even cultivars. Species that are classified as drought-avoiding plants are more likely to reduce their transpirational water loss by employing root signals. In contrast, plants that can tolerate stress or withstand a moderate tissue dehydration may rely on mechanisms other than chemical signals, such as osmotic adjustment (Schultz & Matthews, 1993). Smart & Coombe (1983) argued that grapevines only fit the drought-avoiding category, yet the range of observed responses of different cultivars suggests that there are some grapevine cultivars in each category (Düring & Scienza, 1980). Schultz (1997) has recently drawn attention to the expression of different drought response mechanisms by two *Vitis vinifera* cultivars. He argued that because of their different geographical origin (Shiraz of mesic origin from the Rhone valley and Grenache of hot climate Mediterranean region), and their different leaf water relations, Shiraz could be classified as a drought enduring (drought tolerant) cultivar, whilst Grenache acts more as a drought avoider. The same author reported that, in excised leaves, stomates of Grenache responded faster and showed a more rapid decline in leaf water potential relative to Shiraz leaves which were essentially incapable of complete closure. In his study he did not investigate chemical signals in either variety but suggested that stomata in Grenache are primarily hydraulically regulated, inferring that this variety may be less dependent on chemical signals from the roots.

According to this classification of plant adaptation to stress, varieties mainly used during my study (Cabernet Sauvignon either grafted to Ramsey rootstock or on own roots, and own-rooted Shiraz and Chardonnay) can be classified as drought-avoiding cultivars and were therefore ideal test material to investigate responses to root-borne chemical signals. Stomatal closure in these cultivars appears to respond sensitively to changes in root derived chemical signals thereby adjusting their stomatal aperture. The non-uniform stomatal closure, which was observed to be more evident in PRD field-grown vines than in the conventionally irrigated control vines, may play a vital role in minimising transpirational water loss. The ability to regulate stomatal aperture non-uniformly may be of ecological advantage because a plant can adjust stomates according to differences in the leaf micro

environment (Terashima, 1992). For example, due to leaf overlapping or difference in leaf positioning, there may be gradients of CO₂ partial pressure or VPD. Non-uniform stomatal closure is known to be an early adaptation to such environmental inputs. Stimuli such as light, partial pressure of CO₂ (Beyschlag *et al.*, 1992) or changes in air humidity (Düring & Loveys, 1996) have been reported to stimulate non-uniform stomatal closure. As a consequence some of the stomata might be closed whilst others are open. This needs to be taken into consideration when interpreting calculated data that underestimation of photosynthesis and assimilation under these conditions may occur (Downton *et al.*, 1988a; Terashima *et al.*, 1988; Mott & Buckley, 1998).

In addition, the genetic differentiation of stomatal density and size within the family of *Vitis* can be quite variable which might be another important criterion for differences in drought tolerance and could be an area of future research. Results of a survey of stomatal density on 12 *Vitis* spp. and 27 cultivars of *Vitis vinifera* suggested that, within the genus *Vitis*, stomatal density and size of single stomata can vary, for example within the *vinifera* between 140 and 300 stomata mm⁻² of a size of 20 and 30 µm (Shiraishi *et al.*, 1996).

Roots are able to respond to conditions of water deficit by increasing their ABA concentration (Zhang & Davies, 1989). This was also observed in roots of the 'dry side' on PRD vines during this study (Chapter 5). Furthermore, Loveys (1984a) working with two *Vitis vinifera* cultivars (Riesling and Silvaner) suggested that one reason why ABA can be considered to be important in the control of stomatal aperture is that in these cultivars the xylem sap [ABA] is such that only a slight increase in concentration or flux is sufficient to initiate partial stomatal closure. The xylem sap [ABA] of these varieties was found to vary between 150 to 500 pmol mL⁻¹ [ABA] and exogenous application of (+) ABA at a concentration within this range stimulated partial stomatal closure. Similar xylem sap ABA concentrations were found in own rooted Cabernet Sauvignon and Cabernet Sauvignon grafted to Ramsey rootstock in my study.

Compared to other woody species, such as apricots, the xylem sap ABA concentration in grapevine varieties is relatively high. For example, Loveys *et al.*, (1987) measured ABA concentrations expressed from xylem sap of apricot trees between 30 to 90 pmol mL⁻¹. Exogenous application of (+) ABA over a range of concentrations determined from sap

exudates of both species has shown that whilst in grapevines stomatal conductance could be manipulated (Loveys, 1984a) stomatal conductance of apricot leaves responded to a lesser extent, or not at all (Loveys *et al.*, 1987). These results suggest that stomata of different species can vary in their sensitivity to ABA and different species may rely on mechanisms other than chemical signals to regulate their stomatal aperture.

Results of this study also support the idea of the importance of root-derived ABA as a chemical signal in some *Vitis vinifera* cultivars. Furthermore, it was observed that the bulk leaf ABA concentration in grapevine leaves is relatively high compared to xylem sap [ABA] but only a weak correlation exists between stomatal conductance and bulk leaf ABA (Chapter 4). This indicates that the accumulated xylem-derived ABA in leaves may affect stomatal closure less than the prevailing xylem sap [ABA], as has previously been discussed by Jia & Zhang (1999). The xylem-derived pool of ABA may be metabolized faster than intracellular pools which are protected from cytoplasmic degradative enzymes (Hartung *et al.*, 1980). Another reason could be that the initial rate of breakdown of both pools of ABA is different (Gowing *et al.*, 1993). This does not imply, however, that bulk leaf ABA is not available to affect stomatal closure. Popova *et al.* (2000) has recently discussed the importance of foliar compartmentation of ABA, whereby liberation of compartmentalised ABA reserves may provide another source of ABA in the control of stomatal aperture. In addition guard cells may have two other sources for ABA, that is the synthesis of ABA by the guard cells themselves (Cornish & Zeevaart, 1986) and the import via the xylem as a consequence of soil drying (Davies & Zhang, 1991). Indeed, for field-grown vines used during this study, it was possible to estimate that the flux of ABA to the leaves was approximately 30% higher in PRD-treated vines than in control vines at midday and this was found to be sufficient to cause changes in stomatal conductance, but did not greatly influence bulk-leaf ABA.

It is known that changes in pH can affect the availability of ABA and influence the compartmentation of ABA (Hartung *et al.*, 1990). In PRD-treated vines an increase in xylem sap pH was observed (Chapter 5) which may be of importance in the discussion of ABA availability. Insufficient data on changes in xylem sap pH were collected in this study to be able to conclude that pH changes are affecting the apoplastic ABA

concentration, as has been reported by Wilkinson & Davies (1997), thereby influencing the site of action on the plasmalemma in the apoplast surrounding the guard cells (Hartung *et al.*, 1998). The data suggest, however, that increased xylem sap pH can act as another drought signal induced by PRD. Further research relating to pH changes and changes in xylem sap composition is required in order to better understand this phenomenon.

ABA seems to have many of the prerequisites required of a hormone involved in controlling stomatal response to PRD: it is root-sourced and can move from roots to shoots where it affects stomatal aperture. If endogenous signals really are responsible for these physiological responses, it should be possible to mimic or override these effects by exogenous application of substances which complement or antagonise the action of the endogenous compounds. Exogenous application of ABA may not have the same effects as increased ABA biosynthesis due to soil drying since soil drying may also cause other interrelated and independent effects (Trewavas & Jones, 1991). Care needs to be taken, therefore, in interpreting stomatal responses to exogenous ABA application. Considerable evidence from this study suggests that ABA plays a key role in the control of stomatal conductance under PRD conditions. Furthermore, application of cytokinins to leaves can partly override changes in stomatal conductance caused by ABA. These results provide additional evidence for the contrasting influences of ABA and CK on stomatal behaviour. It also supports earlier reports suggesting that stomatal aperture is affected by both groups of hormones (Radin *et al.*, 1982; Blackman & Davies, 1985).

