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PARTITIONING OF DRY MATTER, CARBON, NITROGEN AND INORGANIC IONS OF GRAPEVINES: EFFECT OF PARTIAL



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PARTITIONING OF DRY MATTER, CARBON, NITROGEN AND INORGANIC IONS OF GRAPEVINES: EFFECT OF PARTIAL ROOTZONE DRYING AND RELATIONSHIP WITH RESTRICTED SPRING GROWTH

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1. Abstract

A reduced shoot growth and greatly increased WUE is a characteristic of partial rootzone drying (PRD), but little is known about the effect of PRD on partitioning of C, N and inorganic ions such as K. Our experiments with field-grown Cabernet Sauvignon, where the PRD grapevines were irrigated at half the control rate, and Shiraz where the PRD grapevines were irrigated at same rate as controls, have confirmed that PRD is not simply an irrigation strategy that applies less water—rather it alters the way in which the plant responds to its environment, e.g. PRD alters the sensitivity of the stomatal response to atmospheric conditions and significantly influences enzymes that regulate nutrient accumulation and partitioning. PRD did not change the total amount of carbon and nitrogen on a whole plant basis. However, it caused a significant partitioning of carbon and nitrogen towards the trunk, roots and fruit at the expense of shoot growth. This change in partitioning occurred as a result of altered activity of the enzymes controlling the assimilation of carbon and nitrogen. This hypothesis was reinforced by the fact that berries showed significantly higher levels of hexose early in the season. Although PRD had no significant effect on berry characteristics at harvest such as Brix and pH, it reduced per berry K^+ content and increased total amino acid concentration that may lead to positive outcomes for wine quality.

Restricted spring growth (RSG) is a disorder with sporadic occurrence in mostly the dry inland areas of Australia. Various groups have speculated that RSG is a result of a combination of factors such as climate, cultural and pathological disruptions on nutrient accumulation leaving grapevines vulnerable to unfavourable external conditions. Field experiments included the exposure of young Cabernet Sauvignon to a range of cultural disruptions on carbon and nitrogen status to induce the RSG disorder in a closely monitored environment. Although field experiments had significant effects on shoot growth and nutrient status, no classical RSG disorders could be achieved. However, RSG symptoms encountered in commercial vineyards were associated with an increase in the C/N nutrient status. Similar observations were made in field experiments where vines were exposed to low soil moisture conditions during the winter months.

2. Executive summary

Partial rootzone drying (PRD) is an irrigation management technique designed to reduce water use in grapevines without a decline in yield, thereby increasing water–use efficiency (measured as tonnes/ML) (WUE). The principle of PRD is to keep part of the root system at a constant drying rate to produce soil-derived signals to above-ground plant organs to induce a physiological response. Major PRD effects include a reduced canopy size and greatly increased WUE with possible improvements in fruit quality. Although we have a good understanding of the hormonal physiology of PRD, little is known on the effect of PRD on partitioning of C, N and inorganic ions such as K. The current study broadened our knowledge on the effects of PRD on grapevine field performance, growth and dry matter accumulation as well as its effects on physiology and biochemistry. In field experiments over 3 seasons, partial rootzone drying (PRD) reduced water use in grapevines without a significant decline in yield. PRD effects included reduced shoot growth and greatly increased WUE. Field-grown Cabernet Sauvignon, where the PRD grapevines were irrigated at half the control rate, and Shiraz where the PRD grapevines were irrigated at same rate as controls, confirmed that PRD is not simply an irrigation strategy that applies less water—rather it alters the way in which the plant responds to its environment.

However, many of the vine characteristics remained unaffected by PRD treatment irrespective of the amount of irrigation water applied compared to control. This in itself seems to be significant, because sub-optimal soil moisture conditions would normally have a negative impact on vine physiology by reducing the photosynthetic apparatus and challenging the vine's ability to assimilate carbon and nitrogen for growth. Although PRD did not change the total amount of carbon and nitrogen on a whole plant basis, enough evidence was collected to conclude that PRD partitioned nutrients and dry matter away from the vegetative structure towards the permanent structure. One could speculate that this enabled the plant to better explore the soil environment by investing C and N into root growth and osmoregulation. PRD-treated grapevine roots on the 'wet'- and 'drying'-sides differed greatly in enzyme activity and osmolality. PRD significantly increased osmolality in both wet and drying roots compared to control by increasing total osmolyte concentration that may facilitate the movement of water from wet to dry roots. The increases in osmolality were also associated with increased free polyamine production (spermidine and spermine) in PRD roots that may

be related to increased root growth and density. Consequently, PRD vines would be more tolerant of stressful environments, such as transient periods of drought.

The change in nutrient partitioning occurred as a result of altered activity of the enzymes controlling the assimilation of carbon and nitrogen. PRD significantly reduced nitrate reductase (NR) activity in grapevine leaves—which catalyses the first step in the assimilation of nitrate—irrespective of the amount of water applied. The reduction in NR activity is closely correlated with the development of the PRD cycle and the associated reduction in stomatal conductance. Hence, it was shown in labelled nitrogen experiments that the amount of nitrogen absorbed during the season would be less in PRD-treated grapevines. This may be due to the observed reduction in shoot growth rate and lowered NR activity in leaves. It is possible that the fertilizer needs of PRD vines may therefore be lower than control vines and this would mean a saving in the production costs. A smaller area of wetted soil under PRD may also mean that fertilizers would be lost more slowly than with standard irigation.

PRD also significantly altered the activity of the sucrolytic enzyme that regulates source-sink relationships. PRD caused transient increases in leaf SPS activity (formation of sucrose) compared to control, but significantly reduced leaf neutral invertase (sucrose cleavage) and leaf starch content in both field and potted experiments. This may indicate an increased photosynthetic capacity and a reduction in sink strength for sucrose in favour of organs such as fruit and roots. This hypothesis was reinforced by the fact that berries showed significantly higher levels of hexose early in the season. Although berry sugar content and Brix at harvest was unaffected by PRD treatment, PRD reduced per berry K^+ content and increased total amino acid concentration which may lead to positive outcomes for wine quality.

Restricted spring growth (RSG) is a disorder with sporadic occurrence in mostly the dry inland areas of Australia. The disorder is associated with delayed budburst, restricted growth in young shoots and loss in yield. Various groups have speculated that RSG is a result of a combination of factors such as climate, cultural and pathological disruptions on nutrient accumulation leaving grapevines vulnerable to unfavourable external conditions. In the current study, field experiments included the exposure of young Cabernet Sauvignon to a range of cultural disruptions on carbon and nitrogen status to induce the RSG disorder in a closely monitored environment because of the unknown probability to encounter the natural occurrence of the disorder in commercial vineyards. Although field experiments had significant effects on shoot growth and nutrient status, no classical RSG disorders could be

achieved. Potted experiments indicated that exposure of dormant shoots to subzero temperatures will subsequently delay budburst and shoot growth with an associated decrease in tissue C/N ratio. However, it was not possible to induce typical RSG symptoms by treatments that may potentially impair storage of C and N reserves. However, RSG symptoms encountered in commercial vineyards were associated with an increase in the C/N nutrient status. Similar observations were made in field experiments where vines were exposed to low soil moisture conditions during the winter months. The results of the current study support the current recommendations on the avoidance of RSG. Growers should be wary of overcropping young grapevines and forcing the growing season into late autumn. Also they should avoid very low soil moisture conditions during the winter months, especially when associated with very low temperatures. Ensuring adequate starch reserves before the onset of winter may be vital in the survival of above-ground plant organs during winter while adequate soil moisture may also reduce the effect of sudden temperature changes on tissue damage.

3. Background

Grapevines under PRD irrigation have shown substantial improvements in water use efficiency (Dry *et al.*, 2001) by reducing the amount of irrigated water compared to control, without causing a significant reduction in yield and berry size that is often associated with other deficit irrigation strategies. In addition, PRD has the potential to improve wine quality by improving the canopy microclimate and light penetration by reducing the growth rate of the main and lateral shoots, especially the latter. The current study aimed to further explore aspects of PRD effects on the accumulation of dry matter and the partitioning of C, N and inorganic ions. The potential exists that PRD causes a shift in the partitioning of C from shoots to fruit that may explain the decrease in vegetative growth and the increase in mass of secondary metabolites, such as phenolic compounds and G-G, on a per berry basis. For example, it was thought that PRD may cause a shift in partitioning of C from shoots to roots. Preliminary results from pot experiments (Dry *et al.*, 2000a) and field vines (Stoll *et al.*, 2000a) suggest that drying half of the root system stimulates an increase in root growth of the wetted half. Therefore, it is possible that the increased water-use efficiency measured with PRD-treated vines in the field may be due, in part, to the creation of a more exploratory root system that may be able to exploit a greater soil volume.

One of the main effects of PRD is a reduction in lateral shoot growth. Research by Stoll *et al.* (2000a) has shown that this is associated with decreased production and/or transport of cytokinins (CK) in roots in drying soil. There is a link between N supply and CK production: N may be involved in regulation of CK synthesis in roots (Takei *et al.*, 2002). Changes in N uptake in response to PRD may have implications for the supply of amino acids available to yeast during fermentation. PRD has been shown to reduce juice and wine pH relative to well-irrigated vines (Dry, 1997). Morris *et al.* (1983) have shown that high juice pH in Concord berries was associated with excessive berry K content that produced problems in colour stability of wine during storage. During winemaking, high K increases the precipitation of tartrate in salt form and therefore decreases free tartrate, reducing the tartrate:malate ratio which is undesirable in the production of high quality wines (Mpelasoka *et al.*, 2003).

It is important to address the question of the different volumes of irrigation water used in the experiments of the current study and in other PRD experiments by the Waite-based group. The main aims in the current commercial use of the PRD irrigation system are to firstly save

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

However, with an equal amount of water applied to different soil surface areas a different set of variables may arise that may be difficult to account for. Firstly, the actual speed of penetration and distribution of water within the rootzone may be different in the PRD treatment compared to control. Therefore it may be possible, especially in heavy soils like the one in the current study, that the designated "dry side" would be smaller due to lateral soil moisture movement. Secondly, the level of water saturation of the soil can be measured, but the amount of water leaching past the rootzone, into deeper soil layers that would not be accessible to the plant, may not be so readily measured. Constant upward movement of soil moisture into the drier layers would then further confound the comparison between wetted soil volumes of PRD to control. This effect was seen in an experiment not included in this study where an unaccounted source of soil moisture confounded the PRD treatment. It seems therefore important to achieve the same depth of wetting and hence a difference in the volume of water applied per plant per unit time. Thirdly, higher application rates may mean greater runoff of irrigated water at the penetration site and greater evaporation rates compared to control.

These points suggest that even if volumes of irrigation water are equal in the PRD and control treatments, plant available water may differ. It may therefore be very difficult to devise an appropriate control for the PRD treatments. Nevertheless, in these experiments we have assumed that when equal water volumes are applied, physiological indicators such as a reduction in stomatal conductance are indicative of a PRD effect, independent of water volume effects.

The second area of research in this project aimed to establish a relationship between carbohydrate reserves in the permanent structures of the vine and the occurrence of Restricted

Spring Growth (RSG). The symptoms of RSG are uneven budburst, retarded shoot growth, and reduced set and yield. The cause is unknown: there is anecdotal evidence that stress (overcropping of young vines, lack of water or nutrients, low temperature) in one season may reduce the level of reserves available for remobilisation in the following season(s). Although the possible link between RSG and pathogens such as AGY has been thoroughly investigated (F. Constable, pers. comm.), the relationship between C and N partitioning and RSG has not been studied in depth (Wilson and Hayes, 1999).

4. Project Aims and Performance Targets

4.1 Objectives

- Investigate the influence of PRD on partitioning of dry matter, carbon, nitrogen and inorganic ions of grapevines
- Acquire information on the production of free α - amino acids under PRD conditions
- Develop a better understanding of the mechanisms by which PRD is able to increase water use efficiency (measured as tonnes of fruit/ML irrigation applied) and wine quality, particularly in relation to resource allocation within the plant.
- Investigate the relationship between the partitioning of carbon and nitrogen, and restricted spring growth of grapevines
- Acquire information that will lead to a better understanding of the factors controlling the incidence of RSG

4.2 Outcomes

- An improved framework of knowledge on which to build irrigation schedules with PRD or other irrigation strategies
- More efficient operation and improved management of PRD
- More efficient use of nutrient inputs under PRD conditions
- More efficient use of irrigation water
- Knowledge that will lead to a better understanding of the factors controlling the incidence of RSG
- Knowledge that will lead to recommendations for appropriate management practices to minimise the impact of RSG

Outputs and Performance Targets

Outputs	Performance Targets	Date
1. Information on the mechanism by which PRD may affect partitioning of C, N and other inorganic nutrients	1. Measurement of quantitative changes in various tissues	May 2002
2. Information on the effect of PRD on dry weight partitioning and root development	2. Measurement of tissue weights in several experiments where crop load is varied under PRD and conventional irrigation regimes	May 2002
3. Information on production of free α - amino acids under PRD conditions	3. Measurement of amino acids, polyamines in various experiments.	May 2002
4. Recommendations on use of PRD to manipulate yield and fruit composition	4. Collection and interpretation of data from all current and past experiments.	April 2004
4. Information on relationship between the expression of RSG symptoms and the status of C and N.	5. Data collected from pot and field experiments.	April 2004

5. Materials and methods

5.1 Sites and conditions

All experiments were conducted on potted or field-grown grapevines (*Vitis vinifera* L.) between November 2000 and November 2003. Potted vines were grown in open shade houses (Cabernet Sauvignon and Chardonnay) or in temperature controlled greenhouses (Cabernet Sauvignon) at the Waite campus of the University of Adelaide. All potted plants were on own roots. Potting mixes varied with experimental design. Potted grapevines used for PRD (Cabernet Sauvignon) had a split-root system and were grown in two pots and sizes varied with experimental design. In all PRD experiments irrigation water was applied either to one side of the root system at any time (PRD; Figure 1 A) or to both sides (control; Figure 1 B).

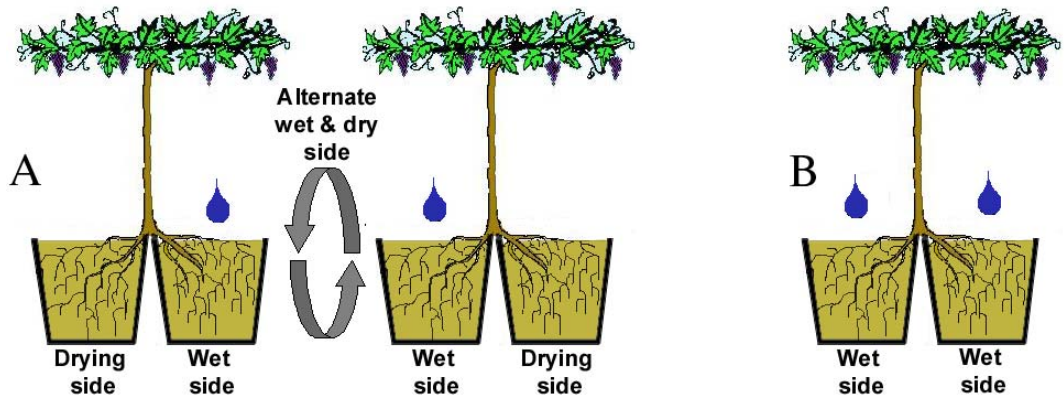


Figure 1 Implementation of PRD irrigation set up in pots: A)PRD: water withheld from one side; B) control: water on both sides.

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

Cabernet Sauvignon and Shiraz vines in the Coombe vineyard were planted in 1992 at the Waite Campus of the University of Adelaide. The vineyard had a vertical shoot positioning (VSP) trellis system and was spur pruned according to the measured mass of pruning weight (30nodes/kg). Shiraz: PRD and Control vines received the same amount of water (2 L/h). Cabernet Sauvignon: PRD received half the amount of water (2 L/h) as control (4 L/h). Amount and timing of additional irrigations and frequency of switches in the wetting pattern were determined by evaluating observed rates of soil drying and soil water contents.