The sensitivity of stomata to hormonal changes which accompany PRD might be one of the reasons for the success of this irrigation technique in grapevines. As was shown in Chapter 4, when total water application rates were low, there was a reduction in water penetration to deeper soil layers for control vines. This resulted in a reduction of stomatal conductance compared to PRD-treated vines which received the same amount of water to a smaller soil surface area thereby facilitating deeper water penetration. This can be seen as a more efficient irrigation strategy when a small amount of water is available, particularly as it does not result in yield penalties, as has been shown in Chapter 8. This highlights the importance of stimulating root-derived chemical signals by manipulating soil water conditions to reduce stomatal aperture, which can have dramatic effects on the water use

efficiency of a vine. ABA evidently plays an important role as a potent, endogenous anti-transpirant. As previously discussed, however, this cannot be generalised for all *Vitis* cultivars or for *Vitis* species other than *V. vinifera*. In relation to other *Vitis* species, Dry *et al.* (2000a) have provided some evidence for the control of leaf gas exchange by chemical signals with cultivars such as Kober 5 BB (*Vitis berlandieri* x *Vitis riparia*) and 110 Richter (*Vitis berlandieri* x *Vitis rupestris*). In order to make profound recommendations on the amount of irrigation water required for specific cultivars, the response of a range of other varieties, rootstocks or graft combinations to PRD needs to be tested.

9.2 Effect on growth

Regulation of transpirational water loss is one of the crucial aspects of PRD and can be inextricably linked to the growth of vines as demonstrated during this and previous studies by Loveys *et al.* (1998) and Dry *et al.* (2000a). The biggest impact on changes in shoot growth were achieved using PRD on high capacity vines with a high shoot vigour. Lateral shoot growth was the vegetative component most affected by PRD and the reduction in lateral growth was related to a decrease in root-borne cytokinins which were found to become altered during alternated soil drying. Concentrations of zeatin, zeatin riboside and isopentenyladenine in roots of field-grown vines were observed to decrease on the 'dry' side and increase again after re-watering. By contrast, root ABA levels, which increased during root drying, decreased again after re-watering. Although the tight relationship between ABA concentration (in roots and xylem sap) and stomatal conductance support the view that ABA is an important root signal affecting growth indirectly or directly, the involvement of CK cannot be excluded.

Using a horizontally-divided root system of rice where protruding roots were allowed to air dry over 24 h, similar changes in ABA and CK concentrations have been found (Bano *et al.*, 1993). In that experiment the increase in ABA in drought stressed roots was less pronounced than the changes in the ratio of ABA / CK which increased dramatically during the water stress period. Similar responses were found in this study. This suggests that

changes in ABA / CK ratio may be relevant for both changes in leaf gas exchange and growth.

The relationship between reduction in lateral growth and reduced supply of cytokinins also supports earlier results of Chang & Goodin (1974) and Richards & Rowe (1977), who studied the role of root-produced cytokinins on the growth of lateral buds in pea and peach seedlings respectively. These authors found that, by excising the root system, lateral growth was suppressed in both species and that in rootless shoots, lateral growth only occurred when CK were externally applied to the buds. During the current study, cytokinin concentrations in the roots, prompt buds and lateral shoots were reduced in response to soil drying and exogenous application of CK overcame the general suppression of lateral shoot growth observed under PRD. The results highlight the importance of root-derived signals.

In many other horticultural crops, particularly apples, measurements of cytokinins are employed to select dwarfing rootstocks, which are used to reduce growth in high density orchards (Kamboj & Quinlan, 1998). With a better knowledge of chemical signals involved in the root to shoot communication, it would be possible to test grapevine rootstocks and scion/rootstock combinations for such breeding criteria. Future research could test the hypothesis that, in different *Vitis* cultivars or species, lower vigour is a result of variations in concentration of, or sensitivity to chemical signals, particularly cytokinins.

Since growth and transpirational water loss are inextricably linked, different varieties could be tested for their sensitivity to ABA. This may also help to select varieties better suited for specific production conditions. In hot dry climatic regions, such chemical signals may be stimulated through manipulation of soil water conditions as has been shown during this study.

In cool, wet climatic regions the control of excessive vegetative growth is even more important. Site selection, labor intensive shoot positioning and trellising are the most common practices to avoid detrimental effects on fruit quality. A better knowledge of the effects of chemical signals may provide a tool for manipulating undesirable growth under conditions where natural manipulation of endogenous signals is difficult to achieve by irrigation management. Foliar application of plant growth retardants (triazole and norbornandiazetidine) considerably increased the endogenous ABA levels when applied to

oilseed rape (*Brassica napus*), thereby influencing transpiration by regulating ABA metabolism (Häuser *et al.*, 1990). If such treatments do not affect other secondary metabolites, an artificial change in chemical signals may be a possible means for reducing undesirable vine growth.

9.3 Effects on fruit composition and yield

Changes in shoot growth affects canopy structure, thereby influencing the light penetration into the canopy. Only the exterior leaves of grapevine canopies are exposed to direct sunlight. Thus they are the main contributors to total canopy photosynthesis (Smart, 1985). A small fraction of total radiation penetrates deep into the canopy. This study found that the berry anthocyanin and phenolic concentration was negatively correlated with leaf area and positively correlated with light interception by the bunch zone (Chapter 8). A recent study by Mabrouk & Sinoquet (1998) suggested that anthocyanin concentration and bunch exposure are not linearly related. Using 3D digitising techniques to recreate a virtual canopy of Merlot, they described the relationship between the solar radiation penetrating inside the canopy and the anthocyanin concentration as a “quadratic relationship” with a bell shape. Therefore bunches receiving the lowest and highest amounts of solar radiation had the lowest anthocyanin concentrations. According to their results the highest anthocyanin concentration was achieved when 9 to 11% of ambient solar radiation penetrated to the inside of the canopy. Results of this study and others on grapevines using PRD (Loveys *et al.*, 1998; Dry, 1997) showed that, due to a reduction in lateral growth, PRD-treated vines had significantly higher light penetration inside the canopy. The light penetration measured in PRD-treated vines was in many cases between 8 and 10% of the ambient solar radiation compared to control vines which had between 4 and 8%. This further highlights the potential improvement that can be achieved using PRD to manipulate canopy architecture.

A slight decrease in yield as a response to halving the amount of irrigation water may be acceptable if fruit quality improves. Compared to other deficit irrigation techniques (McCarthy, 1997a) the yield reduction measured under PRD conditions relative to control during this study and other recent experiments (Dry, 1997) was minor. The yield per vine, however, can be quite variable and is dependent on the number of bunches per vine, berries per bunch and weight per berry. Pruning level and the number of nodes retained per vine is one of the main determinants of yield (Freeman *et al.*, 1980). In this study, the number of nodes retained at winter pruning was generally adjusted to the same level, for both control and PRD-treated vines (e.g. Cabernet Sauvignon grafted on Ramsey rootstock or on own roots). In most cases the berry weight of PRD-treated vines and control vines did not differ even though the number of bunches per vine showed some variation. Further experiments should be conducted whereby the number of bunches is adjusted at an early stage of berry development to the same level for both control and PRD in order to examine the effect of PRD on other yield components, such as berry weight and berry number per bunch.

When the amount of irrigation water applied to half the rate normally used in vineyards in the Adelaide region, such that control and PRD-treated vines received the same amount but to a different soil surface area (Shiraz, own roots), yield of control vines was detrimentally affected relative to PRD. The reduction in yield of control vines was mainly due to a reduction in berry weight. Williams & Matthews (1990) found that berry weight can be a very responsive to water stress. The period when berries are most susceptible to water stress, thereby causing a reduction in berry size is post flowering and before veraison (McCarthy, 1997b). It can be concluded that if only a limited amount of water is available, PRD is more efficient than conventional irrigation, allowing water penetration to deeper soil layers thereby avoiding water stress. Thus part of the root system can maintain a relatively high soil water content during the critical periods of berry development. This cannot be achieved if the same amount of water is applied to a larger soil surface area or only a limited amount of water is available.

PRD can be used as a tool for grape growers to improve water use efficiency. This may also lead to a reduced risk of water stress, maintain or increase yield and improve berry composition by altering anthocyanin and phenolic concentration which can be linked to the

wine quality. In this study, wines with higher anthocyanins and phenolics concentration had a higher quality ranking.

Another potential positive effect of reduced shoot growth rate and a more open canopy is the reduced incidence of foliar fungal diseases which are widespread in dense canopies.

9.4 Soil-vine interaction

Intensive measurements of soil water content using capacitance probes provided detailed data on changes in the soil water content for both control and PRD-treated vines. PRD was initiated at the beginning of the season (2 to 4 weeks after bud burst) when the soil water content started to decrease. The level of soil water deficit determining the alternation of the irrigation sides was defined by monitoring the slope of the soil water content curves on the dried side of the vines. From previous experience (Dry, 1997) the irrigation sides were alternated when no further decrease in soil water content on the 'dry' side was observed. The amount of plant-available water, however, differs greatly between different soil types. To better define the water deficit required to stimulate root-derived signals, an attempt was made to relate the soil water content readings to soil water matric potential. Towards the end of the study, when experiments on the field-grown vines of the Alverstoke vineyard were complete and effects due to disturbing the root system did not further affect the experiment, a trench was dug close to the EnvironScan® capacitance probes. Gypsum blocks and tensiometers were installed close to the sensors of capacitance probes at various soil depths. Due to early rainfall in this season, however, it was not possible to establish a calibration curve relating the soil water content data to soil water potential. Dry (1997) reported in his experiments at the same vineyard site that the soil water matric potential, when 'dry' and 'wet' were alternated, was in the range between -75 kPa to -100 kPa at 0.2 to 0.4 m depth (determined from the water release curve for this soil). Other studies, where severe water stress was induced, reported that the soil water potential can be much more negative and vines can survive a soil matric potential of $-1,000$ kPa to $-1,500$ kPa (McCarthy, 1997b; van Zyl & Weber, 1981).

The process whereby roots transport and release water from soil layers with a higher soil water potential to soil layers with a lower water potential is generally described as hydraulic lift (Richards & Caldwell, 1987; Smith *et al.*, 1999). A similar phenomenon under PRD conditions was observed during my study when water movement from ‘wet’ to ‘dry’ roots was detected. This process of nocturnal water movement to the dried side of the root system was demonstrated by applying deuterium enriched water to the irrigated side of a split-root vine. Changes in the isotopic ratio of water of roots in the ‘dry’ side were detected. In potted, split-root vines this phenomenon started to occur approximately 8 days after withholding water from one side when no further decrease in soil water content were observed. Not only does this maintain the ‘dry’ roots in a viable status, it may also play a role in sustaining the transport of chemical signals from the ‘dry’ roots to the shoots.

Blackman & Davies (1985) have shown that excision of the dried root-system of a split-root plant removes the source of the proposed signals released from the dried side of the root system. Withholding water from one side of potted, split-root vines for longer than 25 days resulted in the same effect (Dry, 1997). Experiments with mature field vines have also shown that continuous drying of one side results in a reduction in the effectiveness of the treatment. These findings were of great importance in developing the PRD protocol which utilises an alternation of ‘wet’ and ‘dry’ zones such that a sustained effect is achieved.

In grapevines, drying of only a small proportion of the root system seems to be sufficient to affect the whole vine physiology. Grapevines tend to have a highly branched root system which can explore a large soil volume. Depending on environmental factors such as soil type, access to soil water, cultural practices such as type of the irrigation system, the root density can vary considerably. Van Zyl & Weber (1981) reported that a maximum concentration of roots of *Vitis vinifera* cv. Chenin Blanc occurred at the 0.30 to 0.45m depth zone, and approximately 90% of the total number of roots was found above 0.9 m. Soil water penetration has been found to be crucial in the grapevine response to PRD. It is important therefore to ensure that part of the root system stays well watered to avoid severe water stress conditions in the whole vine. How effectively grapevines can respond and adjust stomatal conductance has been shown in the experiment where there was a variation

in soil water penetration to the root system (Chapter 4). Continuous monitoring of soil water conditions to greater depth (that is at least 1 m) is advisable especially immediately after alternating the irrigation sides.

9.5 Practical application

Field-grown vines with a split-root system were used for most of the experiments in this study. The two sides of the root system were separated by a vertically buried plastic sheet, which ensured that lateral water movement did not affect the 'test vine'. In addition, each 'test vine' was flanked by buffer vines, which further reduced the impact of the other treatment. From a scientific point of view, such a separation was very important as it constituted a model system which was not affected by soil water movement. PRD can only become useful for commercial application where vines do not have a split-root system achieved by artificial means. Experiments using field-grown vines without the artificial separation were also established and used for this study. The use of field-grown vines was thought to be of importance, since neither shoot nor root growth becomes limited under such conditions. Furthermore, canopy sizes and yield components were comparable to commercial vineyards.

There are many ways to design a commercial irrigation set-up for PRD, with either subsurface or above ground systems:

1. Possibly the most efficient type in terms of the least evaporational water loss, is a subsurface system, where two drip lines per vine row spaced about 0.4 m on either side of the planting line and at a depth of about 0.3 m are installed. Depending on the soil type the time required for the installation may vary. One advantage of the subsurface installation experienced during this study was that weed control during summer becomes unnecessary, since the top soil layer in the whole vineyard stays dry during the whole irrigation season. However, any leaks of the system are hard to detect.

2. An above-ground drip irrigation system (Figure 9.1) with two drip lines has the advantage that an existing system can be readily converted to PRD. Furthermore, any maintenance of the system is easily performed due to accessibility of the lines. The drip lines can be attached to wires (0.3 m above ground) that are normally used in commercial irrigation.

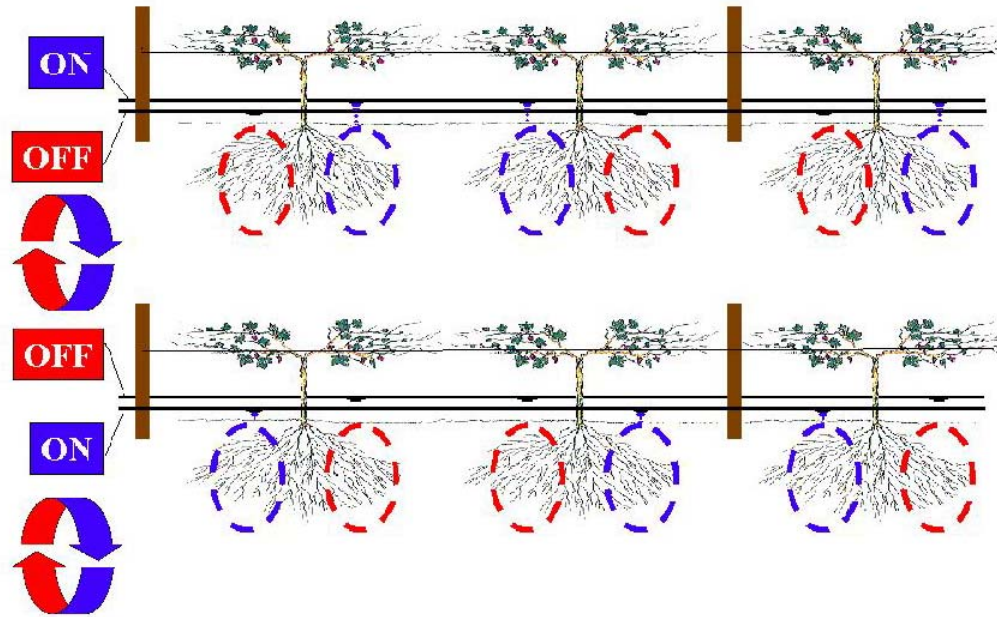


Figure 9.1 Implementation of an above ground drip irrigation system

3. Microjet sprinkler irrigation is also possible using half circle microjets with two separated lines. Compared to drip emitters, however, sprinkler systems apply water to a larger surface area thus more water is needed to penetrate deeper soil layers and the evaporational water loss will be higher. This may reduce the water use efficiency of microjet sprinklers compared to drip emitter systems.