Shiraz vines grown under PRD at Nuriootpa were subjected to three pruning levels as well as to PRD treatments. The vineyard had a single wire trellis system and was spur pruned. PRD vines received half the amount of water of control vines. Vines were subjected to two irrigation treatments (PRD and Control at 2 L/h) and three pruning levels (30, 60 and 120 nodes/vine).

RSG field and potted experiments were conducted in the Alverstoke vineyard at the Waite campus of the University of Adelaide. Grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon clone CW44) used for field experiments were planted in 2000 and irrigated with 4 L/h dripper emitters. Potted vines were propagated from thick cuttings (*Vitis vinifera* L. cv. Chardonnay) taken from the Coombe vineyard. Cuttings (0.35-0.45 m long) were selected in the winter and callused in a heat bed (25°C) until bud-break was observed. Cuttings with well-developed root systems were selected and planted in standard potting mixture. Two-year-old rooted cuttings (*Vitis vinifera* cv. Chardonnay) were also purchased from a commercial nursery in 2001 and planted in standard potting mixture.

All replicates at the Waite campus consisted of 3 vines; the centre vine was used as the test vine and the two buffer vines, one on either side, were not used for sampling. PRD experiments in the Coombe vineyard consisted of seven replicates. The RSG field experiment in the Alverstoke vineyard consisted of five replicates per treatment. The experiment at Nuriootpa consisted of five replicates for each of the 6 treatments.

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

5.2 Soil moisture measurements

Soil moisture in the Coombe vineyard was initially measured using gypsum blocks (Measurement Engineering Ltd., Adelaide, South Australia) at depths of 0.4 m, 0.6 m, 0.8 m and 1 m in a 15 cm circular pattern around the dripper, 0.4 m away from the trunk. However, in 2002 a Diviner 2000®, (Sentek, Adelaide, South Australia) was used to measure soil water content in the Coombe vineyard. One access tube was installed 0.3 m away from the vine trunk underneath the dripper in every treatment and in both cultivars (Cabernet Sauvignon and

Shiraz) to monitor the soil water contents. One more access tube was installed for both cultivars on the PRD opposite side to measure the alternation in wetting pattern. Volumetric soil water content was measured at every 0.1 m depths from 0 to 1m with a portable capacitance probe on either side of the vine row at a distance of 0.4 m from the vine trunk.

Soil matric potential was measured in potted experiments using a portable tensiometer ("Quickdraw" series 2900 soil moisture probe, Soil Moisture Equipment Corp., Santa Barbara, USA). Soil moisture was also measured in potted experiments by the Model 6050X1 Trase system (Soil moisture equipment corp., California, USA) that uses Time Domain reflectometry (TDR) to measure instantaneously the volumetric water content of the soil.

5.3 Leaf gas exchange and photosynthesis

Stomatal conductance of leaves was measured (in units of $mmolm^{-2}s^{-1}$) using a portable AP4 diffusion porometer (DELTA-T Devices Ltd, Cambridge, UK). Measurements was made on mainly cloudless days between 10am and noon or 2pm and 4pm on mature, sun exposed leaves on the outside of the canopy. Assimilation of carbon dioxide and stomatal conductance were measured using a LICOR open photosynthesis system (Li 6400, Lincoln, Nebraska, USA) with an infrared gas analysis instrument (IRGA). This instrument measures differential or absolute changes caused by leaf gas exchange and calculates photosynthesis.

5.4 Xylem sap collection

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

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

5.5 Fruit sampling and measurements

Berries were sampled once a week from the beginning of veraison until harvest. Berry samples of 50 were randomly chosen from the test vine at different positions within the bunch and within the canopy. When fruit was more mature (higher that 21°Brix) and at harvest, sample size increased to 100 to 300 berries. Before berries were frozen for later analyses of sugars, amino acids, K and minerals, polyamines and total C and N contents, berry mass was determined as well as total soluble solid analyses (TSS) and pH. Berries were crushed and pressed in a citrus press and the juice transferred to centrifuge vials (10 mL) and centrifuged for 5 min at 1500g. 50 µL of the supernatant was then used for the measurement of TSS (°Brix) using a digital refractometer (BRX242, Erma Inc., Tokyo, Japan) that was zeroed using distilled water. The pH was measured using a standard pH meter (Activon 110. Thornleigh, NSW).

5.6 Soluble sugars analysis

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

gas free it was passed through an in-line degasser. Flow rate was maintained at 0.6 mL/min. The mobile phase is deionised, degassed (vacuum filtration through a Millipore HA 0.45 mm filter) and bacteria free Millipore water containing 5 mg/L Ca-EDTA. Prior to the initial use and after running about 200 samples, the column was reconditioned by passing 1 L of 500 mg/L Ca-EDTA solution. The column was then washed with the mobile phase (5 mg/L EDTA) until the base line was stable.

5.7 Amino acid analysis

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

5.8 Free polyamine analyses

Free polyamine analysis was done as described by Flores and Galston (1982). Crude extracts was prepared from tissue extracted in cold 5% $HClO_4$ at a ratio of about 100 mg fresh weight/mL $HClO_4$ for 1 hour in an ice bath. After centrifugation at 48,000 g for 20 min the supernatant fraction was used for polyamine analyses by HPLC. Samples stored at -20°C will remain stable for at least 2 months (Flores and Galston, 1982). Extracts were analysed by HPLC according to the benzoylation procedure modified by Flores and Galston (1982). One mL of 2N NaOH was mixed with 250–500 µL of $HClO_4$ extract. After the addition of 10 µl benzoyl chloride, vortexing for 10 sec, and incubation for 20 min at room temperature, 2 mL saturated NaCl was added. Benzoyl-polyamines were then extracted in 2 mL diethyl ether (anhydrous grade; Baker). After centrifugation at 1500 for 5 min, 1 mL of the ether phase was collected and evaporated to dryness under a stream of nitrogen and re-dissolved in 100 µL methanol (Baker; HPLC grade). Standards of putrescine, spermine, spermidine and cadaverine were treated in the same way with up to 50 nmol of each polyamine in the reaction mixture. Stored benzoylated samples are stable at -20°C for several months. Separation, identification and quantification were done with a High Pressure Liquid Chromatography system (Hewlett Packard LC1100) equipped with a 250 x 4 mm Exsil ODS C_{18} column (SGE, Adelab, Adelaide, South Australia) filled with silica spheres with a particle size of 3 µm. Benzoyl-polyamines are eluded with 64% methanol at a flow rate of 1 mL/min and detected at 254 nm.

5.9 Inorganic mineral analyses

Potassium (K), calcium (Ca), magnesium (Mg), Phosphorus (P) and sulphur (S) analyses were done on plant tissue that was dried in a forced air oven for at least 2 weeks at 60°C. All plant tissues were powdered in a commercial coffee grinder and samples of 0.25 g were weighed into open top 75 mL digestion tubes for plant digest (Zarcinas and Cartwright, 1983; Zarcinas *et al.*, 1983). A standard wheat sample of known mineral content and a blank sample were placed within the samples on a regular interval of 30 samples. After the addition of 3 mL concentrated Nitric acid (HNO_3) the digestion tubes were placed in a digestion block and an automatic digestion sequence initiated. The digestion sequence increased the block temperature in steps to 70°C for 60 min, 90°C for 180 min, 100°C for 30 min, 110°C for 30 min, 120°C for 30 min and 125°C for 210 min. The digest typically ran overnight and by the morning the samples had cooled. It was important that the sample had boiled down to 1 mL before further analysis. The digested samples were then diluted to 20 mL with distilled water and vortexed for 10 sec. Samples that may have contained silica were filtrated through

Whatman no. 42 filter paper. The supernatants were then drawn off into an ICP for optical emission spectrometry (CSIRO Div. Soils).

5.10 Starch analysis

Samples (500 mg) were washed with 5 mL 80% ethanol and centrifuged at 3000 g for 10 min. The supernatant was decanted and the pellet rewashed. The pellet retained was oven-dried (60°C) and after the addition of 2 mL 0.5N NaOH samples were boiled for 5 min. The samples were then neutralized with 0.5 N acetic acid to a pH of 6.5 and centrifuged at 3000 g for 10 min. The supernatant was taken and 100 units of α-amylase (in MOPS buffer, pH 6.5) added and incubated for 1 h at 30 °C in a water bath. The pH was then adjusted to 5.1 with Na-acetate buffer, 100 units of aminoglucosidase added and incubated for 1 h at 30 °C. The solution was cooled to room temperature and 50 μL taken to be analysed for glucose. Glucose is analysed by the addition of 950 μL GOPOD reagent and incubated for 30 min at 30 °C. GOPOD reagent consisted of a mixture of 3.45 g $Na_2HPO_4 \cdot H_2O$, 1.6 g $NaH_2PO_4 \cdot H_2O$, 2350 U Glucose oxidase, 375 U Peroxidase and 125 mg ABTS in 250 mL distilled water. The samples were then transferred to spectrophotometer cuvettes, diluted with 1 mL distilled water and the absorption read at 436 nm. Standard absorption curves were created using cornstarch and the same method. The measured absorption in samples was then compared to standard absorption curves and the amount of starch calculated in the original sample.

5.11 Enzyme assays

i) Sucrose Synthase (SucSy) activity

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

Sucrose synthase (SucSy) activity was assayed using 50 µL of the desalted enzyme extract and adding 200 µL reaction mixture (Claussen *et al.*, 1985). The final reaction mixture contained 30 µM Tris HCL (pH 8.7), 3 µM Fructose and 5 µM $MgSO_4$ in both active enzyme and zero samples. To initiate the reaction 50 µL of 1 µM UDPGlucose was added to the active samples or 50 µL H_2O in the zero samples. At this time 200 µL 0.2 M NaOH was added to the zero samples. All samples were then incubated in a water bath for 60 min at 30°C. At the end of 60 min the reaction in the active enzyme samples was stopped by the addition of 200 µL 0.2 M NaOH. All samples were then closed and boiled for 10 min to destroy Fruc-6-P. After cooling, 100 µL was taken to determine the formation of sucrose by adding 250 µL 0.1% Resorcinol in 95% ethanol and 750 µL 30% HCL. The mixture was incubated for 8 min at 80°C. After cooling for 40 min the A_{520} was measured and compared to standard absorption curves of sucrose content.

ii) Invertase activity

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

Neutral invertase (NI, pH 7.5) activity was assayed using 25 µL of the desalted enzyme extract and adding 25 µL 1 M Na-acetate (pH 7.5). After the addition of 50 µL 120 mM Sucrose the mixture was incubated for 60 min at 37°C. Zero samples were done in the same manner as active enzyme samples. Boiling the capped samples for 3 min stopped the reaction. Before the measurement of the amount of glucose formed the sample was dissolved by adding 1 mL H_2O. The amount of glucose formed was measured using 25 µL of the sample and 450 µL of GOPOD reagent. GOPOD reagent consisted of dissolving 3.45g $Na_2HPO_4 \cdot 2H_2O$, 1.6g $Na_2HPO_4 \cdot H_2O$, 2350 U Glucose oxidase, 375 U Peroxidase and 125mg ABTS in 250 mL distilled water. The mixture was incubated at 30°C for 30 min in a water bath, dissolved with 500 µL distilled water and the A_{436} was measured and compared to standard absorption curves of glucose content.

iii) Sucrose Phosphate Synthase (SPS) activity

Sucrose phosphate synthase (SPS) activity was assayed by measuring fructose-6-P dependent sucrose-P (+sucrose) formation from UDPG (Kerr *et al.*, 1984). Plant tissues were frozen in liquid nitrogen, powdered with either a mortar and pestle or commercial coffee grinder and stored at –80°C until needed. The powder was extracted in 4 mL of extraction buffer (8 mL/g) for 30 s using a homogeniser. The extraction buffer contained 50mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2% (w/v) PEG-20, 1% (w/v) BSA, 5 mM DTT and 2 mM GSH. The brei was filtered through Miracloth and the filtrate centrifuged at 38000 g at 4°C for 10 min. The supernatant was immediately used for enzyme activity analyses. The assay mixture (70 µL) containing 7.5 mM UDPG, 7.5 mM fructose-6-P, 15 mM $MgCl_2$, 50 mM Hepes-NaOH (pH 7.5) and an aliquot of enzyme extract was incubated at 25°C for 10 min and terminated by addition of 70 µL 1.0 N NaOH. Heating the mixtures in a boiling water bath for 10 min destroyed unreacted fructose-6-P. After cooling, 0.25 mL of 0.1% (w/v) resorcinol in 95% ethanol and 0.75 mL of 30% HCl were added and the mixtures were incubated at 80°C for 8 min. The tubes were then cooled to room temperature and the A_{520} is measured and compared to standard absorption curves of sucrose content.

iv) Glutamine synthase (GS) activity

The activity of GS was determined by the method described by Lin and Kao (1996). Plant tissue was homogenized with 10 mM Tris-HCl buffer (pH 7.6), containing 1 mM $MgCl_2$, 1 mM EDTA and 1 mM 2-mercaptoethanol in a chilled pestle and mortar. The homogenate was centrifuged at 15000 g for 30 min and the supernatant was used for the enzyme assay. The whole extraction procedure was carried out at 4°C. GS assay was done on the supernatant by the method described by Oaks *et al*. (1980). The reaction mixture contained of a final volume of 1 mL, 80 µM Tris-HCL buffer, 40 µM L-glutamic acid, 8 µM ATP, 24 µM $MgSO_4$ and 16 µM NH_2OH (final pH was 8.0). Reactions were started by the addition of the enzyme extract and after incubation of 30min at 30°C the reaction was stopped by the addition of 2 mL 2.5% (w/v) $FeCl_3$ and 5% (w/v) trichloroacetic acid in 1.5 M HCl. The mixture was centrifuged at 3000 g and the absorbance of the supernatant was read at 540 nm. One unit of GS activity was defined as 1µmol L-glutamate γ-monohydroxamate formed per min.

v) Nitrogen reductase (NR) activity assay by infiltration method

NR activity was determined in leaves, berries and shoots by the method described by Hunter and Ruffner (1997). After the removal of leaf veins, leaves were cut into 2 mm^2 disks. Berries

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

vi) Nitrogen Reductase (NR) assay by extraction method

NR was extracted (Foyer *et al.*, 1998) from leaf tissue powdered in liquid nitrogen. The extraction buffer (50 mM Mops-KOH, pH 7.8, 5 mM NaF, 1 μM Na_2MoO_4, 10 μM FAD, 1 μM Leupeptin, 1 μM microcystin, 0.2 g^{-1} fresh weight PVP, 2 mM β-mercaptoethanol and 5 mM EDTA) was then added to the leaf tissue powder (1 mL/50mg fresh weight). The homogenate was centrifuged at 4°C for 5 min at 12000 g. NR activity was measured immediately on the supernatant. The reaction mixture consisted of 50 mM Mops-KOH buffer, pH 7.5, supplemented with 1 mM NaF, 10 mM KNO_3, 0.17 mM NADH, and either 10 mM $MgCl_2$ or 5 mM EDTA. The reaction was terminated after 8 or 16 min by the addition of an equal volume of sulfanil-amide (1% w/v in 3 N HCl) and then naphthylethylene-diamine dihydrochloride (0.02% w/v) to the reaction. The A_{540} was then measured in a spectrophotometer. The activation state of NR was defined as the activity measured in the presence of 10 mM MgCl divided by the activity measured in the presence of 5 mM EDTA (expressed as a percentage).

5.12 Leaf area and canopy measurements

Leaf area (m^2) in the Nuriootpa Shiraz and Alverstoke Cabernet Sauvignon was assessed after whole shoots were harvested from buffer vines and the shoot length and the lengths of all leaves were measured. The leaf area for every leaf was then calculated using a leaf length: leaf area formula specific to Shiraz and Cabernet Sauvignon vines, and summed to produce total leaf area per shoot. Plotting the total leaf area to shoot length for all harvested shoots per

treatment produced an equation to calculate leaf area using any known shoot lengths. To calculate mean shoot length of experimental vines, five shoots chosen at random were measured per plot to produce the mean shoot length per treatment. After the shoot count the average canopy size was then calculated by multiplying the shoot number per vine with average leaf area per shoot for any given length.