It was demonstrated in this study that PRD improves the water use efficiency. The adoption of the PRD irrigation technique by the wine industry has been extraordinarily

rapid, with large areas of vineyards now converted to this technique. PRD is now passing from the experimental to implementation stage and commercial vineyards in many of the world's grape growing regions are experimenting with PRD (Peter Dry, pers. comm.). As part of this study, a survey of the first commercial experiences with PRD in several winegrowing regions in Australia was conducted in the 1999/2000 season (Table 9.1). Differences in rainfall amount between regions were not considered. The water use efficiency (t/ML) for commercial application of PRD compared to conventional irrigation practices (control) was, on average, increased by 75%. The amount of water was reduced by 47% compared to conventional application whilst the yield was reduced on average by 7%. This further supports the results of my study and earlier observations (Dry *et al.*, 1996; Loveys *et al.*, 1998). These results indicate that by using PRD the amount of irrigation water applied can be substantially reduced and furthermore offers the important advantage of reducing vegetative growth. PRD therefore offers a very useful technique for growers to produce fruit to specification.

Table 9.1 Commercial experience with PRD in different regions in Australia.

region		TSS (°Brix)	pH	TA (g/L)	yield (t/ha)	irrigation water applied (ML/ha)	water use efficiency (t/ML)
McLaren Vale Shiraz	control	13.4	3.7	6.7	20	2	13
	PRD	13.3	3.7	6.9	19	1	25
	% diff				-6	-50	86
Sunraysia Shiraz	control	13.1	3.8	5.6	29	7	4
	PRD	13.1	3.6	6.0	27	4	6
	% diff				-7	-40	55
Padthaway Shiraz	control	13.0			13	4	3
	PRD	13.3			10	2	4
	% diff				-28	-44	29
Adelaide Cabernet Sauvignon	control	13.0	3.4	6.6	10	1	17
	PRD	13.3	3.4	7.0	11	0	37
	% diff				7	-50	113
Riverland Riesling	control	9.7			38	5	7
	PRD	10.2			37	3	13
	% diff				-2	-49	90

PRD is compared to conventional irrigation practice (control) and the differences are expressed PRD as % of control (Stoll *et al.*, 2000a).

Even if the costs of irrigation water only accounts for a small proportion of total production costs, it is becoming an increasingly valuable resource. With increasing environmental pressure, expanding planted areas and changes in water policies, improved irrigation practices will be required in future.

9.6 Future directions

Vitis vinifera cultivars respond differently to water stress. To better match irrigation input and maximise water use efficiency it may be necessary to define a particular rootstock or scion rootstock combination in terms of its ability to produce and respond to root signals. An index based on soil water potential rather than one calculated from soil water content data should be introduced to make the schedule valuable for different sites. In addition, to better estimate the water requirement, sap flow data in combination with measurements of transpiration should be used to establish a model for vine water use and improve the recommendation for whole plant requirements. Although soil water availability seems to be the major driving force in stimulating the synthesis of root signals not much knowledge is available on other components which may influence their production. Signals could be stimulated by either a nutrient deficiency, an increase in soil salinity, changes in soil pH or various other factors after the soil starts to dry.

Berry weight has been discussed as one crucial factor influenced by water stress but also in influencing fruit quality. There are now different irrigation strategies available to manipulate berry size. In one respect, a combination of deficit irrigation (e.g. RDI) to manipulate berry size in combination with PRD to maintain yield, is conceivable. Combinations of different irrigation strategies will therefore become another future challenge for experimental design and commercial application. In another respect, retaining more nodes per vine at pruning and thereby increasing the number of bunches per vine in combination with PRD may also be a means to reduce berry size and maintain high yield.

The first results using Shiraz have shown that application of the same amount of water to different soil surface area of control and PRD-treated vines will detrimentally reduce yield of control vines. Questions can be asked as to whether changes in berry deformability or berry weight are reflective of secondary metabolites concentration in berries. Perhaps changes in berry deformability will provide an economical indicator of the degree of ripening and thus be useful as another criterion to determine fruit quality or the harvest date.

Evidence of root signals involved in PRD, emanating mainly from the 'dry' side of the root system has come from experiments where the dried part of the root system was either excised (Gowing *et al.*, 1990) or dried out for a long time (Dry *et al.*, 2000a). Both cases led to a recovery in growth. The contribution to increased growth resulting after excising the dried part of the root system needs to be assessed in relation to carbohydrate sinks. The results showing that PRD reduces shoot growth but also stimulates root growth suggest that PRD may affect carbon partitioning of the vine. This might also be due to a reduction in nutrients (especially nitrogen) which may divert carbon allocation into roots.

Apart from grapevines, PRD as a novel irrigation technique, will almost certainly find a wider range of application in the near future in other horticultural crops.

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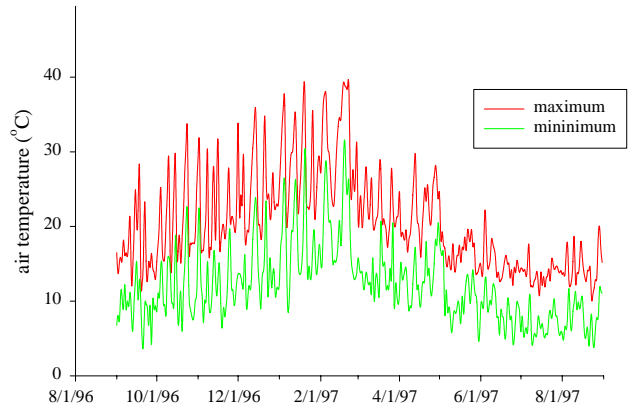
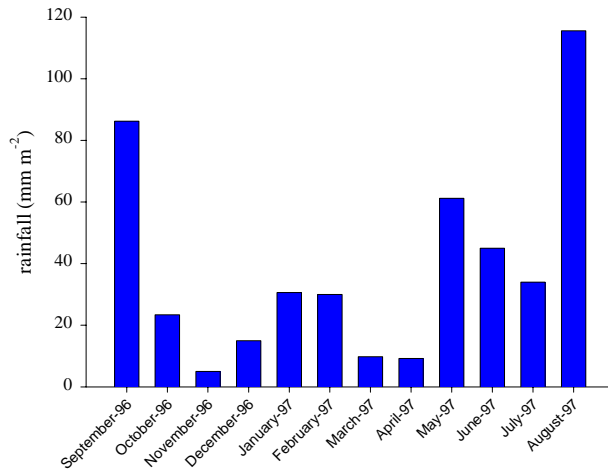
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Appendix 1

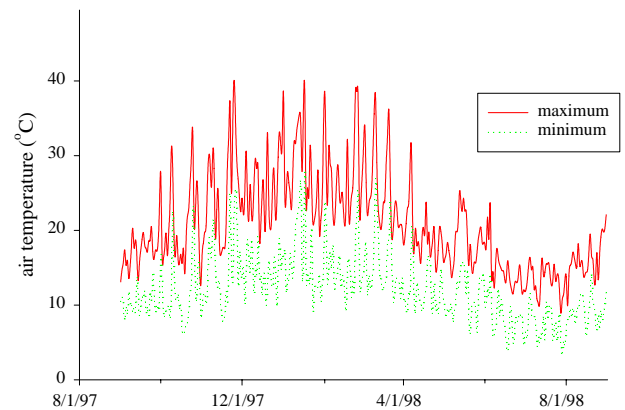
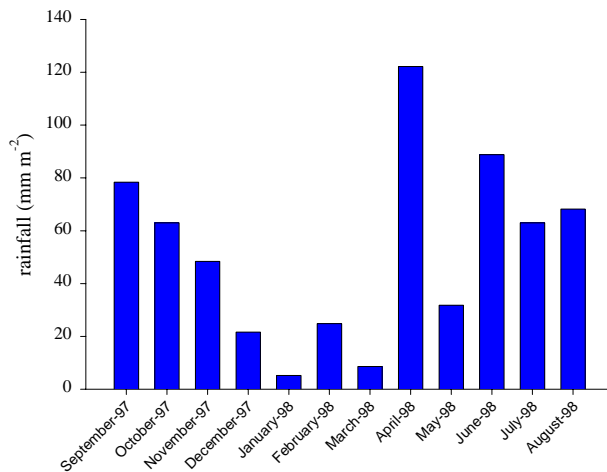
Climatic data September 1996 to August 1997 (Adelaide, Waite Campus)



date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)
09/01/96	16.5	6.8	60	0	10/01/96	15.3	8.6	69	3	11/01/96	29.3	19.3	24	0	12/01/96	33.8	13.6	36	1.2
09/02/96	13.7	8.1	76	5.8	10/02/96	17.7	11.1	83	2	11/02/96	31.1	22	22	0	12/02/96	21.7	13.7	68	0
09/03/96	15.2	7.4	83	1.2	10/03/96	20.9	10.5	71	0	11/03/96	18.5	13.3	83	0.8	12/03/96	24.2	13.3	51	0
09/04/96	15.8	11.2	58	0	10/04/96	25.2	14.6	42	0	11/04/96	16.8	10.8	48	0	12/04/96	23	12.3	66	0
09/05/96	15.4	10.6	82	0.6	10/05/96	17.7	14.5	91	5	11/05/96	16.8	7.1	67	0.8	12/05/96	29.7	12.2	45	0
09/06/96	18.2	9	57	0	10/06/96	15.5	9.7	63	0	11/06/96	19.4	10.1	59	0	12/06/96	19.8	16.2	77	0
09/07/96	16.2	12.2	70	0	10/07/96	16	9.5	61	0	11/07/96	22.6	10.1	62	0	12/07/96	17.9	10	41	0
09/08/96	16.5	8.9	91	0	10/08/96	20.8	8.2	55	0.2	11/08/96	30.3	15.3	28	0	12/08/96	19.5	9.6	32	0
09/09/96	16	10	75	0.4	10/09/96	25	14.8	43	0	11/09/96	17.1	10.2	60	0	12/09/96	19.4	12	55	13.4
09/10/96	18.5	9.3	66	0	10/10/96	29.1	15.9	42	0	11/10/96	17.2	8.5	55	0	12/10/96	23.5	11.5	53	0
09/11/96	21	11.2	38	0	10/11/96	16.8	14.8	75	0	11/11/96	17.6	9.6	64	0	12/11/96	27.1	13.1	34	0
09/12/96	12.6	8.3	66	4.2	10/12/96	14.4	10.2	68	0	11/12/96	26	13.3	61	0	12/12/96	30.5	18.3	30	0
09/13/96	14.4	6	75	7	10/13/96	20.1	9.7	80	0.6	11/13/96	26.6	16.8	33	0	12/13/96	33.3	21.4	26	0
09/14/96	19.4	9.9	83	2.6	10/14/96	25.7	10.1	47	0	11/14/96	18.2	13.5	66	0	12/14/96	35.9	23.9	29	0
09/15/96	24.9	12.9	43	0	10/15/96	29.3	18.7	25	0	11/15/96	26.1	10.7	54	0	12/15/96	29.4	20.9	41	0
09/16/96	20.2	15.2	67	0	10/16/96	16.9	13.7	77	4.2	11/16/96	31.2	13.4	27	0	12/16/96	20.9	14.6	65	0
09/17/96	22.1	10.2	62	0	10/17/96	16.7	7.1	55	0	11/17/96	18.3	14.9	69	0	12/17/96	20.3	11.8	63	0.4
09/18/96	28.1	12.9	35	0	10/18/96	18.7	8.8	69	0	11/18/96	17.9	9.7	59	3.4	12/18/96	18.4	9	58	0
09/19/96	13.6	9.9	89	9.4	10/19/96	15.1	11.1	64	3.2	11/19/96	19.5	6.4	70	0	12/19/96	19.9	13.1	51	0
09/20/96	12.4	4.9	57	2.8	10/20/96	18.5	6	60	0	11/20/96	20.2	9.1	75	0	12/20/96	27.8	14	55	0
09/21/96	15.6	4.2	71	3	10/21/96	21.9	11.5	43	0.2	11/21/96	18.4	7.9	76	0	12/21/96	34.8	17.4	50	0
09/22/96	23.3	9.4	39	0	10/22/96	27.4	14.8	28	0	11/22/96	19.6	9.3	82	0	12/22/96	26.6	23.3	41	0
09/23/96	15.4	9.6	69	9.4	10/23/96	31.1	20.7	22	0	11/23/96	23.5	12	54	0	12/23/96	23.7	12.2	53	0
09/24/96	12.8	9	91	6	10/24/96	32.6	21.8	23	0	11/24/96	27.8	17.8	33	0	12/24/96	24.2	12.2	57	0
09/25/96	15.3	6.8	60	0.6	10/25/96	17.6	12.1	95	4.6	11/25/96	21.1	19.1	61	0	12/25/96	21.1	15.8	54	0
09/26/96	14.5	9.4	87	2	10/26/96	17.4	9.1	75	0	11/26/96	21.1	12.2	64	0	12/26/96	22.7	11.9	42	0
09/27/96	16.4	4.2	50	3.6	10/27/96	17.6	8.2	88	0	11/27/96	21.1	11.6	69	0	12/27/96	27.2	13.6	28	0
09/28/96	14.4	10.1	80	1.2	10/28/96	17.8	7.5	65	0	11/28/96	19.9	9.5	58	0	12/28/96	23.3	13.9	44	0
09/29/96	13.9	8.9	92	11.6	10/29/96	17.7	8.1	74	0	11/29/96	19.4	10.5	57	0	12/29/96	22.4	10.6	40	0
09/30/96	12.9	9.4	72	14.8	10/30/96	19.4	10.2	53	0.4	11/30/96	24.4	12.6	57	0	12/30/96	23	11.7	56	0
					10/31/96	25.5	11.4	29	0						12/31/96	22.8	11.6	62	0
Sep.	16.8	9.2	68.1	86.2	Oct.	20.7	11.7	59.2	23.4	Nov.	21.9	12.2	56.6	5	Dec.	24.8	14.2	48.5	15