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

5.13 Labelled nitrogen experiments

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

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

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

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

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

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

5.14 Determination of nitrate

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

5.15 Total carbon and nitrogen analyses by dry combustion

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

5.16 Root osmolality

Root sap osmolality was measured by a freezing point depression method using an osmometer (Fiske® ONE TEN™ osmometer, Fiske Associates, Norwood, MA). Root sap was extracted from root material that was powdered in liquid nitrogen. Frozen root powder was allowed to thaw and transferred to 1 mL modified eppendorf tubes. The eppendorf tubes were perforated in the bottom by a needle and a small amount of non-absorbent cotton wool was compacted on the bottom to stop any material escaping. The 1 mL eppendorf was then placed inside a 2 mL eppendorf and centrifuged at 12000 rpm for 10 min and the root sap in the lower tube was used for analysis. The osmometer was calibrated before operation to manufacturer's specifications. 15 μL of root sap was loaded into the osmometer and the freezing point measured by super cooling of the sample. The freezing point of the sample was then compared to the standard curve for solutions of known osmolality and the results reported in mOsm/kg H_2O.

6 Results and discussion of PRD research

PRD is being adopted at an increasing rate within the Australian wine industry as a means to improve water use efficiency in grape production. However, the initial aim of PRD was to control excessive shoot growth in irrigated vineyards (Dry *et al.*, 1996). The reduction in shoot growth rate was successfully achieved by drying one part of the root system while irrigating the other, thereby creating two 'zones' that are irrigated in a one to two week rotation.

Although we have now a greater understanding of the physiology behind the PRD system than when first started (Dry *et al.*, 1996), there are still some aspects of its physiology that we do not understand and cannot predict in different environments. There is also a danger that the technique will be inappropriately used and thus lead to negative results. Therefore there were three major focus points of this study into the effect of PRD on grapevine physiology. Firstly, the **acquisition of carbon** that would include the accumulation of dry weights, sugars and starch and the role that sucrolytic enzymes play in regulating the source-sink relationship. Secondly, the **assimilation of nitrogen** and its partitioning into various nitrogen-containing compounds and amino acids, which is highly regulated by specific enzymes. Thirdly, the accumulation and partitioning of **inorganic ions** in various plant organs, especially K, in the berries of PRD vines that may influence grape quality.

6.1.1 PRD effects on growth and the accumulation of dry matter

Transient reductions in shoot growth rate in response to soil drying were found in earlier work (Dry *et al.*, 1996) and as a consequence it was proposed that a constant effect on shoot growth could be maintained by alternating the drying zones. Since then, significant work by Dry and Loveys (1999) and Stoll (2000) has provided evidence that PRD treatment in this way reduces shoot growth rate by 18% to 30% in field-grown grapevines over a whole season. Results in this study support the findings of those authors. PRD decreased grapevine shoot growth when irrigated with either half or the same volume of water and predominantly affected lateral shoot growth (Figures 2 and 3).

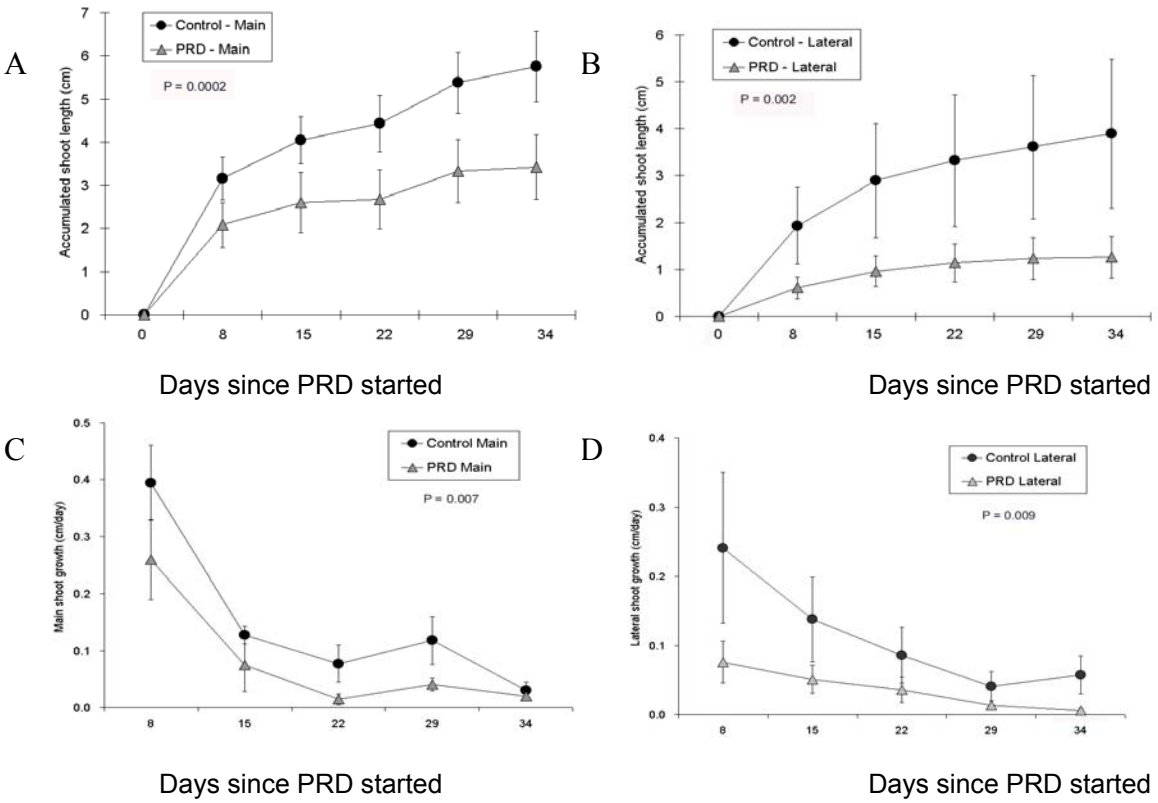


Figure 2 PRD effect on shoot growth in Waite Cabernet Sauvignon during the 2000/1 season. **(A+B)** accumulated main and lateral shoot length and **(C+D)** daily shoot growth rates of main and lateral shoots. PRD received half the amount of water as control. (means n=7; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

Vigorous lateral shoot growth is undesirable, because this may lead to dense canopies and an imbalance favouring vegetative growth versus fruit production (Smart, 1985). It is speculated that a reduction in the strength of this non-harvestable sink may increase the relative sink strength of the fruit (Dry *et al.*, 1996). Furthermore, in crops like tomato the developing side shoots may be a competing sink alongside developing fruit (Davies *et al.*, 2000) and it is speculated that a reduction in the strength of this non-harvestable sink may increase the relative sink strength of the fruit (Dry *et al.*, 1996).

Stoll (2000) observed that PRD-treated vines had canopies with lower leaf area and became more open, resulting in higher light intensities inside the canopy compared to canopies of control vines with a larger leaf area. The exterior leaves of grapevine canopies are exposed to direct sunlight and therefore they are the major contributors in canopy photosynthesis (Smart, 1985). Only a small fraction of total radiation penetrates deep into the canopy and therefore higher light penetration into dense canopies may have significantly positive effects on berry quality.

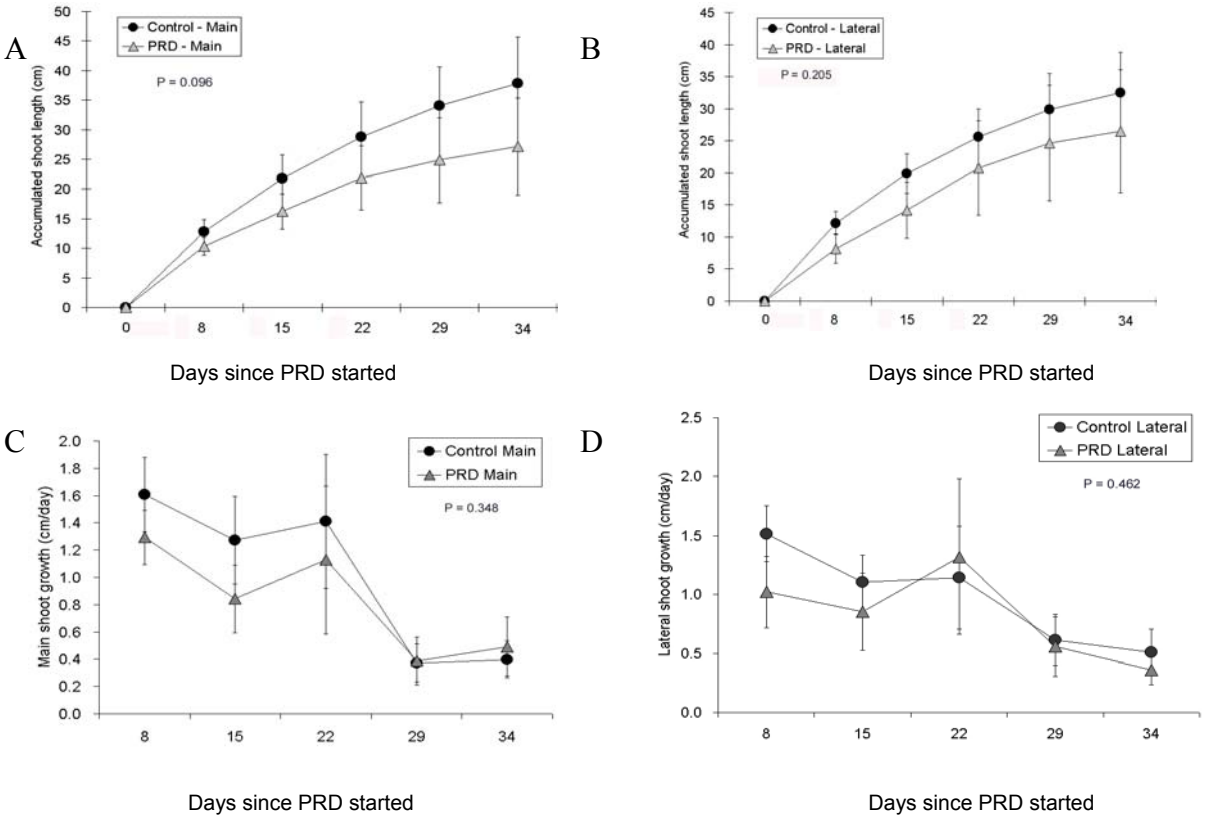


Figure 3 PRD effect on shoot growth in Waite Shiraz during the 2000/1 season. **(A+B)** accumulated main and lateral shoot length and **(C+D)** daily shoot growth rates of main and lateral shoots. PRD received the same amount of water as control. (means n=7; Vertical bars indicate standard errors of the mean; P value indicate the significance level of the main effect of irrigation)

Mabrouk and Sinoquet (1998) suggested that anthocyanin and bunch exposure is not linearly related, but could be described as a "quadratic relationship" with a bell shape. Bunches that received the lowest and highest amounts of solar radiation had the lowest anthocyanin concentration. According to their results, the highest amounts of anthocyanin concentration were achieved with 9% to 11% of ambient solar radiation. In the current study, PRD treatment increased the light penetration into the bunch zone of vines in the Coombe Vineyard (Adelaide, South Australia) irrespective of canopy hedging or the volume of water applied (Figure 4).

The reduction in shoot growth caused by PRD also significantly reduced the canopy size of Shiraz vines at Nuriootpa by 26% on average compared to control vines (Table 1). These findings are in agreement with the conclusions of Dry *et al.* (2001) and the findings of dos Santos *et al.* (2003). Those authors found that the effect of PRD on vegetative growth was characterised by lower total leaf area, lower leaf layer number, decreased canopy wideness and decreased number of water shoots.

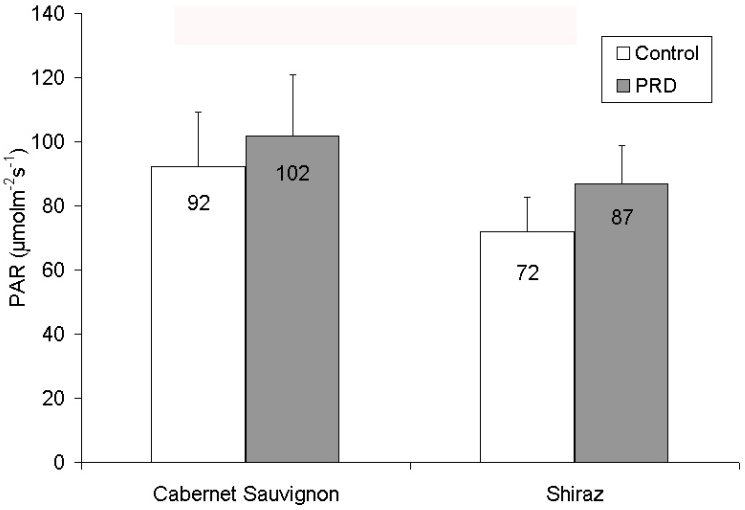


Figure 4 Light penetration into the bunch zone at harvest in Cabernet Sauvignon (2002) and Shiraz vines. (means n = 7; Vertical bars indicate standard errors of the average).

Table 1 PRD and pruning level effects on shoot growth components and leaf area at harvest in Nuriootpa Shiraz (2001) (Control: vines received water on both sides; PRD: water withheld on one side at any time and received half the amount of control irrigation. Pruning levels consisted of retaining 30, 60 or 120 nodes per vine; means n = 5; ± s.e.; n.s. = not significant (P<0.05))

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

Vegetative growth can also be quantified by measuring the pruning weight. Stoll (2000) found that PRD treatment significantly reduced the mean shoot weight and pruning weight in grapevines compared to control, as a consequence of a reduction in shoot growth. In this study, the measurement of the total accumulated seasonal dry matter by winter pruning weight of field-grown vines were compromised in field-grown vines by mechanical hedging during the growing period, removing more accumulated dry weight from control vines than PRD

vines. However, the mean shoot weight of PRD-treated vines was reduced compared to control vines (Table 2) when irrigated with half the volume of water as control. PRD Shiraz receiving the same volume of irrigation water as control had variable results. PRD treatment increased the water use efficiency of Cabernet Sauvignon on average by 89% over the three years due to the significant decrease in irrigation water use without significantly compromising yield.

It is very difficult to estimate the accumulation and partitioning of dry matter in field grown vines without excavating the whole root system, and therefore pot grown grapevines were preferably used. Experiments with pot grown grapevines have shown that both PRD and exogenous ABA application reduced the accumulation of dry matter in both leaves and one-year old shoots, the vegetative section of the grapevine, while slightly increasing dry matter of permanent wood compared to control vines (Table 3).

Table 2 Effect of PRD on grapevine shoot growth components and water use efficiency of Coombe Cabernet Sauvignon and Shiraz (Control: vines received water on both sides; PRD Cabernet Sauvignon received half the volume of control irrigation while Shiraz received the same volume of irrigation water as control; means n = 7; ± s.e.; n.s. = not significant (P<0.05))

Cabernet Sauvignon	**Control**	**PRD**	**%Diff.**	**Sig.**
Shoot weight (g)				
2001	37.79 ± 2.72	36.60 ± 3.39	- 3.1	n.s.
2002	56.84 ± 1.88	54.55 ± 1.51	- 4.0	n.s.
2003	32.49 ± 3.95.	30.81± 2.38	- 5.2	n.s.
Winter pruning weight (kg fresh wt)				
2001	2.12± 0.27	2.28 ± 0.26	+ 7.6	n.s.
2002	2.59± 0.21	2.97 ± 0.22	+ 14.4	<0.05
2003	2.03 ± 0.29	1.92 ± 0.22	- 5.8	n.s.
Irrigation (mm set to harvest)				
2000/1	107	53	- 50	
2001/2	114	57	- 50	
2002/3	90	45	- 50	
WUE (t/100mm)				
2001	8.13 ± 1.50	14.36 ± 2.12	+ 77	<0.01
2002	9.69 ± 0.96	17.82 ± 1.09	+ 84	<0.01
2003	11.4 ± 1.92	23.47 ± 3.41	+ 106	<0.01

Shiraz	**Control**	**PRD**	**%Diff**	**Sig.**
Shoot weight (g)				
2001	40.40 ± 2.72	35.95 ± 2.35	- 11	n.s.
2002	51.09 ± 1.70	52.58 ± 2.47	+ 2.9	n.s.
2003	38.37 ± 3.09	39.39 ± 2.60	+ 2.7	n.s.
Winter pruning weight (kg fresh wt)				
2001	2.38 ± 0.23	2.67 ± 0.16	+ 12.3	n.s.
2002	2.91± 0.14	3.25 ± 0.24	+ 11.4	n.s.
2003	2.25 ± 0.19	2.37 ± 0.21	+ 5.2	n.s.
Irrigation (mm set to harvest)				
2000/1	107	107	0	
2001/2	127	127	0	
2002/3	147	147	0	
WUE (t/100mm)				
2001	10.3 ± 0.61	12.9 ± 0.61	+ 25	<0.05
2002	21.5 ± 1.54	19.5 ± 1.12	- 9.3	n.s.
2003	12.0 ± 1.18	11.6 ± 0.99	- 2.8	n.s.