Appendix 1

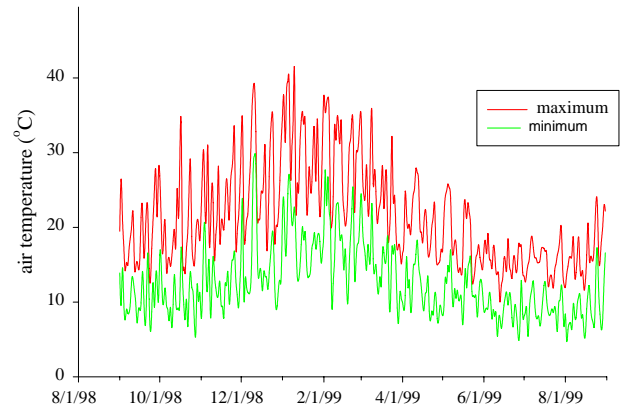
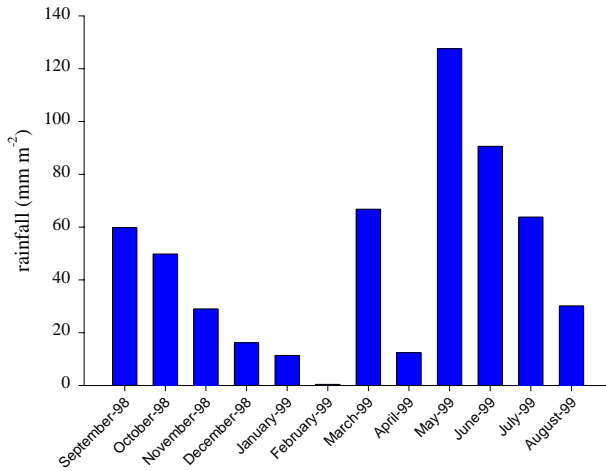
Climatic data September 1997 to August 1998 (Adelaide, Waite Campus)



date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)					
09/01/97	13.1	10.5	76	0	10/01/97	27.7	16.7	30	0	11/01/97	16.7	9.5	62	31.8					
09/02/97	14.8	11.1	93	20.8	10/02/97	16.9	13.5	85	0	11/02/97	18.3	11.1	83	0					
09/03/97	15.8	9.6	68	0	10/03/97	16.2	8.8	54	0	11/03/97	19.8	11.5	71	0					
09/04/97	17.4	8	66	0.6	10/04/97	17.6	8.7	53	0	11/04/97	20.6	10.1	57	0					
09/05/97	15.3	9.8	87	4.2	10/05/97	19.6	9.3	49	0	11/05/97	26.4	12.7	46	0					
09/06/97	16	8.8	67	0	10/06/97	16.6	9.5	74	0	11/06/97	29.9	18.9	41	0					
09/07/97	13.6	11.9	86	10.6	10/07/97	19.9	9.8	60	0	11/07/97	29.2	16.9	47	0					
09/08/97	15.1	10.2	91	2	10/08/97	23.9	8.2	76	0	11/08/97	25.8	17.1	49	0					
09/09/97	17.7	10.3	95	2.4	10/09/97	31	13.2	33	0	11/09/97	30.5	21.2	42	0					
09/10/97	20.3	9	80	0	10/10/97	28	22.6	29	0	11/10/97	28.3	19.9	52	0					
09/11/97	17.7	9.6	63	0	10/11/97	19.1	14.2	60	0	11/11/97	19.1	14.4	90	0					
09/12/97	18.7	9.3	51	0	10/12/97	16.6	11.3	61	0.2	11/12/97	22.8	12.4	85	0					
09/13/97	17	13.7	61	0	10/13/97	19.3	10.6	81	0	11/13/97	25.3	12.9	52	0					
09/14/97	13.3	11.6	92	9	10/14/97	16.2	11.5	81	0	11/14/97	18.4	13.1	82	16.4					
09/15/97	15.5	8.6	81	7.2	10/15/97	15.6	9.4	73	1.4	11/15/97	16.7	9.9	54	0					
09/16/97	17.3	9.8	74	0	10/16/97	16.8	10.1	78	0	11/16/97	17.3	8.2	62	0					
09/17/97	18.8	11.2	51	0	10/17/97	15.6	6.6	60	0	11/17/97	17.7	7.8	52	0					
09/18/97	19.7	10.9	49	0	10/18/97	17.6	6.5	52	0	11/18/97	17.6	8.8	64	0					
09/19/97	18	11.9	87	15.2	10/19/97	14.2	6.4	53	0	11/19/97	20.6	11.9	83	0					
09/20/97	17.6	9	85	0	10/20/97	18.7	7.5	61	0	11/20/97	28.8	10.5	37	0					
09/21/97	17.1	9.8	82	0	10/21/97	21.8	7.6	64	0	11/21/97	32.8	17.8	25	0					
09/22/97	18.6	8.6	67	0	10/22/97	22.9	9.5	65	0	11/22/97	36.9	25	23	0					
09/23/97	18.7	11.8	40	0	10/23/97	26.5	12	65	0	11/23/97	25	15.7	47	0					
09/24/97	20.5	10.6	44	0	10/24/97	30.2	13.7	33	0	11/24/97	34.2	14.1	45	0					
09/25/97	17.2	14.4	48	0	10/25/97	33.3	22.8	22	0	11/25/97	40.1	22.8	28	0					
09/26/97	16.2	11.8	88	4	10/26/97	21.3	19.8	65	0	11/26/97	35.3	25.5	39	0					
09/27/97	17.3	9.1	72	1.4	10/27/97	22.6	11.7	80	0	11/27/97	29.6	22.7	49	0					
09/28/97	17.2	10.1	63	1	10/28/97	26.6	11.3	51	0	11/28/97	26.9	12.5	59	0					
09/29/97	18.4	9.3	58	0	10/29/97	23.6	15.1	74	1.4	11/29/97	24.4	17.1	52	0					
09/30/97	22.9	11.2	43	0	10/30/97	16.7	14.5	78	3	11/30/97	22.5	14.2	60	0.2					
					10/31/97	12.6	8.6	95	57										
Sep.	17.2	10.4	70.3	78.4	Oct.	20.8	11.6	61.1	63.0	Nov.	25.3	14.9	54.6	48.4	Dec.	25.5	14.4	60.6	21.6

Appendix 1

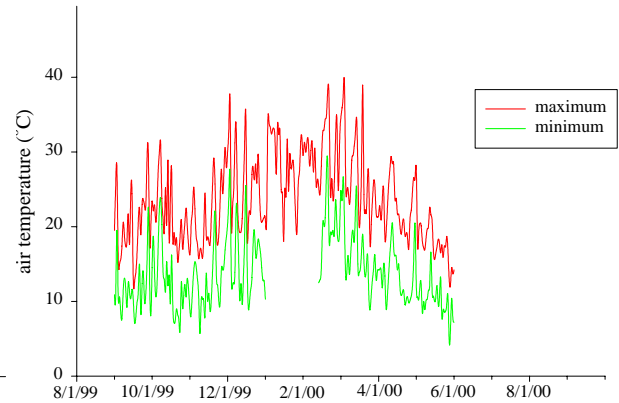
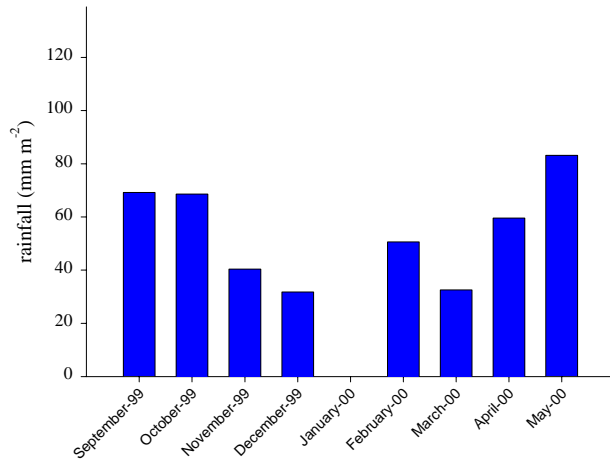
Climatic data September 1998 to August 1999 (Adelaide, Waite Campus)



date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)
09/01/98	19.5	13.9	46	0	10/01/98	26.9	16.3	29	0	11/01/98	22.3	7.9	44	0
09/02/98	26.5	9.6	50	0	10/02/98	21.2	15.4	90	1.2	11/02/98	27.6	11.2	30	0
09/03/98	21.3	14.6	68	2.8	10/03/98	16.5	10.5	71	6.4	11/03/98	30	19.2	21	0
09/04/98	17.7	10.2	84	0	10/04/98	18.9	10.1	55	0	11/04/98	22	19.5	28	0
09/05/98	14.2	7.6	59	0	10/05/98	20.7	11.9	37	0	11/05/98	22.8	12.4	79	0.6
09/06/98	15.3	9	72	2.8	10/06/98	14.3	9.6	57	21.8	11/06/98	31	12.6	48	0
09/07/98	14.9	8.5	54	0.2	10/07/98	15.2	9	89	2.6	11/07/98	18.8	15.5	87	6.2
09/08/98	17.1	8.6	46	0	10/08/98	15.9	7.5	63	0	11/08/98	19.8	8.1	55	0
09/09/98	18.7	10	50	0	10/09/98	14.8	9.3	65	1.6	11/09/98	25.5	11.8	48	0
09/10/98	20.5	13.1	65	0	10/10/98	16.1	6.6	68	1.4	11/10/98	23.3	16.1	29	0
09/11/98	21.9	13	63	0	10/11/98	18.2	9.7	62	0	11/11/98	15.6	13.8	89	17.6
09/12/98	15	11.5	85	4.8	10/12/98	19.6	13.7	90	0	11/12/98	19	11.9	65	0.2
09/13/98	14.6	9.9	60	1.6	10/13/98	17.4	10.1	47	0.2	11/13/98	21.7	10.7	76	0
09/14/98	15.4	7.2	75	0	10/14/98	25.2	9	41	0	11/14/98	28.2	11.2	49	0
09/15/98	14.4	9	70	1.4	10/15/98	21.2	9.2	57	1.6	11/15/98	18.3	12.8	72	0.8
09/16/98	15.7	9	88	5.6	10/16/98	28.8	10.1	56	0	11/16/98	20.5	9.6	61	0
09/17/98	21.3	9.9	63	0	10/17/98	34.1	17.3	32	0	11/17/98	20.6	9.4	59	0
09/18/98	22.6	14.1	35	0	10/18/98	16.9	13.4	63	0	11/18/98	19.7	7.9	51	0
09/19/98	16.2	10.6	68	1	10/19/98	14.7	9.7	90	2.2	11/19/98	23	12.4	52	0
09/20/98	18.3	6.9	59	0	10/20/98	13.8	7.7	65	3.8	11/20/98	26.5	12.2	56	0
09/21/98	22.3	10.2	35	0.2	10/21/98	17.2	10.4	75	0.4	11/21/98	24	14.1	42	0
09/22/98	22.3	16.6	69	0	10/22/98	19.5	6.7	66	0	11/22/98	21	9.9	56	0
09/23/98	14.5	10.4	74	33.4	10/23/98	25.5	11.3	43	0	11/23/98	25.6	11.7	54	0
09/24/98	12.9	6.1	63	2	10/24/98	28.8	14.1	33	0	11/24/98	27.8	15.4	31	0
09/25/98	17.2	8.7	42	0	10/25/98	18.8	12	69	0	11/25/98	30.3	15.7	38	0
09/26/98	21.2	12.6	43	0	10/26/98	15.6	11.2	61	0	11/26/98	32.7	16.8	29	0
09/27/98	25.2	9.3	48	0	10/27/98	15	7	60	6.6	11/27/98	17.9	13.1	82	3.4
09/28/98	27.6	13.6	76	4	10/28/98	18.8	5.9	45	0	11/28/98	19.2	11	42	0.2
09/29/98	21.4	12.9	74	0	10/29/98	20.9	12.4	32	0	11/29/98	21.2	9.2	58	0
09/30/98	27.4	10.1	60	0	10/30/98	20.4	10.1	56	0	11/30/98	25.3	12.4	53	0
					10/31/98	18.2	11.3	52	0					
Sep.	19.1	10.6	61.5	59.8	Oct.	19.6	10.6	58.7	49.8	Nov.	23.4	12.5	52.8	29.0
										Dec.	26.5	15.3	56.6	16.2

Appendix 1

Climatic data September 1999 to May 2000 (Adelaide, Waite Campus)



date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)	date	max air temperature (°C)	min air temperature (°C)	av relative humidity (%)	rain (mm m ⁻²)
09/01/99	19.5	10.9	65	0.6	10/01/99	23.4	14.5	29	0	11/01/99	20.2	8.3	60	0
09/02/99	25.5	10.7	48	0	10/02/99	22.1	18.7	50	0	11/02/99	22	11.2	46	0
09/03/99	27.8	19.5	30	0	10/03/99	22.9	13.8	64	31.8	11/03/99	25.3	14	40	0
09/04/99	16	10.7	95	23	10/04/99	20.6	10.7	64	0	11/04/99	22.4	15.3	46	0
09/05/99	14.9	10.6	64	5	10/05/99	22.7	12	51	0	11/05/99	18.7	14.7	73	5.2
09/06/99	15.6	8.5	85	2.8	10/06/99	27.5	17.5	41	0	11/06/99	17.7	13	45	1.2
09/07/99	17.4	7.7	70	0.2	10/07/99	30.1	22.6	29	0	11/07/99	15.7	10.5	54	0.4
09/08/99	20.6	11.4	29	0	10/08/99	31.2	23.8	26	0	11/08/99	16.8	5.7	36	3.4
09/09/99	18.7	13.1	65	0	10/09/99	23.8	20.3	82	0	11/09/99	16.8	10	40	4.8
09/10/99	17.4	11.6	70	0	10/10/99	21.1	13.9	86	14.6	11/10/99	15.8	10.3	42	3.6
09/11/99	18.2	9.2	52	0	10/11/99	19.3	12.9	89	4.2	11/11/99	18.9	10	25	0
09/12/99	21.7	12.5	39	1	10/12/99	25.2	11.3	58	0	11/12/99	24.5	8	32	0.2
09/13/99	17.6	11.3	79	1.6	10/13/99	20.2	15.3	78	8	11/13/99	19	13.8	42	0
09/14/99	24.5	10.4	42	0	10/14/99	28.9	12.1	78	3.2	11/14/99	18.6	10.1	66	0
09/15/99	24.4	11.6	56	0	10/15/99	18.1	13.5	72	0.6	11/15/99	18.1	11.1	41	0
09/16/99	13	10.2	58	5.8	10/16/99	23.9	9.7	57	0	11/16/99	17.6	8.7	40	0
09/17/99	12.6	7.2	65	15	10/17/99	27.7	16.2	63	0	11/17/99	21.1	10.1	31	0
09/18/99	13.8	7.9	60	2	10/18/99	18.2	9.6	67	0	11/18/99	25.4	13.9	22	0
09/19/99	16.5	9.9	49	0	10/19/99	19.2	7.2	67	0	11/19/99	29.2	18.6	15	0
09/20/99	20.7	11.4	57	0	10/20/99	17.5	7.4	63	0	11/20/99	25.7	21.9	13	0
09/21/99	22.4	14.9	30	0	10/21/99	18.5	9	59	0	11/21/99	19.5	13.7	80	11.4
09/22/99	18.9	8.3	63	0	10/22/99	15.3	8.2	54	0.2	11/22/99	17.5	12.2	69	10.2
09/23/99	23	12.4	60	0	10/23/99	16.6	7.5	47	0	11/23/99	19	10	50	0
09/24/99	23.5	13.7	58	0	10/24/99	18.7	6.2	57	0.2	11/24/99	23.3	9.7	59	0
09/25/99	22.8	9.9	61	0	10/25/99	20.9	12.6	66	0.8	11/25/99	27.7	14.2	59	0
09/26/99	23	11.2	58	0	10/26/99	17.1	9.2	57	1	11/26/99	24.5	14.3	51	0
09/27/99	29.6	15.9	50	0	10/27/99	18.6	10.8	68	0.8	11/27/99	27	14.6	46	0
09/28/99	29.4	22.6	29	0	10/28/99	20.7	12.2	79	0	11/28/99	30.6	16.9	40	0
09/29/99	17.8	12.8	77	12.2	10/29/99	22.5	10.6	67	0	11/29/99	27.9	18.6	54	0
09/30/99	20.1	8.2	58	0	10/30/99	19.7	13.1	85	2.4	11/30/99	30.2	20.5	46	0
					10/31/99	16.2	8.9	55	0.8					
Sep.	20.2	11.5	57.4	69.2	Oct.	21.6	12.6	61.5	68.6	Nov.	21.9	12.8	45.4	40.4
										Dec.	25.1	15.7	58.6	31.8