Table 3 Organ dry-weights for pot-grown Cabernet Sauvignon (2003). PRD received half the volume of irrigation water as control vines but only on one side at a time. ABA treated vines received the same amount of irrigation water as control vines on both sides. Means indicated with different letters are significantly different ($P<0.05$) and means without letters are not significant different ($P<0.05$).

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

Both treatments however had no effect on the dry weight of roots. According to the 'functional equilibrium' of Porter and Nagel (2000), the physiological reaction of plants to the reduction of shoot growth in response to unfavourable soil moisture conditions, is to partition nutrients to increase root volume in order to overcome the 'most limiting' factor, i.e. low soil moisture, by exploring deeper layers of the soil. Mingo *et al.* (2004) found such an increase in the root activity of tomato subjected to PRD, increasing the root biomass by 55%. In the current study however, the volume of soil used in the experiment may have restricted the growth and utilization of the excess nutrients in both PRD and exogenous ABA treated grapevines that may have led to greater root activity in the field environment. Stoll *et al.* (2000a) have shown that PRD-treated grapevines roots grew to greater depths in the field and pot environment while Dry *et al.* (2000a) and Kang *et al.* (2002) found that, in half-dried pot vines, there was a relative increase in root development in the moist soil layers in the 'wet' side as a whole and the deeper layers of the 'dry' side compared to control. This increase in root activity was also found in the current study on the biochemical level.

6.1.2 PRD effects on C and N assimilation at the biochemical level

i) Shoot growth and function

Growth and the accumulation of dry matter are inextricably linked to the regulation of transpirational water loss, as demonstrated in the current study and earlier work by Loveys *et al.(1998)* and Dry *et al.* (2000b). Significant reductions in both shoot growth and stomatal conductance in response to soil drying took place without any change in shoot water relations

(data not shown), suggesting the involvement of a non-hydraulic signal originating from the roots.

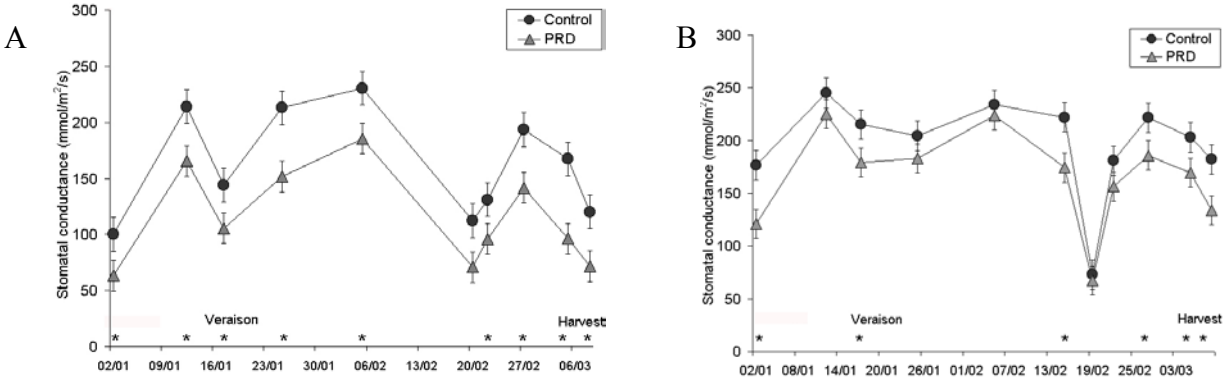


Figure 5 Stomatal conductance of Coombe A) Cabernet Sauvignon and B) Shiraz in 2001. PRD received half the volume and the same volume of water as control respectively. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

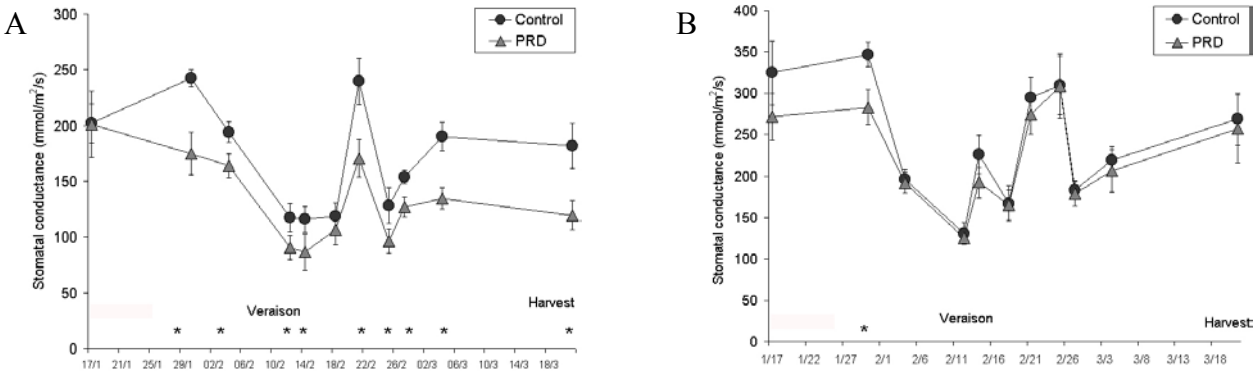


Figure 6 Stomatal conductance of Coombe A) Cabernet Sauvignon and B) Shiraz in 2002. PRD received half the volume and the same volume of water as control respectively. (Vertical bars indicate standard errors of the average. * = significantly different (P<0.05)).

Huber *et al.* (1992a) demonstrated that the enzymes of nitrate reduction (NR) and sucrose synthesis (sucrose phosphate synthase; SPS) share common features and are most likely coordinated with one another and the rate of photosynthesis. However, in this study the SPS activity in pot and field-grown experiments was not significantly affected on most days and, on occasion, the leaf SPS activity was actually significantly increased in response to PRD. (Figure 7)

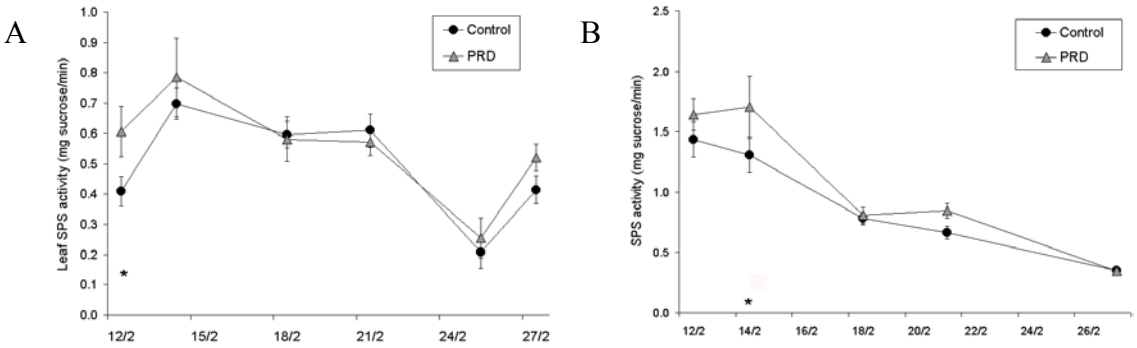


Figure 7 Leaf SPS activity of field-grown (A) Cabernet Sauvignon and (B) Shiraz in the Coombe vineyard over one PRD cycle (2002). (Cabernet Sauvignon PRD received half and Shiraz PRD the same amount of irrigation as control respectively on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different ($P < 0.05$)

Exogenous ABA application to the roots of pot-grown vines however significantly reduced stomatal conductance, but caused a 4-fold increase in leaf SPS activity compared to control vines (data not shown). Therefore, it may be possible that the transient increases found in SPS activity and associated decreases in leaf starch in the leaves of field-grown PRD-treated vines were caused by increased ABA and associated with improved photosynthetic capacity in response to soil drying conditions. A PRD-induced increase in leaf sucrose efflux may be caused by a shift in the sucrose:starch fraction by an increase in leaf SPS activity. Mild water stress in spinach (Quick *et al.*, 1989) has been shown to cause a marked change in sucrose:starch partitioning and the authors consequently proposed that the first site at which leaves respond to a rising water deficit is the activation of SPS. The partitioning of photosynthates responds very sensitively to water stress and there is a progressive stimulation of sucrose synthesis and inhibition of starch synthesis when the leaf fresh weight is decreased by 8% to 10%. Quick *et al.* (1989) also noticed that the shift occurred in a region where there was no marked change in the CO_2-saturated photosynthesis. Galtier *et al.* (1995) similarly showed that the over-expression of SPS activity in tomato leaves decreased leaf starch content, but resulted in a 2 to 3-fold increase in sucrose level.

PRD treatment in the current study had no effect on the sugar levels of grapevine leaves throughout the growing season from veraison until harvest compared to control (data not shown). The amount of sucrose in leaves is regulated by the sucrolytic enzyme acid invertase (AI) (Hammond *et al.*, 1984; Huber, 1989) and it is proposed that high vacuolar AI activity would decrease the amount of sucrose available for both export and storage, while low AI activity would favor sucrose accumulation. In the current study, the AI activity in leaves was unaffected by PRD treatment. Because AI is located exclusively in the vacuole it is concluded

that AI activity did not contribute to the turnover of sucrose in grapevine leaves in response to PRD treatment but rather played a role in maintaining glucose and fructose concentrations.

However, the neutral invertase (NI) activity in both the basal and apical leaves of field-grown PRD-treated vines was significantly reduced compared to control (Table 4). NI is located in the cytosol and probably involved in the channeling of sucrose into metabolism for growth (Winter and Huber, 2000). A reduction in leaf metabolism of sucrose in response to PRD treatment supports the observed reductions in vegetative growth in PRD-treated grapevines in the current study and by several authors in earlier work (Dry *et al.*, 1996; Dry and Loveys, 1999; Stoll, 2000).

Table 4 Sucrolytic enzyme activity in apical and basal leaves of field-grown Coombe Cabernet Sauvignon grapevines (2003). (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

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

However, whether the reduction of NI is the cause of the reduced growth rate in PRD-treated grapevine shoots or just a consequence is not known. The reduced metabolism of PRD-treated leaves may greatly reduce the overall diurnal demand for sucrose in the grapevine, consequently the amount of stored carbohydrates in leaves as starch was found to be less in response to PRD treatment (data not shown).

Using split-rooted plants, Gowing *et al*. (1990) demonstrated that many effects of water stress could be explained by the transport of chemical signals from the roots to shoots and that the excising of the drying rootzone would eliminate the source, thereby leading to the recovery of water stressed plants. Drying soils have been correlated with increases in root abscisic acid (ABA) and much evidence has been accumulated that drying roots under PRD conditions are the source of ABA, which is involved in reducing stomatal aperture and shoot growth (Loveys, 1984; Düring *et al.*, 1996; Dry *et al.*, 2000b). Stoll *et al.* (2000b) have found that the

cytokinin concentrations in roots, shoot tips and buds of PRD-treated vines significantly decreased compared to well-watered vines. This decrease in cytokinins may have contributed to the reduction in shoot growth associated with PRD treatments.

Leaves are the major factor in determining the bulk of the vine nitrogen content and most fast growing plants reduce nitrate in the leaves where the main reducing power is derived directly from photosynthesis (Huber *et al.*, 1992b; de Cires *et al.*, 1993). Foyer *et al.* (1998) found that water stress caused the inhibition of photosynthesis and was correlated with a marked decrease in total NR activity. In the current study, leaf stomatal conductance and NR activity were significantly reduced in response to PRD treatment in both field-grown Cabernet Sauvignon (Figure 8) and Shiraz (Figure 9) grapevines regardless of the amount of irrigation water applied.

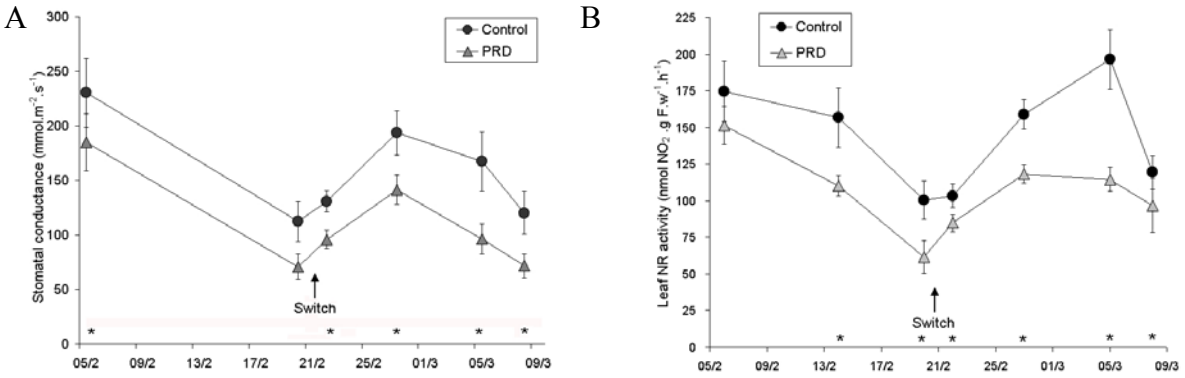


Figure 8 Effect of PRD treatment on **(A)** stomatal conductance and **(B)** NR activity in leaves of field-grown Cabernet Sauvignon over one PRD cycle in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different (P < 0.05))

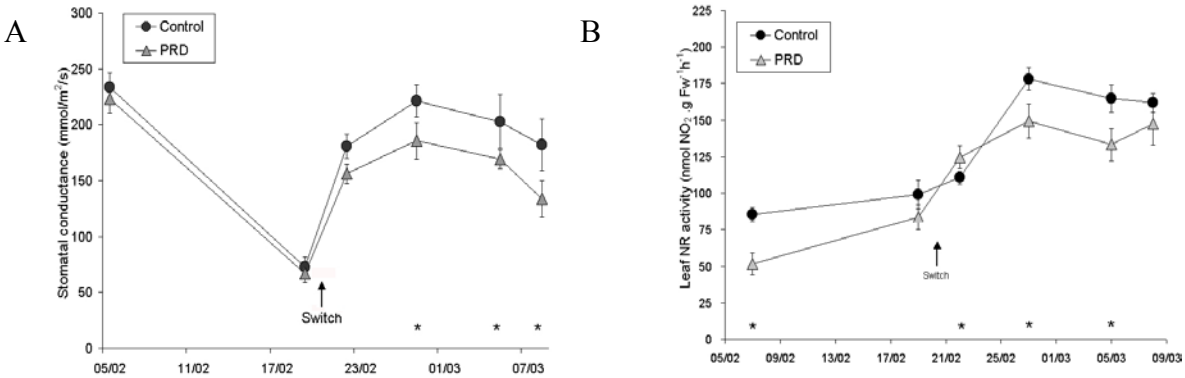


Figure 9 Effect of PRD treatment on **(A)** stomatal conductance and **(B)** NR activity in leaves of field-grown Shiraz over one PRD cycle in 2001. (PRD received the same amount of irrigation as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = Significantly different (P < 0.05)

A strong hyperbolic relationship was found between stomatal conductance and NR activity (data not shown), but PRD may have had independent effects on stomatal conductance and leaf NR activity, because transient increases in NR activity in field vines were not directly

associated with increases in stomatal conductance (data not shown). Exogenous ABA treatment provided further evidence that stomatal conductance and leaf NR activity are independently affected by PRD treatment since ABA action on its own reduced stomatal conductance without influencing leaf NR activity (Figure 10).