Appendix 2

A) Soil description and test results:

Alverstoke vineyard of the University of Adelaide

(Field planting: *Vitis vinifera* L. cv. Cabernet Sauvignon grafted *Vitis champini* cv. Ramsey rootstock (split-root))

Easting 284 175

Northing 6127 675

Mapsheet 6628-3 (Adelaide)

Describer James Hall

General Soil Description

Dark brown clay with shale fragments, grading into red-brown mottled clay; overlying olive-brown mottled cracking clay.

Australian Soil Classification

Mottled Eutrophic Red Dermosol / Brown Vertosol; very thick, gravelly, clay loamy/clayey, deep. [the “/” indicates one soil overlying another]

Site Description

Geology colluvium: shale fragments & soil; overlying crackly clay

Position mid-slope Slope 6%

Relief/Modal Slope undulating rises Aspect 300°

Landform pediment Elevation 135m

Surface Condition hard

Surface Stone 2-10% coarse gravelly shale (2-6 cm)

Drainage well drained

Soil Profile Description

0-10 cm	A11 horizon: dark brown (7.5YR2.5/2), clay loam, with cloddy structure (5-10 cm), pH 6.5 (field test), 10-20% medium gravelly shale fragments (0.6-2 cm), and a clean boundary to:
10-35 cm	A12 horizon: dark red brown (5YR2.5/2), light clay, with prismatic structure (5-10 cm), pH 6.5 (field test), 2-10% medium gravelly shale fragments (0.6-2cm), and a clean boundary to:
35-60 cm	AB horizon: dark red-brown (5YR3/3), clay loam, with weak polyhedral structure (0.5-1 cm), pH 7.0 (field test), 2-10% medium gravelly shale fragments (0.6-2cm), and a clear boundary to:
60-75 cm	BA horizon: dark brown (7.5YR4/4), light clay, with weak polyhedral structure (0.5-1cm), pH 8.0 (field test), 50-9-% coarse gravelly shale fragments (2-6cm), and a gradual boundary to:
75-125 cm	Btg horizon: red-brown (5YR4/6), medium clay with some mottles, with weak polyhedral structure (1-2 cm), pH 9.0 (field test), and with a horizontal thin band of shale & 20-50% partially weathered rock (0.6-2 cm). This B horizon has a wavy upper boundary which varies from 30 to 100cm depth; and a gradual lower boundary to:
125-140 cm	Dgss horizon: olive-brown (2.5Y4/4), medium clay with mottles & slickensides, with lenticular structure (1-2 cm), and pH 9.5 (field test). This horizon consists of moist cracking clay with slickensides and seems to be unrelated to the soil above. This horizon has a wavy upper boundary.

Notes: The soil profile also includes minor quartz & quartzite fragments. The depth of topsoil above the red clay B horizon varies from 30 to 100 cm. The areas with deeper topsoil are very stony, for example, the middle of the trench has very gravelly/stony topsoil over red clay B at 100 cm. The southern end of the excavated trench has signs of greater wetness (mottling & duller colours) than the middle or northern end of the trench.

Samples:

CH117-1 -horizon 1
CH117-2 -horizon 2
CH117-5 -horizon 5
CH117-6 -horizon 6

CH117-X -20m to north; sample from 45cm; from red clay B horizon (B horizon upper boundary@ 30cm)

CH117-Z -20m to north; sample from 100cm; from red BC horizon with some stony fragments and some weathered rock

Site – Land Quality Ratings

Note. The site is the land area within a radius of 20 m of the described soil profile, and similar in characteristics to the described soil profile. Site ratings are based on a land class system with ratings from 1 to 8 – the higher the rating the greater the limitation. The ratings below have mostly been determined using morphological data, and experience with laboratory chemical analyses of similar soils, but are not supported by site specific laboratory chemical analyses.

Waterlogging	2w	moderately well drained
Surface Condition	2c	hard-setting surface
Subsoil Condition	2p	hard & coarsely structured subsoil
Root Zone Water Holding Capacity	2m	moderate
Inherent Fertility	1n	
Subsoil Toxicities	1t	
Acidity	1h	
Alkalinity	1-2I	neutral with alkaline lower subsoil
Salinity	1s	
Scalding	1z	
Water Erosion Potential	2e	moderately low
Wind Erosion Potential	1a	
Water Repellence	1u	
Rockiness	2r	some rocks
Gullying/Tunnelling	1g	
Flooding Potential	1f	

B) Soil description and test results:

Alverstoke vineyard of the University of Adelaide

(Field planting: *Vitis vinifera* L. cv. Cabernet Sauvignon on own roots (split-root))

Easting 284 175

Northing 6127 675

Describer James Hall

Dark brown (7.5YR 3/3) sandy loam with 10-20% fine gravel (2-6 mm).

No fine carbonate effervescence detected using 1 molar HCl. (That is, soil is non calcareous).

Inoculo Laboratories field test kit pH = 6.5 (slightly acidic).

Structure seems to be mostly single grain with some 5-10 mm polyhedral peds.

Appendix 3

Summary of irrigation and rainfall for Waite and Coombe vineyard

Alverstoke Cabernet Sauvignon on Ramsey rootstock (split-root vines)

	1996/1997	1997/1998	1998/1999
irrigation period	26.11. – 28.03.	19.10. - 03.04.	30.10. – 11.03.
irrigation water applied:			
control (ML/ha)	1.9	1.4	0.9
PRD (ML/ha)	2	0.7	0.5
no of irrigation events	54	46	38
no of irrigation cycles	9	8	8
rainfall			
during irrigation period (mm)	85.4	170.0	130.4
from 1 st July to 30 th of June (mm)	538.4	642.4	595.1

Coombe vineyard: Shiraz on own roots

	1997/1998	1998/1999
irrigation period	18.10. - 03.04.	30.10. – 11.03.
irrigation water applied:		
control (ML/ha)	1.4	1.0
PRD (ML/ha)	1.4	1.0
no of irrigation events	46	40
no of irrigation cycles	12	10
rainfall		
during irrigation period (mm)	170.0	130.4
from 1 st July to 30 th of June (mm)	642.4	595.1