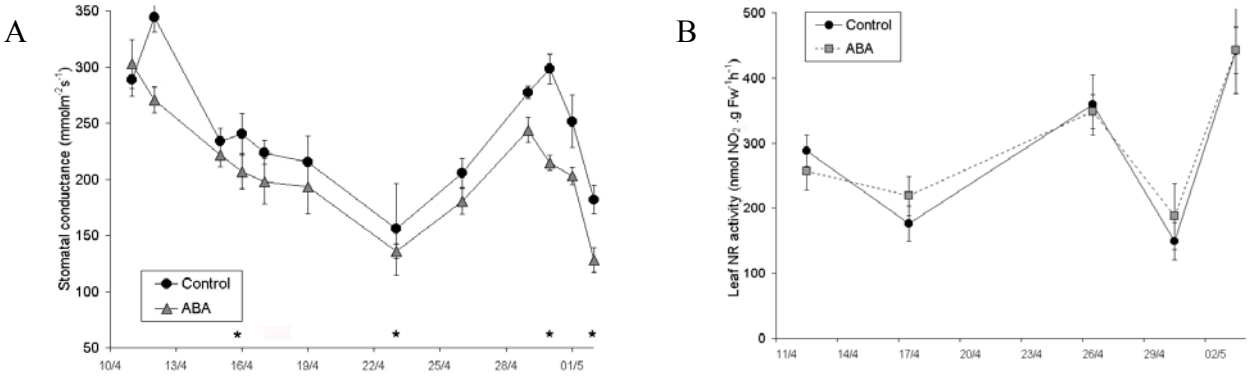


Figure 10 Effect of exogenous ABA on **(A)** stomatal conductance and **(B)** NR activity in leaves of split-rooted Cabernet Sauvignon in 2002 (Control: water on both sides; ABA: water on both sides with additional 10 µM ABA on one side). (means n = 5; Bars represent the standard error of the mean)

It is proposed that PRD effect on NR activity may be associated with a reduction of cytokinin production that may start to recover after extended periods of soil drying and cause transient increases in leaf NR activity. Alternatively, a significant decrease in PRD xylem sap NO_3^- concentration may be due to a significant decrease in the 'dry' side root nitrogen assimilation as measured by its NR activity (Figure 11). During periods of extended soil drying, there may be a change in root physiology to increase production of NO_3^- in roots that are well watered, thereby increasing leaf NR activity without affecting stomatal conductance. This could be an area of further research that could have consequences for recommendations on the N fertiliser needs of PRD-treated vines.

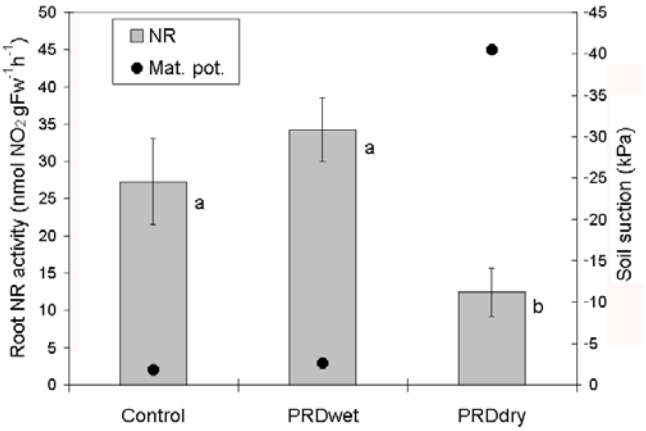


Figure 11 Soil matric potential and root NR activity after 8 days of PRD treatment of split-rooted 'double-pot' Cabernet Sauvignon grapevines. PRDwet = PRD pot currently under irrigation; PRDdry = PRD pot with water withheld. (Control: vines received water on both sides; PRD: water withheld from one side at any time. (means n = 5; vertical bars represent the standard error of the mean; Bars with different letters are significantly different ($P<0.05$).

The effect of PRD on xylem sap nitrate (NO_3^-) concentration in field-grown Cabernet Sauvignon and Shiraz in the Coombe vineyard during the 2002 growing season is shown in Figure 12. PRD is known to reduce sap flow and growth in grapevines irrespective of the amount of irrigation water applied (Stoll, 2000; de Souza *et al.*, 2003) and therefore an increase in xylem sap NO_3^- concentration may be expected in PRD vines due to lower N utilization, especially those that received the same amount of irrigation water as control. This was found to be the case in PRD Shiraz with a significant increase in xylem sap NO_3^- compared to control. However, PRD Cabernet Sauvignon that received half the amount of irrigation water as control had a significant decrease in xylem sap NO_3^- compared to control. The reduction in xylem sap NO_3^- may have a direct effect on the biochemical processes that regulate vegetative growth, but rate of delivery may complicate the interpretation of results. Unfortunately, both the changes in xylem sap NO_3^- and rate of delivery were not examined in depth in the current study, but may be an area of interesting further research.

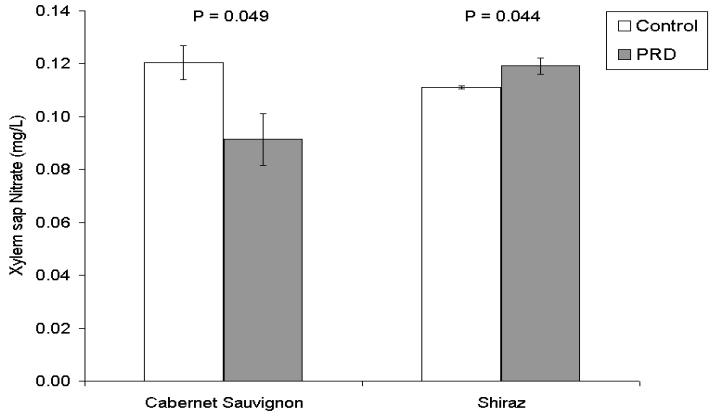


Figure 12 The PRD effect on xylem sap nitrate concentration in Coombe Cabernet Sauvignon and Shiraz grapevines during the 2001/2 growing season (15/03/2002). PRD Cabernet Sauvignon received half the amount of control irrigation and PRD Shiraz received the same amount of irrigation as control. (means n = 7 ± s.e.)

It is therefore hypothesized that the inhibition of NR in PRD vines may be due to: 1) because half of the root system is faced with a diminishing soil water content, the nitrogen absorption of the roots may be decreased, thereby reducing NR activity because of its ability to be substrate inducible (Gojon *et al.*, 1991). In terms of root to shoot communication, nitrate itself is the primary signal molecule triggering the activation of transcription of nitrate assimilation and related genes (Takei *et al.*, 2002). 2) Furthermore, nitrogen availability could commonly modulate cytokinin metabolism and translocation in higher plants (Takei *et al.*, 2002). It is possible that NR activity may be directly influenced by the change in the ABA/cytokinin balance in PRD vines. The major phytohormone that influences NR is cytokinin (for review see Gaudinova (1990)). NR activity is greatly increased in leaves in response to the treatment

with the cytokinin benzyladenine (BA) (Kende *et al.*, 1971; Yu *et al.*, 1998) and suppressed by ABA (Lu *et al.*, 1992).

ii) Root growth and function

In the current study, PRD treatment significantly increased root osmolality in both the PRD 'wet'- and 'dry'-side roots compared to control roots (Figure 13).

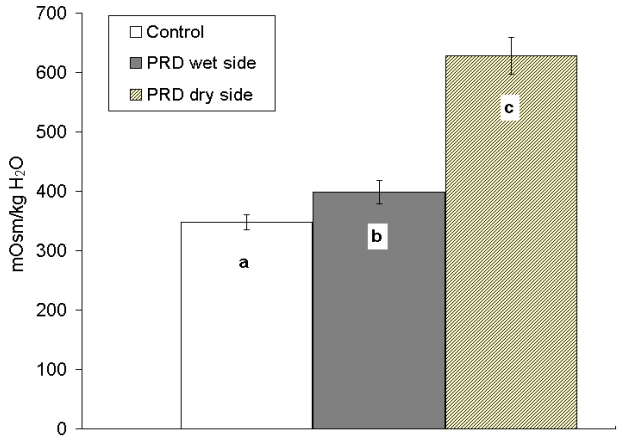


Figure 13 Root sap osmolality of split-rooted 'double-pot' Cabernet Sauvignon grapevines (8/04/2002). Eight days after the switch of wetting pots in PRD. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 ± s.e).

The increased osmolality was associated with significant increases in sugars, nitrogen-containing compounds and amino acids that indicated an active osmoregulation of roots in response to lowered soil moisture conditions (Table 5). PRD 'dry' side roots also significantly accumulated higher amounts of sugars and osmolytes than its own 'wet' side, indicating a concentration gradient for water towards the 'dry' roots. Stoll *et al.* (2000b) have shown that water moves from the 'wet' side roots towards the 'dry' side in PRD-treated grapevines to maintain the hormonal signal under low soil moisture conditions. It was also found that very little water is absorbed by 'dry' side roots at low water potentials and that the dry roots are mostly reliant on the water derived from the wet side. It is therefore proposed that the increase in root osmolality by the accumulation of solutes in the 'dry' side roots of PRD-treated plants serves as a water potential gradient that facilitates the water movement from wet to dry roots.

Table 5 Root sugars and osmolytes (μMol/g fresh wt) for split-rooted pot-grown Cabernet Sauvignon (8/04/2002). PRD received water in only one pot while water was withheld from the other at any given time. Control received water in both pots. 'PRD average' is the mean between PRD 'dry' side and PRD 'wet' side roots. (means n = 10 ± s.e.; means indicated with different letters are significantly different (P<0.05)).

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

Solute accumulation and the increases in root osmolality may be related to observed increases in sucrolytic enzyme activities of PRD-treated roots. In the current study, responses in root SucSy activity indicated that both the PRD 'wet' and 'dry' side roots increased their sink strength and/or growth rate compared to control roots within 2 days of treatment; PRD 'wet' side roots substantially more so than the 'dry' side (Figure 14). This may be an important finding that supports the reports of increased root growth in response to PRD by a number of authors (Dry *et al.*, 2000a; Kang *et al.*, 2002; Mingo *et al.*, 2004).

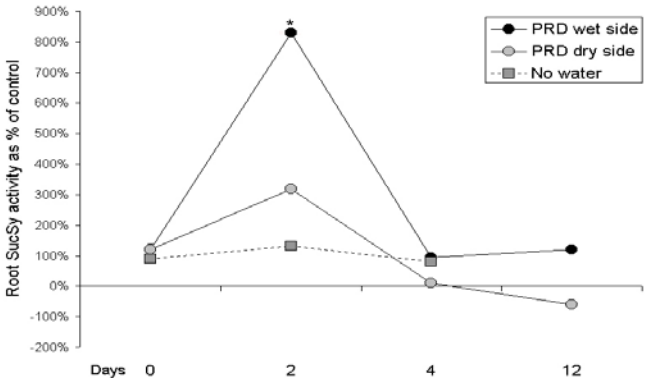


Figure 14 Root SucSy activity of non-irrigated, control, PRD 'wet' and 'dry'-side roots in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.; * = significantly different (P<0.05)). Non-irrigated vines received no water for the duration of the experiment; means n = 2)

PRD 'wet' side roots may increase growth rate to compensate for the 'dry' side that showed reduced rates of growth compared to control, thereby causing a more exploratory root system. Enhanced rooting, especially adventitious root formation, is associated with the accumulation of polyamines, especially spermine and spermidine (Jarvis *et al.*, 1985; Shyr and Kao, 1985; Kakkar and Rai, 1987; Faust and Wang, 1992). In the current study, PRD roots accumulated significantly higher amounts of spermine and spermidine compared to control roots (Figure 15).

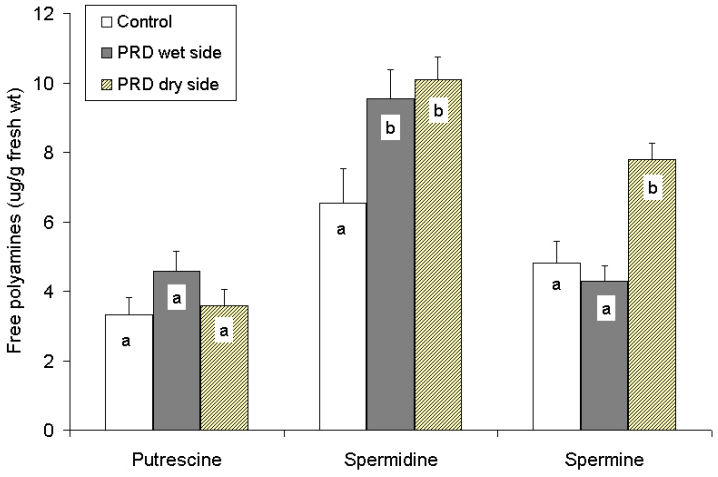


Figure 15 Free polyamine contents of control, PRD 'wet' and 'dry'-side roots of split-rooted 'double-pot' Cabernet Sauvignon grapevines (8/04/2002). (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 10 + s.e.; bars with different letters are significantly different ($P<0.05$)).

Spermine and spermidine are synthesized from putrescine by the addition of one or two aminopropyl groups respectively from S-adenosylmethionine, an intermediate of ethylene biosynthesis (Faust and Wang, 1992). It could therefore be speculated by its association with polyamine production, that ethylene biosynthesis in PRD roots may be involved in the conditioning of roots to increase density in order absorb more water per volume of soil. This hypothesis supports the findings of Dry *et al*. (2001) who reported a significantly higher abundance of roots of the 1 mm to 3 mm diameter at the 0.4 m to 0.7 m depth in PRD roots of field-grown grapevines compared to control vines. The implication is that a more exploratory root system of PRD-treated vines may contribute to greater water stress tolerance and a better soil environment for root growth. Stoll *et al.* (2000a) found evidence of a more exploratory root system in field-grown PRD-treated vines with roots that grew to greater depths than control.

The soluble invertases are proposed to have limited involvement in root growth, but serve a function in osmoregulation, cell enlargement and channelling sucrose into metabolism (Sturm and Tang, 1999). In the current study, PRD 'dry' side roots showed significant increases in

both AI and NI activity compared to control that may be involved in osmoregulation by regulating sugar accumulation or an increased sugar flow to respiration in order to tolerate the stressful conditions (Figure 16). Alternatively the increased invertase activity in the PRD 'wet' side roots may be associated with increased growth rates and hence the required increase in phloem unloading compared to control roots.

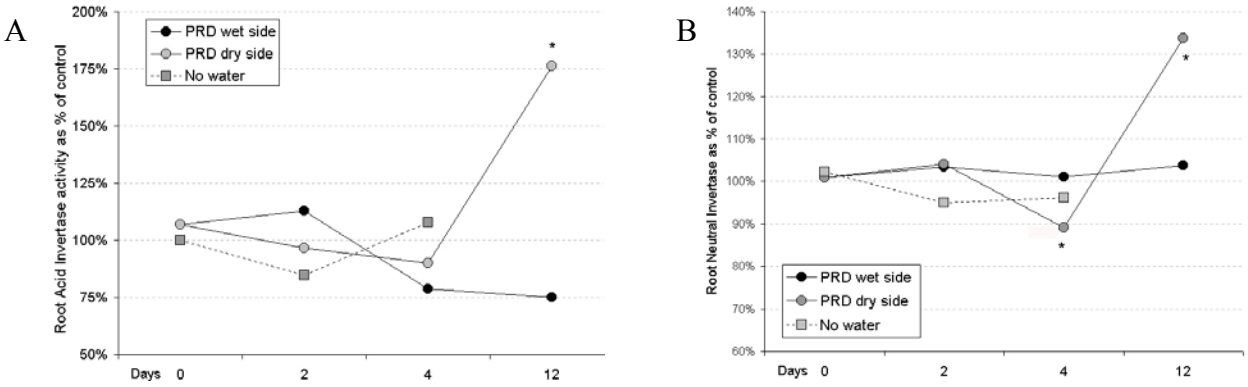


Figure 16 Root A) AI and B) NI activity (as % of control) of PRD 'wet' and 'dry'-side and non-irrigated roots in split-rooted 'double-pot' Cabernet Sauvignon grapevines under glasshouse conditions in 2003. (Control: vines received water on both sides; PRD: water withheld from one side at any time; means n = 5 ± s.e.; * = significantly different ($P<0.05$)). Non-irrigated vines received no water for the duration of the experiment; means n = 2)

iii) Berry growth and function

Hunter and Ruffner (1997) showed that the effect of canopy management that caused an increased light penetration into the bunch zone had only a minor effect on berry NR activity compared to control. Berry NR activity was expected to be higher in response to the canopy manipulation. They concluded that the increase in leaf amino acid formation in response to treatment increased amino acid transport from leaves to berries and may have reduced the induction of nitrate reductase. In this study, PRD marginally decreased berry NR activity when PRD vines received half the amount of irrigation water as control (Table 6). This supports the observed reduction of PRD berry sizes measured in the 2000/1and 2001/2 seasons compared to control (Figure 17). However, the reduction in berry growth was not observed in Shiraz that received the same amount of irrigation water as control (data not shown) and therefore it is concluded that the reduction in berry growth and berry NR activity may have been related to the reduction in irrigation water. However, the NR activity in berries may only have a small contribution to berry amino acid content because the major source of amino acids is the leaves.

Table 6 NR activity measured in berries of field-grown Cabernet Sauvignon grapevines in the Coombe vineyard (7/02/2001) (PRD received half the amount of control irrigation; means n = 7 ± s.e.; n.s. = Not Significant ($P<0.05$); NR activity measured as nmol $NO_2.gFw^{-1}.h^{-1}$)

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

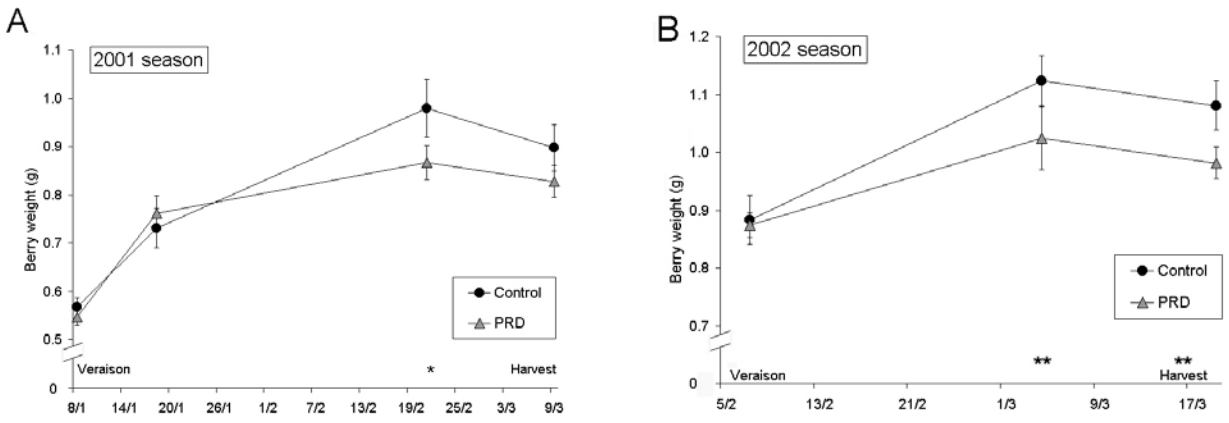


Figure 17 Effect of PRD on the increase in berry weight (g fresh wt) of field-grown Coombe Cabernet Sauvignon vines during the **A)** 2000/1 and **B)** 2001/2 season from veraison to harvest (PRD received half the amount of irrigation water as control on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significant ($P<0.05$); ** = significant ($P<0.01$)).

PRD treatment of Cabernet Sauvignon vines reduced the berry acid invertase (AI) and sucrose synthase (SucSy) activities compared to control when irrigated with half the volume of irrigation water compared to control (Table 7). This may have been an indication of reduced sink strength in response to a reduction in berry growth. At the same time, the neutral invertase (NI) activity in PRD-treated berries was greatly increased compared to control irrespective of the volume of irrigation water applied. The function of NI activity is still unclear, but reported to be localized in the cytosol, highly substrate specific for sucrose (Sturm *et al.*, 1999) and proposed to channel sucrose into metabolism (Sturm and Tang, 1999; Winter and Huber, 2000). Furthermore, in conditions where AI and SucSy activities are low, the NI is considered to be a 'maintenance' enzyme that is involved in sucrose degradation. It is therefore hypothesized that the increase in NI activity in response to PRD-treatment serves as a way to increase the metabolic function of berries in response to the ABA signal induced in response to PRD (Stoll *et al.*, 2000b). Whether or not this increase in NI activity in response to PRD has any effect on berry metabolism and the production of amino acids, phenolics, anthocyanins, etc. is still unknown.

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

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

6.1.3 PRD effects on the partitioning of carbon and nitrogen

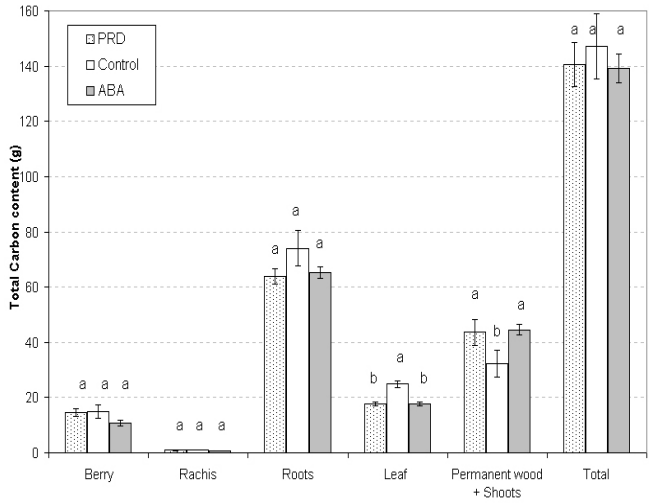
Clarkson and Hanson (1980) argued that the quantity of nutrient absorbed by a plant over a given period of time is an integration of the growth that has occurred and that the rate of absorption from a given concentration is determined by the "demand" for nutrients created by growth. In the current study, In the current study, PRD treatment of pot-grown grapevines had no significant effect on the total vine accumulation of C or N compared to control. However, the partitioning of N and C was significantly affected by increasing the accumulation in permanent wood and shoots at the expense of the leaf canopy (Figure 18 and 19).

The investigation into C and N nutrition in field grown grapevines in the current study provided inconclusive results. No significant effect of PRD treatment on the accumulation of nutrients or in the C/N ratio of all the different plant tissues measured compared to control could be found (data not shown). It is proposed that, unlike the pot environment where roots have constraints in terms of volume and moisture, the excess nutrients partitioned to permanent structures in field-grown grapevines would be used to enlarge its root structure to exploit the deeper layers of soil.

Labelled nitrogen (^{15}N) measurements are often used to elucidate the absorption and partitioning of newly absorbed N during a certain period of time (Conradie, 1986; Glad *et al.*, 1994). The grapevine's demand for seasonal nitrogen is fairly well known, but by using conventional methods it is not possible to distinguish between nitrogen absorbed during different periods within one growing season. Apart from the permanent structure, leaves and

shoots are important transitional reservoirs for absorbed nitrogen and up to harvest nitrogen is turned over at a relatively fast rate in these organs with large contributions to fruit, even though the total nitrogen content does not change (Conradie, 1991). In the current study, PRD-treated vines absorbed significantly less ^{15}N than exogenous ABA-treated and control vines during the ripening season that extended from veraison until harvest (Table 8). This was manifested in lower ^{15}N abundances in berries, leaf canopy, one-year old shoots and thin roots. However, the total amount of ^{15}N partitioned to the fruit was the same. As for the rest of the plant, similar to the accumulation of starch and inorganic minerals, PRD and exogenous ABA increased the partitioning of ^{15}N to the permanent structure, specifically to the older wood at the expense of the vegetative structure.

A



B

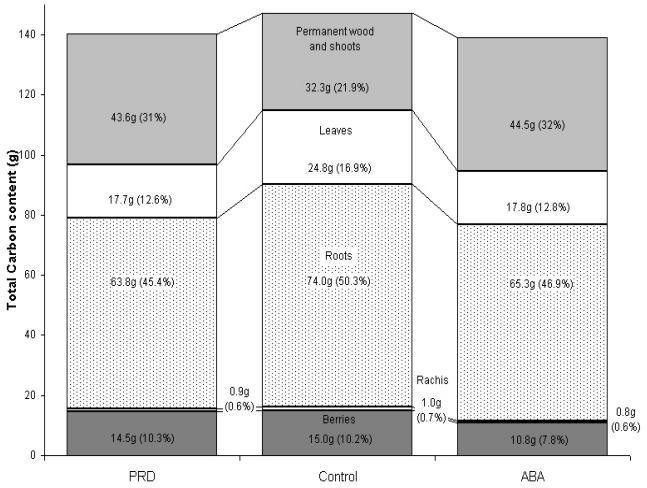
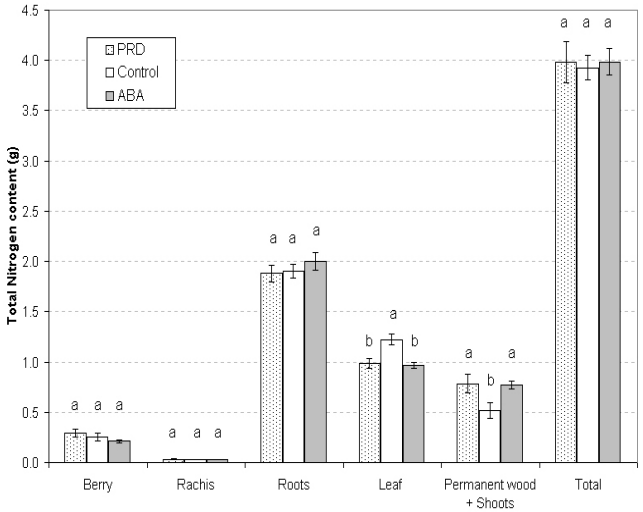


Figure 18 A+B) Effect of PRD and exogenous ABA on accumulated organ carbon content (g) of split-rooted Cabernet Sauvignon vines (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 µM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different (P<0.05). Percentages represent the relative distribution of total carbon between organs

A



B

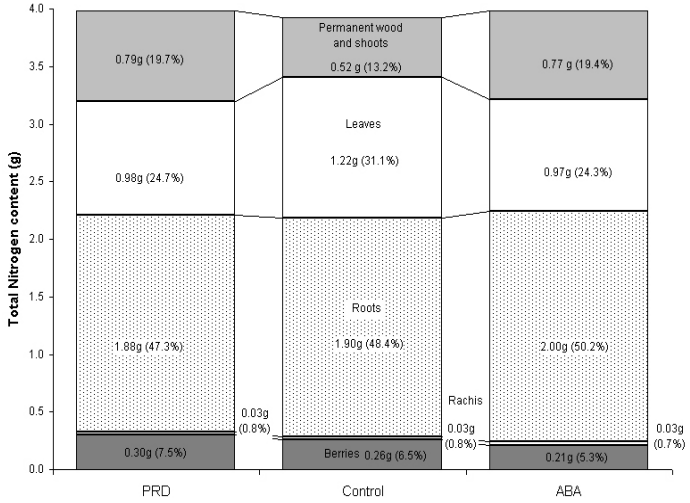
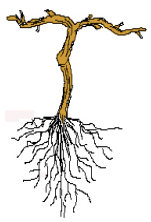
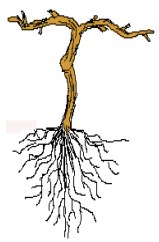
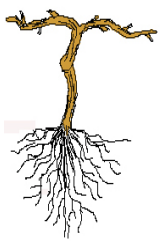


Figure 19 A+B) Effect of PRD and exogenous ABA on accumulated organ nitrogen content (g) of split-rooted Cabernet Sauvignon vines (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 µM ABA on one side). Bars represent means of 5 replicates; ± s.e. Bars with different letters are significantly different (P<0.05). Percentages represent the relative distribution of total nitrogen between organs.

Table 8 Total ^{15}N (mg/organ) of split-rooted Cabernet Sauvignon (Control: water on both sides; PRD water withheld from one side; ABA: water on both sides with additional 10 μM ABA on one side).

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

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

It is therefore clear that PRD-treated vines absorb less nitrogen during the ripening period than control and therefore fertilization needs may be lower than control vines. The effect of PRD treatment on the partitioning of C and N between organs however shows the same trend and is supported by the differences found in enzyme activities involved in the assimilation and partitioning processes at the biochemical level. Vegetative organs of PRD-treated vines that showed significant reductions in growth and accumulation in C and N were associated with significant inhibitions of NR activity and sucrolytic enzyme activity, while organs such as roots and fruit that had favoured partitioning of nutrients had increased activities of selected enzymes compared to control vines.

Soluble sugars (sucrose) and amino acids are formed in photosynthetically-active leaves and transported to organs in order of their sink strength. A change in the organ sink strength or production in source leaves may have an effect on the partitioning of C and N in the vine as a whole. In general, soluble sugar content tends to be maintained in the leaves of droughted plants, in spite of lower rates of carbon assimilation (Du *et al.*, 1998; Chaves *et al.*, 2003). This is achieved at the expense of starch, which drastically declines (Chaves, 1991). This response favours osmoregulation and enhances desiccation tolerance. Similarly, PRD treatment in this study had no effect on the evolution of sugars (sucrose, glucose and fructose), sugar-alcohols (pinitol and mannitol) or nitrogen-containing compounds (glycine betaine, methyl proline and proline) in leaves of field-grown vines during the season compared to control, irrespective of the amount of irrigation water applied (data not shown). The most abundant amino acids were arginine in leaves and proline and arginine in shoots. This indicated that there was no detrimental effect of PRD on the production and accumulation of amino acids in grapevine shoots compared to control even though PRD-treated vines received half the amount of irrigation water as control vines.

6.1.4 PRD effects on berry characteristics and the accumulation of inorganic ions

The ultimate goal of irrigation and canopy management is to benefit fruit quality in a sustainable way without disturbing growth and development in other sinks. In the current study, the volume of irrigation water applied affected the characteristics and composition of berries more than the PRD treatment did.

Irrigating PRD-treated grapevines with half the volume of irrigation as control advanced maturity but significantly reduced berry growth and maturation later in the ripening period (See section 6.1.2). Other berry characteristics at harvest, such as juice pH and total soluble solids were unaffected by PRD treatment. The PRD effect on berry size also influenced the accumulation of hexose during the ripening period. Although PRD berries showed significantly higher hexose concentrations during most of the ripening period (data not shown), the hexose content per berry at harvest was significantly reduced when irrigated with a reduced volume of irrigation water compared to control (Table 9).

A

B

C

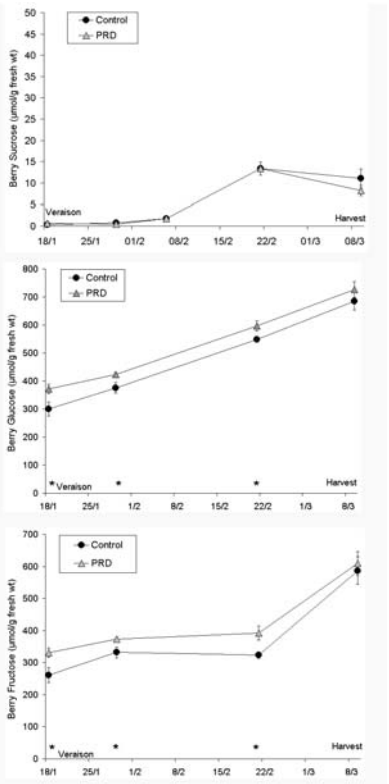


Figure 20 The evolution in berry **(A)** sucrose, **(B)** glucose and **(C)** fructose concentration (μMol/g fresh wt) of field-grown Coombe Cabernet Sauvignon vines during the 2000/1 season from veraison to harvest. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.; * = significantly different ($P<0.05$))

Table 9 Sugars and NCCs (μMol per berry) in berries of field-grown Coombe Cabernet Sauvignon grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7 ± s.e.)

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

According to Ollat and Gaudillere (1996) the sink strength of the berry may be estimated by carbon import and accordingly it may be deduced that in some cases berry sink strength was reduced in PRD berries compared to control. However, when PRD treatment did not affect berry size no significant effects on hexose accumulation and sink strenght could be found compared to control. In fact, the results of PRD Nuriootpa Shiraz indicated that hexose accumulation in the berries can be increased by PRD in situations where the appropriate cultural practices is used to balance vegetative growth with crop level (Table 10). Average pruning levels showed the potential to increase the accumulation of hexose under PRD conditions while higher than normal pruning levels tended to increase the effect of PRD treatment on berry size. Consequently, the amount of hexose accumulated per berry in PRD-treated vines was significantly less compared to control. It is therefore concluded that environmental factors and cultural practices may have a strong influence over the PRD effect on berry size and sink strength.

Table 10 Sugars and NCCs (μMol/g fresh wt) in berries of field-grown Nuriootpa Shiraz grapevines at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 5 ± s.e.)

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

Amino acids, the first products of ammonium assimilation, are the building blocks of proteins in grapevines and play a major role in the fermentation of musts (Roubelakis-Angelakis and Kliewer, 1992). Both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine (Stines *et al.*, 2000). Stines *et al.* (2000) concluded that the basic pattern of amino acid composition in mature fruit is determined by genetic factors and that environmental and cultural factors have only a modifying effect. In the current study, the volume of irrigation water had a significant effect on the accumulation of amino acids in berries (Table 11).

Table 11 The evolution in the concentration of free amino acids (nMol/g fresh wt) in Coombe Cabernet Sauvignon berries during the ripening period in 2001 (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n = 7; [a] = significantly different (P<0.10); * = significantly different (P<0.05)).

Days after veraison	0		12		21		36	
	Control	PRD	Control	PRD	Control	PRD	Control	PRD
Proline	18801	17448	19162	19212	21236	28559 [a]	22120	24849 *
Arginine	1227	1254	1552	1085 [a]	7837	8373	9020	9378
Histidine	1439	1664	1602	1578	2744	2880	2430	2396
Tyrosine	984	1194	1271	951	2092	1856	1834	1863
Methionine	223	301	378	118	869	759	1189	780 *
Glutamic acid	474	509	285	346	759	689	268	218
Valine	297	520	191	73	1347	1381	747	1026
Asparagine	373	379	231	270	227	196	651	556
Threonine	282	335	385	329	426	447	460	435
Leucine	302	267	331	362	383	367	423	467
Aspartic Acid	299	300	100	118	338	298	47	29
Alanine	324	347	365	266 *	219	224	73	76 *
Glycine	50	61	29	0	185	195	194	197
Hydroxy-L-Pro	52	59	56	58	153	126	73	76 *
Glutamine	1458	1294	1445	1424	230	313	147	149
Isoleucine	36	52	52	49	109	105	132	137
Cystine	1213	503	795	367	378	231	74	141
Norleucine	0	6	0	12	0	16	49	27
Serine	5236	3990	6267	6569	53	70	46	43
Tryptophan	5	19	49	0	44	19	46	52
Phenylalanine	3	9	27	7	22	24	36	30
Lysine	14	11	41	13	19	29	23	16
Total	33973	31516	36461	36280	38455	39026	40996	44256 *

PRD-treated vines that received half the volume of irrigation water accumulated proline in berries to significantly higher concentrations compared to control in the later stages of ripening. Together with slightly higher arginine concentrations the PRD-treated berries had a significantly higher total amino acid concentration at harvest compared to control. In comparison, PRD-treated Shiraz vines that received the same amount of irrigation water as control showed no significant differences in the total amino acid concentration in berries, although there were some significant decreases in minor amino acids compared to control (data not shown). In conclusion, the significant increase in berry amino acid concentration in response to PRD treatment may be

solely due to the reduced volume of water applied compared to control and the reducing effect on berry size that probably caused increased concentration of amino acids.

Sugar is the major solute that accumulates in ripening berries, but minerals such as potassium (K), calcium (Ca), sodium (Na), copper (Cu), magnesium (Mg), manganese (Mn) and phosphate (P) can contribute significantly as osmotic components. K is by far the major cation in ripe berries (Mpelasoka *et al.*, 2003) and plays the most important role in osmoregulation and plant water relations compared to other inorganic ions, especially under conditions of low sugar accumulation. Excessive K in berries can cause problems in the vinification process by decreasing free acids by precipitation of tartrate and increasing overall pH that may cause problems in colour stability during storage (Morris *et al.*, 1983). Increased shoot growth and dense canopies caused by high soil moisture conditions would generally increase bunch shading. Jackson *et al.* (1993) found that fruit from internally shaded canopies produced wines with high pH and K that exhibited poorer characteristics. Reduced shoot growth and better light penetration in response to PRD treatment may have positive effects on berry pH and K content. In the current study, PRD-treatment did not significantly affect the berry K concentration relative to control, irrespective of the volume of water applied (Figure 21).

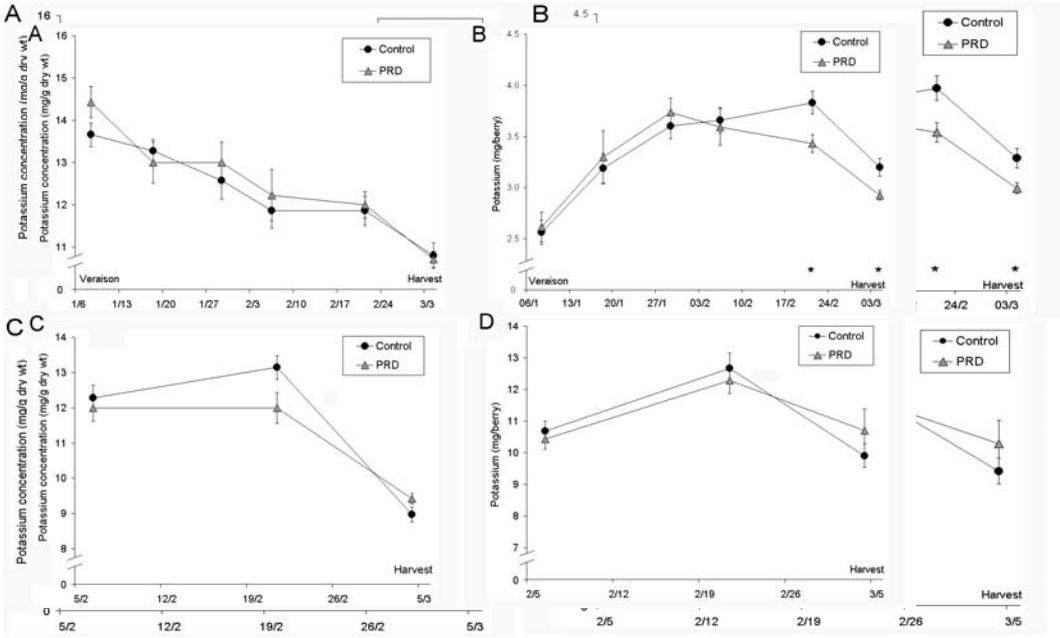


Figure 21 The changes in K ion concentration (mg/g dry wt) and ion content (mg/berry) respectively in **(A+B)** Coombe Cabernet Sauvignon and **(C+D)** Shiraz berries during the ripening period in 2000/1 between veraison and harvest. (PRD Cabernet Sauvignon received half the volume and Shiraz the same volume of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=7 ± s.e.; * = significantly different ($P<0.05$)).

However, in situations where PRD treatment reduced berry size, the K content per berry was reduced by a similar degree. Therefore, the main effect of PRD treatment on berry K was due to the effect on berry size in response to the amount of water applied. Furthermore, PRD-treatment of Nuriootpa Shiraz vines pruned to higher pruning levels had significantly lower ion contents per berry, especially K, Mg and S, than control vines (Table 12). PRD treatment may have a greater effect on berry pH and ion accumulation when vines are grown with dense canopies. The PRD effect on the minor inorganic ions that included Ca, P, S and Mg was irregular over the 3 year study and mostly insignificant compared to control (data not shown).

Table 12 The Ca, K, Mg, P and S content (μg/berry) of Nuriootpa Shiraz berries at harvest in 2001. (PRD received half the amount of irrigation water as control by irrigating on only one side at any time; control received water on both sides; means n=5 ± s.e.)

Ca				
Nodes/vine	Control	PRD	% Diff	Sig.
30	364 ± 22	391 ± 22	+ 7	n.s.
60	365 ± 25	402 ± 28	+ 10	n.s.
120	390 ± 20	363 ± 17	- 7	n.s.
K				
Nodes/vine	Control	PRD	% Diff	Sig.
30	3329 ± 182	3479 ± 449	- 4	n.s.
60	3342 ± 283	3175 ± 235	- 5	n.s.
120	3073 ± 278	2389 ± 107	- 22	<0.05
Mg				
Nodes/vine	Control	PRD	% Diff	Sig.
30	151 ± 4	153 ± 9	+ 1	n.s.
60	156 ± 10	156 ± 6	0	n.s.
120	157 ± 7	132 ± 6	- 16	<0.05
P				
Nodes/vine	Control	PRD	% Diff	Sig.
30	360 ± 20	386 ± 36	+ 7	n.s.
60	339 ± 20	358 ± 17	+ 6	n.s.
120	319 ± 25	284 ± 7	-11	n.s.
S				
Nodes/vine	Control	PRD	% Diff	Sig.
30	195 ± 7	203 ± 19	+ 4	n.s.
60	202 ± 14	196 ± 13	- 3	n.s.
120	194 ± 14	160 ± 4	- 18	<0.05

7 Results and discussion of the RSG research

RSG has been reported to substantially reduce growth and yield in several inland Australian vineyards (Mattschoss, 1995; Wilson, 1995; Wilson and Hayes, 1996) that may have serious financial implications to producers at harvest and during vineyard management. Severe cases of RSG may result in total loss of yield and in some cases the retraining and replacement of young vines. The investigation into the RSG disorder by the National Task Force (Wilson, 1995) supported the theory that the restricted growth is not the result of a single or specific cause, but rather a combination of factors such as climate, environmental, cultural and pathological disruptions (Wilson, 1995; Wilson and Hayes, 1996; Freeman, 1999) resulting in 'soft wood' and insufficient starch reserves before the onset of winter. According to Mattschoss (1995), cultural practices that have a diminishing effect on starch and nitrogen resources may have a compounding effect when combined with adverse environmental conditions, leaving the vine vulnerable to winter chilling damage.

Results of experiments in the current study have provided evidence that some cultural practices and adverse environmental conditions may have a negative impact on vine nutrition and growth. Defoliation plus fertilizing in combination with low soil moisture conditions during the winter significantly reduced spring growth in field-grown vines (Table 13) that was associated with a significant reduction in amino acids (Table 14), but increased sugars in bleeding sap compared to control (Table 15). However, although vine nutrition and growth were adversely affected, no characteristic RSG symptoms could be reproduced in any field experiments.

Table 13 Shoot length of Alverstoke Cabernet Sauvignon grapevines on 24/11/03. DN = vines was minimally pruned and received fertilizer, additional water and 80% defoliation before winter; DND= vines received the same treatments as DN vines + soil was kept dry by plastic covering. DNDR= vines received the same treatments as DND + roots were pruned. Control received normal irrigation and was spur pruned. (means n = 5 ± s.e.; means with different letters are significantly different (P<0.05))

	C	DN	DND	DNDR	P
Shoot length (cm)	$104.0^{a} \pm 5.9$	$104.1^{a} \pm 3.8$	$88.0^{a} \pm 4.0$	$62.7^{b} \pm 7.2$	0.0001

Table 14 Amino acid concentration (nMol/ml) in the bleeding sap of Alverstoke Cabernet Sauvignon in the spring of 2002. DN = vines were 'minimally' pruned and received fertilizer, additional water and 80% defoliation before winter; DND= vines received the same treatments as DN soil was kept dry by plastic covering. Control received normal irrigation and was spur pruned (means n = 5 ± s.e.; means with different letters are significantly different (P<0.05)).

	Control	DN	DND	P
Glycine	3753.8^{a} ± 376.1	2780.3^{ab} ± 286.0	2350.8^{b} ± 380.2	**0.026**
Proline	195.5^{a} ± 23.5	123.0^{b} ± 6.4	126.5^{b} ± 21.5	**0.017**
Phenylalanine	191.9^{a} ± 45.3	87.2^{b} ± 21.2	62.3^{b} ± 16.9	**0.013**
Valine	157.0^{a} ± 35.9	91.7^{ab} ± 24.9	68.7^{b} ± 21.3	0.088
Histidine	128.5^{a} ± 28.4	69.1^{ab} ± 14.6	52.6^{b} ± 15.8	**0.036**
Tyrosine	122.6^{a} ± 25.6	53.1^{b} ± 13.2	35.9^{b} ± 6.5	**0.003**
Asparagine	106.9^{a} ± 26.7	41.4^{b} ± 5.7	36.5^{b} ± 11.4	**0.012**
Glutamine	104.5 ± 14.3	73.3 ± 7.6	79.3 ± 15.3	0.210
Isoleucine	92.2 ± 23.6	59.5 ± 15.6	42.3 ± 10.9	0.143
Arginine	51.6 ± 10.9	70.4 ± 17.9	88.0 ± 17.5	0.280
Threonine	45.6^{a} ± 11.8	24.1^{ab} ± 4.3	21.2^{b} ± 3.6	0.059
Aspartic acid	40.3 ± 4.4	32.7 ± 3.1	35.3 ± 4.2	0.390
Norleucine	39.7 ± 2.5	43.3 ± 1.9	42.5 ± 6.7	0.830
Methionine	38.4^{a} ± 11.7	14.8^{b} ± 5.4	5.4^{b} ± 0.6	**0.012**
Leucine	29.1^{a} ± 5.5	14.3^{b} ± 3.0	11.8^{b} ± 2.8	**0.009**
Alanine	28.1 ± 7.2	24.7 ± 5.5	21.5 ± 6.9	0.781
Cystine	23.1 ± 5.3	16.1 ± 2.3	17.6 ± 3.7	0.435
Serine	14.2 ± 1.9	14.1 ± 1.0	16.2 ± 2.2	0.636
Tryptophan	10.0^{a} ± 2.0	4.8^{b} ± 0.3	5.1^{b} ± 0.7	**0.010**
Glutamic acid	2.7^{b} ± 0.5	2.6^{b} ± 0.9	6.5^{a} ± 1.3	**0.011**
Hydroxy-L-pro	2.5 ± 0.5	2.2 ± 0.4	2.1 ± 0.5	0.823
Lysine	2.0 ± 0.8	1.5 ± 0.5	2.1 ± 0.9	0.819
Total	5218.7^{a} ± 625.9	3684.5^{b} ± 384.3	3200.7^{b} ± 528.7	**0.029**

Table 15 Sugar and osmolyte concentration (μMol/ml) in the bleeding sap of Alverstoke Cabernet Sauvignon in the spring of 2003. DN = vines were minimally pruned and received fertilizer, additional water and 80% defoliation before winter; DND= vines received the same treatments as DN soil was kept dry by plastic covering. DNDR= vines received the same treatments as DND + roots were pruned. Control received normal irrigation and was spur pruned (means n = 5 ± s.e.; means with different letters are significantly different (P<0.05); n.d. = not detected).

	Control	DN	DND	DNDR	P
Sucrose	0.25^{b} ± 0.16	0.48^{b} ± 0.20	1.44^{a} ± 0.38	1.17^{a} ± 0.10	**0.010**
Glucose	0.26^{bc} ± 0.15	0.06^{c} ± 0.03	0.54^{ab} ± 0.11	0.87^{a} ± 0.11	**0.001**
Mannose	n.d.	0.73^{a} ± 0.47	n.d.	1.81^{a} ± 0.74	0.097
Fructose	n.d.	0.01^{b} ± 0.01	0.20^{a} ± 0.06	0.25^{a} ± 0.08	**0.009**
Mannitol	37.14^{c} ± 4.21	42.81^{bc} ± 7.58	61.30^{ab} ± 4.74	68.96^{a} ± 7.91	**0.008**
Hydroxy Me Pro	0.26 ± 0.06	0.18 ± 0.04	0.31 ± 0.11	0.43 ± 0.09	0.287
Glycine betaine	8.36 ± 0.62	8.02 ± 0.67	6.90 ± 0.86	7.04 ± 2.5	0.896
Methyl Proline	0.76 ± 0.18	0.42 ± 0.06	0.63 ± 0.13	0.40 ± 0.17	0.382
DL-Proline	0.11 ± 0.01	0.12 ± 0.01	0.23 ± 0.07	0.20 ± 0.03	0.131

Winkler (1974) observed that a disturbance of the C/N balance in grapevines would have negative effects on vine growth and productivity. Poor vegetative growth and limited fruit bud formation are often associated with vines showing high carbon and low nitrogen tissue content that is typically found when grown in soils with low nitrogen content. In the current study, cultural treatments and low soil moisture conditions significantly decreased the N concentration of plant tissues without affecting the C concentration (Table 16), thereby increasing the C/N ratio and affecting spring shoot growth. This increase in the C/N ratio was also found in the commercial environment where characteristic symptoms of RSG were found (Table 17).

Table 16 Total N and C (% dry wt) concentration of young leaves and shoots of Alverstoke Cabernet Sauvignon in the spring of 2003. DN = vines were minimally pruned and received fertilizer, additional water and 80% defoliation before winter; DND= vines received the same treatments as DN soil was kept dry by plastic covering. DNDR= vines received the same treatments as DND + roots were pruned. Control received normal irrigation and was spur pruned (means n = 5 ± s.e.; means with different letters are significantly different (P<0.05)).

	Control	DN	DND	DNDR	P
N%	$4.59^{a} \pm 0.07$	$4.31^{b} \pm 0.09$	$4.14^{b} \pm 0.03$	$3.81^{c} \pm 0.05$	**0.0001**
C%	$45.67^{a} \pm 0.18$	$44.34^{a} \pm 1.19$	$44.80^{a} \pm 0.27$	$45.05^{a} \pm 0.38$	0.5412
C/N ratio	$9.97^{c} \pm 0.12$	$10.28^{c} \pm 0.18$	$10.81^{b} \pm 0.03$	$11.83^{a} \pm 0.13$	**0.0001**

Table 17 N and C concentration (% dry wt) and the C/N ratio of leaves, permanent wood and berries in field-grown Chardonnay in Waikerie in the spring of 2002. (means n=3 ± s.e.).

Leaves	Normal	RSG symptom	% Diff	P
N	2.45 ± 0.06	2.17 ± 0.04	- 11	0.099
C	42.92 ± 0.14	42.57 ± 0.32	- 1	0.470
C/N ratio	17.56 ± 0.40	19.64 ± 0.32	+ 12	0.091
Permanent wood	Control	RSG	% Diff	P
N	1.11 ± 0.13	1.07 ± 0.07	- 4	0.847
C	40.05 ± 0.17	40.04 ± 0.14	0	0.970
C/N ratio	36.99 ± 3.88	37.85 ± 2.66	+ 2	0.906
Berries	Control	RSG	% Diff	P
N	2.43 ± 0.07	2.26 ± 0.05	- 7	0.006
C	44.25 ± 0.12	43.96 ± 0.20	- 1	0.314
C/N ratio	18.21 ± 0.50	19.46 ± 0.37	+ 7	0.010

In comparison, characteristic symptoms of RSG such as delayed budburst, reduced spring growth and the incidence of water shoots could be induced by low temperature treatments of young pot-

grown Chardonnay vines. Low temperature treatments of shoots and trunks upset the vine nutritional balance by decreasing the C/N ratios in shoot tissues due to a significant decrease in C concentration compared to control. High N and moderate C concentration tends to increase growth in grapevines (Winkler *et al.*, 1974) and therefore in time, the shoot growth of treated vines that survived would have caught up to that of control vines. Whether or not the vines would set fruit however is not known and it would therefore be useful to repeat the experiment with older, fruiting vines in future research.

8 Outcomes/Conclusions

This study has broadened our knowledge of the physiological aspects of the PRD irrigation system. It was shown that not only does PRD affect the vegetative growth of grapevines, but also the biochemical processes that influence the assimilation and partitioning of C and N. Enough evidence was collected to conclude that PRD partitioned nutrients and dry matter away from the vegetative structure towards the permanent structure in order to explore the soil environment by investing C and N into root growth and osmoregulation. Consequently, PRD vines would be more tolerant of stressful environments, such as transient periods of drought. However in the current study, it was shown in labelled nitrogen experiments that the amount of nitrogen absorbed during the season would be less in PRD-treated grapevines. This may be due to the reduced growth rate and lowered NR activity in leaves. It is possible that the fertilizater needs of PRD vines may be lower than those irrigated in a normal manner which would mean a saving in the production costs. A smaller area of soil wetted with PRD may also mean that fertilizers would be lost more slowly than control vines.

One of the main questions that has been asked of the PRD irrigation system is why an alteration of wetting pattern is necessary. Would a simple reduction in irrigation water volume not have the same effect? The main differences that were encountered in the current study between PRD treatments that received half and the same volume of irrigation water as control respectively, are summarised in Table 18. This will help us understand the effects attributable to water volume as opposed to a ‘real’ PRD effect. Many of the vine characteristics remained unaffected by PRD treatment irrespective of the amount of irrigation water applied compared to control. This in itself seems to be significant, because sub-optimal soil moisture conditions would normally have a negative impact on vine physiology by reducing the photosynthetic apparatus and challenging the vine’s ability to assimilate carbon and nitrogen for growth. Rabe (1990) concluded in his review that water stress usually causes the accumulation of nitrogen containing compounds (NCCs) in plant tissues. The NCCs that most commonly accumulate in response to water stress are proline, glycine betaine and putrescine.

Table 18 Various physiological responses of grapevines on PRD treatment (% change compared to control). (n.s. = not significant)

Response	PRD gets 50% water of control	PRD gets 100% water of control
Shoot growth rate (Main)	Reduced by 12% to 34%	Reduced by 8% to 18%
Shoot growth rate (Lateral)	Reduced by 22% to 74%	Reduced by 16% to 19%
Winter pruning weight	Increased by 5% (n.s.)	Increased by 10% (n.s.)
Winter shoot weight	Reduced by 4% (n.s.)	Reduced by 2% (n.s.)
Harvest leaf area (canopy size)	Reduced by 26%	Reduced by 30%
Light penetration into the bunch zone	Increased by 11%	Increased by 21%
Stomatal conductance	Reduced by 22% to 41%	Reduced by 7% to 16%
Leaf water potential	Reduced by 8% (n.s.)	Increased by 1% (n.s.)
Leaf starch	Reduced by up to 40%	?
Leaf sugars and amino acids	No significant difference	No significant difference
Shoot sugars and amino acids	No significant difference	No significant difference
Berry and leaf polyamines	No significant difference	No significant difference
Shoot polyamines	Increased putrescine	No difference
Yield	Reduced by 5% (n.s.)	Increased by 4% (n.s.)
Berry weight/size	Reduced by 8%	No significant difference
Berry TSS	Increased by 1% (n.s.)	Increased by 1% (n.s.)
Berry hexoses conc.	No significant difference	No significant difference
Hexoses/berry	Reduced by 8 – 10%	Increased by 9% (n.s.)
Berry pH	Increased by 0.3% (n.s.)	Reduced by 1.4% (n.s.)
Berry total amino acid conc.	Increased by 8 – 30%	Reduced by 3 – 13%
Berry K concentration	No significant difference	No significant difference
K/berry	Reduced by 7%	No significant difference
WUE (t/100mm)	Increased by 77 - 106%	Increased by 4% (n.s.)
Berry N/C ratio	Increased by 16.8%	Reduced by 4% (n.s.)
Leaf N/C ratio	Reduced by 0.4 % (n.s.)	Reduced by 0.3% (n.s.)
Shoot N/C ratio	Reduced by 4% (n.s.)	Increased by 4% (n.s.)
Berry NR activity	Reduced by 11% (n.s.)	?
Berry AI activity	Reduced 12 - 32%	Increased by 21%
Berry NI activity	Increased by 146%	Increased by 116%
Berry SucSy activity	Reduced 24 - 48%	Increased by 50%
Leaf NR activity	Decreased up to 29%	Decreased up to 9%
Leaf SPS activity	Transient inc. up to 48%	Transient inc. up to 30%
Leaf NI activity	Reduced by 57%	?
Leaf AI activity	Reduced by 2% (n.s.)	?
Leaf SucSy activity	Increased by 9% (n.s.)	?

However, PRD-treated vines in the current study showed no reductions in tissue sugars and amino acids or accumulations in NCCs or polyamines compared to control, irrespective of the amount of irrigation water applied. This study and previous work by Stoll (2000) have shown that large changes in stomatal conductance had only minor effects on assimilation rate under PRD.

The higher water loss from fully open stomata without an increase in CO_2 assimilation could be considered a luxurious consumption and an inefficient use of water if no other processes were affected. A reduction in stomatal aperture without a concurrent reduction in assimilation rate would greatly increase the grapevine's water use efficiency. Furthermore, it may be possible that the transient increases found in leaf SPS in response to PRD were caused by an ABA-induced effect to improve the photosynthetic capacity in response to soil drying conditions.

The reduction in shoot growth, accumulation of dry matter, stomatal conductance and NR activity are common to the two different rates of irrigation, and similar to many earlier reports (Loveys *et al.*, 2000; Stoll *et al.*, 2000b; de Souza *et al.*, 2003; dos Santos *et al.*, 2003), it is concluded that PRD is mainly responsible for the effect on vegetative growth. Canopies with lower leaf area become more open, resulting in higher light intensities inside the canopy compared to canopies with a larger leaf area.

PRD treatment in our experiments had no significant effect on either yield or fruit quality. However increased light penetration into the fruiting zone may have beneficial effects for berry quality. PRD-treated vines that received half the volume of irrigation water as control had, on occasion, smaller berry sizes that resulted in higher amino acid concentrations and lower K/berry. This may have positive ramifications for the vinification process and the quality of the wine. Lowered amount of K would prevent high pH due to the precipitation of tartrate in salt form during vinification and also enhance colour stability during storage (Mpelasoka *et al.*, 2003). Stines *et al.* (2000) reported that both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine. The evaluation of individual amino acids on wine quality is however a highly specialised area of research that falls beyond the scope of the current study. However, this may be an area of future research.

Results obtained from RSG field and pot experiments indicate that cultural practices may have a significant effect on the C/N balance in young developing grapevines that would significantly reduce spring growth compared to control vines. It is advisable that producers be wary of overcropping young vines and to maintain an adequate irrigation schedule during winters of low monthly rainfall. Ensuring adequate starch reserves before the onset of winter may be vital in the

survival of above-ground plant organs during winter, however soil moisture may also reduce the effect of sudden temperature changes on tissue damage.

9 Recommendations

It has been shown, in the current study, that PRD reduced shoot growth by its inhibitory effects on NR activity in grapevine shoots. However, the principle factor responsible for the reduction in NR activity has not been clearly elucidated. The possibility exists that in our experiments there was a slight recovery in cytokinin production before the switch in PRD irrigation that might have caused an increase in leaf NR activity. On the other hand, soil drying under PRD conditions may have influenced the amount of nitrate absorbed and delivered to shoots. Both explanations are supported by experimental results in the current study and by earlier CK work that was done by Stoll (2000). Further experimentation that should include the manipulation of CK levels and resultant effect on leaf NR activity under PRD conditions. Furthermore, the analysis of PRD treatment on the concentration of NO_3^- in the xylem sap of field-grown grapevines should be done on a larger scale over a longer duration.

The fertilizer needs of PRD vines during the pre-veraison and post-harvest period is still unknown and is an area of further research. In future investigations into the effect of PRD on the partitioning of C and N, a larger population of fruiting vines may be used for more detailed total C and N analyses. We started this experiment in 2002 with 80 split-rooted Cabernet Sauvignon, but unfortunately the vines did not bear fruit. The effect of PRD treatment on the distribution of these macronutrients can then be elucidated by periodically harvesting smaller samples of vines throughout the growing season. Destructively harvesting vines and measuring all plant weights and nutrient status would show the detailed effect of PRD on the accumulation and partitioning of nutrients during specific periods in the growing season. This will allow a more detailed explanation of the effect of PRD on the growth and development of treated vines compared to control and a more comprehensive recommendation could be given on fertilizer needs.

It was concluded that PRD has the potential to reduce the K content of berries in situations of highly vigorous growth that resulted in dense canopies. This may be an area of future research, because the control vines in the current study had moderate shoot vigour. More significant results may be obtained by using excessively vigourous vines under PRD conditions.

Stines *et al.* (2000) reported that both the concentration and type of nitrogenous compounds present in musts can affect the rate of fermentation and the 'fermentation bouquet' of the resultant wine. PRD showed significant increases in some amino acids, while reducing others compared to control. There were also very different combinations of amino acids from year to year that may influence wine characteristics. The effect of different combinations and individual amino acids on wine quality may be an area of future research that may differentiate wines made from PRD grapevines from those of control vines.

We conclude that environmental factors and cultural practices may have a strong influence on the PRD effect on berry size and sink strength. Higher pruning levels that influence canopy architecture and increase sink demand from growing shoots have negative effects on berry growth. This may be an area of future research to determine the PRD effect under different conditions of site and cultural practices. PRD may possibly modulate rootstock and scion interactions to form unique interactions with the soil environment.

Cultural treatments on young Cabernet Sauvignon have shown that the C/N balance of grapevines can be easily upset. In addition, low temperature treatments decreased the C/N ratio, significantly delayed budbreak and reduced spring growth. Whether or not the vines would have set fruit is not known and it would therefore be useful to repeat the experiment with older, fruiting vines in future research. Field vines that showed retarded growth were associated with significantly lower K levels in xylem sap. Saayman (1983) warns that soils that are low in K and combined with inadequate irrigation may contribute to the occurrence of the growth disorder. In such cases, it may be useful to experiment with the use of foliar K_2SO_4 sprays on affected vines to ameliorate the vine K status. Saayman (1983) also found that affected vines had significantly higher N contents in leaves and shoots. If naturally occurring RSG symptoms could be found, it would be interesting to measure the types of nitrogenous compounds that accumulate. The accumulation of polyamines would be of great interest due to their hormone-like activities and anti-senescence effects.

Appendix 1: Communication

PhD thesis: "The effect of Partial Rootzone Drying on the partitioning of dry matter, carbon, nitrogen and inorganic ions of grapevines", University of Adelaide. Submitted July 2004.

Du Toit, P.G., Dry, P.R. and Loveys, B.R. (2003) A preliminary investigation on partial rootzone drying (PRD) effects on grapevine performance, nitrogen assimilation and berry composition. *S. Afr. J. Enol. Vitic.* 24 (2): 43-54

Du Toit, P.G., Dry, P.R. and Loveys, B.R. (2004) Partial rootzone drying (PRD): irrigation technique for sustainable viticulture and premium quality grapes. *Wineland.* April 2004: 84-87

Du Toit, P.G., Dry, P.R. and Loveys, B.R (2004) "Partial rootzone drying (PRD): strategic irrigation management as viticultural tool affecting plant physiology and berry quality". Paper presented at the Joint International Conference on Viticultural Zoning held in Cape Town, South Africa, November 2004.

Appendix 2: References

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Appendix 3: Staff

- Assoc. Prof. Peter Dry, University of Adelaide
- Affil. Prof. Brian Loveys, CSIRO, Plant Industry, Adelaide
- Suzanne du Toit who contributed with casual work.
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